

# American College of Veterinary Internal Medicine



Research Abstract Program of the 19<sup>th</sup> Annual ACVIM Forum  
Denver, CO, May 23-26, 2001

## Index of Abstracts

### ORAL PRESENTATIONS – Wednesday, May 23

Time	#	Presenting Author	Abstract Title
<b>SMALL ANIMAL – GASTROENTEROLOGY</b>			
2:00 p.m.	1	Matthew Krecic	Comparison of 24-Hour 2-Sugar, 4-Sugar, and 5-Sugar Gastrointestinal Permeability and Mucosal Function Testing in Healthy Cats
2:15 p.m.	2	Nashwa E. Waly	Epithelial Expression of Major Histocompatibility Complex Class II in Cats with Inflammatory Bowel Disease
2:30 p.m.	3	G.M. Rutz	Antibodies Against Bovine Serum Albumin (BSA) in Serum from Cats with Gastrointestinal Disease
2:45 p.m.	4	Craig Ruaux	Biochemical Markers of Cobalamin Deficiency Accompanying Severe Hypocobalaminemia in the Cat
3:00 p.m.	5	Andrea Grieter	Quantitative Evaluation of Proinflammatory Cytokine Upregulation in the Stomachs of <i>Helicobacter Pylori</i> Infected Cats
3:15 p.m.	6	Don Westfall	Evaluation of the Passage of Tablets and Capsules Through the Esophagus in Cats
3:30 p.m.	7	Christopher Jones	<i>In vivo</i> Effect of a COX-2 Selective and Nonselective Nonsteroidal Antiinflammatory Drug (NSAID) on Gastric Mucosal and Synovial Fluid Prostaglandin Synthesis in Dogs
3:45 p.m. – 4:15 p.m.		BREAK	
4:15 p.m.	8	Jan S. Suchodolski	Serum $\alpha$ 1-Proteinase Inhibitor/Typsin Complex as a Marker for Canine Pancreatitis
4:30 p.m.	9	Joerg Steiner	Serum Canine Pancreatic Lipase Immunoreactivity (cPLI) Concentrations in Dogs with Exocrine Pancreatic Insufficiency (EPI)
4:45 p.m.	10	Joerg Steiner	Serum Canine Pancreatic Lipase Immunoreactivity (cPLI) Concentrations in Dogs with Spontaneous Pancreatitis
5:00 p.m.	11	Michael Leib	Treatment of <i>Helicobacter Spp.</i> in Dogs with Chronic Vomiting
5:15 p.m.	12	Clive Elwood	Expression of Gluten Sensitive Enteropathy [GSE] of Irish Setter Dogs May Be Age Dependent
5:30 p.m.	13	Bert Mohr	Effect of Early Enteral Nutrition On Intestinal Permeability, Intestinal Protein Loss, and Outcome in Dogs with Parvovirus
5:45 p.m.	14	Albert Jergens	Flow Cytometric Analysis of Mucosal Lymphocytes in Dogs with Inflammatory Bowel Disease

**Boldface type** indicates presentation by graduate student or resident

\*Presentation times are subject to change

**SMALL ANIMAL – HEMATOLOGY**

- 2:00 p.m. 15 Polly Foureman Canine Leukocyte Adhesion Deficiency: Presence of a Cys36Ser Missense Mutation in the  $\beta 2$  Integrin Gene in an Affected Irish Setter from the US and in Irish Red and White Setters
- 2:15 p.m. 16 Mary Beth Callan Correction of Canine Phosphofructokinase Deficiency by Bone Marrow Transplantation

**SMALL ANIMAL – INFECTIOUS DISEASE**

- 2:30 p.m. 17 Cynda Crawford FeLV, FIV, and FIP: Epidemiology and Clinical Disease Syndromes
- 2:45 p.m. 18 Edward Breitschwerdt Molecular Evidence of *Ehrlichia Canis* Infection in Cats from North America
- 3:00 p.m. 19 Tommaso Furlanello Concurrent Coinfections in Dogs Detected By Serology During A Survey For *Rickettsia Rickettsii*: Results From 1093 Serum Samples Collected in Italy
- 3:15 p.m. 20 Douglass Macintire *Babesia Gibsoni* Infections in Dogs from Alabama and Georgia
- 3:30 p.m. 21 Liesel van der Merwe Opportunistic Disseminated Mycoses in Dogs: 1993 - 2000
- 3:45 p.m. – 4:15 p.m. BREAK
- 4:15 p.m. 22 Valeria Scorza Detection of *Cryptosporidium Parvum* in Feline Feces Using A Polymerase Chain Reaction Assay
- 4:30 p.m. 23 R. Dino Rulli Safety of Repeated Treatments with Moxidectin Canine SR (Sustained Release) Injectable

**SMALL ANIMAL - RESPIRATORY**

- 4:45 p.m. 24 Lynelle Johnson Clinicopathologic and Radiographic Findings in 24 Dogs with Coccidioidomycosis: 1995-2000
- 5:00 p.m. 25 Carol Norris Cytokine Profiles in Peripheral Blood Mononuclear Cells and Bronchoalveolar Lavage Cells in Cats with Experimental Feline Asthma
- 5:15 p.m. 26 Claire Weigand Effects of Sublethal Endotoxin Administration on Phagocytosis and Pathology in Feline Lung and Liver
- 5:30 p.m. 27 Cécile Clercx Immunoglobulin Levels and Lymphocyte Subsets in Peripheral Blood and Bronchoalveolar Lavage Fluid in Dogs with Eosinophilic Bronchopneumopathy

**SMALL ANIMAL - THERIOGENIOLOGY**

- 5:45 p.m. 28 Brenda Griffin Effects of Melatonin Implants on Ovarian Function in the Domestic Cat

**SMALL ANIMAL - CARDIOLOGY**

- 2:00 p.m. 29 Eran Dvir The Effect of Electrocardiographic Filters on the R-Amplitude of Canine Electrocardiograms
- 2:15 p.m. 30 Sydney Moise Noise Reversion in Paced Dogs
- 2:30 p.m. 31 Philip Fox Clinical, Echocardiographic, and ECG Findings in 232 Sequentially Examined Irish Wolfhounds
- 2:45 p.m. 32 Lee Pyle Evaluation of Pulsed Wave Doppler Technique for Determining Cardiac Index in Normal and Pharmacologically Stressed Dogs
- 3:00 p.m. 33 Jelena Ristic A Retrospective Study of 26 Dogs with Pulmonic Stenosis
- 3:15 p.m. 34 Bradley A. Green Relationship Between Severity of Subvalvular Aortic Stenosis and Ventricular Ectopia in Young Newfoundland Dogs
- 3:30 p.m. 35 Kathy Wright Clinical Spectrum of Congenital Tricuspid Valve Malformations in an Extended Family of Labrador Retrievers
- 3:45 p.m. – 4:15 p.m. BREAK
- 4:15 p.m. 36 Matthias Schneider Echocardiographic Measurement of Patent Ductus Arteriosus
- 4:30 p.m. 37 Scott Brown Evaluation of Doppler Ultrasonic and Oscillometric Estimates of Blood Pressure in Cats
- 4:45 p.m. 38 Kathryn Meurs Myomesin, a Sarcomeric Protein, is Reduced in Main Coon Cats with Familial Hypertrophic Cardiomyopathy
- 5:00 p.m. 39 Tony Glaus Pulmonary Hypertension Associated with Hypoxemia Due to Residence in Moderate Altitude in Dogs
- 5:15 p.m. 40 Denise Schwartz Response to Active Head-Up Tilt During Progression of Rapid-Pacing Induced Heart Failure in Dogs
- 5:30 p.m. 41 Remo Lobetti Cardiac Troponins in Canine Babesiosis

- 5:45 p.m. 42 **Deborah M. Fine** **The pH of Pericardial Effusion Does Not Reliably Distinguish Between Idiopathic and Neoplastic Effusions**
- FOOD ANIMAL – INTERNAL MEDICINE**
- 2:00 p.m. 43 **Dusty Nagy** Colostral Immunoglobulin G Concentrations Vs. Subsequent Serum Immunoglobulin G Concentrations in Naturally Suckled Llama and Alpaca Crias
- 2:15 p.m. 44 **Ahmed Ahmed** **Effect of Cimetidine and Ranitidine on Abomasal pH in Milk Fed Calves**
- 2:30 p.m. 45 **Masie E. Dawes** **Effects of Supplemental Lactoferrin at Birth on Neutrophil Oxidative Metabolism, Serum IGG and Growth of Holstein Calves**
- 2:45 p.m. 46 **Nicole M. Holloway** **Serum Immunoglobulin G Concentrations in Calves Fed Fresh and Frozen Colostrum**
- 3:00 p.m. 47 **Gilles Fecteau** Bacteriological Contamination of Colostrum Fed to Newborn Calves in Quebec Dairy Herds
- 3:15 p.m. 48 **Susan Cushing** Biological Characteristics of Enteric and Respiratory Coronavirus in Dairy Calves
- 3:30 p.m. 49 **Daryl Nydam** **Total Number of Cryptosporidium Oocysts or Giardia Cysts Shed By Dairy Calves Following Natural Infection**
- 3:45 p.m. – 4:15 p.m. **BREAK**
- 4:15 p.m. 50 **Peter Constable** Clinical Assessment of Acid-Base Status in Adult Cattle: Calculation of Plasma Atot and Ka Values For Use in the Strong Ion and Simplified Strong Ion Models
- 4:30 p.m. 51 **Dawn Morin** Acid Base and Electrolyte Balance in Postparturient Holstein Cows with Experimentally Induced Primary Ketosis
- 4:45 p.m. 52 **Joachim Berchtold** Acid-Base, Cerebrospinal Fluid, and Cardiovascular Effects of Rapid Intravenous Hypertonic Sodium Bicarbonate Administration in Calves with Experimentally Induced Respiratory and Metabolic Acidosis
- 5:00 p.m. 53 **Gilles Fecteau** Cardiomyopathy in 7 Young Ayrshire Cattle; A New Clinical Entity?
- 5:15 p.m. 54 **Christopher Cebra** Effects of Exogenous Insulin On Glucose Tolerance in Alpacas
- \*Also see Food Animal – Internal Medicine abstract #88**
- EQUINE**
- 2:00 p.m. 57 **Jonathan Foreman** Clinical Progression of Neurological Signs and Cerebrospinal Fluid Values in Horses with Experimentally Induced Leukoencephalomalacia
- 2:15 p.m. 58 **Gillian Perkins** Plasma Adrenocorticotropin (ACTH) Concentrations and Clinical Response in Horses Treated For Equine Cushing's Disease with Cyproheptadine or Pergolide
- 2:30 p.m. 59 **Mary Durando** Correlation of Echocardiographic Stress-Tests with Left Ventricular Pressure Dynamics
- 2:45 p.m. 60 **Stephanie Valberg** Glucose Clearance in PSSM Horses Determined by Euglycemic Hyperinsulinemic Clamp
- 3:00 p.m. 61 **Erica McKenzie** **Serum CK Activity in Thoroughbred Horses with Recurrent Exertional Rhabdomyolysis Consuming Diets Varying in Fat, Starch and Bicarbonate Content**
- 3:15 p.m. 62 **Harold Schott** Ionized Calcium Concentration During Endurance Exercise
- 3:30 p.m. 63 **Eduard Jose-Cunilleras** **Pre-Exercise Feeding Alters Substrate Utilization and Glucose Flux in Exercising Horses**
- 3:45 p.m. – 4:15 p.m. **BREAK**
- 4:15 p.m. 64 **Thomas Goetz** Nasal Dilator Strips Do Not Affect Arterial Hypoxemia and Hypercapnia, Lactate and Ammonia Production, or the Occurrence of EIPH During Short-Term High-Intensity Exercise in Thoroughbred Horses
- 4:30 p.m. 65 **Clayton D. Chilcoat** **The Effect of cAMP Modulation on Equine Neutrophil Activation by Immune Complexes**
- 4:45 p.m. 66 **Peter Morresey** **Endotoxin-Neutralizing Activity in Blood After Intravenous Administration of Polymyxin B in the Horse**
- 5:00 p.m. 67 **Ray Geor** Effect of Corn Oil on Solid Phase Gastric Emptying in Horses
- 5:15 p.m. 68 **Rachel Dionne** **Prevalence of Gastric Ulcers in Standardbred Racehorses in Quebec**
- 5:30 p.m. 69 **Jenifer Nadeau** Volatile Fatty Acid Injury in the Nonglandular Region of the Equine Stomach: Implications in the Pathogenesis of Gastric Ulcer Disease
- 5:45 p.m. 70 **Rodney L. Belgrave** **Effects of Oxyglobin® Administred to Ponies with Normovolemic Anemia**

**SMALL ANIMAL - NEUROLOGY**

2:00 p.m.	71	Charles Vite	Myelin Abnormalities in Feline Alpha-Mannosidosis
2:15 p.m.	72	Dilip Bhalleran	Molecular Screening for Myotonia Congenita in Miniature Schnauzers
2:30 p.m.	73	John McDonnell	Is There a Myelopathy Associated with Feline Leukemia Virus?
2:45 p.m.	74	Urs Giger	Mucopolysaccharidosis Type IIIB (Sanfilippo B Syndrome) in Schipperke Dogs
3:00 p.m.	75	Boyd Jones	Muscular Dystrophy in Japanese Spitz Dogs
3:15 p.m.	76	Richard Goldstein	Effects of Short Term Steroid Administration on Acetylcholinesterase Activity in Healthy Dogs
3:30 p.m.	77	Richard Goldstein	Canine Acetylcholinesterase: Molecular and Functional Characterization

3:45 p.m. – 4:15 p.m. BREAK

4:15 p.m.	78	<b>Robert L. Bergman</b>	<b>Matrix Metalloproteinases in Normal Canine CSF</b>
4:30 p.m.	79	Dominik Faissler	Electroencephalic Findings in Ten Dogs with Hydrocephalus
4:45 p.m.	80	<b>Sonia Añor</b>	<b>Evaluation of Jitter by Stimulated Single Fiber EMG in Normal Dogs</b>

**SMALL ANIMAL - ONCOLOGY**

5:00 p.m.	81	Stuart Helfand	Malignant Cells from Dogs with Lymphoma and Leukemia Express Receptors for Interleukin-2
5:15 p.m.	82	Gregory MacEwen	Genetically Modified Salmonella for Canine Cancer: A Phase I Study
5:30 p.m.	83	Eric Hoots	Characterization of Genetic Alterations in Feline Vaccine-Associated Sarcoma Using Whole Chromosome Painting Probes
5:45 p.m.	84	Elizabeth McNeil	Characterization of Chromosomal Aberrations in Feline Vaccine-Associated Sarcoma Using Comparative Genomic Hybridization

**ORAL PRESENTATIONS – Thursday, May 24**

**EQUINE**

8:00 a.m.	85	<b>Adrienne P. Bentley</b>	<b>Antimicrobial-Induced Endotoxin and Cytokine Activity in an <i>In Vitro</i> Model of Foal Septicemia</b>
8:15 a.m.	86	<b>Allison Stewart</b>	<b><i>Actinobacillus Spp.</i> Bacteremia in Foals: Clinical Signs and Prognosis</b>
8:30 a.m.	87	<b>Shaun A McKane</b>	<b>Changes in Collagen Content and Morphometric Characteristics of the Equine Lung Following Intrapulmonary Blood Inoculation</b>
8:45 a.m.	88	<b>Marie-France Haddad</b>	<b>Effects of Intramammary Hypertonic Saline Infusion in Cows with Experimentally Induced Coliform Mastitis</b>
9:00 a.m.	89	<b>Vincent Gerber</b>	<b>Equine Mucin Genes: MUC 5AC but not MUC 2 is Expressed in Horse Airways</b>
9:15 a.m.	90	<b>Stephanie Jacks</b>	<b>Pharmacokinetics of Azithromycin in Foals and Concentration of the Drug in Serum, Body Fluids, and Bronchoalveolar Cells</b>
9:30 a.m.	91	<b>Jennifer L. Davis</b>	<b>Pharmacokinetics of Azithromycin in Foals</b>

9:45 a.m. – 10:45 a.m. BREAK

**SMALL ANIMAL - HEPATOLOGY**

10:45 a.m.	92	<b>Pedro Boria</b>	<b>Effect of S-Adenosyl Methionine on Bile Induced Apoptosis in MDCK-NTCP Cells</b>
11:00 a.m.	93	<b>Erik M. Moeller</b>	<b>Comparison of Different Doses of <sup>13</sup>C-aminopyrine for a <sup>13</sup>C-aminopyrine Demethylation Blood Test in Clinically Healthy Cats</b>
11:15 a.m.	94	Sharon Center	Copper, Iron, and Zinc Concentrations in Liver Tissue From Dogs with Various Liver Disorders
11:30 a.m.	95	Cynthia Gaskill	Alanine Aminotransferase and Alkaline Phosphatase Activity in Liver Tissue From Phenobarbital-Treated Epileptic Dogs
11:45 a.m.	96	Nicola Mason	The Use of Carboxy Fluorescein Diacetate Succinimidyl Ester (CFSE) to Determine the Proliferative Capacity of Lymphocyte Subsets in the Cat

**SMALL ANIMAL - TOXICOLOGY**

12:00 p.m.	97	Jill Richardson	Ingestion of Zolpidem in Dogs: 33 Cases (January 1998-July 2000)
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**SMALL ANIMAL - EDUCATION**

12:15 p.m.	98	Hylton Bark	Objective Structured Clinical Examination As a Component of the Small Animal Graduating Examination
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**SMALL ANIMAL - ENDOCRINOLOGY**

- 8:00 a.m. 99 Margarethe Hoenig Influence of Glucose Dosage On Interpretation of Intravenous Glucose Tolerance Tests in Lean and Obese Cats
- 8:15 a.m. 100 **Melanie D. Thompson** **Comparison of Blood Glucose Values in Blood Obtained Using a Marginal Ear Vein Lance Technique (capillary blood) Versus Peripheral Vein Collection in Healthy Cats and in Cats with Diabetes Mellitus**
- 8:30 a.m. 101 **Linda Fleeman** **Comparison of Serial Blood Glucose Curves Performed On Consecutive Days in Diabetic Dogs**
- 8:45 a.m. 102 Claudia Reusch Alteration in the Growth Hormone-Insulin-Like Growth Factor Axis in Cats with Diabetes Mellitus
- 9:00 a.m. 103 Deborah Greco Comparison of A Low Carbohydrate Versus High Fiber Diet in Cats with Diabetes Mellitus
- 9:15 a.m. 104 **Catharina Broemel** **Ultrasonographic Evaluation of the Thyroid Gland in Golden Retrievers**
- 9:30 a.m. 105 Peter Graham A 12-Month Prospective Study of 234 Thyroglobulin Antibody Positive Dogs Which Had No Laboratory Evidence of Thyroid Dysfunction

9:45 a.m. – 10:45 a.m. BREAK

- 10:45 a.m. 106 Ian Ramsey Increased Parathyroid Hormone Concentrations in Dogs with Hyperadrenocorticism
- 11:00 a.m. 107 Michael Herrtage Plasma 17-Hydroxyprogesterone Concentrations in the Diagnosis of Canine Hyperadrenocorticism
- 11:15 a.m. 108 Cynthia Ward The Inhibitory G Protein, G<sub>i2</sub> Shows Decreased Expression in Adenomatous Thyroid Tissue from Hyperthyroid Cats
- 11:30 a.m. 109 **Kamil Tomsa** **<sup>99m</sup>Tc-Perthchnetate Scintigraphy in Hyperthyroid Cats with Normal Serum Thyroxine Concentrations**
- 11:45 a.m. 110 Harriet Syme Evaluation of Proteinuria in Hyperthyroid Cats
- 12:00 p.m. 111 **Stacey B. Hoffman** **Evaluation of Dosing Regimen in Hyperthyroid Cats Treated with Metrimazole**
- 12:15 p.m. 112 **Gaby Hoffmann** **Topical Methimazole Treatment in Cats with Hyperthyroidism**

12:30 p.m. – 2:00 p.m. LUNCH

**SMALL ANIMAL - NEPHROLOGY**

- 2:00 p.m. 113 Wayne Jensen Prevalence of Microalbuminuria in Dogs
- 2:15 p.m. 114 Barrak Pressler Prevalence of Microalbuminuria in Dogs Evaluated at a Referral Veterinary Hospital
- 2:30 p.m. 115 Shelly Vaden Longitudinal Study of Microalbuminuria in Soft-Coated Wheaten Terriers
- 2:45 p.m. 116 Abigail Stevenson The Effect of Dietary Sodium on Urine Composition of Healthy Miniature Schnauzers
- 3:00 p.m. 117 Vincent C. Biourge Increased Dietary NaCl Significantly Increases Urine Volume But Does Not Increase Urinary Calcium Oxalate Relative Supersaturation in Healthy Cats
- 3:15 p.m. 118 **C. Lekcharoensuk** **Canine Calcium Oxalate Urolithiasis: Epidemiological Association Between Dietary Calcium and Risk of Urolith Formation**
- 3:30 p.m. 119 Brigitte Smith *Ex Vivo* Canine Struvite Stone Dissolution

3:45 p.m. – 4:15 p.m. BREAK

- 4:15 p.m. 120 Larry Cowgill Changes in Blood, ECF & ICF Volumes in Response to Fluid and Lasix Administration to Normal Dogs
- 4:30 p.m. 121 **Thierry Francey** **Assessment of Electrical Properties in Normal and Uremic Dogs by Multi-frequency Bioimpedance Spectroscopy**
- 4:45 p.m. 122 **Paige Langdon** **Effect of Sedation with Ketamine/Diazepam or Propofol On Measurement of Glomerular Filtration Rate By Renal Scintigraphy in Cats**
- 5:00 p.m. 123 Goy-Thollot Isabelle Estimation of Glomerular Filtration Rate (GFR) Using Plasma Clearance of Iohexol: Establishing Normal Values in Dogs
- 5:15 p.m. 124 Harriet Syme Feline Plasma Pro-Atrial Natriuretic Peptide Measurement: Correlation with Systolic Blood Pressure and Renal Function
- 5:30 p.m. 125 John Kruger Clinical Trial of Amitriptyline For Management of Acute Nonobstructive Feline Idiopathic Lower Urinary Tract Disease
- 5:45 p.m. 126 **Laurie J. Blanco** **Evaluation of Blood Agar Plates As A Transport Medium For Aerobic Bacterial Urine Cultures**

## ORAL PRESENTATIONS – Saturday, May 26

### SMALL ANIMAL – PHARMACOLOGY

8:00 a.m.	127	Mary Boy	Clinical Pathology Results in Dogs Administered Carprofen (Rimadyl®) Perioperatively
8:15 a.m.	128	Terrence Clark	The Steady-State Pharmacokinetics of Carprofen (Rimadyl®) Administered Orally and Subcutaneously in Dogs
8:30 a.m.	129	Linda Kidd	Variability in Azathioprine Metabolism in Dogs
8:45 a.m.	130	Curtis Dewey	Pharmacokinetics of Single-Dose Oral and Intravenous Mycophenolate Mofetil Administration in Normal Dogs
9:00 a.m.	131	Heather Streppa	Novel <i>In Vitro</i> Assessment of Cyclooxygenase (COX) Selectivity of Nonsteroidal Antiinflammatory Drugs (NSAID) in Canine Whole Blood
9:15 a.m.	132	Eran Lavy	Evaluation of A Novel Sustained Release Gastroretentive Dosage Form of Riboflavin in Dogs
9:30 a.m.	133	John Huhn	The Effect of Injection Volume On the Pharmacokinetics of Carprofen (Rimadyl®) Administered Subcutaneously in Dogs

### SMALL ANIMAL – NUTRITION/METABOLISM

8:00 a.m.	134	Daniel Gingerich	Milk Protein Concentrate From Hyperimmunized Cows Expresses Anti-Inflammatory Activity and Clinical Utility in Osteoarthritis
8:15 a.m.	135	Brigitte Smith	Longterm Feeding of an Acidifying Diet to Cats: Effect on Bone Density
8:30 a.m.	136	John E. Bauer	<i>In Vitro</i> Alteration of Canine Neutrophil Membrane Fatty Acid Composition, Fluidity, and Function: A Model for Dietary Intervention
8:45 a.m.	137	Gary Davenport	Effect of Dietary Protein On Body Composition and Metabolic Responses of Geriatric and Young-Adult Dogs
9:00 a.m.	138	Lynn Guptill	Effects of Dietary Oxidized Lipids On Growth and Immune Function of Young Specific Pathogen-Free Cats

## POSTER PRESENTATIONS

**On Display:** Wednesday, May 23, 2:00 p.m. – 10:00 p.m.; Thursday & Friday, May 24 & 25, 7:00 a.m. – 10:00 p.m.; Saturday, May 26, 7:00 a.m. – 5:00 p.m.

**Attended by Author:** Thursday, May 24, 6:00 p.m. – 7:00 p.m.; Friday, May 25, 3:45 p.m. – 4:15 p.m.

#	Presenting Author	Abstract Title
140	Jennifer Steele	Effects of ACE-Inhibition on the renin-Angiotensin-Aldosterone System and Blood Pressure in Cats with Hypertension Associated with Chronic Renal Disease
141	Scott Brown	Evaluation of Doppler Ultrasonic and Oscillometric Estimates of Blood Pressure in Conscious Dogs
142	Alan W. Spier	Signal-Averaged ECG in the Assessment of Arrhythmogenic Cardiomyopathy in Boxers
143	Alexander Biondo	Comparative Sequence of Feline Endothelin-1 (ET-1)
144	Jonathan Abbott	Aortic Valve Disease in Boxers with Physical and Echocardiographic Findings of Aortic Stenosis
145	Greg J. Ferguson	Severe Hypertrophic Cardiomyopathy in 10 Young Ragdoll Cats
146	Eran Dvir	Cardiac Histopathologic and Electorcardiographic Changes in Canine Babesiosis
147	Gaby Hoffmann	Measurement of Corticoid Concentrations in Non-extracted Canine Urine by Chemiluminescence
148	Ray Nachreiner	Comparison of Blood Spot and Serum Samples to Detect TgAA and Assessment of Heredity of Thyroiditis in Welsh Springer Spaniels
149	Leah Cohn	Comparison of Glucose Measuring Devices in Cats
150	William Monroe	Efficacy and Safety of Caninsulin®, Purified Porcine Insulin for Treating Diabetic Dogs
151	Tracey Gillespie	Evaluation of Total and Ionized Serum Magnesium and Calcium Concentrations in Acidotic and Non-Acidotic Diabetic Dogs
152	Shannon Parsons	Endogenous Serum Insulin Concentration in Dogs with Diabetic Ketoacidosis
153	Ray Nachreiner	Comparison of Protease Inhibitors for Stabilizing Parathyroid Hormone and Free T4 by Dialysis in Serum Samples

- 154 Craig Ruaux Development and Validation of Assays for Compounds in Feline Serum Relevant to Single Carbon Metabolism
- 155 Craig Ruaux Postprandial Alterations in Serum Unconjugated Bile and Concentrations in Normal Dogs
- 156 **Gabriele M. Rutz** **Oral Bleeding Due to Pancreatic Extract in Three Dogs with Exocrine Pancreatic Insufficiency**
- 157 Joerg Steiner Development and Validation of an Enzyme-linked Immunosorbent Assay (ELISA) for the Measurement of Canine Pancreatic Lipase Immunoreactivity (cPLI) in Serum
- 158 Joerg Steiner Serum Canine Pancreatic Lipase Immunoreactivity (cPLI) in Dogs with Experimentally Induced Chronic Renal Failure
- 159 Joerg Steiner Determination of a Control Range for a 5-Sugar Gastrointestinal Permeability and Mucosal Function Test in Clinically Healthy Dogs
- 160 **Jan S. Suchodolski** **Development and Validation of an Enzyme-linked Immunosorbent Assay for Measurement of  $\alpha$ 1-Proteinase Inhibitor/Trypsin Complexes in Canine Sera**
- 161 Sheila Gummingier Role of Apoptosis in Pancreatic Acinar Atrophy in Dogs
- 162 **Dalia Strauss-Ayali** ***Helicobacter Spp.* Infection in Cats: Evaluation of the Humoral Immune Response and Prevalence of Gastric *Helicobacter Spp.***
- 163 Vivien Rolfe Systemic Changes During Canine Non-Specific Dietary Sensitivity
- 164 Eric Schrauwen Anal Cutaneous Lupus Erythematosus as a Cause of Chronic Severe Dyschezia in the Dog
- 165 Jamie Burkitt Intestinal Intussusception In Twenty Cats
- 166 **Tonatiuh Melgarejo** **Erythromycin Stimulates Contractions of Canine, but Not Feline, Longitudinal Colonic Smooth Muscle**
- 167 **Matthew Krecic** **Kinetics of Urinary Recovery of Five Orally Administered Sugars in Healthy Cats**
- 168 Anthony Carr Hemostatic Parameters in 7 Dogs with a Protein Losing Enteropathy
- 169 **Erik M. Moeller** **Comparison of Different Doses of  $^{13}\text{C}$ -aminopyrine for a  $^{13}\text{C}$ -aminopyrine Demethylation Blood Test in Clinically Healthy Dogs**
- 170 **Erik M. Moeller** **Kinetics of  $^{13}\text{C}$ -aminopyrine Demethylation in Clinically Healthy Cats**
- 171 Sharon Center Urine Sulfated Bile Acids in Cats with and without Liver Disease
- 172 **Kerry Heuter** **Leptin as a Marker for the Development of Hepatic Lipidosis in Cats**
- 173 Jody Gookin *Trichomonas foetus* and not *Pentatrichomonas hominis* is the Causative Agent of the Recently Described Feline Intestinal Trichomonosis
- 174 **Jennifer E. Stokes** **Prevalence of Serum Antibodies to Six *Leptospira* Interrogans Serovars in Asymptomatic Dogs from the Lower Peninsula of Michigan**
- 175 Amy Grooters Production of Polyclonal Antibodies for the Immuno-Histochemical Identification of *Pythium Insidiosum*
- 176 Helio de Moraes Detection of Canine Distemper Virus by RT-PCR in Serum, Leukocytes, Cerebro-Spinal Fluid (CSF), and Urine in Dogs with Distemper Encephalitis: 11 Cases
- 177 Abigail Stevenson A Comparison of Urine Composition of Labrador Retrievers and Cairn Terriers
- 178 Barrak Pressler Detection of Canine Microalbuminuria Using Semi-Quantitative Test Strips Designed for Use in Human Urine
- 179 Rosama Pusoonthornthum The Possible Role of Urolith-Induced Feline Lower Urinary Tract Disease in Thailand: An Asian Point of View
- 180 John Kruger Genetic Characterization of a Feline Calicivirus Isolated from Urine Obtained from a Cat with Obstructive Idiopathic Lower Urinary Tract Disease
- 181 Alain Le Garrères Comparison of Plasma Clearances of Creatinine and Iohexol for Glomerular Filtration Rate Assessment in Cats
- 182 Valerie Chetboul Comparison of Surgical Methods for Gene Delivery into Canine Kidneys
- 183 Todd Axlund Hypophysectomy in Dogs Using an Ultrasonic Aspirator and a New Surgical Approach
- 184 **William Bush** **Age, Neurological Examination, and Cerebrospinal Fluid Analysis as Predictions of Outcome of MRI Scanning in 115 Dogs with Seizures**
- 185 **Thomas Flegel** **Computed Tomographic Imaging Guided Brain Biopsy Using a Disposable Real-time Stereotactic System**
- 186 **Marguerite Knipe** **Intervertebral Disc Extrusion In Six Cats**
- 187 Miyoka Saito Status Epilepticus in Dogs with Idiopathic Epilepsy: A Long-Term Evaluation of 32 Cases
- 188 Erin Streiff **A Comparison of the Nutrition of Home-Prepared and Commercial Diets for Dogs**
- 189 **Brent Dunbar** **Characterisation of  $\Delta$ -6-Desaturase Activity in Dog Liver Microsomes**
- 190 **Gabriele M. Rutz** **The Effect of Dietary Medium Chain Triglycerides on Dogs with Exocrine Pancreatic Insufficiency**
- 192 **Mary Alexis Seguin** **Pharmacokinetics of Enrofloxacin, a Fluoroquinolone Antibiotic, in Neonatal Kittens**

- 193 **Julia K. Veir** **Feline Nasopharyngeal Polyps: Historical, Clinical, and PCR Findings for Feline Calici Virus and Feline Herpes Virus-1 In 21 Cases**
- 194 **John E. Stein** **Bacterial Culture Results in Cats with Upper and Lower Airway Disease: 255 Cases (1995-1999)**
- 195 Brenda Griffin A Model for Non-invasive Monitoring of Ovarian Function in the Domestic Cat
- 196 Brenda Griffin Lessons in Reproductive Physiology and Behavior in Domestic Queens from Non-invasive Monitoring
- 197 Brenda Griffin Endogenous Melatonin Concentrations in the Domestic Cat
- 198 Francesco Porciello Relationship Between Racing Performance and Echocardiographic Measurements Before and After Training in Thoroughbred Horses
- 199 Margaret Sleeper Chronic Clenbuterol Administration Negatively Alters Cardiac Function in Horses
- 200 Daniel Jean Myocardial Disease in Horses: Retrospective Study in 25 Cases (1998-2000)
- 201 Mary Durando Effect of Cardiac Catheterization on cTNI And CK-MB in Exercising Horses
- 202 Catherine McGowan Efficacy of Trilostane in the Management of Equine Cushing's Disease
- 203 **Nigel Campbell** **The Role of NSAIDs in Recovery of Bile-Injured Equine Colon**
- 204 Gillian Perkins Serum IgM Levels in Healthy and Sick Adult Horses
- 205 Melissa Hines Flow Cytometric Detection of Intracellular Interferon-Gamma in Normal Horses and in Horses Challenged with *Rhodococcus Equi*
- 206 Debra Ruffin Methicillin-Resistant Staphylococcus Aureus Outbreak in a Veterinary Teaching Hospital
- 207 **Geof Smith** **Cardiovascular Dysfunction is Present in Horses with Experimentally Induced Leukoencephalomalacia**
- 208 Sally Vivrette Cardiorespiratory and Thermoregulatory Effects of Endophyte-Infected Fescue in Exercising Horses
- 209 Harold C. Schott Assessment of Changes in Body Fluid Volumes During Dehydration and Rehydration Using Bioelectrical Impedance Measures
- 210 **Lisa Katz** **Vasodilator Responses of Equine Digital Blood Vessels to Peptide Neurotransmitters**
- 211 Sarah Gardner Characterization of the Pharmacodynamic Properties of the Angiotensin-Converting Enzyme Inhibitor, Enalapril, in Horses
- 212 Simon Bailey Amines from the Equine Hindgut May Cause Digital Vasoconstriction by Direct or Indirect Mechanisms
- 213 Gabriela Stark Clinical Efficacy of a Leukotriene Receptor Antagonist (Montelukast) in Equine Chronic Obstructive Pulmonary Disease (COPD): A Pilot Study
- 214 **Geof Smith** **Atypical Parapoxvirus Infections in Sheep**
- 215 **Christie Malazdrewich** **Pharmacological Inhibition of Inflammatory Cytokine Production by Bovine Alveolar Macrophages**
- 216 **Christie Malazdrewich** **Pulmonary Expression of Inflammatory Cytokines in Experimental Bovine Pneumonic Pasteurellosis**



- 1** COMPARISON OF 24-HOUR 2-SUGAR, 4-SUGAR, AND 5-SUGAR GASTROINTESTINAL PERMEABILITY AND MUCOSAL FUNCTION TESTING IN HEALTHY CATS. M.R. Krecic,\* J.M. Steiner,<sup>†</sup> M.R. Kern,\* J.R. Cardwell,<sup>†</sup> and D.A. Williams.\*  
\*College of Veterinary Medicine, Mississippi State University, Mississippi State, MS; <sup>†</sup>GI Laboratory, Texas A&M University, College Station, TX.

Gastrointestinal permeability and mucosal function testing is a sensitive method for evaluating gastrointestinal integrity and function. Testing consists of oral administration of a sugar solution and determination of the percent urinary recovery of the sugars administered. The goal of this project was to evaluate whether the simultaneous use of 2, 4, or 5 different sugars would lead to alterations in the urinary recovery of any one of the other sugars within a 24-hour sampling period.

Ten clinically healthy male experimental cats were evaluated. An indwelling urethral catheter was placed in each cat. Cats received 30 ml of an isotonic solution of 2 sugars (1.0 g lactulose [L] and 1.0 g rhamnose [R]), 60 ml of 4 sugars (1.0 g L, 1.0 g R, 0.5 g methylglucose [M], and 1.0 g xylose [X]), or 100 ml of 5 sugars (1.0 g L, 1.0 g R, 0.5 g M, 1.0 g X, and 4.0 g sucrose [S]) via nasoesophageal tubes. Urine samples were collected aseptically from a closed urinary collection system prior to administration of the solution, every 2 hours for 12 hours, and again at 24 hours. Total urine volume was recorded for each time-point. Urinary recoveries of the sugars were determined by high-pressure anion exchange liquid chromatography with pulsed amperometric detection. Mean cumulative urinary recovery for each sugar was calculated and compared.

Cumulative 24-hour urinary recovery for S in the 5-sugar test was  $1.2 \pm 0.7$  (mean  $\pm$  SD). Mean cumulative recovery of L was not significantly different between the 2- ( $3.2 \pm 1.8$ ), 4- ( $5.0 \pm 5.3$ ), and 5- ( $3.0 \pm 1.6$ ) sugar tests ( $p = 0.21$ ). Mean cumulative urinary recovery of R was significantly different between the 2- ( $11.6 \pm 4.5$ ), 4- ( $14.7 \pm 5.4$ ), and 5- ( $8.9 \pm 3.6$ ) sugar tests ( $p = 0.0016$ ). Mean cumulative urinary recoveries of M ( $p = 0.07$ ) and X ( $p = 0.16$ ) were not significantly different between the 4- ( $42.3 \pm 14.5$  and  $23.4 \pm 9.8$ , respectively) and 5- ( $32.0 \pm 11.0$  and  $18.8 \pm 7.9$ , respectively) sugar tests.

We conclude that in healthy cats intestinal permeability of L and R within 24 hours of oral application is unaffected and affected, respectively, by concurrent administration of M, X, and S. Mucosal absorption of M and X within 24 hours of oral application to healthy cats is unaffected by concurrent administration of S. Different control ranges for 24-hour urinary recovery of R for the 2-, 4-, and 5-sugar tests may need to be established for cats.

- 2** EPITHELIAL EXPRESSION OF MAJOR HISTO-COMPATIBILITY COMPLEX CLASS II IN CATS WITH INFLAMMATORY BOWEL DISEASE. N.E. Waly,<sup>1</sup> T. J. Gruffydd-Jones,<sup>1</sup> C.R. Stokes,<sup>1</sup> AND M.J. Day.<sup>2</sup>  
<sup>1</sup>Department of Clinical Veterinary Science, <sup>2</sup>Department of Pathology and Microbiology, University of Bristol, UK.

The aim of this study was to define the epithelial expression of major histocompatibility complex (MHC) class II in the small intestine of healthy cats and cats with inflammatory bowel disease (IBD).

Endoscopic duodenal biopsies were collected from cases referred to the Feline centre, Department of Clinical Veterinary Science for investigation of chronic vomiting and/or diarrhoea ( $n = 21$ ). The diagnosis of IBD was based on the clinical investigations as well as the histopathological interpretation. Duodenal biopsies were also collected from a group of cats presented with similar signs but were diagnosed with disorders other than inflammatory bowel disease, and were considered as control ( $n = 7$ ). The results from this group of cats were also compared with samples collected from specific pathogen-free, healthy cats ( $n = 16$ ). Immunohistochemical labelling was used to detect MHC class II expression by the villus and crypt epithelium of the duodenal biopsies. The expression was then scored in a semi-quantitative fashion in the upper and lower crypt and villus epithelium (0 = no expression to 10 = intense expression).

Samples from 90.4% of IBD cats were positive for epithelial expression of MHC class II (19/21) with mean intensity of expression =  $6.9 \pm 2.9$  (median = 7.0), whilst only 14.3% of non-IBD cats were positive for epithelial expression (1/7) and the level of intensity of this expression was mild (grade 2). None of the 16 SPF cats had epithelial expression of MHC class II.

MHC class II is expressed by antigen presenting cells and this combination of MHC-antigenic peptide may be recognized by the T cell receptor of CD4<sup>+</sup> helper T cells. The finding of this study suggests that intestinal epithelial cells may have an important role in presentation of luminal antigen, and may therefore be involved in the pathogenesis of inflammatory bowel disease. The results also suggest that epithelial expression of MHC class II could be used as an aid in diagnosis of feline IBD.

- 3** ANTIBODIES AGAINST BOVINE SERUM ALBUMIN (BSA) IN SERUM FROM CATS WITH GASTROINTESTINAL DISEASE. GM Rutz, CG Ruaux, L Jobe, JM Steiner and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, Texas.

Chronic exposure of the gut to antigens may lead to the development of a cytopathic immune response, a possible cause of chronic intestinal disorders in cats. Many vaccines contain trace amounts of BSA as a result of vaccine virus culture. Repeated exposure at annual vaccinations thus might sensitize cats to BSA that may be encountered in the diet. The goal of this project was to develop and validate an enzyme-linked immunosorbent assay (ELISA) for the measurement of anti-BSA antibodies in feline serum, and to determine if anti-BSA titers differ between clinically healthy cats and cats with gastrointestinal disease.

A direct ELISA was developed. ELISA plates were coated with pure BSA. Feline sera were applied in doubling dilutions across the plate. Anti-BSA antibodies were detected with biotinylated goat anti-feline IgG antibody. Bound antibody was detected with streptavidin-horseradish peroxidase conjugate, using a trimethyl benzidine substrate (TMB Substrate, Pierce Chemical). Specificity of binding of the feline anti-BSA antibodies was demonstrated by immunoblotting. Precision and reproducibility of the assay were evaluated. End-point titers of anti-BSA antibodies were determined in sera from 11 clinically normal cats, 17 cats with low serum folate concentrations ( $3.5\text{--}9.6 \mu\text{g/L}$ ), 16 cats with high serum fTLI concentrations ( $90\text{--}923 \mu\text{g/L}$ ) and 16 cats with low serum fTLI concentrations ( $<2\text{--}8 \mu\text{g/L}$ ). For statistical analysis, the antibody titers were  $\log_2$  transformed and compared using a two-tailed Student's *t*-test.

Intraassay coefficients of variation (CVs) of three different serum samples ranged from 0% to 3.3%. Interassay CVs of 12 different serum samples ranged between 0% and 5.7%. Anti-BSA titers in the group of cats with low serum fTLI concentrations were significantly lower than in normal cats ( $P < 0.05$ ). No significant differences were detected between any other groups.

It is concluded that this assay is valid for the determination of end-point antibody titers against BSA in cats. No evidence was found for a role of anti-BSA antibodies in perpetuation of gastrointestinal disease in cats. A role in disease initiation or disease in the perivaccinal period can not be ruled out.

- 4** BIOCHEMICAL MARKERS OF COBALAMIN DEFICIENCY ACCOMPANYING SEVERE HYPO-COBALAMINEMIA IN THE CAT. CG Ruaux, JM Steiner and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, Texas.

Cobalamin (Vitamin B<sub>12</sub>) acts as a cofactor for enzymes involved in the transfer of single carbon functional groups during normal cellular metabolism. All cells require cobalamin for normal cellular metabolism. The purpose of this study was to investigate the presence of biochemical markers of cobalamin depletion in feline samples with very low serum cobalamin concentrations.

Sera from cats with presumed gastrointestinal disease and hypcobalaminemia (serum cobalamin  $< 100 \text{ ng/L}$ ,  $n = 40$ ) were selected from accessions to the Gastrointestinal Laboratory. Control sera, with cobalamin within our control range ( $290\text{--}1500 \text{ ng/L}$ ), were from student-owned, volunteered cats ( $n = 24$ ). Serum cobalamin was measured using a commercially available chemiluminescent immunoassay system. Serum concentrations of methylmalonic acid (MMA, a marker of tissue cobalamin availability) and sulfur amino acids (methionine, homocysteine, cystathionine, cysteine) were measured using stable isotope dilution gas chromatography/mass spectrometry. Data were analyzed with a statistical software package (GraphPad Prism 3.0), using two-tailed Student's *t*-tests, with values of  $P < 0.05$  considered significant.

Hypcobalaminemic cats exhibited dramatic increases in serum MMA concentrations compared to the controls. There was a significantly higher mean serum concentration of methionine in the cobalamin deficient cats, and significantly lower mean serum cystathionine and cysteine concentrations. No significant difference in serum homocysteine concentrations was detected. Asterisks indicate statistical significance, \* $P < 0.05$ , \*\* $P < 0.01$ .

Analyte	MMA (nmol/L)	Methionine (μmol/L)	Homocysteine (μmol/L)	Cystathionine (nmol/L)	Cysteine (μmol/L)
Affected	9,607**	133.8*	3.67	449.6**	142.3*
Control	448	101.1	3.97	573.2	163.9

These data demonstrate that cats with extremely low serum cobalamin concentrations have significant biochemical abnormalities as a result of tissue-level cobalamin deficiency. The lack of difference in homocysteine, contrary to findings in human beings with cobalamin deficiency, suggests that there may be a species difference in metabolism of this amino acid.

5

QUANTITATIVE EVALUATION OF PROINFLAMMATORY CYTOKINE UPREGULATION IN THE STOMACHS OF *HELICOBACTER PYLORI* INFECTED CATS. <sup>1</sup>R.K. Straubinger, <sup>1</sup>A. Grieter, <sup>1</sup>A. Gerold, <sup>1</sup>A. Straubinger, <sup>2</sup>E. Scanziani, <sup>1</sup>K.W. Simpson. <sup>1</sup>Cornell University and <sup>2</sup>University of Milan.

The goal of this study was to further characterize the gastric mucosal inflammatory response of cats to *H. pylori* infection by quantitative evaluation of proinflammatory cytokine upregulation.

Five clinically healthy cats with naturally acquired *H. pylori* infection (*cagA*, *picB*) and 7 *Helicobacter*-free cats were evaluated. Gastric colonization was determined by tissue urease activity, light microscopy, culture and PCR. The mucosal inflammatory response was evaluated by light microscopy, and by quantitative real-time RT-PCR of the proinflammatory cytokines IL-1 $\beta$ , IL-6, IL-8 and interferon- $\gamma$  in gastric mucosa using primers and probes designed and validated for use in cats.

*H. pylori* colonized the pylorus and fundus less densely than the cardia. Bacteria were observed free in the lumen of gastric glands and were also tightly adherent to epithelial cells. Lymphoid follicle hyperplasia was observed primarily in *H. pylori* infected cats, with the pylorus most severely affected. Neutrophilic and eosinophilic infiltrates, and globular leukocytes were observed solely in infected cats. Up-regulation of IL-1 $\beta$ , IL-8 and interferon- $\gamma$  was consistently detected in mucosa from the pylorus, fundus and cardia of *H. pylori* infected cats. Upregulation was most marked in the pylorus where mean values ranged from 37 to 130 fold higher than uninfected cats.

The pattern of colonization and the mucosal inflammatory response in cats with naturally acquired *H. pylori* are broadly similar to those in infected people, particularly children, and non-human primates. The upregulation of IL-8 in infected cats was independent of *cagA* and *picB*.

6

EVALUATION OF THE PASSAGE OF TABLETS AND CAPSULES THROUGH THE ESOPHAGUS IN CATS. DS Westfall, DC Twedt, PF Steyn, EB Oberhauser, JW VanCleave. From the College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

We have reported tablet induced focal esophagitis and esophageal stricture formation in cats secondary to doxycycline administration. The proposed mechanism is abnormal esophageal tablet retention resulting in focal esophagitis and subsequent stricture formation. The objective of this study was to evaluate the passage of tablets and capsules when given alone (dry swallow) and when followed by a water bolus (wet swallow).

Thirty healthy cats of various ages were used in this study. Health status of the cats was verified by physical examination, CBC, serum chemistry panel, urinalysis, and FeLV/FIV testing. Cats were excluded if they had a history of gastrointestinal disease or chronic oral medication administration. Each cat was given a 20 mg barium tablet and a 190 mg (size 4) capsule both as a dry and wet swallow. A wet swallow consisted of immediately following administration with 6.0 ml of water orally via syringe. A specially designed Plexiglass box was used to restrain the cats during the study. Fluoroscopy was utilized to evaluate tablet or capsule passage at 30, 60, 90, 120, 180, and 300 seconds following administration. Dry swallows and wet swallows were evaluated. Successful passage was defined as complete passage into the stomach at a given time interval. The Z proportional test was used to evaluate statistically significant differences between the two treatments (dry versus wet swallow) for each time period.

The percentage of dry tablet swallows that successfully passed into the stomach was 0.0% at 30 and 60 seconds, 6.7% at 90 seconds, 13.3% at 120 seconds, 26.7% at 180 and 240 seconds, and 36.7% at 300 seconds. Wet tablet swallows successfully passed 90.0% of the time at 30 seconds, 93.3% at 60 seconds, and 100.0% of the time thereafter. The percentage of dry capsule swallows that successfully passed was 16.7% at each time interval. Wet capsule swallows successfully passed 96.7% of the time at 30 seconds and 100% of the time thereafter. For each time interval, wet swallows achieved significantly greater percentage passage into the stomach when compared to dry swallows ( $P < 0.05$ ).

The results of this study show that tablets or capsules given as a dry swallow have prolonged retention in the esophagus. A water bolus following tablet or capsule administration results in significantly faster passage through the esophagus. Based on this study, we recommend the routine administration of a water bolus to cats receiving oral tablets or capsules to prevent possible medication associated esophagitis.

7

IN VIVO EFFECT OF A COX-2 SELECTIVE AND NONSELECTIVE NONSTEROIDAL ANTI-INFLAMMATORY DRUG (NSAID) ON GASTRIC MUCOSAL AND SYNOVIAL FLUID PROSTAGLANDIN SYNTHESIS IN DOGS. CJ Jones, HK Strep-pa, SC Budsberg, BG Harmon. University of Georgia. Athens, GA.

NSAID selective against cyclooxygenase (COX)-2 have provided the potential for antiinflammatory effects with a decreased gastrointestinal toxicity. Selectivity is expressed as a ratio of the inhibitory capability of a particular NSAID, using *in vitro* methods, against each isoform. Problems with these ratios include poor correlation between studies, species differences making extrapolation between species difficult, and limited data on the correlation between *in vitro* selectivity and *in vivo* activity. Our goal was to investigate the *in vivo* activity of a known selective COX-2 inhibitor (meloxicam) and a nonselective NSAID (aspirin) on specific target tissues, the gastric mucosa and the joints, in the dog and correlate this activity to COX-1 and COX-2 inhibition in the blood.

Twelve dogs with unilateral osteoarthritis of the stifle were used. Each dog was treated in a crossover design with aspirin or meloxicam for 21 days with a 6 month "washout" between treatment periods. PGE<sub>2</sub> levels were determined measured at days 0 (baseline), 7, and 21 of each treatment period in LPS-stimulated whole blood, synovial fluid collected by arthrocentesis, and endoscopic gastric mucosal biopsies. TXB<sub>2</sub> was also evaluated in whole blood on days 0, 7, and 21 of each treatment period. TXB<sub>2</sub> and PGE<sub>2</sub> levels were measured using an ELISA. PGE<sub>2</sub> and TXB<sub>2</sub> levels were evaluated over time with a repeated measure ANOVA ( $p < 0.05$ ).

Aspirin significantly suppressed PGE<sub>2</sub> in blood, gastric mucosa, synovial fluid, and TXB<sub>2</sub> in blood at days 7 and 21. Meloxicam significantly suppressed PGE<sub>2</sub> in blood and synovial fluid at days 7 and 21 but had no effect on TXB<sub>2</sub> in blood or PGE<sub>2</sub> in gastric mucosa.

Suppression of LPS-stimulated PGE<sub>2</sub> in blood and PGE<sub>2</sub> in synovial fluid by both aspirin and meloxicam is consistent with activity against the COX-2 isoenzyme. Suppression of PGE<sub>2</sub> in the gastric mucosa and TXB<sub>2</sub> in blood by aspirin is consistent with activity against COX-1. Meloxicam, in contrast, had minimal effect on functions mediated by COX-1. This *in vivo* model verifies the effect of a COX-2 selective inhibitor, meloxicam, on target tissues, sparing gastric prostaglandin synthesis while retaining anti-prostaglandin effects within the inflamed joint.

8

SERUM  $\alpha_1$ -PROTEINASE INHIBITOR/TRYPsin COMPLEX AS A MARKER FOR CANINE PANCREATITIS. JS Suchodolski,<sup>1</sup> CG Ruaux,<sup>1</sup> JM Steiner,<sup>1</sup> JC Collard,<sup>1</sup> KW Simpson,<sup>2</sup> DA Williams.<sup>1</sup> <sup>1</sup>GI Laboratory, Texas A&M University, College Station, TX; <sup>2</sup>College of Veterinary Medicine, Cornell University, Ithaca, NY.

We recently developed and validated an ELISA for measurement of  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -PI) complexed with canine cationic trypsin (cCT) in canine serum. During pancreatitis  $\alpha_1$ -PI binds reversibly to trypsin. The concentration of serum  $\alpha_1$ -PI/trypsin has been evaluated as a diagnostic and prognostic marker for pancreatitis in human beings. The aim of this study was to evaluate serum  $\alpha_1$ -PI/cCT in experimentally induced pancreatitis (IP) and spontaneous pancreatitis (SP) in dogs.

16 healthy research dogs were used in this study. Mild, moderate, and severe pancreatitis was induced using 3 different models: IV injection of cholecystokinin (CK,  $n = 3$ ), pancreatic duct ligation (DL,  $n = 4$ ), and intraductal injection of enterokinase (EK) in combination with autologous bile into the pancreas ( $n = 5$ ), respectively. Two dogs were sham operated, and 2 dogs served as nonoperated controls. Sera were collected at 0, 1, 2, 3, 4, and 5 hours. Sera from 10 dogs with histopathologically confirmed SP were also evaluated. Concentrations of  $\alpha_1$ -PI/cCT were measured by ELISA. Data were analyzed with a statistical software package (GraphPad Prism 3.0). Variations in concentrations of  $\alpha_1$ -PI/cCT in dogs with IP were analyzed by time-point relative to baseline using one-way, repeated measures ANOVA. Mean  $\alpha_1$ -PI/cCT of normal dogs ( $n = 45$ ) and dogs with SP was compared using a two tailed *t*-test. Statistical significance was assigned for values of  $p < 0.05$ .

Concentration of  $\alpha_1$ -PI/cCT varied significantly between baseline serum samples and serum taken after the induction of pancreatitis in all three experimental models. Concentration of  $\alpha_1$ -PI/cCT in dogs with CK induced pancreatitis was significantly elevated after 1 hour (150% of baseline,  $p < 0.05$ ) but was not significantly different from baseline thereafter. In dogs with DL and EK induced pancreatitis  $\alpha_1$ -PI/cCT increased steadily over time (425% and 1918% of baseline after 5 hours). No significant differences were detected in sham and control dogs. While mean  $\alpha_1$ -PI/cCT concentration in dogs with SP was greater than in control dogs, the difference was not statistically significant ( $p = 0.236$ ).

We conclude that serum  $\alpha_1$ -PI/cCT is increased early in the course of experimentally induced pancreatitis and reflects disease severity. This transient increase may explain why  $\alpha_1$ -PI/cCT was elevated in dogs with experimentally induced pancreatitis, but that the increase in dogs with spontaneous pancreatitis was not significant different when compared to normal dogs.

## 9 SERUM CANINE PANCREATIC LIPASE IMMUNOREACTIVITY (cPLI) CONCENTRATIONS IN DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY (EPI). JM Steiner, SR Gumminger, GM Rutz, and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, TX

Exocrine pancreatic insufficiency (EPI) is caused by a lack of synthesis and secretion of digestive enzymes by the exocrine pancreas. Serum canine trypsin-like immunoreactivity (cTLI) has been shown to be both highly sensitive and specific for EPI in dogs. Recently, an enzyme-linked immunosorbent assay (ELISA) for the measurement of canine pancreatic lipase immunoreactivity (cPLI) has been developed and validated and a reference range has been established in 74 healthy control dogs (2.2–102.1 µg/L). The goal of this project was to determine whether this assay is a specific marker for exocrine pancreatic function.

74 clinically healthy dogs and 25 dogs with EPI were enrolled in the study. A diagnosis of EPI was made on the basis of clinical signs compatible with EPI, a severely decreased serum cTLI concentration (cTLI ≤ 2.0 µg/L), and response to enzyme replacement therapy. Mean serum lipase activity (data sets passed normality test) and median serum cPLI concentration (data sets failed normality test) were compared using a two-tailed *t*-test and a Mann-Whitney test, respectively.

Serum lipase activity (mean ± SD) was not significantly different between clinically healthy dogs (319.1 ± 1146.7 U/L) and dogs with EPI (341.1 ± 145.1 U/L; *p* = 0.5186). Median serum cPLI concentration was significantly lower in dogs with EPI (0.1 µg/L) than in clinically healthy dogs (16.3 µg/L; *p*-value < 0.0001). Furthermore, all of the dogs with EPI had serum cPLI concentrations that were below the lower limit of the reference range with the highest concentration being 1.4 µg/L. One of the normal dogs also had a serum cPLI concentration of 1.4 µg/L.

In conclusion serum lipase activity is not specific for exocrine pancreatic function. In contrast serum cPLI concentration is highly specific for exocrine pancreatic function. However, there was a minimal overlap of cPLI between clinically healthy dogs and dogs with EPI. Such an overlap was not observed for serum cTLI in the same group of patients. Thus serum cPLI may be slightly inferior to cTLI for the diagnosis of EPI in the dog. However, deficiencies of single digestive enzymes have been reported in human beings and serum cPLI may be useful for a diagnosis of such cases in the dog.

## 10 SERUM CANINE PANCREATIC LIPASE IMMUNOREACTIVITY (cPLI) CONCENTRATIONS IN DOGS WITH SPONTANEOUS PANCREATITIS. JM Steiner,<sup>1</sup> J Broussard,<sup>2</sup> CS Mansfield,<sup>3</sup> SR Gumminger,<sup>1</sup> and DA Williams.<sup>1</sup> <sup>1</sup>Gastrointestinal Laboratory, Texas A&M University, College Station, TX; <sup>2</sup>Animal Medical Center, New York, NY; <sup>3</sup>Faculty of Veterinary Medicine, Dublin, Ireland.

Pancreatitis is the most common exocrine pancreatic disorder in dogs. Serum lipase activity, which has been used for the diagnosis of canine pancreatitis for decades lacks both sensitivity and specificity. Recently, an enzyme-linked immunosorbent assay (ELISA) for measurement of canine pancreatic lipase immunoreactivity (cPLI) has been developed and validated and a reference range has been established in 74 clinically healthy dogs (2.2 to 102.1 µg/L). Also, this assay has recently been shown to be highly specific for exocrine pancreatic function. The goal of this project was to examine the sensitivity of serum cPLI concentration for the diagnosis of pancreatitis in dogs.

74 clinically healthy dogs and 11 dogs with a histopathologically-confirmed pancreatitis were enrolled. Serum was evaluated for lipase activity (LA), trypsin-like immunoreactivity concentration (cTLI), and canine pancreatic lipase immunoreactivity concentration (cPLI). Mean LA (data sets passed normality) and median cTLI and cPLI (data sets failed normality) were compared between both groups using a two-sided *t*-test and a Mann-Whitney test, respectively.

LA (mean ± SD) was significantly higher in dogs with pancreatitis (319.1 ± 146.7 U/L) than in clinically healthy dogs (4,512.0 ± 5,375 U/L; *p* < 0.0001). However, only 7 dogs had LA above the upper limit of the reference range and only 6 above an empirical cut-off value of 3 times the upper limit of the reference range (sensitivity 54.5%). Median cTLI was significantly higher in dogs with pancreatitis (28.5 µg/L) than in clinically healthy dogs (10.7 µg/L; *p* = 0.0004). However, cTLI was only increased above the upper limit of the reference range in 4 dogs and above the currently recommended cut-off value of 50 µg/L in 4 dogs (sensitivity 36.4%). Median cPLI was significantly higher in dogs with pancreatitis (676.8 µg/L) than in clinically healthy dogs (16.3 µg/L; *p* < 0.0001). Furthermore, cPLI was increased above the upper limit of the reference range in all 11 dogs and above an empirical cut-off value of 250 µg/L in 9 dogs (sensitivity 81.8%).

We conclude that serum cPLI concentration is highly sensitive for the diagnosis of spontaneous pancreatitis in the dog.

## 11 TREATMENT OF *HELICOBACTER SPP.* IN DOGS WITH CHRONIC VOMITING: PRELIMINARY RESULTS 4 WEEKS AFTER TREATMENT. M.S. Leib, R.B. Duncan. Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA.

*Helicobacter pylori* is a common cause of gastritis and peptic ulceration and increases the risk of gastric cancer in humans. Although *Helicobacter spp.* are commonly found in the stomachs of dogs and cats, their role in causing disease is unclear. Many therapies have been described in humans that result in long-term eradication of the organisms and relief of clinical signs. In research dogs and cats, a variety of antibiotic combinations have been ineffective in eradicating the organisms after 4–6 weeks. Better results have been obtained in a group of vomiting pet dogs. The purpose of this study was to evaluate the effectiveness (bacterial eradication rates and clinical response) of triple therapy, with and without acid suppression, in dogs with chronic vomiting and gastric *Helicobacter spp.* infection.

Ten dogs with vomiting of at least 2 weeks duration and *Helicobacter spp.* infection were randomized to receive BID treatment with amoxicillin 15 mg/kg, metronidazole 10 mg/kg, and Pepto Bismol on a sliding scale, with or without famotidine 10 mg/kg for 2 weeks. Diagnosis of *Helicobacter spp.* was based on identification of the organisms in gastric biopsy samples obtained during endoscopy. If organisms were not seen with a routine H&E stain, a Steiner's silver impregnation technique was used. Dogs were further stratified on the basis of normal biopsies, gastritis, and / or inflammatory bowel disease. Owners maintained a daily record of clinical signs. Four weeks after completing treatment, endoscopy was performed and biopsy samples collected for histopathological evaluation. The frequency of vomiting after treatment was compared to historical levels and ranked from 1 (no change) to 6 (at least a 90% reduction). Data was analyzed by Chi square and Mann-Whitney testing.

Eradication of bacteria occurred in 2 of 4 dogs treated with famotidine and 3 of 6 treated without famotidine. These results were not different (*p* > 0.5). Vomiting frequency decreased in dogs in which bacteria were eradicated (median score 6) while overall it was unchanged in those still infected (median score 2), *p* = 0.26. However vomiting frequency also decreased greatly in 2 dogs still infected, suggesting that other factors besides gastric helicobacters caused the vomiting.

After 4 weeks, both treatments eradicated bacteria in 50% of dogs. Dogs in which bacteria were eradicated 4 weeks after treatment tended to have less vomiting than those still infected. In order to further validate these data, additional cases should be enrolled into this study and a longer followup period evaluated.

## 12 EXPRESSION OF GLUTEN SENSITIVE ENTEROPATHY (GSE) OF IRISH SETTER DOGS MAY BE AGE DEPENDENT. C. M. Elwood, P. F. Moore\* and R. M. Batt. Department of Small Animal Medicine and Surgery, The Royal Veterinary College, University of London, UK and \*VM Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, California.

GSE is defined by a mucosal pathologic response to dietary gluten that resolves upon gluten withdrawal and recurs upon re-challenge. This study evaluated early and late mucosal immune responses to gluten challenge in adult Irish setters (IS) that were assigned as gluten sensitive because of villus atrophy and increased intraepithelial lymphocyte counts (IEL) whilst receiving dietary gluten as juveniles.

Nine IS (5M, 4F, age 4.5–7y) and four beagles (2M, 2F, age 3.5–4.5y), fed a gluten-free diet for at least 3 months, were challenged intra-duodenally with 10g peptic-tryptic gluten digest. Biopsies were obtained from the proximal small intestine before, at 24 and 72h after challenge and after 3 months of dietary gluten (0.5g/kg BW/day). Biopsies were divided between Bouin's fixative for gross morphology and total IEL counts per 100 epithelial cells, in acid-alcohol for measuring villus heights by dissection microscopy (VH, µm) or snap-frozen for immunocytochemistry and counting of CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, TCRαβ<sup>+</sup> and TCRγδ<sup>+</sup> cells per 0.0025mm<sup>2</sup> mid-villus lamina propria or per 100 epithelial cells. MHC Class II expression on the epithelium and in the lamina propria was scored on a four-point scale (0 = no staining, 1 = faint staining, 2 = obvious staining, 3 = marked staining).

No clinical or gross pathologic response to gluten challenge was seen in either group. Total IEL counts were significantly increased from baseline at 3 months in IS (Mean ± SD, 22.4 ± 7.2 v 46.9 ± 13.7, *p* < 0.001) but not in controls (33.8 ± 9.0 v 45.3 ± 6.2). VH were not significantly different from pre-challenge values in IS (756 ± 181) or controls (877 ± 119) at any point. In the lamina propria and epithelium, numbers of CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, TCRαβ<sup>+</sup> and TCRγδ<sup>+</sup> cells and MHC Class II expression did not differ from baseline in either group.

Increased IEL counts without other evidence of mucosal pathology in these adult IS contrasts with the mucosal immune response and severe disruption seen following challenge of adult humans with GSE (celiac disease). This emphasises differences between these dogs and humans with GSE and suggests that expression of GSE in IS may be influenced by age.

- 13** EFFECT OF EARLY ENTERAL NUTRITION ON INTESTINAL PERMEABILITY, INTESTINAL PROTEIN LOSS, AND OUTCOME IN DOGS WITH PARVOVIRUS. A.J. Möhr, A.L. Leisewitz, L.S. Jacobson (University of Pretoria, Pretoria, RSA), J.M. Steiner, C.G. Ruaux, and D.A. Williams (GI Lab, Texas A&M University, College Station, TX).

A prospective, randomized clinical trial was performed to investigate the effect of early enteral nutrition (EEN) on intestinal permeability, protein-losing enteropathy (PLE), clinico-pathologic parameters, and clinical outcome in 30 puppies with parvovirus confirmed by electron-microscopy.

Dogs were randomly assigned to 2 groups. 15 dogs received nil per os until vomiting had ceased for 12 hours, after which a low fat diet was fed (group NPO). 15 dogs were fed immediately (Pedigree® Canine Concentration Instant Diet) by naso-esophageal tube (EEN). All other treatments were identical. Intestinal permeability was assessed using urinary lactulose and rhamnose recoveries (%L and %R) and L/R ratio. Fecal  $\alpha_1$ -proteinase inhibitor concentrations ( $\alpha_1$ -PI) quantified PLE.

Median time to normalization of habitus and appetite, and resolution of vomiting and diarrhea was 1 day shorter for the EEN group for each parameter. Body weight remained stable in dogs in the NPO group, while EEN was associated with a mean weight increase of 11.5% by day 5 and 8.4% by day 6. Hct decreased significantly for all days in NPO ( $p < 0.02$ ), while no significant Hct decreases occurred in EEN. However, there was no significant difference of Hct between the groups over time.

Compared with reference values, urinary %Ls were elevated, %Rs reduced, and L/R ratios increased throughout the study for both groups. %L behaved significantly differently between groups ( $p = 0.035$ ), with a progressive decrease of %L in the EEN group vs. a progressive increase in the NPO group. %R progressively decreased, and L/R ratios increased significantly over time in both groups. Fecal  $\alpha_1$ -PI was increased throughout the study in both groups, but significant decreases were seen in the NPO group on days 2, 4, and 6, and on day 6 in EEN. There was no significant difference of serum albumin, %Rs, L/R ratios, or fecal  $\alpha_1$ -PI between the two groups over time. 13 of 15 NPO dogs (87%) and all of the EEN dogs (100%) survived ( $p = 0.48$ ).

In conclusion, in dogs with parvovirus EEN was associated with earlier improvement of clinical variables. The significantly decreased lactulose permeability in the EEN vs. the NPO group, although not reflected in L/R ratios, might indicate improved gut-barrier function due to EEN. This could limit bacterial translocation and distant organ dysfunction. However, the more rapid decrease in intestinal protein loss in the NPO group may conflict with this speculation, although there was no significant difference for fecal  $\alpha_1$ -PI, serum albumin, or Hct between the 2 groups.

- 14** FLOW CYTOMETRIC ANALYSIS OF MUCOSAL LYMPHOCYTES IN DOGS WITH INFLAMMATORY BOWEL DISEASE. AE Jergens,<sup>1</sup> IM Sonea,<sup>1</sup> LK Kaufman,<sup>1</sup> PF Moore,<sup>2</sup> TJ Benson.<sup>1</sup> Iowa State University,<sup>1</sup> Ames, IA and University of California,<sup>2</sup> Davis, CA.

Qualitative and quantitative assessment of intestinal lymphocytes may provide new insights into the immunopathology of canine inflammatory bowel disease (IBD). In the present study, phenotypic characterization of lymphocytes in the small intestinal mucosa were investigated in healthy dogs and dogs with IBD by flow cytometry. Tissue specimens obtained endoscopically from the small intestine of 10 control (CTL) dogs and 10 diseased (IBD) dogs were evaluated. Isolation of mucosal lymphocytes was performed as previously described (Sonea et al, 2000), yielding predominantly a population of intraepithelial lymphocytes which were subsequently immunostained for cell surface expression of canine-specific CD3, CD45, CD4/CD8 $\alpha$ , CD8 $\alpha$ /CD8 $\beta$ , TCR $\alpha\beta$ /TCR $\gamma\delta$ , and CD21 antigens. Flow cytometric data was obtained using a sorting-grade flow cytometer which identified two distinct populations of lymphocytes: (1) small mucosal lymphocytes with characteristics similar to peripheral blood lymphocytes and (2) larger, more granular mononuclear cells. Statistical analysis allowed comparison of mean rank cell counts between the dog groups.

Most mucosal lymphocytes were CD3+, with the majority being CD8 $\alpha$ +, and these were accompanied by lesser numbers of CD4+ and CD4+/CD8 $\alpha$ + cells. Significant differences between dog groups for these cell types were not observed. Staining for CD8 $\alpha$ +/CD8 $\beta$ + revealed nearly equal populations of CD8 $\alpha$ +/CD8 $\alpha\beta$ + and CD8 $\alpha\beta$ + cells in CTL and IBD dogs. Mature B-lymphocytes (CD21+) were extremely rare. Small intestinal T cells in both groups predominantly expressed TCR $\alpha\beta$ +, while large TCR $\gamma\delta$ + cells were significantly ( $P = 0.02$ ) decreased in dogs with IBD in comparison to CTL dogs. Within the IBD group, both large and small lymphocytes expressing TCR $\gamma\delta$ + were significantly ( $P = 0.02$  and  $P = 0.01$ , respectively) less numerous as compared to TCR $\alpha\beta$ + lymphocytes.

In conclusion, we provide the first report describing flow cytometric analysis of small intestinal mucosal lymphocytes obtained by endoscopic biopsy in dogs with IBD.

These data show that dogs with IBD have altered expression of TCR $\gamma\delta$ + cells as compared to healthy dogs, and this imbalance includes both small and large granular T lymphocytes. The significance of decreased TCR $\gamma\delta$ + cells in IBD remains unknown; however, this observation offers new perspective into the aberrant mucosal immune response characteristic of canine IBD.

- 15** CANINE LEUKOCYTE ADHESION DEFICIENCY: PRESENCE OF A CYS36SER MISSENSE MUTATION IN THE  $\beta 2$  INTEGRIN GENE IN AN AFFECTED IRISH SETTER FROM THE U.S. AND IN IRISH RED AND WHITE SETTERS. P. Fourreman, U. Giger. Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

Canine leukocyte adhesion deficiency (CLAD) is a primary immune disorder inherited as an autosomal recessive that is caused by a dysfunction of leukocyte adhesion with, among other effects, the inability of neutrophils to extravasate to sites of infection. Fourteen years ago we reported the first CLAD in a cross-bred Irish Setter from Pennsylvania and recognized that the previously described canine granulopathy syndrome in an Irish Setter from Washington State may have been the same disease. Recently, CLAD was also identified in Irish Setter puppies from Sweden and the responsible missense mutation (Cys36Ser) was found in the  $\beta 2$  integrin gene that codes for a common subunit (CD18) of several dimeric adhesion molecules. We describe here the clinical course and molecular basis of the disease in our originally described Irish Setter. We also surveyed a closely related breed, Irish Red and White Setters, in the United States for the presence of the  $\beta 2$  integrin mutation.

The proband presented with the now classic signs of recurrent bacterial infections, gingivitis, severe leukocytosis ( $> 100,000/\mu\text{L}$ ), lack of pus formation and poor wound healing. Infections responded slowly to antibiotics and rapidly recurred. Despite nearly continuous antibiotic therapy, this dog developed an ascending osteomyelitis that led to the amputation of the affected right hind limb, pyometra, rhinosinuites, pneumonia, multiple skin papillomas, and furunculosis. In contrast with other dogs described, which were euthanized before 6 months of age, this patient was medically managed with a good quality of life for 4 years.

DNA was extracted from the affected dog's lymphocytes that had been previously frozen. The DNA fragment containing the site of the  $\beta 2$  integrin mutation was amplified and sequenced, and it was found that this dog had the same mutation as seen in the Swedish Irish Setters. We developed a test based on a *Hinf*I restriction digest that yields an internal control fragment and different length fragments in the affected and control animals. In a group of 19 Irish Red and White Setters from the U.S. tested to date, we found two littermates whose father was British that are carriers for the same  $\beta 2$  integrin mutation. Irish Setters carrying this mutation have also been found in several European countries and in Australia. In conclusion, this study documents the presence of the same  $\beta 2$  integrin mutation seen in Europe and Australia in the first dog described with CLAD as well as in the Irish Red and White Setter breed.

- 16** CORRECTION OF CANINE PHOSPHOFRUCTOKINASE DEFICIENCY BY BONE MARROW TRANSPLANTATION. Callan MB, Griot-Wenk ME, McCully K, Rajpurohit Y, Goldschmidt MH, Smith BF, Weil M, Evans SM, Haskins ME, Giger U. University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA.

Phosphofructokinase (PFK) is a key regulatory enzyme in anaerobic glycolysis, the essential energy-producing pathway of erythrocytes and muscle. An autosomal recessive inherited muscle-type PFK deficiency characterized predominantly by chronic compensated hemolytic anemia with intermittent hemolytic crises and to a lesser extent exertional myopathy has been identified in the English springer spaniel (ESSP). Canine PFK-deficient erythrocytes have reduced 2,3-diphosphoglycerate (DPG) content, which increases their hemoglobin-oxygen affinity and alkaline fragility. With noninvasive near infrared and magnetic resonance spectroscopy, impaired muscle function and oxygen extraction during muscle exercise have been documented.

We examined the effects of bone marrow transplantation (BMT) in 4 PFK-deficient dogs (3 male, 1 female) on their hematologic and muscle function. Five to 12 week old puppies were given a single dose of 7 Gy total body irradiation, and approximately  $1-5 \times 10^8$  nucleated cells/kg body weight were collected from histocompatible normal ( $n = 3$ ) or heterozygous ( $n = 1$ ) littermates and administered intravenously to affected dogs. Engraftment of donor hematopoietic cells was observed within 9 to 11 days in 3 of 4 dogs. Severe graft-versus-host disease involving the skin was observed in 1 dog 1 month after BMT. The 2 other dogs tolerated the BMT procedure well without significant adverse events. The fourth dog showed no evidence of engraftment after receiving bone marrow cells 5 times during a 3-month period. At necropsy, multifocal vascular dystrophic mineralization and severe multifocal chronic active necrosis with mineralization involving the heart, subcutis, and muscle were noted in this dog that failed to engraft. Correction of the erythrocyte M-PFK deficiency was documented by measuring erythrocyte PFK activity and 2,3-DPG levels and by a PCR that identified normal, heterozygous, and mutant PFK alleles. M-PFK activity in muscle tissue remained absent, however, the change in erythrocyte function improved the ability to deliver oxygen to muscle during work as assessed by near infrared spectroscopy. The 3 dogs that were engrafted were monitored for 4 to 24 months, had normal complete blood cell counts, and did not experience any hemolytic crises.

Bone marrow transplantation, albeit associated with morbidity and mortality, can completely correct the hematologic abnormalities and improve aerobic muscle function during work in dogs with PFK-deficiency.

# 17 FeLV, FIV, AND FIP: EPIDEMIOLOGY AND CLINICAL DISEASE SYNDROMES. P.C. Crawford, J.K. Levy, J.M. Bicknell, C.R. Layton, T.A. Hannaka. University of Florida, Gainesville, FL.

The purpose of this study was to characterize epidemiological trends in diagnosis and concurrent diseases associated with feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), and feline infectious peritonitis (FIP) since the first cases were reported to a national data base.

This was a retrospective study using cases reported from veterinary medical teaching hospitals to the Veterinary Medical Data Base (VMDB) at Purdue University. Information analyzed for these cases included sex, breed, age, year reported, and concurrent diagnoses. Statistical analysis was performed with the exact Chi-square test and the crude odds ratio (OR) to determine if there was a significant ( $p < 0.05$ ) association between each viral infection and sex, breed, age, and concurrent diseases.

From 1964–1998, 8,715 cats were diagnosed with FeLV out of a total of 445,672 feline cases. From 1987–1998, 871 cats were diagnosed with FIV out of 228,093 feline cases. From 1979–1998, 1851 cats were diagnosed with FIP out of 449,910 feline cases. The prevalence of cats with FeLV increased from 1970–1985 to a peak of  $>3\%$  of the total cases, but has since declined to approximately 1% of the total cases. The prevalence of cats with FIV increased from 1987–1991, and has stabilized at 1% of the total cases. The prevalence of cats with FIP increased from 1979–1983, and has also stabilized at 1% of the total cases. For all 3 virus infections, there was an increased prevalence in male cats. Mixed breed cats were at increased risk for FeLV and FIV, while purebred cats were at higher risk for FIP. Cats  $<2$  years of age were at higher risk for FIP, middle-aged cats (4–10 years) were at higher risk for FeLV, and older cats (10–15 years of age) were at higher risk for FIV. The most common concurrent diseases in FeLV-infected cats were anemia, lymphosarcoma, and upper respiratory infections. The most common concurrent diseases in FIV-infected cats were FeLV, periodontal disease, upper respiratory infections, anemia, kidney disease, lymphosarcoma, and anterior segment ocular diseases. The most common concurrent diseases in cats with FIP were FeLV, inflammatory ocular diseases, and anemia.

The reported prevalence of FeLV, FIV, and FIP at veterinary medical teaching hospitals is low. Although the viruses occurred in all breeds, ages, and sexes, each virus had a characteristic signalment for cats at highest risk. Each viral infection was also associated with markedly increased risk for specific concurrent diseases. Cats with either FIV or FIP were also at risk for coinfection with FeLV.

# 18 MOLECULAR EVIDENCE OF *EHRlichia CANIS* INFECTION IN CATS FROM NORTH AMERICA. CATS FROM NORTH AMERICA. E. Breitschwerdt,<sup>1</sup> A. Abrams-Ogg,<sup>2</sup> S. Hancock,<sup>1</sup> S. Cowan,<sup>1</sup> J. Cloutier,<sup>2</sup> and B. Hegarty.<sup>1</sup> College of Veterinary Medicine, North Carolina State University,<sup>1</sup> Raleigh, NC and the Ontario Veterinary College,<sup>2</sup> Guelph, Canada.

Although antibodies to *E. canis* antigens have been detected in sera from North American cats, no *Ehrlichia* species has ever been cultured from a naturally-infected cat. Also, to our knowledge, *E. canis* DNA has not been amplified and sequenced from cat blood or tissue samples. The purpose of this report is to describe the clinicopathologic, serologic and molecular findings in three cats that were naturally-infected with *E. canis*.

The cats originated from Greensboro, NC (C-1), Guelph, ONT (C-2), and Norval, ONT (C-3). At the time of admission in May, March and October respectively, they were 31, 12 and 12 months of age and the duration of illness was 14, 2, and 3 days. All 3 cats were examined because of acute onset of lethargy and inappetence. Cryptococcosis was diagnosed in C-1 and systemic lupus erythematosus in C-2 at 18 and 7 months of age. Physical examination abnormalities included fever (41.1, 40.2 °C) in C-1 & C-2, moderate stifle joint effusion (C-1), generalized lymphadenopathy (C-2) and pallor, petechiae and blindness (C-3). Ocular abnormalities included chemosis, conjunctivitis and protrusion of the third eye lid in C-1, and multifocal retinal hemorrhage with bilateral retinal detachment in C-3. Clinicopathologic testing identified numerous abnormalities including neutrophilia with a regenerative left shift and neutrophilic polyarthritis in C-1, pancytopenia with reactive lymphadenopathy in C-2, and a normocytic normochromic non-regenerative anemia and thrombocytopenia in C-3. A core bone marrow biopsy revealed erythroid and megakaryocytic hypoplasia in both C-2 and C-3. Due to the predominance of early myeloid precursors in C-2, acute myeloid leukemia or immune-mediated myelodysplasia were the main differential diagnoses. All 3 cats were negative for FeLV, FIV and various other infectious diseases. Antinuclear antibodies were detected in the two cats tested (C-1 & C-2). By IFA testing, reciprocal antibody titers to *E. canis* antigens were  $<16$  for C-1 and C-2. Using primers ECCmod and HE3R mod, 380 bases of the 16S rRNA gene were amplified, cloned and sequenced. The resulting sequence from all three cats was identical, except for 2 bases in C-1, to the homologous sequence for *E. canis*. Treatment with doxycycline, and other drugs, elicited clinical improvement in all 3 cats, but the concurrent use of immunosuppressive drugs for the presumptive diagnoses of immune-mediated polyarthritis, systemic lupus erythematosus and immune-mediated anemia and thrombocytopenia may have interfered with therapeutic elimination of the organism. We conclude that cats can be infected with *E. canis*, without developing an IFA *E. canis* antibody response.

# 19 CONCURRENT COINFECTIONS IN DOGS DETECTED BY SEROLOGY DURING A SURVEY FOR RICKETTSIA RICKETTSII: RESULTS FROM 1093 SERUM SAMPLES COLLECTED IN ITALY. Tommaso Furlanello,<sup>\*</sup> Marco Caldin,<sup>\*</sup> George Lubas,<sup>®</sup> Filippo Tognin.<sup>\*</sup> Veterinary Clinic San Marco, Padova, Italy; <sup>®</sup>Dipartimento di Clinica Veterinaria, Università di Pisa, Pisa, Italy

A high incidence of seroconversions to *Rickettsia rickettsii* (etiological agent of Rocky Mountain Spotted Fever, RMSF) in samples collected in dogs coming from all over Italy has been recently described by our working group (Furlanello *et al.*, Proc. 10th ESVIM Congr, 115, 2000). From this preliminary study, the concurrent presence of bacterial tick born diseases (TBDs) and protozoal infections in dogs seropositive to *R. rickettsii* appeared of great interest. In this abstract we will present our findings in a wider number of cases.

In the period from July, 1999, to November, 2000, we tested 1621 serum samples as part of diagnostic testing in the clinical study of diseased dogs, presumably affected by one or more TBDs, for infection with *R. rickettsii* (or related disease) *Ehrlichia canis* (Canine Monocytic Ehrlichiosis, CME), the agent of Canine Granulocytic Ehrlichiosis/Human Granulocytic Ehrlichiosis (CGE/HGE) or *Leishmania infantum* (Canine Leishmaniasis, CL). The samples were tested with immunofluorescence tests (IFAT), commercially available, validated for the canine species, run with standard technique and detecting IgG. The cut-off for a positive test was 1:80.

Main results are the following: among 1621 samples, 1093 dogs were seropositive for *R. rickettsii* (67.4%). Among those, 928 were tested also for CME and 389 were positive (41.9%), 138/205 (67.3%) were positive for CGE/HGE, and 400 were tested for CL (209 seropositive, 52.2%). So we had the following combination: 189 samples tested for RMSF, CME, and CGE/HGE (69 positive, 36.5%); 320 samples tested for RMSF, CME, and CL (75 positive, 23.4%); 37 samples tested for RMSF, CGE/HGE and CL (15 positive, 40.5%); and finally 36 samples tested for RMSF, CME, CGE and CL (7 positive, 19.4%).

Such high incidence of concurrent infections is of great importance and to our knowledge has been never reported in the canine species to this extent. Considering that cross reactions are not expected for these infections and the high number of determinations carried out, we can state that exposure to various TBDs and leishmaniasis is a common event for Italian dogs. Obviously, only a portion of these dogs are affected by the clinical form of one or more of the infections examined. Further studies on effects on the canine immune system by concurrent infections should be considered, and hopefully, the true nature of the spotted fever agent able to induce antibody production against *R. rickettsii* should also be clarified.

# 20 BABESIA GIBSONI INFECTIONS IN DOGS FROM ALABAMA AND GEORGIA. DK Macintire, MK Boudreaux, C Bourne, J Wright. Auburn University, Auburn, AL.

The purposes of this study were to determine whether Pit Bull terriers living in Alabama and Georgia are at increased risk for *Babesia gibsoni* infection compared to other breeds of dogs living in the same geographic area, and to determine whether the gene sequencing for *B. gibsoni* in Alabama dogs matches previously reported isolates.

Blood samples for PCR analysis were obtained from 33 Alabama Pit Bull terriers (20 from a commercial breeder and 13 pet dogs), 37 Foxhounds from a Georgia kennel, 12 dogs from the local humane society, and 38 dogs of varying breeds admitted to Auburn University over the same time period. All positive PCR samples were sequenced and compared to sequences of previously isolated small babesia samples submitted to Genbank.

Nine of the 33 samples from Pit Bull dogs were PCR negative; 15/33 (45%) were positive and 9/33 (27%) were suspicious (faint band). Blood samples from the other 87 dogs tested were all negative. Small babesia organisms were identified on the blood smears in 9/15 PCR positive dogs and in 2/9 PCR suspicious dogs. The genetic sequence of the Alabama isolate is identical to RNA subunit sequences of isolates taken from dogs in Okinawa, Oklahoma, North Carolina, Indiana, and Missouri; but it differs from the genetic sequence of the California isolate. Only 1 dog was clinically ill at the time blood was drawn, but 4 of the Pit Bulls had a history of previous hemolytic illness. Subclinically infected dogs had significantly lower platelet counts ( $p < 0.05$ ), lower hematocrits ( $p < 0.05$ ), and increased mean platelet volumes ( $p < 0.05$ ) compared to PCR negative dogs.

Pit Bull terriers from Alabama appear to be at a high risk for subclinical infection with *Babesia gibsoni*, whereas other breeds in the same geographic area do not. This study provides further evidence that more than one type of small babesia infects dogs, and also suggests that infection with this type of babesia is often clinically inapparent.

## 21 OPPORTUNISTIC DISSEMINATED MYCOSES IN DOGS: 1993–2000. MJ Booth,<sup>1</sup> LL van der Merwe,<sup>2</sup> <sup>1</sup>University of Liverpool, United Kingdom. <sup>2</sup>University of Pretoria, South Africa.

Mycology records of the Faculty of Veterinary Science, University of Pretoria, were reviewed for fungal cultures of body fluids or tissue samples. Clinical records were subsequently reviewed. Cases with 2 or more non-contiguously affected organs were classified as disseminated mycoses.

Seventeen case records were sufficiently complete to allow inclusion. Breeds involved were: German shepherd dogs (14), Bull terriers (2) and a Boxer. Females accounted for 12 cases (GSD = 11). Mean age was 4 years–2 months. Common clinical signs were depression (10), anorexia (9), pain (8), hindquarter neurological defects (9), emaciation (7), forelimb/cranial nerve signs (5). Median duration of signs was 42 days (range 7–475). Discospondylitis was radiologically evident in 10 cases. The thoracolumbar spine was most commonly involved (34/38 sites). Intervertebral disc aspirate cytology was diagnostic in 4/5 cases, disc culture in 2/4 cases. Fungi were demonstrable in the urine of all disc-aspirated cases. Urine sediment and/or culture were diagnostic in 9/13 cases. Blood culture was diagnostic in 1/8 cases. Where urine and blood were tested (n = 7), blood culture was negative in all and urine evaluation was diagnostic in 4/7 cases. Organs commonly affected on post mortem evaluation (14) included: kidneys (13), spleen (13), intervertebral discs (8) and myocardium (6). Fungi isolated were: *Aspergillus* spp (13), *Paecilomyces variotti* (2), *Xylomyces bantianum* (1) and *Candida* spp (1). Neutrophilia was present in 12/16 cases (range 14.4–42.74 × 10<sup>9</sup>/l), 9/12 exhibited a neutrophilic left shift (range 0.88–6.49 × 10<sup>9</sup>/l). Monocytosis (range 1.55–9.96 × 10<sup>9</sup>/l) was evident in 12/16 cases. Anemia was present in 7/16 cases (RBC, range 1.29–5.07 × 10<sup>12</sup>/l). Hyperproteinemia was found in 3/11 cases and hyperglobulinemia in 10/11 (range 3.73–5 g/dl). Creatinine was elevated in 11/15 cases (range 1.47–12.47 mg/dl), urea in 10/15 (range 36.7–184.52 mg/dl). All cases with azotemia had fungi demonstrated in the kidneys. Three cases died, 9 were euthanized after diagnosis and 5 after unsuccessful antifungal treatment. The median survival time from diagnosis in treated patients was 40 days (range 9–252).

This study confirms that young adult, female, German shepherd dogs are predisposed to opportunistic disseminated fungal infections. The data also indicates that urine evaluation is a sensitive, cost effective and non-invasive diagnostic test.

## 22 DETECTION OF *CRYPTOSPORIDIUM PARVUM* IN FELINE FECES USING A POLYMERASE CHAIN REACTION ASSAY. V Scorza and MR Lappin. College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

The purpose of this study was to compare the use of a polymerase chain reaction (PCR) assay and a commercially available monoclonal antibody-based immunofluorescence assay (IFA; Meridian Diagnostics, Cincinnati, Ohio) for detection of *C. parvum* in feces from experimentally inoculated cats.

Eight, young, *C. parvum*-naïve, DSH cats were inoculated with 1 × 10<sup>6</sup> oocysts of a *C. parvum* isolate initially recovered from an infected human. Feces were collected from all cats before inoculation, daily for the next 30 days, and then twice weekly until day 85. Methylprednisolone acetate was administered at 20 mg/kg, IM on days 85, 92, and 99. Feces were then collected daily from days 86–115 and then up to twice weekly until day 126. Feces were refrigerated until assayed by IFA and then frozen at –70 °C until assayed by PCR. PCR primers were designed to detect all sequenced isolates of *C. parvum*. On days 110–114, all cats were given paromomycin at 150 mg/kg, PO, q24hr for intractable diarrhea.

All cats were negative for *C. parvum* in feces by IFA and PCR prior to inoculation. After inoculation, *C. parvum* was detected in feces from all cats by day 9 by both IFA and PCR. *C. parvum* was not detected by IFA in feces of any cat from day 30 to day 85 but was detected by PCR intermittently in feces of 4 cats. After methylprednisolone was given, feces from all cats were IFA and PCR positive for varying time periods. *C. parvum* was detected by IFA or PCR in feces of 3 cats after treatment with paromomycin. Overall, *C. parvum* was detected by PCR in 100 of 374 samples and by IFA in 52 of 374 samples; 27 samples were PCR positive, IFA positive; 73 samples were PCR positive, IFA negative; 25 samples were PCR negative, IFA positive; and 249 samples were PCR negative, IFA negative.

Results of this study suggest that PCR is more sensitive than IFA for the detection of *C. parvum* in cats.

## 23 SAFETY OF REPEATED TREATMENTS WITH MOXIDECTIN CANINE SR (SUSTAINED RELEASE) INJECTABLE. R. Dino Rulli (1), Donald Montgomery (2), William E. Barton (3), Craig Trammel (3), Tyler Holstein (3), Denise Gomez (3), and Kathleen Heaney (1) 1. Fort Dodge Animal Health, Princeton, NJ, 2. Texas Veterinary Medical Diagnostic Lab, Amarillo, TX, 3. CAVL, Amarillo, TX.

Moxidectin is a macrocyclic lactone that has broad-spectrum antiparasitic activity. A single, subcutaneous injection of a novel microsphere sustained release (SR) formulation provides a minimum of 6 months protection from heartworm (*Dirofilaria immitis*) disease in dogs. This study evaluated the safety of up to six injections at the proposed dose rate (0.17 mg moxidectin / kg body weight) given at six month intervals.

Three groups of four dogs each received moxidectin canine SR injectable at 6 month intervals. Serum was periodically collected and analyzed for moxidectin content after the first treatment. Two dogs in each group were injected on the right side of the neck at each treatment; the other two were injected on alternative sides of the neck. One group was necropsied 6 months after their 2nd injection, one group was necropsied 6 months after their 4th injection, the third group was not necropsied but had moxidectin serum levels measured before and periodically after their 6th treatment.

Peak serum values of moxidectin were approximately 5 ppb about 7 to 10 days after the first treatment. Prior to the 6th treatment (six months after the 5th treatment) three dogs had moxidectin serum levels below the limit of quantification (0.5 ppb), whereas one dog had a serum level of 0.89 ppb. The highest moxidectin serum level detected following the 6th treatment was 3.47 ppb at seven days posttreatment. These data indicate that moxidectin serum levels do not accumulate to appreciable levels even after 6 injections at 0.17 mg moxidectin/kg body weight administered at six month intervals.

Clinically, no injection site swelling or other adverse reactions associated with the injections were noted for any animal. Microscopic examination of the injection sites at necropsy revealed macrophages, lymphocytes, and increases in collagen, which were interpreted as a reaction to the microspheres.

In conclusion, moxidectin canine SR injectable, administered at 0.17 mg moxidectin/kg body weight at six month intervals, did not cause significant adverse reactions in dogs given up to 6 injections. Moxidectin serum levels do not accumulate even after six injections. Microscopically, the injected areas from dogs injected up to four times generally had macrophages, lymphocytes, and increases in collagen.

## 24 CLINICOPATHOLOGIC AND RADIOGRAPHIC FINDINGS IN 24 DOGS WITH COCCIDIOIDOMYCOSIS: 1995–2000. LR Johnson, University of California, Davis, CA 95616

Coccidioidomycosis, a fungal infection caused by *Coccidioides immitis*, is characterized by pulmonary and bone involvement and is endemic to southwestern USA. Medical records from 1995–2000 at University of California Veterinary Medical Teaching Hospital were searched for a clinical diagnosis of coccidioidomycosis in dogs, and 24 cases were identified. Historical features and clinical findings were abstracted.

Dogs were from 1 to 10 years in age at the time of diagnosis, and 10 of 24 dogs were between 1 and 3 years of age. Male (11 of 24) and female (13 of 24) dogs were equally represented, and most were large breed dogs (median weight 20.5 kg). Historical complaints included cough (8 of 24 dogs), lameness (7 of 24 dogs) and head or neck pain (3 of 24 dogs). Twelve of 20 dogs were febrile on presentation (temperature > 102.5°F).

A complete blood count was performed in 19 dogs. Anemia and leukocytosis were present in 10 dogs each. Neutrophilia (11 dogs) and monocytosis (7 dogs) were found most commonly, and plasma fibrinogen was increased in 7 of 14 dogs tested. Blood chemistry panels were available in 15 dogs. All dogs were hypoalbuminemic (range 1.3–2.8), hyperglobulinemia was present in 8 of 15 dogs, and mild hypercalcemia was noted in 2 dogs. Thoracic radiographs were made in 23 of 24 cases, and 6 were normal. Pulmonary infiltrates were reported in 15 cases; diffuse bronchointerstitial patterns (8 of 15 dogs), patchy alveolar infiltrates (5 of 15 dogs), and nodular densities (2 of 15 dogs) were described. Hyperinflation and airway mineralization were seen in one dog each. Hilar lymphadenopathy was present in 10 of 23 cases. Abdominal ultrasound was performed in 14 dogs and was normal in 7. Mesenteric or sublumbar lymphadenopathy was reported in 3 dogs. IgM and/or IgG antibody responses to *C. immitis* were evaluated in 20 dogs by using agar gel immunodiffusion. One dog was positive for IgM only, five were positive for IgM and IgG, and 14 were positive for IgG only. Quantitative IgG titers were reported in 14 dogs and ranged from 1:2 to 1:128 (median and mode 1:32). In 6 cases, biopsy samples revealed fungal spherules ranging from 8–70 µm in diameter. Transtracheal wash or bronchoscopy was performed in 2 cases but did not reveal fungal organisms in either case.

Diagnosis was based on serology in this group of dogs. Pulmonary coccidioidomycosis appeared to be common, however airway samples were rarely collected. Evaluation of airway lavage samples is useful in characterizing many respiratory diseases, however *C. immitis* organisms are reportedly rare in cytologic specimens, and the diagnostic efficacy of airway sampling in coccidioidomycosis is unknown.

## 25 CYTOKINE PROFILES IN PERIPHERAL BLOOD MONONUCLEAR CELLS AND BRONCHOALVEOLAR LAVAGE CELLS IN CATS WITH EXPERIMENTAL FELINE ASTHMA. CR Norris, CM Leutenegger, LJ Gershwin, and DM Hyde. University of California, Davis.

Allergic asthma in humans and cats is associated with development of reversible airway obstruction, nonspecific airway hyperreactivity, and histologic evidence of airway remodeling. We hypothesize that allergic airway responses in cats, similar to humans, are associated with CD4+ T cells predominantly expressing Th2 cytokines. Cats sensitized to house dust mite allergen (HDMA; 3 cats) or bermuda grass allergen (BGA; 3 cats) were used to determine the cytokine pattern of peripheral blood mononuclear cells (PBMCs) and bronchoalveolar lavage (BAL) cells using real-time TaqMan reverse transcriptase polymerase chain reaction (RT-PCR) to quantify cytokine messenger RNA (mRNA) transcription. Baseline collection of PBMCs and BAL cells was performed prior to sensitization with parenteral and intranasal allergen over a 4 week period. Aerosolization of allergen in awake cats in a sealed chamber was then performed twice weekly for 7 treatments, and 48 hours after the last aerosol challenge, PBMCs and BAL cells were collected. Cytokines were quantitated from unstimulated BAL cells and PBMCs. Extracted RNA was reverse transcribed to create complementary DNA (cDNA) which was subsequently amplified using a real-time TaqMan PCR system. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as the endogenous control. Profiles of feline IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-12 p40, IL-18, IFN- $\gamma$ , IFN- $\alpha$ , TNF- $\alpha$ , MIP-1 $\alpha$  and RANTES were determined. Statistical comparisons between baseline values and post-sensitization and challenge were performed using a Mann-Whitney U test; a P value <0.05 was considered significant.

Results showed significantly increased transcription of the type 2 cytokines IL-4, IL-6, and IL-10 in the BAL cells and PBMCs of sensitized and challenged cats, whereas the transcription of the type 1 cytokines IL-18 and IFN- $\gamma$  were significantly downregulated. In both BAL cells and PBMCs, the IL-12 p40 transcription was not affected. In addition, BAL cells for sensitized and challenged cats showed significantly suppressed transcription for the CD4 chemotactic cytokine IL-16 and increased transcriptional activity for the monocyte chemoattractant RANTES. Our preliminary data suggest cats sensitized with either HDMA or BGA display a type 2 cytokine profile, similar to what is reported in humans with asthma. Therapeutic intervention to manipulate the Th2 response may be useful in the modulation of allergic airway responses in the cat.

## 26 EFFECTS OF SUBLETHAL ENDOTOXIN ADMINISTRATION ON PHAGOCYTOSIS AND PATHOLOGY IN FELINE LUNG AND LIVER. C.M. Weigand, Rowley Memorial Animal Hospital, Springfield, MA. A.R. Dillon, N.R. Cox, Auburn University College of Veterinary Medicine, Auburn, AL.

Pulmonary intravascular macrophages (PIM) are resident phagocytic cells found in pulmonary capillaries of certain species of animals. These cells are important in the induction of acute lung injury (ALI) due to their location, phagocytic abilities, and secretory abilities. Sepsis is a major risk factor for acute lung injury, particularly in most species that have PIMs. The purpose of this study was to evaluate the effects of serial, sublethal doses of lipopolysaccharide (LPS) on phagocytic abilities and pathology of the lung and liver in the cat.

Eight cats were randomly divided into control and treatment groups. Each cat was sedated on day 0, and a central venous line was placed. Cats in the treatment group (n = 4) were administered 1 mcg/kg of LPS IV on days 1, 3 and 5. Cats in the control group (n = 4) were administered saline IV on days 1, 3 and 5. On day 6, each cat was administered 1 mCi Tc99 labeled sulfur colloid IV. Twenty-four hours later, the cats were humanely euthanized. Phagocytosis was evaluated based on tissue uptake of Tc99 in a well-type gamma scintillation counter of multiple samples. Percent phagocytosis was calculated based on organ weights for lung, liver, spleen, kidney, heart, skeletal muscle, bone marrow, blood, and urine. Light microscopy of lung and liver and transmission electron microscopy (TEM) of lung were independently reviewed by two investigators and assigned a numeric score. The scintillation counts and pathology scores were compared using one way ANOVA and box plot variance analysis.

Vomiting, which was the only clinical sign, was induced in three of the four treatment cats within 20 minutes after LPS administration. There was no statistical difference in the pathology scores between the control and treatment groups. Phagocytosis was significantly affected. In the treatment group, pulmonary uptake of Tc99 labeled sulfur colloid was significantly decreased as compared to the control group (45% vs 62%, p = 0.01). Hepatic uptake of Tc99 labeled sulfur colloid was significantly higher in the treatment group (34% vs 51%, p = 0.01).

We conclude that systemic intravascular exposure to serial, sublethal doses of LPS significantly decreased phagocytic capabilities in the lung of the cat without induction of significant pathologic changes. The doses administered in this model, which induce ALI in sheep and are fatal in other species, did not induce pathology in the lung or liver of the cat. Repeated systemic LPS exposure was associated with decreased phagocytosis in the lung and a relative increase in hepatic phagocytosis.

## 27 IMMUNOGLOBULIN LEVELS AND LYMPHOCYTE SUBSETS IN PERIPHERAL BLOOD AND BRONCHOALVEOLAR LAVAGE FLUID IN DOGS WITH EOSINOPHILIC BRONCHOPNEUMOPATHY. C. Clercx, D. Peeters, A. German, K. McEntee K, Y. Khelil, A. Vanderplaschen, F. Schyns, M.J. Day. University of Liège, Belgium and University of Bristol, England.

Immunological parameters in dogs with eosinophilic bronchopneumopathy (EBP) have not been extensively evaluated. The aim of this study was to determine immunoglobulin levels and to perform phenotypic subtyping of lymphocytes in the bronchoalveolar lavage fluid (BALF) and peripheral blood (PB) in dogs with EBP at the time of the diagnosis (TD), and to compare them with those obtained in healthy dogs, as well as in EBP dogs during corticosteroid treatment (TM).

EBP was diagnosed in 12 dogs, based on physical, radiographical, bronchoscopic, cytological and histopathological criteria. Oral corticotherapy was initiated either immediately (6 dogs), or after antibiotherapy (6 dogs), and the dose was tapered to achieve maintenance levels (TM). Four ml of BALF and 2 ml of PB were used to study lymphocyte subpopulations. Purified mononuclear cells were incubated with monoclonal antibodies reacting with CD4 or CD8. The percentages of CD4+ and CD8+ T cells were determined. Matched samples of serum and BALF were used to determine immunoglobulin (Ig) concentrations (IgG, IgM and IgA) by capture ELISA. The proportion of secreted Ig was determined using a secretory index (SI).

There was no significant difference in serum Igs in EBP dogs, when compared to controls. After corticotherapy, all serum IgG and IgA values were lower than control. Absolute values of BALF IgG, IgM and IgA were significantly higher in EBP dogs at TD than in controls. However, the secretory index in dogs with EBP at TD did not differ significantly from control. In EBP dogs, treatment with corticosteroids led to a decrease in all BALF Ig values (IgG, M and A) as well as in the SI for IgA.

In the PB of dogs with EBP at TD, there were significantly raised percentages of CD4+ and CD8+ T cells in comparison to controls, but there was no significant difference in CD4:CD8. In the BALF of dogs with EBP at TD, there were significantly higher percentages of CD4+T cells and significantly lower percentages of CD8+T cells than in controls, with a significantly higher CD4:CD8 than control. Following corticotherapy the BALF T cell percentages returned to normal.

Dogs with EBP appear to have a selective increase in the numbers of CD4+T cells within the respiratory mucosa, a finding similar to studies in human asthma. We propose that the influx of eosinophils into the airway of dogs with EBP is at least in part mediated by cytokines derived from these CD4+T cells. Further studies of canine cytokines and chemokines will help determine whether canine EBP involves type I hypersensitivity mechanisms regulated by Th2 lymphocytes.

## 28 EFFECTS OF MELATONIN IMPLANTS ON OVARIAN FUNCTION IN THE DOMESTIC CAT. B. Griffin,<sup>1,2</sup> A. M. Heath,<sup>2</sup> D. W. Young,<sup>2</sup> J.C. Wright,<sup>2</sup> M. D. Rolsma,<sup>2</sup> H. J. Baker,<sup>1,2</sup> Scott-Ritchey Research Center,<sup>1</sup> College of Veterinary Medicine,<sup>2</sup> Auburn University, Auburn, AL.

The pineal gland and melatonin frequently influence reproductive activity in species that are seasonal breeders, including domestic cats where exogenous melatonin administration inhibits ovarian function. The purpose of this study was to determine if continuous melatonin administration (melatonin implants) suppresses ovarian function.

Two colonies of 6 DSH queens were group housed on a stimulatory light cycle (12D:12L). Reproductive cyclicity was documented for each queen by daily observation for behavioral estrus, fecal estradiol concentrations every other day and biweekly serum progesterone concentrations. After 12 months, cats were sedated with torbugesic (0.3 mg/kg), midazolam (0.2 mg/kg) and ketamine (10 mg/kg) SQ for aseptic placement of 12 mg melatonin implants (12 ga, 3/8 inch) (Wildlife Pharmaceuticals, Inc., Ft. Collins, CO) SQ between the shoulder blades using a syringe gun. Four queens received 1 implant (low dose group), 4 queens received 5 implants (high dose group) and 4 queens received placebo implants (control group). Hematology and serum biochemistries were performed prior to implantation and ovariectomy. Plasma was collected biweekly for melatonin concentrations. Body weights were recorded weekly and activity daily. All monitoring was continued for 6 months and queens were ovariectomized.

Major changes in body weight and activity were not seen. Clinicopathologic evaluation was normal. Estrus suppression occurred in 0/4, 2/4 and 3/4 cats in control, low and high dose groups, respectively. Mean biweekly plasma melatonin concentration in control cats was 1.27 pg/ml over the entire study period. Mean biweekly plasma melatonin concentrations ranged from a high of 209.2 pg/ml and 932.3 pg/ml to a low of 43.7 pg/ml and 232.5 pg/ml in low and high dose groups, respectively. Mean length of time from implantation to estrus suppression was 20 days (range 17–26 days). Mean duration of estrus suppression was 75 days (range 53–94 days.) Pre-implant interestrous intervals ranged from 1–4 weeks. No correlation was found between implant dosage or plasma melatonin concentration and estrus suppression. Histopathology of the ovaries and uterus of all 8 implanted queens revealed marked uterine wall thickening, endometrial hyperplasia and frequent cystic change. Tissue cultures were negative.

Melatonin implants may result in short term estrus suppression in some queens, however the dosage required is relatively high and varies dramatically among individual queens. Although the mechanism is unclear, continuous melatonin administration causes uterine pathology and cannot be recommended as a safe contraceptive in cats.



## 29 THE EFFECT OF ELECTROCARDIOGRAPHIC FILTERS ON THE R-AMPLITUDE OF CANINE ELECTROCARDIOGRAMS.

E. Dvir, P. J. Cilliers, R. G. Lobetti, University of Pretoria, RSA.

The goal of this study was to describe the influence of low-pass filtering on R-amplitude in the canine ECG and to propose a simple formula to compensate for these changes. Additionally the correlation between the mass of the dog and the magnitude of the reduction in the R-amplitude by the filtering was evaluated.

Lead II ECGs were recorded in 88 dogs with canine babesiosis ranging in mass from 3 to 50 kg. A commercial direct writing electrocardiograph was used with manual notch filter at 50/60 Hz and a low-pass filter with a cutoff frequency of -3dB at 35 Hz ON, immediately followed by recording with both filters OFF.

The reduction in R-amplitude from filters OFF to filters ON settings was evaluated and ranged from 22-100% (mean = 53 ± 18%, median = 51%). The R-amplitude with filters OFF was related to the R-amplitude with filters ON, providing a possible practical formula to retrospectively correct for the effect of the filters. The R-amplitude reduction was found to be inversely correlated to body mass and to QRS-complex duration. Other known changes induced by filters, such as the elimination of notches and slurring of the junction between the QRS-complex and the ST-interval to a point of coving, were also observed.

These severe R-amplitude reductions indicate that for canine practice an electrocardiograph with better high frequency response than that obtained with filters in an ECG machine designed for humans is needed. Electrocardiograms should preferably be recorded without filters. However, severe electrical noise artefacts sometimes necessitate the use of filters, indicating the need for specifically designed ECG-filters for veterinary use. The inverse relation between dog mass and the magnitude of R-amplitude reduction due to ECG-filters indicate the need for different ECG-machine characteristics for different size animals.

## 31 CLINICAL, ECHOCARDIOGRAPHIC, AND ECG FINDINGS IN 232 SEQUENTIALLY EXAMINED IRISH WOLFHOUNDS.

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To help determine the prevalence of acquired heart disease in Irish wolfhounds, 232 from northwestern Europe were sequentially examined by physical examination, 10-lead ECG, 2D and CFD echocardiography (AV) between (6/1999-9/2000): 152 (65.5%) were normal; 28 (12.1%) had DCM; 11 (4.7%) had LA enlargement; 10 (4.3%) had RV enlargement; 19 (8.2%) had relatively normal echocardiograms with atrial fibrillation [AF]; and 12 (5.2%) had sinus rhythm with intraventricular conduction defects.

In the normal cohort (n = 152; 60 m, 92f), none had detectable cardiac murmurs or gallop rhythms. Ages were 3.0±1.4yrs- m, v. 3.3±1.7yrs- f (NS). Males were 70±7.3kg v. 59.8±6.4kg (f), p<0.001. Heart rate (beats/min) at examination was 124±25- m, v. 123±21- f (NS). Males had larger echo parameters (mm) than females for: LVd, 51.7±6.7 v. 50.2±3.7 (p = 0.019); LVWd, 11±1.7 v. 10.3±1.6 (p = 0.015); IVSd, 11.5±1.8 v. 10.8±1.9 (p = 0.031); IVSs, 16.1±2 v. 15.1±2.3 (p = 0.006); Ao, 34±2.8 v. 32±3 (p<0.001); RA, 39.7±4.7 v. 38.9±4.7 (p = 0.049); RVd, 30.4±3.3 v. 29±3.6 (p = 0.025). There was NS difference (mm) between m v. f in: LVs (33.9±2.8 v. 33.5±4.7); LVWs (16±2.1 v. 15.6±1.9); LAs (31.9±3.3 v. 31.8±3.2); EPSS (6.3±1.5 v. 6.2±1.6); %FS (34.4±3.8 v. 33.4±4.5); EF% (63.3±5.6 v. 62.3±7.3). Comparison of P-QRS-T parameters (m v. f) for leads II, CV<sub>3</sub>RL, CV<sub>3</sub>LL, CV<sub>3</sub>LU, V<sub>10</sub> were NS (except R-wave, CV<sub>3</sub>LU: 2.4±0.8mV- m v. 2.7±1.0mV- f (p = 0.024). Pooled gender P-QRS-T parameters for lead II were: P-wave, 0.24±0.07mV, 44.5±7.8msec; PR, 115±14.6msec; Q-wave, 0.34±0.21mV; R-wave, 2.0±0.58mV; S-wave, 0.16±0.13mV; QRS, 58.3±6.1msec; QT, 297±22.1msec; T-wave, 0.3±0.27mV; salient precordial data was: CV<sub>3</sub>RL R-wave, 1.27±0.78mV; S-wave, 0.62±0.39mV; CV<sub>3</sub>LL R-wave, 1.97±0.92mV; S-wave, 0.38±0.26mV; CV<sub>3</sub>LU R-wave, 2.6±0.97mV, S-wave, 0.42±0.33; V<sub>10</sub> Q-wave, 0.71±0.23mV, R-wave, 0.29±0.15mV.

The 28 dogs with DCM (16m, 12f) were: 4.2±1.9yrs, 67.9±9.8kg; atrial fibrillation (AF) occurred in 19 (147.2±43.2bpm) and sinus rhythm (146.5±37bpm) in 9; 12 had systolic heart murmurs and 7 were in CHF. Excluding arrhythmias, the ECG was abnormal in 3/28 DCM dogs (increased R-wave voltage in left precordial chest leads). FS in 14 dogs receiving therapy was 21.9±3.7% v. 25.7±5.3% in 14 others without therapy.

Nineteen additional dogs had AF: 8/19 (6m, 2f; 2.7-7.9 yrs; HR, 144.8±38.2bpm) had mild-moderate LA enlargement; FS was 32.6±4.6%. In the remaining 11 (4m, 7f; 1.8-8.5 yrs; HR, 148.6±29bpm), DCM had been previously diagnosed (3/11 had CHF), but LVs and LVd had improved to normal with therapy; %FS was 28.0±3.9% (22.2-24.9% in 3).

Structural or functional cardiac derangement was detected in 80/232 (34.5%) dogs (CHF in 10, 4.3%); beyond rhythm assessment, the ECG was generally unremarkable.

## 30 NOISE REVERSION IN PACED DOGS.

N. Sydney Moise and Amara H. Estrada. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Noise reversion is a protective algorithm used in pacemakers that causes asynchronous pacing when there is repetitive sensing in the ventricle that is faster than the noise reversion rate. The purpose is to prevent inappropriate pacemaker inhibition by electromagnetic interference, electrocautery, and myopotentials. Tachycardias can cause pseudo-nonsensing with asynchronous pacing due to noise reversion with the risk of capturing the ventricle during the vulnerable period resulting in ventricular fibrillation. The purpose of this report is to demonstrate that noise reversion pacing is a correctable complication in paced dogs. Four dogs with syncope and bradyarrhythmias that required pacing were studied. Each dog also had a tachyarrhythmia. After pacemaker implantation all dogs experienced inappropriate pacing. Noise reversion was confirmed by the marker channel recording as detected by a Medtronic programmer model 9790 (see figure). To correct the noise reversion the refractory period and sensitivity were optimized (see table) for each dog after amplitude and pulse width strength-duration curves were examined. It was found that shortening the refractory period and decreasing the sensitivity prevented the retriggerable refractory period and inappropriate asynchronous pacing. It is important that this complication of inappropriate programming of pacemakers in dogs be differentiated from undersensing.

Rhythms, QT, and Programmed Parameters During (d) and After (a) Noise Reversion

Dog	Rhythm	Slow/Fast Rate (bpm)	Slow QT (msec)	Fast QT (msec)	Pacemaker Model Pacing Mode
1	3 <sup>rd</sup> /VT	40/315	320	190	Thera S 8964i Unipolar
2	SSS/SVT	30/260	220	180	Preva SR 8089U Unipolar
3	SSS/SVT	50/270	370	200	Legend II 8424 Unipolar
4	AS/SVT	40/150	215	165	Preva SR 8089U Unipolar

Dog	Refractory Period (msec)	Sensitivity (volts)
1	330 <sup>a</sup> /270 <sup>a</sup>	2.8 <sup>a</sup> /2.8 <sup>a</sup>
2	310 <sup>a</sup> /240 <sup>a</sup> /330 <sup>a</sup>	2.8 <sup>a</sup> /5.6 <sup>a</sup> /8.0 <sup>a</sup>
3	475 <sup>a</sup> /220 <sup>a</sup> /330 <sup>a</sup>	2.5 <sup>a</sup> /2.5 <sup>a</sup> /5.6 <sup>a</sup>
4	330 <sup>a</sup> /330 <sup>a</sup>	2.8 <sup>a</sup> /8.0 <sup>a</sup>

3<sup>rd</sup>-third degree heart block, VT-ventricular tachycardia, SSS-sick sinus syndrome. SVT-supraventricular tachycardia, AS-atrial standstill.

## 32 EVALUATION OF PULSED WAVE DOPPLER TECHNIQUE FOR DETERMINING CARDIAC INDEX IN NORMAL AND PHARMACOLOGICALLY STRESSED DOGS.

R.L. Pyle, J.A. Abbott, T.W. Chittenden, D.L. Ward, Virginia Tech, Blacksburg, VA.

Cardiac output (CO) and cardiac index (CI) can be important parameters for evaluating global cardiac performance. A non-invasive, echocardiographic technique for determining CI has potential clinical and research implications.

Twenty healthy, adult dogs weighing 13-32 kg (mean = 22.31 kg) were induced with pentothal and maintained with isoflurane. Two operators used two echocardiographs (Hewlett Packard Sonos 1000 and General Electric System FiVe) to image from the left side down position. Ten of the dogs were pharmacologically stressed by giving intravenous dobutamine to a level that approximately doubled the non-dobutamine CO. Pulsed wave Doppler was used to obtain the aortic velocity profile from the left caudal window. The transducer was oriented to maximize the alignment of the aorta with the Doppler beam. Velocity profiles were traced on three consecutive beats to provide velocity time integrals (VTIs). The aortic area was determined from the two dimensional image in the left cranial, short axis view. Three consecutive area measurements were made in systole using the electrocardiogram and the position of the aortic valve leaflets as reference points. The VTIs were multiplied by the aortic area and heart rate/minute to provide CO. CO was normalized to body surface area to provide CI.

The echocardiographic results were compared to thermodilution (TD) values for CO/CI. A vessel sheath-introducer system was placed percutaneously into the right jugular vein and a 7-7.5 Fr Swan-Ganz catheter was advanced into a pulmonary artery. Catheter placement was guided by monitoring intravascular pressures. A minimum of three TD values were obtained at each event by injecting iced D5W.

Measurements from all dogs before and after stress were pooled to assess and compare the methods. The described method for determining CI consistently provided higher values than TD. Paired t-tests detected positive biases (p<0.0001). Bland-Altman plots revealed a greater bias at higher CI values when dobutamine was administered.

The greatest source of variability in this method appears to be the determination of aortic area (51%). The VTI measurement is a lesser but significant contributing factor (34%). Operator to operator variability was less significant (15%).

Pulsed wave Doppler CO/CI measurements in normal or pharmacologically stressed, healthy, anesthetized dogs cannot be used as a direct substitute for TD measurement of CO/CI.



### 33 A RETROSPECTIVE STUDY OF 26 DOGS WITH PULMONIC STENOSIS. J. M. E. Ristic, C. Marin and M. E. Herrtage. Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, UK.

Pulmonic stenosis is a common congenital heart defect in dogs and treatment using percutaneous pulmonary balloon valvuloplasty (PBV) has produced promising results in short term studies. The aim of this study was to evaluate the longer term effects of PBV in dogs with congenital pulmonic stenosis and to compare the results with a group of untreated dogs.

A retrospective study revealed 26 dogs diagnosed with pulmonic stenosis between 1990–1999. Sixteen dogs were treated with PBV and eight were not treated. The dogs were followed for a period of between 6 months and 9 years (mean 3.2 years).

Twelve of the PBV treated dogs were alive and asymptomatic. One dog died during PBV and three dogs were euthanased for various reasons between 1 month to 2 years post PBV. The mean Doppler pressure gradient across the pulmonic valve before PBV was 98 mmHg, (range 51–144 mmHg). Twenty four hours after treatment, the pressure gradient was reduced to a mean of 65 mmHg (range 16–108 mmHg). In the longer term, the mean Doppler pressure gradient was 64 mmHg (range 16–131 mmHg). Although the reduced mean pressure gradient was maintained, three dogs (17%) re-stenosed, one dog showed a further reduction in the pressure gradient and the remaining dogs maintained a similar pressure gradient in the follow up period.

Four out of the eight untreated dogs were alive 2 to 4.5 years after diagnosis. The mean Doppler pressure gradient at the time of diagnosis for this group was 89 mmHg (range 36–144 mmHg). One dog showed a 50% reduction in the pressure gradient in the longer term.

A retrospective classification of the type of stenosis was possible in 21 dogs. Nineteen dogs had evidence of valvular stenosis of which 64% were dysplastic and 36% were fused. The type of stenosis did not appear to influence the results of PBV, but the number of cases in each group was small.

Despite an apparent improvement in survival following PBV, there was no statistically significant difference in the survival rates between the treated and untreated groups. However, symptomatic dogs with pulmonic stenosis treated with PBV did experience a reduction in clinical signs and an improvement in their quality of life.

### 34 RELATIONSHIP BETWEEN SEVERITY OF SUBVALVULAR AORTIC STENOSIS AND VENTRICULAR ECTOPIA IN YOUNG NEWFOUNDLAND DOGS. Bradley A. Green, Kathryn M. Meurs, Linda B. Lehmkühl, John D. Bonagura, Alan W. Spier, Andrea Nicastro. The Ohio State University, Columbus.

The natural history of moderate to severe subvalvular aortic stenosis (SAS) in dogs often includes sudden death. Progression of ventricular premature complexes (VPC) to ventricular fibrillation is thought to be causative in some of these dogs. Myocardial hypertrophy, fibrosis, and ischemia can serve as substrates for ventricular ectopia, and these abnormalities are related to the severity of outflow tract obstruction. The objective of this retrospective study was to examine the relationship between severity of SAS, as defined by the peak pressure gradient (PG), and the number/complexity of VPC, as recorded on a 24-hour ambulatory electrocardiogram (AECG).

Medical records from Newfoundland dogs between 6 and 18 months of age with a diagnosis of moderate to severe SAS were retrieved. The diagnosis of SAS was confirmed in each case by continuous-wave Doppler echocardiography recorded from the subxiphoid position. Assessment of VPC was based on the 24-hour AECG. Only dogs with AECG recordings exceeding 20 hours were included. Dogs with multiple cardiac lesions or those receiving antiarrhythmic or beta-blocker therapy were excluded from analysis. Age, gender, peak PG, total number of VPC, and maximal grade of VPC were tabulated. Grade of VPC was categorized as follows: 0 = none, 1 = single VPC, 2 = bigeminy, trigeminy, 3 = couplets, triplets, 4 = R on T or ventricular tachycardia. For statistical evaluation, dogs were further categorized based on VPC number per 24 hours: group 1 (<5 VPC), group 2 (5 to 100 VPC), or group 3 (> 100 VPC). A one-way ANOVA was performed to identify differences in peak PG based on group. Correlations between PG and VPC number and between PG and VPC grade were determined by the Spearman rank correlation coefficient.

Thirty-one dogs between 6 and 17 months of age were identified. These included 18 males (58%) and 13 females (42%). Peak PG ranged from 48–228 mmHg. Averaged peak PG for the three groups were 89 mm Hg (group 1), 130 mm Hg (group 2), and 169 mm Hg (group 3). VPC number ranged from 0 to 11,733 and arrhythmia grade ranged from 0 to 4. A modest positive correlation was noted between peak PG and VPC number ( $r = 0.60$ ), and between peak PG and VPC grade ( $r = 0.68$ ). A significant difference ( $p < .001$ ) was observed in peak PG between groups 1 and 3.

These data indicate that Newfoundland dogs with more severe SAS as estimated by PG are more likely to have higher numbers of VPCs and a more severe grade of arrhythmia complexity. Factors that trigger ventricular ectopia in dogs with SAS require further study as does the relationship between the number/complexity of VPC and occurrence of sudden death.

### 35 CLINICAL SPECTRUM OF CONGENITAL TRICUSPID VALVE MALFORMATIONS IN AN EXTENDED FAMILY OF LABRADOR RETRIEVERS. K. N. Wright, M. E. Bleas, D.W. Benson. The Children's Hospital Medical Center, Division of Cardiology, Cincinnati, OH.

Congenital tricuspid valve malformations (CTVM) encompass a broad spectrum of developmental abnormalities involving the tricuspid valve apparatus and architecture of the morphologic right ventricle. We have identified an extended family of Labrador Retrievers in which CTVM appear to be inherited. The purpose of this study was to define the spectrum of auscultation, ECG, and echocardiographic findings in this family of dogs with CTVM. Physical examinations, 12-lead ECGs, and echocardiograms have been performed on 35 relatives to date from four consecutive generations.

Congenital tricuspid valve malformations have been found in approximately 28% of the family members examined thus far. Affected dogs have been identified in all 4 generations. Males and females have been equally affected. Six dogs had audible murmurs of tricuspid regurgitation, varying from grade II-III. No murmurs of tricuspid stenosis were auscultated. Three dogs had detectable abnormalities of their first heart sound. Electrocardiographic abnormalities included right axis deviation in the frontal plane (7 dogs), left cranial axis deviation in the frontal plane (2 dogs), deep S waves in the left precordial leads (4 dogs), and splintering of the QRS complex (4 dogs). No family members to date have had electrocardiographic evidence of right atrial enlargement. The left parasternal apical four-chamber view proved to be the single most informative echocardiographic view, although a number of other views were also helpful in fully defining the anatomy of the tricuspid valve apparatus and right ventricle. Apical displacement of the septal tricuspid valve leaflet (TVL) (6 dogs), tethering of the septal TVL by abnormal chordae tendinae (4 dogs), elongation and redundancy of the parietal TVL (5 dogs), prolapse of the septal and/or parietal TVL (4 dogs), thickening of one or both TVLs (9 dogs), and the presence of hypertrophied and/or fused right ventricular papillary muscles (6 dogs) were found in various combinations in members of this family and ranged from mild to severe. Tricuspid regurgitation was found by color flow Doppler in all affected dogs and again ranged from mild to severe. Tricuspid stenosis has not been identified to date in any members of this kindred.

We conclude that CTVM are inherited in this family of dogs and that a wide spectrum of clinical, ECG, and echocardiographic abnormalities are seen in affected dogs. Studies now are directed at defining the inheritance pattern and performing initial molecular genetic evaluation in this and other kindreds of Labrador Retrievers with CTVM.

### 36 ECHOCARDIOGRAPHIC MEASUREMENT OF PATENT DUCTUS ARTERIOSUS. M. Schneider, N. Hildebrandt, T. Schweigl, M. Wehner. Medical and Forensic Veterinary Clinic, Department of Small Animal Internal Medicine, Justus-Liebig-University of Giessen, 35392 Giessen, Germany.

The aim of the study was to compare the echocardiographic and angiographic measurements of the minimal diameter of the patent ductus arteriosus (PDA) in dogs.

40 dogs with a natural occurring PDA were explored prospectively (May 1995 to December 1998). Measurements of the minimal PDA diameter in two-dimensional and color flow Doppler echocardiography were made from the left cranial parasternal window by means of electronic sector probes (2.5–7.5 MHz). The maximum value was calculated by threefold determination. As control the angiographic determination of PDA minimal diameter in the lateral projection was performed.

The measurements of the PDA minimal diameter in two-dimensional and in color flow Doppler echocardiography were successful in 38 of 40 dogs (95%). At these 38 dogs the angiographic PDA minimal diameter ranged from 1.5 to 6.9 mm (median 3.3 mm). In the echocardiographic study the median value was 2.9 mm (range 1.5–7.5 mm) in two-dimensional technique and 3.8 mm (range 2.3–8.7 mm) in color flow Doppler. The median difference to the angiographic measurement was 0.1 mm (range –1.6–1.4 mm) and 0.8 mm (range –0.9–2.8 mm) respectively. There was a strong correlation between angiography and two-dimensional echocardiography ( $R = 0.904$ ,  $p < 0.001$ ) as well as color flow Doppler ( $R = 0.885$ ,  $p < 0.001$ ).

For a skilled observer the echocardiographic measurement presents a good possibility to estimate the PDA minimal diameter for a more precise planning of a catheter intervention.

- 37** EVALUATION OF DOPPLER ULTRASONIC AND OSCILLOMETRIC ESTIMATES OF BLOOD PRESSURE IN CATS. Scott Brown, Christopher Haberman, and Jefferson Morgan, College of Veterinary Medicine, University of Georgia, Athens, GA.

To evaluate the utility of methods for the indirect measurement of systemic arterial pressure in cats, results of over 300 measurements by an oscillometric device (Dinamap Model 8300) or a Doppler ultrasonic flowmeter (Parks Model 811) were compared to simultaneously obtained values using radiotelemetry (Model TA11PA-C40 Implants, Data Sciences International) in 14 cats.

In conscious cats, the strength of correlations of the indirect devices with direct measurement by radiotelemetry was dependent upon site selection and device with values for  $R^2$  ranging from 0.822 to 0.826 with the ultrasonic Doppler device and ranging from 0.000 to 0.349 with the oscillometric device. Averaging 5 consecutive values improved the strength of correlations between the indirect estimates and direct measurements, with an  $R^2$  of 0.839 with the ultrasonic Doppler device and values for  $R^2$  ranging from 0.362 to 0.550 with the oscillometric device ( $P < 0.0001$  for all). In anesthetized cats these correlations were improved with an  $R^2$  value of 0.928 with the ultrasonic Doppler device and values for  $R^2$  ranging from 0.602 to 0.853 with the oscillometric device ( $P < 0.0001$  for all). The mean difference between the direct measurements and indirect estimates ranged from 11.5 to 25.6 mm of Hg in the conscious cats and 11.5 to 27.0 mm of Hg in the anesthetized cats.

Both of the indirect devices produced errors in accuracy and precision in conscious cats and results obtained by indirect devices in conscious or anesthetized cats should be interpreted cautiously. The ultrasonic Doppler device provided the strongest correlations with directly measured blood pressure, particularly in conscious cats.

- 39** PULMONARY HYPERTENSION ASSOCIATED WITH HYPOXEMIA DUE TO RESIDENCE IN MODERATE ALTITUDE IN DOGS. T.M. Glaus, B. Reiner, M. Casella, D.M. Glaus, C. Reusch. University of Zurich, Zurich, Switzerland.

Pulmonary hypertension is a well recognized entity in dogs, and among other causes can be due to pulmonary thromboembolism, left sided heart failure, and chronic respiratory disease. Up till now the influence of permanent living in high altitude on pulmonary artery pressure in dogs is not known. Hence, in order to evaluate the effect of residence in moderately high altitude on pulmonary artery pressure in dogs, a colony of clinically and biochemically normal greenland sled dogs ( $n = 19$ ) living permanently  $> 2300$  m above sea level was examined.

Laboratory parameters to evaluate degree of hypoxemia were packed cell volume (PCV) and arterial blood gas. The cardiovascular system was evaluated with 6-lead-ECG, blood pressure measurement, and cardiac ultrasound. Systolic pulmonary artery pressure was estimated by color flow doppler guided continuous wave doppler examination of tricuspid regurgitation (TR) using the modified Bernoulli equation. Pulmonary hypertension was defined as TR peak velocity  $> 2.8$  m/s.

Laboratory findings were median PCV of 48.5 % (range 41–59), median arterial PO<sub>2</sub> of 63 mmHg (range 47–76), and median arterial oxygen saturation of 91.5 % (range 81–96). No abnormalities were found on ECG. Median systolic, diastolic and mean blood pressure were 155 mmHg (range 109–192), 101 mmHg (range 66–131), and 116 mmHg (range 80–153). In all dogs 2-D- and m-mode cardiac ultrasound were unremarkable. TR was detectable by color flow doppler in 8 of the 19 dogs. Median TR peak velocity in these 8 dogs was 2.85 m/s (range 1.8–3.4 m/s). Five of the 8 dogs had pulmonary hypertension.

Dogs living permanently in moderate altitude have moderate arterial hypoxemia with resultant mild decrease in oxygen saturation, and evidence of pulmonary hypertension. The pulmonary hypertension is considered mild, and does not appear to cause cardiovascular problems. However, in association with other diseases causing pulmonary hypertension, cardiovascular changes due to residence in moderate altitude may be of clinical relevance.

- 38** MYOMESIN, A SARCOMERIC PROTEIN, IS REDUCED IN MAINE COON CATS WITH FAMILIAL HYPERTROPHIC CARDIOMYOPATHY. KM Meurs,<sup>1</sup> MD Kittleson,<sup>2</sup> PJ Reiser,<sup>1</sup> AL Maganon,<sup>1</sup> JA Towbin<sup>3</sup> The Ohio State Univ., Columbus, OH,<sup>1</sup> Univ. of California-Davis, CA,<sup>2</sup> Baylor College of Medicine, Houston, TX.<sup>3</sup>

Hypertrophic cardiomyopathy (HCM) is a familial disease inherited as an autosomal dominant trait in the Maine Coon (MC) cat. The disease has many similarities to familial HCM in humans, including inheritance pattern, clinical presentation, and histopathologic changes. Over 125 mutations in nine genes encoding sarcomeric proteins (beta myosin heavy chain ( $\beta$ -MHC),  $\alpha$  tropomyosin, troponin I, troponin T, myosin light chains, myosin binding protein C, alpha cardiac actin, and titin) are known to cause familial HCM in humans. The objective of this study was to evaluate myocardial sarcomeric proteins from MC cats with familial HCM.

Left ventricular samples, obtained and frozen at  $-70^\circ\text{C}$  immediately post-mortem from 5 affected MC cats, 1 unaffected MC, and 3 unaffected mixed breed cats, were evaluated by SDS-PAGE.

A 180-kDa protein was reduced or absent in all affected MC cats but present in all unaffected cats. Evaluation of this protein by mass spectrometry and Western blot analysis determined it to be myomesin, an M band sarcomeric protein. Additionally in the affected cats, the amount of a 250-kDa protein was markedly increased in a reciprocal fashion with a decrease in  $\beta$ -MHC. The 250-kDa protein was determined to be anomalously-migrating  $\beta$ -MHC.

We conclude that myomesin, a sarcomeric protein, is reduced or absent in myocardium from MC cats affected with familial HCM. Further, there is an alteration in  $\beta$ -MHC in the same cats. This information supports the theory that familial HCM in MC cats is a disease of the sarcomere, and it provides the rationale for additional evaluation of the feline myomesin gene.

- 40** RESPONSE TO ACTIVE HEAD-UP TILT DURING PROGRESSION OF RAPID-PACING INDUCED HEART FAILURE IN DOGS. Schwartz DS,\* Miyamoto M; Nishijima Y; Hamlin RL. Dept. of Veterinary Biosciences, OSU, Columbus, OH; \* FMVZ-UNESP-Botucatu, SP, Brazil.

The immediate heart rate (HR) response to standing has a characteristic bimodal increase in the first 20 seconds of postural change in normal dogs. The response can be quantified by the mean increase in HR during tilt, the maximal increase in HR, as well as the relative decrease in HR observed normally around 11 seconds of active head-up tilt (a measure of reflex vagal tone).

The method was applied to 15 dogs ( $30.4 \pm 0.96$  kg) during the progression of tachycardia-induced heart failure (HF) model with the Mayo-Clinic modification. Baseline head-up tilts were performed in the dogs before they were submitted to the pacemaker implant, and after 3 weeks of pacing at 180bpm (I) and 3 weeks after pacing at 200bpm (II). It was possible to detect abnormalities in the HR response as early as stage I, before other symptoms became apparent. The HR fluctuations that are evident in normal dogs decreased significantly during the progression of HF, showing a significant interaction between postural change and stage of HF. The relative bradycardia was significantly decreased, and there was no decrease in heart rate at return to the horizontal position.

These findings indicate that not only the resting vagal tone (measured by vagal tone index) was lower in the dogs of this study, but also that it could not be activated by a reflex stimulus that is present in normal conditions. The results also indicate that the tilt method was able to detect changes in the autonomic control of HR in very early stages of progression in this model of tachycardia-induced HF. This method should also be useful in the assessment of baroreflex changes in clinical dogs.

#### 41 CARDIAC TROPONINS IN CANINE BABESIOSIS R. Lobetti, E. Dvir and J. Pearson. Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa.

This study investigated myocardial injury diagnosed by histopathology, cardiac troponins, and electrocardiography (ECG), and compared the sensitivity of ECG and cardiac troponins to predict cardiac histopathological changes, clinical severity and survival in canine babesiosis.

One control group (n = 9) and 4 groups of babesiosis cases were studied: mild uncomplicated (n = 8), severe uncomplicated (n = 9), complicated (n = 8), and concurrent immune-mediated hemolytic anemia (IMHA) (n = 9). A one-minute lead II ECG was recorded. Cardiac troponin I (cTnI) and T (cTnT) concentrations were determined by standard methods. Five dogs that died had full necropsies and cardiac histopathology performed.

Cardiac troponin I concentrations were significantly higher in the complicated and concurrent IMHA groups and in the 3 dogs that succumbed to the disease. These 3 non-survivors had the highest cardiac troponin (I and T) concentrations and most severe cardiac histopathological changes, but no arrhythmia and minimal other ECG changes. Troponin T concentrations were below the upper limit in all dogs, but with significantly higher concentrations in the non-survivors.

This study showed that dogs with babesiosis could develop a variety of ECG changes. Most of the changes were, however, not associated with severity, outcome and cardiac troponin concentrations. The exception was the presence of VPCs, which were associated with elevated cardiac troponin concentrations. Histopathological changes were found in 4 of the 5 dogs that died and included pericardial effusion, hemorrhage, necrosis, inflammatory infiltrates and fibrin thrombi.

This study showed an association between cTnI concentration and histological changes, clinical severity and survival, and no correlation between ECG abnormalities and histological changes or biochemical evidence of myocardial damage as reflected by cTnI concentrations. From this study, it was concluded that the analysis of serum cTnI is a feasible and sensitive test and superior to cTnT and ECG analysis to diagnose cardiac involvement in dogs with babesiosis.

#### 42 THE pH OF PERICARDIAL EFFUSION DOES NOT RELIABLY DISTINGUISH BETWEEN IDIOPATHIC AND NEOPLASTIC EFFUSIONS. D.M. Fine, A.H. Tobias, K.A. Jacob. University of Minnesota, St. Paul, MN.

The prognosis of dogs with pericardial effusion (PE) depends upon the underlying etiology. Neoplastic PE is generally associated with short survival times, while idiopathic PE usually has a good prognosis. This study examined the utility of pH measurement to determine the etiology of PE.

Dogs were classified as having idiopathic PE (n = 10) if the diagnosis of pericarditis was made on histopathology (n = 4), or where there was no echocardiographic evidence of recurrent PE for at least 6 months following pericardiocentesis (n = 6). Dogs were classified as having neoplastic PE (n = 14) if a cardiac tumor was confirmed on histopathology (n = 4), or a discrete mass was visualized associated with either the right atrial or ventricular free wall, or the aorta on echocardiography (n = 10).

The pH of PE from each of the affected dogs was measured by placing a 10 cc aliquot in an additive-free vacutainer tube. Samples were centrifuged and pH of the supernatant was measured using a Corning Chekmate pH-10 meter. The pH meter was calibrated prior to each measurement, and results were reported at 25°C. In dogs with multiple recurrences of PE, only the pH measurement from the first occurrence was included in the statistical analysis. Data were not normally distributed and were therefore analyzed using the Mann-Whitney rank-sum test.

The lowest pH measurement (6.40) was found in a dog with idiopathic PE and the highest pH measurement (7.85) was found in a dog with neoplastic PE. However, there was considerable overlap of the pH data from the 2 groups, and the median pH from the idiopathic and neoplastic groups were not significantly different (7.32 and 7.42, respectively; P = 0.11). Further, second pH measurements were obtained from 3 dogs with recurrent idiopathic PE and they differed from the first measurements by between 0.3 and 1.0 pH units. We obtained results in 5/24 cases that suggest that particularly low PE pH (< 6.5; n = 1) is idiopathic, whereas particularly high PE pH (> 7.5; n = 4) is neoplastic. Due to the degree of overlap, pH data from the remaining 19/24 cases were not useful in making the distinction between idiopathic and neoplastic PE.

#### 43 COLOSTRAL IMMUNOGLOBULIN G CONCENTRATIONS VS. SUBSEQUENT SERUM IMMUNOGLOBULIN G CONCENTRATIONS IN NATURALLY SUCKLED LLAMA AND ALPACA CRIAS. D.W. Nagy,<sup>a</sup> J. Chakwenya,<sup>b</sup> J.W. Tyler,<sup>b</sup> J. Holle.<sup>b</sup> <sup>a</sup>Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL 61802 and <sup>b</sup>Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO 65211.

Llamas and alpacas rely on the passive transfer of maternal antibody through the ingestion of colostrum to provide humoral immunity in the neonate. Currently, no study has examined the relationship between maternal colostrum IgG concentration and serum IgG concentration achieved in the cria. Colostrum samples were taken from 18 llamas immediately postpartum, prior to allowing crias to suckle their dam. Serum samples for IgG determination were taken from each cria at 48 ± 3 hours for the determination of serum IgG concentration. Only crias that were able to stand and nurse their dam on their own within 4 hours were included in the study. Colostrum and serum IgG concentrations were determined by radial immunodiffusion. Regression models were developed to attempt to predict 48 hour cria serum IgG as a function of colostrum IgG concentration or a transformed measurement of colostrum IgG concentration. The model which best predicted cria serum IgG concentration was as follows:

$$\text{Serum IgG} = 1510.69 + [0.00000135 (\text{colostrum})^2].$$

Colostrum IgG was significantly (P = 0.075) correlated with serum IgG concentration (R<sup>2</sup> = 0.185). The strength of the relationship between colostrum and serum IgG concentrations was similar to that observed in cattle.

#### 44 EFFECT OF CIMETIDINE AND RANITIDINE ON ABOMASAL pH IN MILK FED CALVES. A.F. Ahmed, P.D. Constable, N.A. Misk, College of Veterinary Medicine, University of Illinois, Urbana, IL USA; Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

Abomasal ulceration occurs commonly in milk fed calves, and severe ulceration can result in abomasal perforation, peritonitis, and death. An important therapeutic goal in treating gastric ulceration in monogastric animals is maintaining gastric pH >3.5 (to facilitate ulcer healing) by administering H<sub>2</sub>-receptor antagonists, proton pump inhibitors, or oral antacids. Because the efficacy of H<sub>2</sub>-receptor antagonists in increasing abomasal pH is unknown in cattle, the purpose of this study was to determine the effect of cimetidine and ranitidine on abomasal pH in calves.

Five male dairy calves (aged 5 to 17 days) with abomasal fundic and antral cannulas were administered the following treatments in a randomized crossover design with a 24h wash out period between treatments: milk replacer (60 ml/kg, q 12h; untreated control), or milk replacer with cimetidine (50 mg/kg, PO, q 8h), cimetidine (100 mg/kg, PO, q 8h), ranitidine (10 mg/kg, PO, q 8h), or ranitidine (50 mg/kg, PO, q 8h). Fundic and antral pH were measured every second for 24h using a flexible pH glass electrode and pH values were digitized for offline analysis.

Mean fasting fundic pH was 1.41 ± 0.70 (mean ± SD). There was no difference between fundic and antral pH during digestion (pH difference, -0.01 ± 0.55) or fasting (pH difference, +0.04 ± 0.42), indicating lack of pH compartmentalization in the abomasum of the milk fed calf. Suckling of milk replacer immediately increased fundic pH from 1.4 to 6.1 (pH of milk replacer) followed by a gradual decrease in pH to fasting values by 6h. Both cimetidine and ranitidine caused a significant (P < 0.05) dose-dependent increase in abomasal pH. The percent of the 24h recording period that fundic pH >3.5 was greater for the 4 treatments: cimetidine 100 mg/kg (76 ± 13%); ranitidine 50 mg/kg (72 ± 24%); cimetidine 50 mg/kg (67 ± 19%); ranitidine 10 mg/kg (55 ± 9%), than for milk replacer (39 ± 8%). Fundic pH was >3.5 for longer in calves treated with cimetidine 100 mg/kg or ranitidine 50 mg/kg, compared to calves treated with ranitidine 10 mg/kg. Ranitidine (50 mg/kg) increased fundic pH for a similar duration to cimetidine (50 mg/kg), suggesting similar potency.

The results indicate that compared to adult monogastric animals, high doses of cimetidine and ranitidine are required to increase abomasal pH in milk fed calves.

**45** EFFECTS OF SUPPLEMENTAL LACTOFERRIN AT BIRTH ON NEUTROPHIL OXIDATIVE METABOLISM, SERUM IGG AND GROWTH OF HOLSTEIN CALVES. Dawes ME, Lakritz J, Tyler JW, Marsh AE, Chakwenya J, Hostetler DE. Dept. Vet. Med. and Surgery, University of Missouri-Columbia 65211.

Lactoferrin (LF) is an iron-binding protein, present in mucosal secretions, including the colostrum and milk. Recent work in our laboratory has demonstrated that colostral LF may improve neutrophil (PMN) function in calves. LF is present in colostrum; however, its relative absorption compared to immunoglobulins (Ig) is not known. Previous studies demonstrated that pasteurization of colostrum reduces serum concentrations of LF and Ig, and PMN oxidative burst.

This study was designed to address the following questions: 1) Is colostral LF absorbed by the neonate? 2) Does LF supplementation of colostrum alter absorption of Ig? 3) Does exogenous LF alter PMN oxidant production in colostrum-deprived neonates? 4) Do colostrum-deprived neonates receiving exogenous LF grow similar to colostrum replete calves?

Fifteen calves were enrolled at the time of birth by administering colostrum (C; n = 6), colostrum supplemented with LF (C+LF 1g/kg; n = 4), or milk replacer supplemented with LF (MR+LF 1g/kg; n = 5). Calves were bled immediately after birth, prior to feeding for PMN isolation and collection of serum. Calves were fed 4L of C, C+LF, or MR+LF by 4 hours of birth. Each calf was also bled at 24 hours of age to repeat measurements.

Serum LF measurements in C, C+LF, MR+LF calves were: 200±140 ng/ml pre, 650±130 ng/ml post; 100±47 ng/ml pre, 980±200 ng/ml post, and 330±70 ng/ml pre, 930±150 ng/ml post, respectively. Serum [IgG] determined by RID at 24 hours were: C = 3050±600 mg/dL; C+LF = 2000±600 mg/dL; MR+LF = <100 mg/dL respectively. Opsonized zymosan induced PMN superoxide determined by reduction of cytochrome c were C = 11.0±1.4 nmol/2x10<sup>6</sup> PMN pre, 8.0±2.3 nmol/2x10<sup>6</sup> PMN post; C+LF 10±1.4 nmol/2x10<sup>6</sup> PMN pre, 8±2.4 nmol/2x10<sup>6</sup> PMN post; MR+LF 7±1 nmol/2x10<sup>6</sup> PMN pre, 12.0±1.9 nmol/2x10<sup>6</sup> PMN post. Growth rates were similar in all three groups. Two MR+LF calves were killed, while others including C, C+LF calves required treatment for diarrhea.

These results suggest LF is absorbed, is associated with reduced serum IgG at high doses, results in elevated PMN superoxide in the absence of colostrum, and appears to reduce morbidity in neonates receiving it (3/5 survived).

**46** SERUM IMMUNOGLOBULIN G CONCENTRATIONS IN CALVES FED FRESH AND FROZEN COLOSTRUM. Nicole M. Holloway,<sup>a</sup> Jeff W. Tyler,<sup>a</sup> Jeff Lakritz,<sup>a</sup> Steven Carlson,<sup>b</sup> Julie Holle.<sup>a,b</sup> Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, Missouri 65211 and <sup>b</sup>Visalia, California.

Although administration of frozen stored colostrum is often recommended as a method to provide immunoglobulins to neonatal calves, no previous study has critically examined serum immunoglobulin concentrations in calves fed fresh and frozen colostrum. A series of experiments were conducted to determine whether calves fed frozen stored colostrum (-20 C) have similar serum IgG concentrations to calves fed refrigerated stored colostrum (4 C). In experiment 1, 10 pairs of Holstein calves (20 calves) were fed 4 l matched aliquots of colostrum, which had either been frozen or stored at refrigerator temperatures at 3 hours of age. Serum IgG concentrations at 2 days of age were compared among the 2 groups using a paired t-test. No significant difference was observed between the two groups. In the second experiment, 26 calves were fed 4 l of either fresh (n = 13) or frozen (n = 13) colostrum. A series of regression models were developed which attempted to predict calf serum IgG concentration or a suitable transformation of serum IgG concentration at 2 days of age as a function of colostral IgG concentration or a transformed measurement of colostral IgG concentration and colostrum storage group. No significant relationship was observed between colostrum storage group and day 2 serum IgG concentration in any of the models explored. The model, which best-correlated serum IgG and colostral IgG concentrations, predicted serum IgG concentration squared as a function of log(colostrum IgG concentration). In conclusion, our data suggests that administration of frozen colostrum is an appropriate strategy to provide immunoglobulins to neonatal calves.

**47** BACTERIOLOGICAL CONTAMINATION OF COLOSTRUM FED TO NEWBORN CALVES IN QUÉBEC DAIRY HERDS. Gilles Fecteau,<sup>a</sup> Paul Baillargeon,<sup>b</sup> Robert Higgins,<sup>c</sup> Madeleine Fortin<sup>a</sup> and Julie Paré<sup>a</sup> a: Faculté de Médecine Vétérinaire, Université de Montréal, Montréal, Canada, b: Clinique Vétérinaire St-Louis, St-Louis de Gonzague, Canada, c: St-Hyacinthe, Canada.

The objectives of this study were: 1) to describe the bacteria isolated from colostrum fed to newborn calves in commercial dairy herds, 2) to quantify the importance of the bacterial contamination 3) to evaluate association between bacterial contamination and the farm of origin, season of birth, calf's gender and dam's parity.

A convenience sample of 234 colostrum samples, collected directly from the nursing bottle immediately prior to first feeding were studied. Samples originated from 6 different farms and were collected over a 24 months period. Routine bacteriological techniques were used to quantify the bacterial load of the colostrum, as well as to identify the bacteria present.

Overall, 221 colostrum samples (94.4%) were contaminated with at least one microorganism. Using the upper tolerance level of 100 000 cfus/ml, 84 samples were considered contaminated (35.9%). *Staphylococcus spp* (57.7%), Gram negative rods and bacilla (47.8%), Coliforms (44.1%) and *Streptococcus uberis* (20.5%), were amongst the most frequent contaminants. Risk of more than 100 000 cfus/ml was significantly greater in warm weather months (RR = 2.42, 95% CI: 1.56-3.76) and in colostrum offered to male calves (RR = 1.55, 95% CI: 1.09-2.20). Bacterial load was also associated with the farm of origin (p < 0.0001).

When assessing colostrum management, bacterial contamination should be taken into consideration. Multiple factors are likely associated with the degree of contamination and farm specific factors may play an important role. Further studies are necessary to evaluate the exact impact on neonatal health.

**48** BIOLOGICAL CHARACTERISTICS OF ENTERIC AND RESPIRATORY CORONAVIRUS IN DAIRY CALVES. S.O. Cushing,<sup>1,\*</sup> R.J.Callan,<sup>1</sup> R.P.Dinsmore,<sup>1</sup> D.C.VanMetre,<sup>1</sup> J. Carman,<sup>2</sup> <sup>1</sup>Dept. of Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, CO. <sup>2</sup>Colorado State Veterinary Diagnostic Laboratory, Fort Collins, CO.

Calfhood enteric and respiratory infections account for significant production and economic losses in both the dairy and beef industries. Bovine coronavirus (BCV) is considered endemic in the United States cattle population and one of the primary etiologic agents in bovine enteric disease. Studies looking at coronavirus in feedlot cattle suggest there may be a respiratory strain of BCV and that it is a significant player in the pathogenesis of feedlot respiratory disease. In dairy cattle, the significance of bovine respiratory coronavirus (BRCV) as a separate entity from bovine enteric coronavirus (BECV) remains undetermined. The objectives of this study were to prospectively compare BRCV isolation, and BECV isolation with regard to clinical symptoms in dairy calves and hemagglutination (HA) characteristics.

Nasal and fecal samples were collected from 66 calves over a period of 12 weeks and inoculated onto human rectal tumor cells, clone 18G (HRT-18G). The cells were examined for cytopathic effect and the supernatants were used for HA assays on rodent and chicken red blood cells to detect BCV. Fecal samples were also analyzed by electron microscopy for presence on coronavirus. The calves were monitored twice weekly for signs of gastrointestinal and/or respiratory diseases.

The results found that at any time point within the study, at least 30% of the calves displayed morbidity. No association was found between respiratory clinical signs and nasal virus isolation for BCV. No association was found between enteric clinical signs and fecal virus isolation for BCV. Additionally, no association was found between enteric clinical signs and positive fecal electron microscopy. Over the entire sampling period, BCV was exclusively isolated from the respiratory tract in 8% of the calves. In 9% of the calves, BCV was isolated from both the respiratory and enteric tracts. 39% of the calves had enteric infections without respiratory isolates being detected.

This data suggests that BRCV is a minor respiratory pathogen in Northern Colorado dairy calves. Similar HA activity between enteric and respiratory isolates suggests these are the same or similar strains with both respiratory and enteric tropisms.

## 49 TOTAL NUMBER OF CRYPTOSPORIDIUM OOCYSTS OR GIARDIA CYSTS SHED BY DAIRY CALVES FOLLOWING NATURAL INFECTION. DV Nydam, SE Wade, HO Mohammed. Cornell University, Ithaca, New York.

*Cryptosporidium parvum* and *Giardia spp.* are zoonotic protozoan parasites that have relatively recently come to the attention to livestock producers and public health officials. No data is available to describe the dynamics of shedding of these 2 organisms from large populations of naturally infected dairy youngstock. The purpose of our study was to determine the total number of *C. parvum* oocysts and *Giardia spp.* cysts shed by dairy calves during their most at risk period so that cost-effective strategies may be devised to control environmental contamination.

Oocysts or cysts were enumerated from fecal specimens from 478 calves naturally infected with *C. parvum* and 1016 youngstock naturally infected with *Giardia*, in a watershed. From the distribution of the (oo)cysts versus age, hypotheses were developed and tested with respect to the best fitting mathematical function. The number of (oo)cysts/gram of feces for a given duration of shedding was computed by determining the area under the curve by numerical integration. The total number of (oo)cysts was calculated by taking the product of the resultant and the expected mass of feces.

The intensity of *C. parvum* oocysts was best described by a 2nd order polynomial function. Shedding increased from 4 days of age, peaked at day 12, after which it declined. An infected 6-day old calf produced a total of  $3.89 \times 10^{10}$  oocysts until 12 days old. The pattern of shedding of *Giardia spp.* cysts was best described by exponential functions. The intensity of shedding increased from 4 days of age, peaked at day 14, after which it declined. A 50-day old calf produced a total of  $3.8 \times 10^7$  cysts until 56 days old.

The large number of (oo)cysts shed, as predicted by these models, indicates that shedding dairy youngstock pose a risk for susceptible calves, people, and environmental contamination. It is particularly staggering to note that the ID50 for seronegative humans is 132 *C. parvum* oocysts. The estimates in this paper will be useful to aid in designing cost-effective strategies to manage this risk.

## 50 CLINICAL ASSESSMENT OF ACID-BASE STATUS IN ADULT CATTLE: CALCULATION OF PLASMA Atot AND Ka VALUES FOR USE IN THE STRONG ION AND SIMPLIFIED STRONG ION MODELS. P.D. Constable, Dept. of Veterinary Clinical Medicine, University of Illinois, Urbana, IL.

Two quantitative mechanistic acid-base models are available for the clinical assessment of acid-base status: the strong ion model (*Can.J.Physiol.Pharmacol.* 61:1444, 1983) and the simplified strong ion model (*J.Appl.Physiol.* 83:297, 1997). Both strong ion approaches require species-specific values for Atot (the total concentration of plasma nonvolatile weak acids) and Ka (the effective dissociation constant for plasma nonvolatile weak acids), but these values have not been determined for bovine plasma. Accordingly, the purpose of this study was to obtain accurate Atot and Ka values for bovine plasma.

Data was obtained from in vitro HCl/NaCO<sub>3</sub> titration of plasma from healthy red-pied cows (*Vet.Med.[Praha]* 14:75, 1969). The simplified strong ion model was applied to reported values for pH, Pco<sub>2</sub>, and strong ion difference (calculated from the reported base excess value), and nonlinear regression was used to solve simultaneously for Atot and Ka. Calculated mean values for normal bovine plasma were: Atot = 25.9 mM/L; Ka =  $0.95 \times 10^{-7}$ . The same approach was applied to data obtained from in vitro CO<sub>2</sub> titration of plasma from 8 healthy cattle and 2 sheep (*Br.Vet.J.*, 126:325, 1970). Calculated mean values using the second data set were: Atot = 27.7 mM/L, Ka =  $0.97 \times 10^{-7}$ . Both sets of calculated values differed from those experimentally determined for horse plasma (Atot = 15 mM/L, Ka =  $2.2 \times 10^{-7}$ ) and those empirically assigned to human plasma (Atot = 17 mM/L, Ka =  $3.0 \times 10^{-7}$ ).

The results suggest that the following Atot and Ka values should be applied to bovine plasma with normal albumin and globulin concentrations: Atot = 27 mM/L, Ka =  $1.0 \times 10^{-7}$ , equivalent to a pKa = 7.00. The results also suggest that for jugular venous blood from adult cattle at normal pH (7.42), Pco<sub>2</sub> (42 mm Hg), and HCO<sub>3</sub> concentration (26.7 mM/L), that net buffer ion charge = 19.6 mEq/L and normal strong ion difference = 46 mEq/L. The calculated values for Atot and Ka should facilitate the clinical application of the strong ion and simplified strong ion models to acid-base disturbances in adult cattle.

## 51 ACID BASE AND ELECTROLYTE BALANCE IN POSTPARTURIENT HOLSTEIN COWS WITH EXPERIMENTALLY INDUCED PRIMARY KETOSIS. D.E. Morin, P.D. Constable, H.M. Dann, and J.K. Drackley. Departments of Veterinary Clinical Medicine and Animal Sciences, University of Illinois, Urbana, IL.

Primary ketosis (PK), a common disorder of dairy cows, is caused by insufficient dietary energy intake to meet the demands of early lactation. Although cows with PK are usually assumed to have metabolic acidosis because of increased blood ketoacid concentrations, the host of metabolic changes accompanying ketosis may have a more complex effect on acid base balance. The objective of this study was to characterize the acid base status and identify mechanisms of acid base alterations in cows with PK.

Primary ketosis was induced in 6 healthy multiparous Holstein cows by restricting feed intake by 50% beginning day 5 postpartum. Feed restriction was continued until cows developed signs of clinical ketosis (anorexia, abnormal behavior) or reached day 14 postpartum. Five healthy multiparous Holstein cows fed the identical ration, but at ad libitum intake, served as controls (CON). Jugular venous blood samples and mid-stream urine samples were collected 15–60 min after the morning feeding on day 14 postpartum, or at the onset of clinical ketosis.

Feed restriction caused metabolic changes consistent with naturally occurring PK, including significantly ( $P < 0.05$ ) lower serum glucose concentration (PK,  $31 \pm 11$ ; CON,  $55 \pm 8$  mg/dl) and higher serum NEFA (PK,  $1637 \pm 571$ ; CON,  $491 \pm 232$  uEq/L), plasma betahydroxybutyrate (BOHB; PK,  $33 \pm 11$ ; CON,  $6 \pm 4$  mg/dl), and urine BOHB (PK,  $153 \pm 137$ ; CON,  $2 \pm 3$  mg/dl) concentrations. Primary ketosis was not associated with acidemia (venous pH; PK,  $7.42 \pm 0.04$ ; CON,  $7.43 \pm 0.02$ ) or metabolic acidosis (extracellular base excess; PK,  $2.9 \pm 2.5$ ; CON,  $4.1 \pm 1.8$  mEq/L), as determined by traditional (Henderson-Hasselbalch) criteria. Urine pH also did not differ for PK and CON cows. Increased anion gap (Na+K-Cl-HCO<sub>3</sub>) in PK cows ( $23.6 \pm 1.2$  mEq/L) compared with CON cows ( $18.3 \pm 2.7$  mEq/L) was due to ketonemia and high NEFA concentrations. Cows with PK had a higher measured strong ion difference (Na+K-Cl;  $51.5 \pm 2.2$  mEq/L) than CON cows ( $47.4 \pm 2.4$  mEq/L), due to higher serum Na concentrations. Serum nonvolatile buffer (albumin, globulin, phosphorus) and creatinine concentrations were not different between groups. Results indicate that experimental induction of PK in periparturient cows was not accompanied by clinically important acid base alterations.

## 52 ACID-BASE, CEREBROSPINAL FLUID, AND CARDIO-VASCULAR EFFECTS OF RAPID INTRAVENOUS HYPERTONIC SODIUM BICARBONATE ADMINISTRATION IN CALVES WITH EXPERIMENTALLY INDUCED RESPIRATORY AND METABOLIC ACIDOSIS. J. Berchtold, P. Constable, G. Smith, S. Mathur, D. Morin, and W. Tranquilli. Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL.

Mixed metabolic and respiratory acidosis occurs frequently in critically ill calves with perinatal asphyxia or diarrhea and dehydration. The objectives of this study were to investigate whether rapid IV administration of 8.4% sodium bicarbonate is safe and effective for the treatment of metabolic and respiratory acidosis in neonatal calves.

Ten halothane anesthetized calves were instrumented to permit cardiovascular monitoring and to determine acid-base status in blood and CSF. Baseline values were obtained with calves mechanically ventilated at normal tidal volume. Calves were then allowed to breathe spontaneously for 30 min to induce respiratory acidosis. Metabolic acidosis was then induced by IV administration of 3 mmol/kg isotonic L-lactate. Calves with experimentally-induced respiratory and metabolic acidosis were then randomly assigned to receive either IV 5 ml/kg 8.4% sodium bicarbonate within 5 min ( $n = 5$ ) or no treatment (controls,  $n = 5$ ) and further observed for 60 min.

Spontaneous ventilation resulted in a significant ( $P < 0.05$ ) increase in blood PCO<sub>2</sub> from  $43 \pm 4$  (mean  $\pm$  SD) to  $81 \pm 8$  mmHg and an associated decline in arterial pH from  $7.44 \pm 0.05$  to  $7.18 \pm 0.06$  and CSF pH from  $7.33 \pm 0.05$  to  $7.17 \pm 0.04$ . Administration of L-lactate caused a further decrease in arterial pH to  $7.09 \pm 0.08$  and development of metabolic acidosis (base excess decreased from  $+3.1 \pm 1.3$  to  $-3.4 \pm 2.2$  mEq/L). Respiratory and metabolic acidosis was accompanied by increased heart rate, cardiac output, mean arterial pressure, mean pulmonary artery pressure, and cardiac contractility (LV dP/dt<sub>max</sub>).

Rapid IV administration of hypertonic sodium bicarbonate corrected the observed metabolic acidosis (sustained increase in pH and base excess in arterial blood) but transiently increased blood PCO<sub>2</sub> to  $93 \pm 15$  mmHg 5 min after administration was completed. The increase in arterial PCO<sub>2</sub> did not induce paradoxical CSF acidosis or alter the PCO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup> concentration in CSF. Sodium bicarbonate administration significantly increased heart rate, cardiac output, and cardiac contractility (LV dP/dt<sub>max</sub>) and decreased mean arterial pressure, as compared to controls. We conclude that rapid IV administration of hypertonic sodium bicarbonate provided an effective and safe method for treating metabolic acidosis in halothane-anesthetized normovolemic calves.

- 53** CARDIOMYOPATHY IN 7 YOUNG AYRSHIRE CATTLE A NEW CLINICAL ENTITY? Gilles Fecteau, Richard Drolet, Nicolas Sattler, Rocky DiFrancia, Claudine Tremblay, Doris Sylvestre, Ted Burnside\* Pascal Dubreuil et André Desrochers, Faculté de Médecine Vétérinaire, Université de Montréal, Montréal, Canada, \* Centre d'insémination artificielle du Québec.

Records from 7 cattle less than 24 months of age presented to the veterinary teaching hospital at the Faculty of veterinary medicine of St-Hyacinthe with congestive heart failure between 1994 and 1998 were studied. All animals were females Ayrshire (6 to 24 months; median = 9 months) originating from 4 different herds. All but one were fed ionophors supplemented feed.

Elevation in the muscular enzymes CK and AST was present in 3 animals. Serum selenium concentration was considered marginally low in three animals. Vitamin E concentration was measured in 2 animals and considered adequate. Echocardiographic examination (n = 4), revealed sub-normal myocardial contractility. All animals died or were euthanized without specific treatment. The lesions found in the myocardium were non specific and compatible with end-stage heart failure or a progressive, low-grade degenerative process. Pedigree of all animals were studied. Six out of the 7 animals were related to the same sire within 3 generations. This common ancestry was a popular AI sire, which thus appears in a large number of Ayrshire pedigrees.

Precise aetiology for this possible new clinical entity remains open for discussion. A breed predisposition seems present but a simple hereditary pattern cannot be identified and seems unlikely with the number of animals studied. The elevation in the muscular enzymes may indicate generalised muscular damage. However the exact role of Vitamin E and selenium deficiency remains uncertain. Possibly, more than one factor is involved in the aetiology of the condition. Multiple aetiology leading to a common outcome of congestive heart failure could also be an explanation but the breed predisposition remains a factor to consider.

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- 54** EFFECTS OF EXOGENOUS INSULIN ON GLUCOSE TOLERANCE IN ALPACAS. C.K. Cebra, S.A. McKane, S.J. Tornquist. College of Veterinary Medicine Oregon State University, Corvallis, OR.

We previously described llamas and alpacas as having slow glucose clearance with a poor insulin response after glucose challenge. Here, we investigated whether exogenous insulin improved glucose clearance.

On each of two subsequent days, 7 fasted adult castrated male alpacas were administered 0.5 g of a 50% glucose solution per kg of body weight by rapid injection through one channel of a double lumen catheter. On one day, determined randomly for each alpaca, 0.2 IU/kg of regular insulin was administered intravenously 15 minutes later. Blood samples were withdrawn through the second channel immediately before glucose injection and 15, 20, 25, 30, 45, 60, 90, 120, 180, and 240 minutes later. Blood samples were analyzed for glucose and lactate content, and fractional turnover rate and half-life of glucose were calculated for all time intervals. Values were compared between alpacas with and without insulin injection.

Insulin caused an increase in fractional turnover of glucose and lactate production, and a decrease in plasma glucose concentrations and half-life. However, even with exogenous insulin, glucose clearance was slower than in many other domestic animals and similar to animals with diabetes mellitus.

These findings suggest that slow blood glucose clearance in alpacas is not due to poor insulin production or release alone, but also is due to tissue insensitivity to insulin. Insulin may be administered with glucose to improve assimilation, but it is likely that insulin protocols used for other animals would have to be adapted to be suitable for alpacas.

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- 57** CLINICAL PROGRESSION OF NEUROLOGICAL SIGNS AND CEREBROSPINAL FLUID VALUES IN HORSES WITH EXPERIMENTALLY INDUCED LEUKOENCEPHALOMALACIA. J.H. Foreman, P.D. Constable, A.L. Waggoner, R.M. Eppley, G.W. Smith, M.E. Tumbleson, and W.M. Haschek, College of Veterinary Medicine, University of Illinois, Urbana, IL USA and U.S.F.D.A., Washington, DC.

Ingestion of fumonisins in moldy corn leads to leukoencephalomalacia and liver disease in horses. Previous studies of fumonisin toxicity in horses have focused on the terminal nervous system derangement (leukoencephalomalacia), but none have described in detail the clinical progression of neurological signs or cerebrospinal fluid (CSF) findings in a number of affected horses. Accordingly, the aims of this study were to describe the clinical progression of neurological signs in horses with leukoencephalomalacia, and to document the CSF values in affected horses.

Seventeen horses were randomly allocated to receive purified fumonisin B<sub>1</sub> at 0.20 mg/kg (n = 4), 0.10 mg/kg (n = 3), 0.05 mg/kg (n = 3), or 0.01 mg/kg (n = 3) IV daily, or 10 ml saline IV daily (controls, C). All horses were examined daily for neurological changes by an investigator (J.H.F.) who was blinded to treatment assignment. At the onset of moderate to severe signs of neurological disease (0.05–0.20 mg/kg doses, n = 11) or study termination (0.01 mg/kg, C), horses were anesthetized with xylazine/ketamine and atlanto-occipital opening CSF pressure measured and CSF obtained for analysis.

Control horses and horses administered fumonisin at 0.01 mg/kg remained neurologically normal until study termination at day 7–28. Horses receiving fumonisin at 0.05–0.20 mg/kg developed neurological signs on days 6–12. Early clinical changes were mild proprioceptive abnormalities including hind limb ataxia, delayed forelimb placing reactions, and decreased tongue tone and movement. These signs rapidly progressed, usually over a few hours (<24h). Behavioral changes were not detected initially, but depression, hyperesthesia, and intermittent dementia became increasingly evident in some horses. Horses were not blind, based on the presence of intact menace and pupillary light responses. Horses with neurological signs had significantly (P<0.05) decreased CSF pressure (20±7 cm H<sub>2</sub>O; C, 28±4 cm H<sub>2</sub>O) and increased CSF protein concentration (197±192 mg/dl; C, 41±13 mg/dl). Leukocyte and erythrocyte concentrations in the CSF of affected horses were the same as control horses. This is the first report characterizing the CSF values and clinical progression of neurological signs in a number of horses with leukoencephalomalacia.

- 58** PLASMA ADRENOCORTICOTROPIN (ACTH) CONCENTRATIONS AND CLINICAL RESPONSE IN HORSES TREATED FOR EQUINE CUSHING'S DISEASE WITH CYPROHEPTADINE OR PERGOLIDE. G Perkins, S Lamb, HN Erb, B Schanbacher, D Nydam, TJ Divers. Cornell University, Ithaca, New York.

The purpose of our study was to determine the value of monitoring ACTH levels when treating equine Cushing's disease (ECD). A prerequisite to this was validation of the chemiluminescent enzyme immunoassay for ACTH (Immulate) along with calculating the sensitivity and specificity of high plasma ACTH (>35 pg/ml) for detecting ECD.

Dilutional parallelism, and intra-assay and interassay co-efficients of variations (CV) were determined for the equine ACTH assay. A retrospective study of hospitalized equids (n = 62) with plasma ACTH measurements was done. The horses were classified as (1) hirsutism; gold standard for ECD (2) possible ECD; no hair-coat abnormality yet other clinical signs of ECD (3) not ECD; no clinical signs of ECD or another confirmed diagnosis. Using chi-square tests, the sensitivity and specificity of ACTH for detecting ECD was determined. To determine the clinical response to therapy and changes in ACTH levels, a survey was sent to veterinarians that submitted an initially high ACTH level (35 pg/ml) with ≥ 2 sample submissions (n = 42). The questions were designed to evaluate the clinical signs, ACTH levels at the time of diagnosis and during therapy with either cyproheptadine (cyp) or pergolide (perg). Non-parametric, rank-sum (two-sample: Mann-Whitney) and Fisher's exact tests were performed. A p value ≤ 0.05 was considered statistically significant.

The ACTH chemiluminescent immunoassay had an immunologic specificity expressed as observed over expected of 99.2%, accuracy of 101% and intra-assay CV of 9.3%. The sensitivity and specificity of high plasma ACTH levels for detecting ECD were 84 and 78%, respectively. From our survey, 32 were treated with cyp and 10 with perg. The median treatment time was 4 months (range 1–15). Horses treated with either drug showed decreases in ACTH (p = 0.03) and a decrease in ACTH to normal was associated with an improvement in hirsutism (p = 0.04). Horses treated with cyp were more likely to show improvement in laminitis than those treated with perg (p<0.01). There was no association between improvement in clinical signs and decreases in ACTH in horses treated with cyproheptadine.

The ACTH assay is valid and is more sensitive than it is specific for ECD. ACTH levels might be helpful when monitoring therapy of ECD, although improvement in clinical signs should be considered most important. ECD horses with laminitis may benefit more from cyp than perg.

- 59** CORRELATION OF ECHOCARDIOGRAPHIC STRESS-TESTS WITH LEFT VENTRICULAR PRESSURE DYNAMICS. MM Durando, VB Reef, and EK Birks. University of Pennsylvania, Kennett Square, PA.

The objectives of this study were to correlate echocardiographic (EC) findings obtained immediately post-exercise with left ventricular (LV) pressure dynamics recorded simultaneously, and to compare the LV dynamic measurements obtained post-exercise with those during maximal exercise.

Seven Thoroughbreds, without evidence of cardiac disease and trained to run on a high-speed treadmill, were used. After a resting EC was obtained, a high-fidelity Millar catheter was passed into the LV via a right carotid artery that had been surgically elevated to a subcutaneous position. Ultrasound and pressure measurements were used to identify catheter location. Horses ran at 110% of the speed necessary to elicit VO<sub>2</sub>max. LV pressure signals were digitized at 500 Hz and recorded continuously on a computer-based data acquisition system (Pentium III computer with CODAS software). LV pressure dynamics were calculated for 10 seconds at maximal speed, immediately after exercise (0), and 30, 60, 70, 80, 90, 100, 110, and 120s following exercise. Within 60–120s after exercise, an EC was obtained, with wall motion (WM) abnormalities and % fractional shortening (%FS) determined. Results were analyzed by ANOVA and post hoc analysis conducted using Tukey's HSD, with values of p<0.05 considered significant.

Mean dP/dtmax at 0 was significantly greater than dP/dtmax at maximal speed (11759.7±522.6 (mean±SEM) vs 9378.5±1103.8 mmHg/s) (p<0.0001). Mean dP/dtmax at 30s was not significantly different from dP/dtmax at maximal speed (p = 0.9026). dP/dtmax at all other time points was significantly less than dP/dtmax at maximal speed (p<0.0001). Mean dP/dtmin at time 0 was significantly greater than dP/dtmin at maximal speed (p<0.01). Mean dP/dtmin at all other time points was significantly less than dP/dtmin at maximal speed. Variability (%CV) was significantly greater from 60–120s vs maximal, 0, and 30s. Mean %FS pre-exercise was 36.6±1.6% and post-exercise was 39.5±2.5%, a 7.4±7.3% increase. However the %CV was 262.3%. Average WM indices were hypokinetic post-exercise.

These data suggest that cardiac function is more accurately measured by pressure dynamics than by EC evaluation. The inter-horse variability for dP/dtmax and min was smallest up to 30s after exercise. After this time, when stress EC are generally performed, variability was significantly greater. The increased %CV in LV pressure measurements after 30s post-exercise were far less than that observed with EC examination. This indicates that conclusions regarding cardiac function made from EC are more prone to error. Additionally, stress EC are performed after completion of exercise, and may not accurately reflect cardiac function during maximal exercise.

- 60** GLUCOSE CLEARANCE IN PSSM HORSES DETERMINED BY EUGLYCEMIC HYPERINSULINEMIC CLAMP. SJ Valberg,<sup>1</sup> JR Mickelson,<sup>2</sup> D Nahey,<sup>2</sup> J Collins,<sup>1</sup> FD De La Corte and ER Seauquist,<sup>3</sup> Dept. Clinical and Population Sciences,<sup>1</sup> Dept. Veterinary Pathobiology,<sup>2</sup> College of Veterinary Medicine, Dept. of Medicine,<sup>3</sup> Medical School, University of Minnesota.

Exertional rhabdomyolysis in a population of related Quarter Horses is caused by a defect that results in excessive glycogen storage and the accumulation of an abnormal polysaccharide in skeletal muscle. The excessive glycogen storage in this polysaccharide storage myopathy (PSSM) is not due to the inability to metabolize glycogen. Previous studies of the effects of a single intravenous bolus of insulin or glucose suggest that horses with PSSM have enhanced glucose clearance due to uptake into skeletal muscle. The purpose of this study was to determine whole body glucose clearance in PSSM horses during a steady state of hyperinsulinemia, using clamping techniques adapted from human diabetic studies.

Four healthy control horses ranging in age from 2–11 yrs and 5 PSSM horses ranging in age from 3–14 yrs were used in this study. Horses were fed hay and stall rested for 1 month followed by a 12 hr fast immediately prior to the study. A right jugular venous catheter was used to collect blood every 5 min for determination of glucose (QID glucometer) and insulin concentrations (RIA). A left jugular catheter was used for controlled glucose and insulin infusion. Insulin was diluted in isotonic saline and homologous blood and infused at 3 mU/min/kg body weight to maintain concentrations at a plateau of 150–300 µU/ml. Blood glucose was monitored every 5 min to determine the rate of glucose infusion necessary in order to maintain blood glucose at approximately 100 mg/dl (range 95 to 110 mg/dl).

The mean rates of glucose infusion (mg/kg body weight/min) necessary to maintain euglycemia during the insulin clamp are shown in the table below. The rate of infusion during steady state 30 min time blocks was significantly higher for PSSM horses than controls (p<0.001).

Group	60-89 min	90-119 min	120-150 min	150-180 min
PSSM	103.9 ± 22.2	98.1 ± 19.1	111.7 ± 12.7	121.2 ± 2.6
Controls	73.9 ± 28.9	52.0 ± 24.8	43.1 ± 9.4	74.4 ± 15.5

These results confirm our hypothesis that greater insulin sensitivity in horses with PSSM results in enhanced blood glucose clearance. We suggest that high muscle glycogen concentrations in PSSM horses are due to enhanced clearance of blood glucose into skeletal muscle, the largest mass of insulin-sensitive tissue in the body.

61

SERUM CK ACTIVITY IN THOROUGHBRED HORSES WITH RECURRENT EXERTIONAL RHABDOMYOLYSIS CONSUMING DIETS VARYING IN STARCH, FAT AND BICARBONATE CONTENT. EC McKenzie,\*<sup>1</sup> SJ Valberg,<sup>1</sup> JM MacLeay,<sup>1</sup> J Khaleel,<sup>1</sup> JD Pagan,<sup>2</sup> RJ Geor,<sup>2</sup> GP Carlson<sup>3</sup> <sup>1</sup>Dept Clinical and Population Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, <sup>2</sup>Kentucky Equine Research, Versailles KY, <sup>3</sup>University of California, Davis CA.

A reduction in grain intake and addition of sodium bicarbonate to the diet are common recommendations for Recurrent Exertional Rhabdomyolysis (RER) in Thoroughbreds. Recently high dietary fat has been advocated for RER to increase caloric intake without additional starch. The purpose of this study was to assess the effects of three isocaloric diets (28 MCal/day) on post-exercise serum CK activity in RER horses.

Horses consumed a similar amount of hay and one of 3 supplements; **HS** was high in starch (21%), and low in fat (3%) with a dietary cation anion balance (DCAB) of +190; **HB** was the same but had 4.2% sodium bicarbonate with a DCAB of +380, and **HF** was low in starch (9%) and high in fat (13%) with a DCAB of +200. Five fit RER horses with a history of high serum CK, and abnormal skeletal muscle caffeine contracture tests were fed each diet for a three-week period in a block design. Horses were exercised with intervals of walk, trot, and canter for 30 minutes five days a week while consuming each diet. Blood samples were taken daily for analysis of pre- and post-exercise serum pH, [Na<sup>+</sup>], [K<sup>+</sup>], [HCO<sub>3</sub><sup>-</sup>], [iCa<sup>++</sup>] and post exercise CK. On the last day of each diet a urine sample was obtained from all horses to calculate urinary fractional excretion (FE) of Ca<sup>++</sup>, P, Mg<sup>++</sup>, Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>, and a near maximal standardized exercise test (SET) was performed, during which serial measurement of blood [lactate] was performed. Results were analyzed using Analysis of Variance (significance P < 0.05).

Post-exercise serum CK activity was above normal limits on **HS** and **HB** (2451 U/L ± 681 and 2554 U/L ± 1595) and was significantly lower on **HF** compared to the other two diets (407 U/L ± 53). Pre- and post-exercise serum pH, [HCO<sub>3</sub><sup>-</sup>] and [lactate] did not differ significantly between the diets. Post-exercise [iCa<sup>++</sup>] was significantly greater on **HF**. Urinary FE did not differ significantly between the diets.

The results indicate a high fat/low starch diet significantly reduces post-exercise CK in RER horses undergoing treadmill exercise. Dietary bicarbonate supplementation appears to have no effect on the occurrence of rhabdomyolysis in RER horses.

62

IONIZED CALCIUM CONCENTRATION DURING ENDURANCE EXERCISE. H.C. Schott II, M.W. Davis, P. Butudom, K.F. Dusterdieck, S.W. Eberhart, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI 48824-1314.

Sweat production during prolonged endurance exercise results in depletion of body fluid and electrolyte stores. As a consequence, horses may develop various medical problems described as the "exhaustive disease syndrome". Warning signs of exhaustion include muscle fasciculations and synchronous diaphragmatic flutter. These signs of neuromuscular irritability (NI) have traditionally been attributed to hypocalcemia and are often treated with small volumes of calcium-containing solutions. Such treatment may counteract NI but does little to replace the large deficits of water and other electrolytes that are more likely the underlying cause. We tested the hypothesis that endurance exercise results in a decrease in [iCa<sup>++</sup>] in two studies in which six 2-year-old Arabian horses exercised on a treadmill. In study 1, [iCa<sup>++</sup>] decreased by 0.37 ± 0.06 mg/dl (p<0.01) during 45 km of treadmill exercise but returned to the pre-exercise value by 20 minutes into the recovery period. Further, [iCa<sup>++</sup>] was correlated (r = -0.21, p<0.01) with blood pH. In study 2, horses performed two 60 km exercise bouts—with and without electrolyte supplementation (NaCl and KCl oral pastes). Without electrolyte supplementation, [iCa<sup>++</sup>] decreased by 0.50 ± 0.16 mg/dl (p<0.01) after 60 km of exercise. In contrast, a decrease in [iCa<sup>++</sup>] was not observed when horses were supplemented with NaCl and KCl. As in study 1, [iCa<sup>++</sup>] was again correlated (r = -0.38, p<0.01) with blood pH. However, supplementation with electrolytes abolished the mild alkalosis that developed during prolonged exercise and, thereby, may have attenuated any decrease in [iCa<sup>++</sup>]. In conclusion, these data suggest that moderate endurance exercise does not produce clinically significant hypocalcemia; rather, the observed decreases in [iCa<sup>++</sup>] may simply be physiochemical consequence of changes in blood pH.

63

PRE-EXERCISE FEEDING ALTERS SUBSTRATE UTILIZATION AND GLUCOSE FLUX IN EXERCISING HORSES. E. Jose-Cunilleras, K.W. Hinchcliff, R.A. Sams, J. Kim, J. Linderman, S.T. Devor. The Ohio State University, Columbus, OH.

Our hypothesis was that the composition of a meal fed before exercise would affect metabolism and substrate utilization during subsequent exercise. Therefore, we compared energy expenditure, rates of substrate oxidation, and glucose utilization by skeletal muscle during moderate intensity exercise in horses denied access to food for 18 hours and then: (1) fed cracked corn (1.7 kg for 450 kg horse, 5.6 Mcal digestible energy), or (2) fed an isocaloric amount of alfalfa cubes (3.0 kg for a 450 kg horse) 2-3 hours before exercise, or (3) not fed before exercise.

In a randomized, balanced, crossover study each of 6 fit, adult Standardbred horses received each treatment 2-3 hours prior to 60 minutes of running on a treadmill at 50% VO<sub>2max</sub>. Plasma glucose, lactate, glycerol, free fatty acid and insulin concentrations were measured before and during exercise. Infusion of dideuterated glucose before and during exercise allowed determination of rate of glucose absorption and production and glucose utilization by skeletal muscle. Energy expenditure and partitioning of substrate oxidation (carbohydrate vs. lipid) were estimated during exercise by indirect calorimetry. Glycogen concentration of middle gluteal muscle was measured before and after each trial via a biopsy sample. Statistical analyses were performed using a two-way ANOVA repeated measures, and values are reported as mean ± SE.

Prior to the start of exercise, when compared with the fasting treatment, pre-exercise feeding with grain resulted in higher plasma glucose (6.6±0.7 vs. 4.5±0.1 mM, p<0.05), higher serum insulin (176±17 vs. 84±6 pM, p<0.05) and lower serum non-esterified fatty acid (0.6±0.2 vs. 1.0±0.2 mM, p<0.05) concentrations. During the exercise bout, when compared with the fasting treatment, pre-exercise feeding with grain resulted in lower plasma glucose (5.8±0.6 vs. 8.5±1 mM, p<0.05), lower serum glycerol (0.7±0.1 vs. 0.9±0.1 mM, p<0.05), lower serum free fatty acid concentrations (0.7±0.1 vs. 1.1±0.1 mM, p<0.05), higher skeletal muscle utilization of blood-borne glucose (56±4 vs. 38±5 umol/(kg×min), p<0.05) and lower rate of lipid oxidation (49±4 vs. 63±1 umol/(kg×min), p<0.05). Pre-exercise feeding with hay resulted in similar results for most variables when compared to feed withholding. No statistical differences were found among treatments for rates of total carbohydrate oxidation and muscle glycogen oxidation.

We concluded that feeding a soluble carbohydrate-rich meal prior to moderate intensity exercise results in increased skeletal muscle utilization of blood-borne glucose and a decreased rate of lipid oxidation, without a muscle glycogen sparing effect.

64

NASAL DILATOR STRIPS DO NOT AFFECT ARTERIAL HYP-OXEMIA AND HYPERCAPNIA, LACTATE AND AMMONIA PRODUCTION, OR THE OCCURRENCE OF EIPH DURING SHORT-TERM HIGH-INTENSITY EXERCISE IN THOROUGHBRED HORSES. T.E. Goetz, M. Manohar, A.S. Hassan, and G. J. Baker. College of Veterinary Medicine, University of Illinois, Urbana, IL.

Human athletes frequently apply adhesive nasal strips to enhance athletic performance. Performance may be enhanced as the strip may reduce work of breathing by decreasing resistance to nasal air flow. Although a clear advantage to the use of nasal strips in athletes remains to be established, based on this rationale, nasal strips (FLAIR™, CNS Inc., Minneapolis, MN) have recently been introduced as a drug-free means of improving nasal air flow in exercising horses. The use of FLAIR™ strips has been approved for racehorses in many states including Illinois. Therefore, this study was undertaken to assess whether application of nasal strips would affect pulmonary gas exchange, anaerobic metabolism (lactate and ammonia production), and the occurrence of exercise-induced pulmonary hemorrhage (EIPH) in exercising horses.

Control and nasal strip experiments were carried out in random order 7 days apart on 7 healthy, exercise-trained horses. Simultaneous measurements of core temperature, arterial and mixed-venous O<sub>2</sub> and CO<sub>2</sub> tensions, pH, O<sub>2</sub> saturation, O<sub>2</sub> content, as well as lactate/ammonia concentrations were made at rest and during exercise at 6, 8, and 14 m/s on a 3.5% uphill grade. Galloping at 14 m/s on a 3.5% uphill grade elicited maximal heart rate and induced EIPH in all horses.

In both experiments, sub-maximal exercise caused hyperventilation and arterial O<sub>2</sub> tension was well maintained. During exercise at 14 m/s, both experimental groups experienced significant decreases in arterial O<sub>2</sub> tension and O<sub>2</sub> saturation, while CO<sub>2</sub> tension increased significantly. Also, in both groups, arterial O<sub>2</sub> content increased significantly (upon splenic contraction), while mixed-venous blood O<sub>2</sub> content decreased markedly as O<sub>2</sub> extraction dramatically increased. Significant exercise-induced increments were also observed in lactate and ammonia concentrations in both groups. There were no statistically significant differences in any of the measured variables between the control and the nasal strip experiments. This exercise protocol induced EIPH in all horses in the control and nasal strip experiments.

Based on these data, we conclude that application of nasal strips neither improved pulmonary gas exchange nor did it diminish anaerobic metabolism or the incidence of EIPH in Thoroughbreds performing short-term high intensity exercise.



## 65

THE EFFECT OF cAMP MODULATION ON EQUINE NEUTROPHIL ACTIVATION BY IMMUNE COMPLEXES. C. D. Chilcoat, Y. Sharief, S. L. Jones. Department of Clinical Sciences, North Carolina State University, Raleigh, North Carolina.

Toxic products such as reactive oxygen intermediates released by activated neutrophils (PMN) have an important role in the pathophysiology of diseases associated with the deposition of immune complexes (IC) in tissues. IC-induced activation of PMN requires adhesion mediated by integrin adhesion receptors. Of the integrins expressed on PMN, the  $\beta_2$  family has been found to be of particular importance for activation of PMN by IC.  $\beta_2$  integrin ligand binding must be activated to enable adhesion to IC. Both activating and inhibitory signals regulate  $\beta_2$  integrin ligand avidity and adhesion. The second messenger cAMP has been demonstrated to inhibit the activation of PMN in response to a variety of stimuli. The purpose of this study is to test the hypothesis that cAMP-dependent signals inhibit  $\beta_2$  integrin dependent adhesion of equine PMN to immobilized IC and subsequent adhesion-dependent activation of respiratory burst activity.

To test this hypothesis, we examined the effect of cAMP modulators on adhesion of purified equine PMN to immobilized IC using a 96 well microtiter plate adhesion assay. Fluorescently labeled PMN were allowed to adhere to immobilized IC or the control substrate serum. The fluorescence in each well was measured using a fluorescence plate reader before and after washing. Adhesion was quantitated as the percent PMN remaining after washing. Activation of respiratory burst activity during adhesion to IC or the control substrate serum was determined using a microtiter plate assay based on the measurement of the  $H_2O_2$ -dependent loss of scopoletin fluorescence.

Treatment of equine PMN with  $\beta_2$  adrenergic agonists isoproterenol or clenbuterol, which trigger an increase in intracellular cAMP concentration, inhibited adhesion of equine PMN to IC in a dose dependent manner (isoproterenol IC50 = 2.5mM, clenbuterol IC50 = 150uM). Similarly, inhibition of cAMP metabolism by the non-specific phosphodiesterase inhibitor pentoxifylline and the phosphodiesterase 4-specific inhibitor rolipram inhibited adhesion of equine PMN to IC (pentoxifylline IC50 = 1mM, rolipram IC50 = 7.5uM). Importantly, co-treatment of equine PMN with rolipram and isoproterenol synergistically inhibited the adhesion of equine PMN to IC. Modulation of intracellular cAMP levels also inhibited IC-induced activation of respiratory burst activity in equine PMN.

Our conclusion is that cAMP negatively regulates  $\beta_2$ -dependent adhesion of equine PMN to IC and subsequent activation of effector functions in horses. Thus, these drugs may be useful as anti-inflammatory agents in IC-mediated diseases. The synergy between  $\beta_2$ -agonists and phosphodiesterase inhibitors may also have clinical importance.

## 66

ENDOTOXIN-NEUTRALIZING ACTIVITY IN BLOOD AFTER INTRAVENOUS ADMINISTRATION OF POLYMYXIN B IN THE HORSE. P.R. Morresey, R.J. MacKay, K.A. Gillis, M.P. Brown. College of Veterinary Medicine, University of Florida, Gainesville, FL.

Polymyxin B (PB) is a cyclic cationic polypeptide antibiotic that binds and neutralizes bacterial endotoxin (lipopolysaccharide; LPS). Administration of a single dose of PB has been shown to protect against effects of IV-administered LPS. In clinical situations, however, administration of the drug is often repeated several times. The purpose of this study was to determine endotoxin-neutralizing activity in blood after single and repeated doses of PB to establish a dosage schedule for PB in endotoxemic horses.

An assay for quantification of endotoxin-neutralizing activity in equine serum was developed. In brief, activity of samples was measured in vitro as ability to suppress LPS-induced production of NO (measured as  $NO_2$  concentration) by interferon gamma-primed cells of the J774A.1 murine macrophage cell line. Concentration of active PB in serum samples was determined by comparison with a standard curve of PB concentration vs.  $NO_2$  concentration generated using serial dilutions of PB in serum.

In a preliminary experiment, active (endotoxin-neutralizing) PB was measured in blood collected from 3 horses at intervals for 24 h after they were given a single IV dose of 1 mg PB sulfate/kg. PB was diluted in 1 L 0.9% saline and infused IV over 15 min. Maximal mean ( $\pm$  sem) serum concentration of active PB was  $2955 \pm 472$  ng/ml 4 min after infusion and mean concentration declined to become undetectable by 16 h. Using Curry's method, these data were used to determine a dosage schedule of 1 mg PB/kg every 8 h. According to this schedule, trough concentration of active PB ( $> 200$  ng/ml) were predicted to neutralize  $>75\%$  of the  $NO_2$ -inducing activity of 1 ng LPS/ml. Five adult horses each were given PB (1 mg/kg) IV every 8 h for 5 successive treatments and blood was collected for measurement of active PB concentration. Maximal mean ( $\pm$  sem) serum concentration of active PB was  $3003 \pm 910$  ng/ml 10 min after the first infusion and declined to  $250 \pm 81$  ng/ml at 7.75 h. The PB concentration profile after the 5th infusion did not differ significantly ( $P < 0.05$ , repeated measures ANOVA) from that for the first infusion. Maximal PB concentration was  $2234 \pm 1017$  ng/ml, declining to  $244 \pm 100$  ng/ml at 7.75 h, and to undetectable levels by 14 h post-infusion. Mean trough concentration after 5 infusions was  $240 \pm 14$  ng/ml and trough concentrations did not differ significantly ( $P < 0.05$ ). No abnormal clinical sign was seen during the experiment.

In conclusion, it appears that PB infused IV to horses at a dosage of 1 mg/kg every 8 h maintains adequate circulating anti-endotoxic activity without accumulation of drug or signs of toxicity.

## 67

EFFECT OF CORN OIL ON SOLID-PHASE GASTRIC EMPTYING IN HORSES. R.J. Geor, \*P.A. Harris, K.E. Hoekstra, J.D. Pagan. Kentucky Equine Research, Versailles, KY and \*Equine Studies Group, WALTHAM Centre for Pet Nutrition, Leicestershire, UK.

The  $^{13}C$ -octanoic acid breath (or blood) test has been recently developed as a non-invasive method for measuring the rate of solid-phase gastric emptying (GE). We used this method to test the hypothesis that GE is delayed following ingestion of a grain plus corn oil meal compared to a meal of grain alone. Four mature (10–12 yr) Arabian horses were studied in a  $2 \times 2$  factorial design. Factor A was the habitual diet, either a control (CON; hay plus sweet feed [SWF]) or an isocaloric fat-supplemented diet (FAT; hay, SWF and corn oil). Factor B was the type of meal consumed for the GE test (SWF; 2 g/kg bwt. vs. SWF 2 g/kg bwt. plus 10% corn oil [OIL]). Each diet period lasted 10 weeks, with 6 weeks in between. GE studies were performed during the 4<sup>th</sup> and 8<sup>th</sup> weeks in each period. Within each dietary period, and in random order, horses were tested in both the SWF and OIL conditions. The 4 treatment combinations being: CON/SWF, CON/OIL, FAT/SWF, and FAT/OIL. For assessment of solid-phase GE, the test meals were labeled with 1 g of  $^{13}C$ -octanoic acid. Blood samples for measurement of plasma glucose concentration and  $^{13}C$ -enrichment were collected at 30 min and immediately before ingestion of the test meal and at frequent intervals thereafter for 7 h. Three indices of blood  $^{13}C$ -enrichment were calculated: half-dose recovery time ( $t_{1/2}$ ), the time to peak blood  $^{13}C$ -enrichment ( $t_{max}$ ), and the gastric emptying coefficient (GEC).

The glycemic response was markedly decreased in the OIL compared to the SWF trials; this effect of corn oil was not altered by habitual diet. In 1 horse for both the CON/OIL and FAT/OIL trials, the blood  $^{13}C$  vs. time curve was altered such that it was not possible to calculate  $t_{1/2}$  and  $t_{max}$ . Excluding data from this horse, addition of corn oil to the meal of SWF was associated with a significant decrease in GEC and a significant increase in  $t_{1/2}$  and  $t_{max}$ , as shown (mean  $\pm$  s.d.):

Treatment	GEC	$t_{1/2}$ (h)	$t_{max}$ (h)
CON/SWF	$2.96 \pm 0.15$	$2.25 \pm 0.55$	$1.20 \pm 0.21$
CON/OIL	$2.10 \pm 0.14$	$3.87 \pm 0.39$	$2.08 \pm 0.30$
FAT/SWF	$3.02 \pm 0.09$	$2.21 \pm 0.45$	$1.24 \pm 0.37$
FAT/OIL	$2.05 \pm 0.21$	$4.11 \pm 0.66$	$2.14 \pm 0.28$

We conclude that: 1) the addition of corn oil to a meal sweet feed results in a delay in solid-phase GE; 2) the effect of oil on GE is not affected by short-term adaptation to a fat-supplemented diet; and 3) the slowing of GE may contribute to the blunted glycemic response following a grain meal containing corn oil. The delayed GE may be due to a direct effect of oil on motility or the resultant increased energy density of the test meal.

## 68

PREVALENCE OF GASTRIC ULCERS IN STANDARDBRED RACEHORSES IN QUEBEC. R. Dionne, A. Vrin, M.Y. Doucet, J. Pare. Université de Montreal, Faculté de médecine vétérinaire, Quebec, Canada.

The purpose of this study was to determine the prevalence of gastric ulcers and to identify risk factors for their presence in Standardbred racehorses. Two hundred seventy-five (275) Standardbred racehorses from five training centers and two racetracks in Quebec, Canada, were included in the study. Historical data from the previous 2 months were recorded for each horse and the presence of gastric ulcers (GU) was determined by gastroscopy using a 3m videoendoscope. All evaluations were done from September to December, 1999. Location and severity (using a scoring system, grades 0 to 3) were recorded. The association between the presence of GU or score and a given risk factor was determined using univariate statistical analysis. Horses with GU (score 1–3) were compared to those without GU (score 0) using logistic regression analysis to identify risk factors. A significance level of  $p < 0.05$  was used for analysis.

The study population was composed of 112 females (41%), 80 geldings (29%) and 83 stallions (30%). There were 160 pacers (58%), 105 trotters (38%) and 10 horses (4%) for which gait was unknown at the time of the data collection. Forty-four horses were at rest, 92 were in training and 139 were actively racing. Overall, 121 horses (44%) had GU while the prevalence of GU in actively racing horses was 63% ( $n = 88$ ). The following factors were significantly associated with the presence of GU: activity status ( $p < 0.0001$ ) for a horse in training (OR = 2.18) or racing (OR = 9.29), gait ( $p = 0.004$ ) for trotters (OR = 2.23) and racetrack stables ( $p = 0.0027$ ). The mean number of lesion sites ( $p < 0.0001$ ) was significantly associated with the gastric lesion score. The only clinical sign significantly associated with the presence of GU was poor body condition ( $p = 0.02$ ). Poor body condition was significantly associated with ulcers located at the cardia ( $p < 0.0001$ ) and with lesions scores  $\geq 2$  ( $p < 0.0001$ ).

The prevalence of GU in Standardbred racehorses in this study was slightly lower than in Thoroughbred racehorses (60–90%). Horses that are actively racing, are living at racetracks, are trotters or have poor body condition are more likely to have GU. Also, GU lesion score (grades 0–3) based on lesion size was found to also reflect the number of sites where lesions were found in the stomach. This further validates the scoring system with regards to its correlation with severity.

## 69 VOLATILE FATTY ACID INJURY IN THE NONGLANDULAR REGION OF THE EQUINE STOMACH: IMPLICATIONS IN THE PATHOGENESIS OF GASTRIC ULCER DISEASE. J.A. Nadeau,<sup>1</sup> F.M. Andrews,<sup>1</sup> C.S. Patton,<sup>1</sup> A. M. Saxton,<sup>1</sup> and R.A. Argenzio.<sup>2</sup> <sup>1</sup> University of Tennessee, Knoxville, TN. <sup>2</sup> North Carolina State University, Raleigh, NC.

The prevalence of Equine Gastric Ulcer Syndrome (EGUS) ranges from 60 to 90% in performance horses and results in decreased performance, loss of revenue and even death due to gastric rupture. The non-glandular mucosa, adjacent to the margo plicatus, of the horse stomach is most commonly affected. In a previous study, a volatile fatty acid (VFA) (acetic acid), a byproduct of carbohydrate fermentation, was shown to cause injury in the nonglandular gastroesophageal mucosa of pigs. Recently, VFAs (propionic, butyric, and valeric acids) were found in high concentration in the horse stomach and at a low stomach pH, these horses had increased numbers and more severe non-glandular gastric ulcers. To determine the effect of acetic, butyric, propionic, and valeric acids on the nonglandular mucosa, thirty horses, free of clinical signs related to gastric disease, were euthanatized and their stomachs removed. The nonglandular mucosa was sharply dissected from the underlying submucosa and muscular layers and a 3.5 cm disc of tissue mounted in each of 12 Ussing's chambers. The mucosal surface was bathed in Ringer's solution (control) or Ringer's solution containing 60 mmol of either acetic, butyric, propionic, or valeric acid and pH of the solutions adjusted to 1.5, 4 or 7. Short circuit current (Isc) and potential difference (PD), indicators of sodium transport and tissue resistance, were recorded every 15 minutes for 3.5 hours. At the end of the experiment, the tissue was removed from the chambers, embedded in paraffin and stained with H&E. Butyric and propionic acids caused a significant ( $P < 0.01$ ) decrease in Isc and PD at a pH of 1.5 and 4, when compared to control, acetic acid, and the same solutions at pH 7.0. On histopathologic examination, butyric, propionic, and valeric acids caused cell swelling in the non-glandular mucosa at pH 4.0 and 1.5. In addition, valeric acid caused cell swelling at pH 7. Butyric, propionic, and valeric acids, because of their high lipid solubility and ability to remain undissociated at a low pH, may diffuse into the nonglandular mucosal cells, causing cell acidification and damage to sodium transport, which results in cell swelling, necrosis, and ulceration. Volatile fatty acids produced in the stomach of horses may contribute to the pathogenesis of EGUS. This study was supported in part by the Grayson-Jockey Club Research Foundation and the Comparative Gastroenterology Society.

## 70 EFFECTS OF OXYGLOBIN® ADMINISTERED TO PONIES WITH NORMOVOLEMIC ANEMIA. RL Belgrave, WM Bayly, MT Hines, RD Keegan, DC Sellon, KJ Wardrop. Washington State University, Pullman, WA.

The development of ultrapurified hemoglobin based oxygen carriers such as Oxyglobin®, has eliminated many of the potential problems seen with whole blood transfusions in other species. We hypothesized that the administration of Oxyglobin® would result in improved hemodynamic parameters in ponies with normovolemic anemia without adverse effects on renal function or coagulation times.

Normovolemic anemia was induced in six (6) healthy adult ponies. Over a 3 day period, at least 45ml/kg of whole blood was withdrawn from each pony, until a target PCV of  $< 12\%$  was attained. Plasma was separated from the red blood cells via centrifugation, and re-administered to the ponies on each day. After the final plasma transfusion, 15ml/kg of hetastarch (control,  $n = 3$ ) or 15 ml/kg of Oxyglobin® (treatment,  $n = 3$ ) was administered at 10ml/kg/hr IV. Hetastarch was chosen to control for the oncotic properties of Oxyglobin®.

Heart rate (HR), cardiac index (CI), plasma hemoglobin concentration (Hgb), PCV, and arterial oxygen content ( $AO_2$ ) were measured 15 minutes after the final plasma transfusion (anemic baseline, AB) and at "0 min" and "120 min" after infusion of either Oxyglobin® or hetastarch. Coagulation times (partial thromboplastin time [PTT] and prothrombin time [PT]) were determined at the same time points. Renal function parameters (serum creatinine concentration and urinalysis) were determined at AB, 24 hours and 48 hours after infusion. Data were analyzed with a two way repeated measures analysis of variance with a p value of 0.05.

A significant difference was observed between both groups for the mean CI\* at time "0" and "120 min" post infusion. No adverse renal or coagulation time effects were observed with Oxyglobin® administration.

Parameters ± SEM	Hetastarch AB	Oxyglobin® AB	Hetastarch t=120	Oxyglobin® t=120
CI (L/min/kg)*	0.09 ± 0.034	0.09 ± 0.005	0.11 ± 0.001	0.07 ± 0.004
HR (bpm)	64.0 ± 4.67	62.0 ± 1.58	87 ± 5.36	48.6 ± 0.19
Hgb (g/dl)	4.7 ± 0.32	4.6 ± 0.02	3.3 ± 0.20	5.3 ± 0.26
$AO_2$ (Vol% $O_2$ )	6.3 ± 0.46	6.3 ± 0.03	4.7 ± 0.49	7.1 ± 0.36
PCV (%)	10.8 ± 0.10	10.6 ± 0.69	8.3 ± 0.19	6.3 ± 0.77

These results suggest that Oxyglobin® is both safe and effective as a blood substitute in horses experiencing a normovolemic anemic insult.

## 71 MYELIN ABNORMALITIES IN FELINE ALPHA-MANNOSIDOSIS. Charles Vite,<sup>1</sup> Joseph C. McGowan,<sup>2</sup> Kyle G. Braund,<sup>3</sup> Margaret Weil,<sup>1</sup> Patty O'Donnell,<sup>1</sup> John H. Wolfe,<sup>1</sup> Mark E. Haskins,<sup>1</sup> School of Veterinary Medicine, University of PA, Philadelphia, PA,<sup>1</sup> Department of Radiology, School of Medicine, University of PA, Philadelphia, PA;<sup>2</sup> Veterinary Neurological Consulting Services, Dadeville, AL.<sup>3</sup>

Alpha-mannosidosis is a disease caused by the deficient activity of alpha-mannosidase, a lysosomal hydrolase involved in the degradation of glycoproteins. The disease is characterized by the accumulation of mannose-rich oligosaccharides within lysosomes. The purpose of this study was to characterize the central and peripheral nervous system abnormalities in cats with a four base pair deletion in the gene encoding alpha-mannosidase.

Three affected and three unaffected cats from two litters were examined weekly from 4 to 18 weeks of age. Progressively worsening neurological signs developed in affected cats that included whole body tremors, loss of balance, and nystagmus. Magnetic resonance imaging of affected cats revealed diffuse white matter signal abnormalities throughout the brain. Quantitative magnetization transfer imaging showed a 8–16% decrease in magnetization transfer ratio in the white matter of affected cats compared to unaffected cats indicating myelin abnormalities while gray matter areas were similar to normal cats. Affected cats showed slow distal motor nerve conduction velocity, increased F-wave latency, and increased central conduction time indicative of demyelination. These data were compared to histologic findings. Demyelination/remyelination was the dominant change found in the peripheral nerves of affected cats and was not seen in unaffected cats. Axonal degeneration was uncommon. Thin sections of peripheral nerves showed a significant increase in the G-ratio (axon diameter/fiber diameter) in all nerves examined from affected cats. The histologic findings confirm previously undescribed myelination abnormalities in both the central and peripheral nervous system in related cats with the four base pair deletion in the gene encoding lysosomal alpha-mannosidase.

## 72 MOLECULAR SCREENING FOR MYOTONIA CONGENITA IN MINIATURE SCHNAUZERS. Dilip P.Bhalerao, Yashoda Rajpurohit, Charles H. Vite, and Urs Giger. Sections of Medical Genetics and Neurology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Myotonia congenita is an inherited muscle disorder characterized by delayed relaxation of skeletal muscles in several species. Myotonic Miniature Schnauzers exhibit hypertrophic muscles, have difficulty rising, exhibit a stiff gait, and may bunny hop when running. Increased respiratory sounds, difficulty in swallowing, and superior prognathism are also noticed. The defect is inherited as an autosomal recessive trait and is biochemically characterized by reduced chloride conductance resulting in delayed relaxation after voluntary contraction. The molecular defect is a C to T missense mutation in the skeletal muscle voltage-dependent chloride channel CIC-1 gene which results in a threonine to methionine substitution within the carboxy terminal region of the D5 transmembrane segment of CIC-1.

We describe a reliable PCR based test for myotonia congenita in Miniature Schnauzers and present the data on the initial frequency of the chloride channel mutation in Miniature Schnauzers. DNA was extracted from either blood or cheek swab samples obtained from Miniature Schnauzers from various parts of the United States and Canada. After amplifying the DNA segment around the mutation with specific primers, the 340 bp PCR product was digested with the restriction enzyme *HpyCH4 III* which cuts the normal allele twice resulting in three fragments of 175, 135 & 30 bp, whereas the mutant allele is cut only once leaving a 175 and 165 bp fragment. The species specific primers do not amplify human DNA. A total of 312 Miniature Schnauzers were screened in the year 2000, and 78% were found clear (homozygous normal) for the CIC-1 mutation, 21% were determined to be carriers and 1% were affected dogs.

In this biased group of dogs the mutant allele frequency was 11% (0.11). Pedigrees were available from 26 of the carriers and all four myotonic dogs. One popular sire known to be a carrier was identified in all pedigrees as a common ancestor. In conclusion, the mutation for myotonia congenita appears to be common in Miniature Schnauzers related to this ancestor. Screening of all breeding Miniature Schnauzers is recommended.

## 73 IS THERE A MYELOPATHY ASSOCIATED WITH FELINE LEUKEMIA VIRUS? JJ McDonnell,<sup>1</sup> KP Carmichael,<sup>2</sup> D Bienze,<sup>3</sup> <sup>1</sup>Tufts University, Grafton, MA. <sup>2</sup>University of Georgia, Athens, GA. <sup>3</sup>University of Guelph, Guelph, ON.

Cats persistently infected with the retrovirus feline leukemia virus (FeLV) are affected by a wide spectrum of proliferative and degenerative diseases. Some diseases such as the leukemia/lymphoma complex and fibrosarcomas are generally accepted to be induced by or associated with FeLV infection. We present evidence that FeLV-infected cats have a novel and unique neurodegenerative process clinically evident as a progressive myelopathy.

We have identified a chronic neurologic syndrome in long-term FeLV-infected cats consisting of paresis that progresses to paralysis. Other signs reported include abnormal vocalization and a hyperesthesia syndrome. Records of nine affected cats were reviewed which showed that all cats were FeLV antigenemic for more than 3 years and had a nonpainful progressive spastic paraparesis. There was no other common physical examination finding or hematological abnormality. Imaging studies consisting of radiography, myelography and/or magnetic resonance imaging were negative for compressive lesions. Cerebrospinal fluid (CSF) analysis was normal. Cats were euthanized due to neurological deterioration. Necropsies of eight cats did not identify gross central nervous system abnormalities. Microscopically, there were profound lesions in the spinal cord and brainstem. The most severe lesions were within the thoracolumbar spinal cord that would explain the clinical signs. Lesions consisting of diffuse white matter degeneration characterized by dilated myelin sheaths and swollen axons were present in the absence of mononuclear cell infiltrates. FeLV antigen was identified immunocytochemically in the spinal cords of affected cats. Proviral DNA was amplified from sections of spinal cord, intestine, spleen and lymph nodes. These findings suggest that in a proportion of chronically FeLV-infected cats, a virus has evolved with an expanded host cell range enabling infection of neurons and glial cells resulting in axonal degeneration.

## 74 MUCOPOLYSACCHARIDOSIS TYPE IIIB (SANFILIPPO B SYNDROME) IN SCHIPPERKE DOGS. U. Giger, P. Wang, N.M. Ellinwood, U. Prociuk, T. Skeen, W. Bush, N.J. Edwards, E. Hardam, M.E. Haskins. Section of Medical Genetics and Laboratory of Pathology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; College of Veterinary Medicine, North Carolina State University, Raleigh, NC; and Capital Region Veterinary Medical Specialists, Albany, NY.

Mucopolysaccharidosis (MPS) are hereditary lysosomal storage diseases characterized by the accumulation of glycosaminoglycans due to a deficiency in catabolic hydrolases. In a group of MPS III disorders referred to as Sanfilippo Syndrome, heparan sulfate is stored and associated with a slowly progressive neuropathy. We have previously described MPS IIIA in dachshunds and report here on schipperke dogs with MPS IIIB, a disorder previously only documented in humans and by us in emus.

A male and a female, both 3-year-old schipperke dogs, presented for hind limb ataxia and dysmetria resulting in wide-based stance, truncal swaying, infrequent stumbling, and falling. Mononuclear blood cells contained granules that stained positively with toluidine blue for mucopolysaccharides. The urinary MPS spot test was also positive due to the presence of heparan sulfate. A dark auburn brown coat color and systolic murmur were also noted. The dogs had no menace, normal papillary light reflexes, and peripheral retinal degeneration. On physical examination, mostly cerebellar signs were present including a fine head intentia tremor and whole body tremor hypermetria and brisk reflexes.

Because of progressive signs the male dog was euthanized at five years of age. Histopathology revealed marked cerebellar atrophy, purkinje cell loss and neuronal and hepatic storage material that stained positively with PAS and toluidine blue. Fibroblasts were cultured from affected dogs for lysosomal enzyme studies. The activity of N-acetyl- $\alpha$ -D-glucosaminidase was <5% of normal when assayed with a fluorogenic substrate documenting an MPS IIIB diagnosis. Furthermore, other measured lysosomal enzyme activities were normal. Pedigree analyses and the study of family members N-acetyl- $\alpha$ -D-glucosaminidase activity supports an autosomal recessive mode of inheritance. In conclusion, a novel MPS IIIB disorder also known as Sanfilippo B syndrome causing a slowly progressive neuropathy was discovered in schipperkes.

## 75 MUSCULAR DYSTROPHY IN JAPANESE SPITZ DOGS. B.R.Jones,<sup>1</sup> J.J.Callanan,<sup>1</sup> C.T.Mooney,<sup>1</sup> E.Engvall,<sup>2</sup> Ling Gu,<sup>2</sup> D.Shelton.<sup>3</sup> University College Dublin, Ireland;<sup>1</sup> The Burnham Institute, La Jolla, California;<sup>2</sup> University of California, San Diego, La Jolla, California.<sup>3</sup>

We report a new muscular dystrophy in five young male Japanese Spitz dogs. Clinical signs were first observed at 10–12 weeks of age. There was excess salivation and dysphagia, a slow onset of exercise intolerance, an abnormal gait and pain on palpation of muscles. The clinical signs progressively worsen and all dogs were dead by 13 months of age. Serum creatine kinase (330–>66,000 iu/L), and alanine aminotransferase (213–292 iu/L) were elevated. Neurological examination was normal. One dog had gastroesophageal reflux, another a hiatal hernia.

Fixed and frozen muscles were evaluated by standard histopathological and histochemical stains. Microscopic examinations revealed clusters of muscle fibers with mild or marked variation in size primarily related to atrophy and occasionally to hypertrophy. There was also prominent myocyte degeneration with occasional necrotic fibers containing coarse basophilic granules, replacement fibrosis and monocyte infiltrations.

Unfixed cryostat sections (8mm) were processed for indirect immunofluorescence using several monoclonal and polyclonal antibodies and fluorescein labelled secondary antibodies. The following antibodies were used: monoclonal antibodies against the rod domain and carboxy terminus of dystrophin, against spectrin, alpha- and beta-sarcoglycan, laminin alpha-2 and gamma-1, and dysferlin. Polyclonal antisera were used against beta-dystroglycan, alpha- and beta-sarcoglycans, and laminins. Labelling was absent with the monoclonal antibody against the rod domain of dystrophin. All other antibody labelling including that against the carboxy terminus of dystrophin was similar to control tissue. Since dystrophin was not absent with both antibodies (DYS1 and DYS2), an attenuated form of dystrophin is suspected similar to Becker's dystrophy in humans. Further analysis of dystrophin by western blot is in progress.

## 76 EFFECTS OF SHORT TERM STEROID ADMINISTRATION ON ACETYLCHOLINESTERASE ACTIVITY IN HEALTHY DOGS. Goldstein RE,<sup>1</sup> Klein O,<sup>1</sup> Milgram J,<sup>1</sup> and Soreq H.<sup>2</sup> 1. Koret School of Veterinary Medicine, Hebrew University of Jerusalem. 2. Institute of Life Sciences, Hebrew University of Jerusalem.

Over-expression and post-transcriptional changes of the acetylcholinesterase (AChE) gene have been associated with deleterious effects of acute and chronic stress including myopathy in mice and possibly post traumatic stress disorder in people. A functional glucocorticoid element has been identified in the promoter region of the human and rodent AChE gene. To explore a possible role for AChE in the response to stress or glucocorticoid administration in dogs we initiated a study evaluating the effect of short-term steroid administration on the AChE activity in muscles, erythrocytes and plasma of healthy dogs.

Ten healthy adult beagles (6 male, 4 female) were divided into 2 groups. The study group included 6 dogs that received 1mg/kg/day of prednisolone subcutaneously (SC) daily for 7 days (days 0–6). The control group included 4 dogs that received an equal volume of saline SC daily. Deltoid muscle biopsies for AChE activity were obtained on days 0 (left side) and 7 (right side). Whole blood in EDTA was drawn on days 0 and 7 for erythrocyte AChE activity, and heparinized plasma was drawn on days 0,1,3,5, and 7 for plasma AChE activity. Total cholinesterase (ChE) activity was assessed by Ellman's method. Butyrylcholinesterase (BChE) activity was inhibited to assess specific AChE activity. Muscle AChE activity was expressed per mass of muscle protein, assayed by a modified Lowry method. Values are expressed as mean  $\pm$  SD.

Plasma AChE increased significantly in the steroid treated dogs from  $339.3 \pm 43$ , to  $408.7 \pm 72.8$  nmol substrate hydrolyzed/min/ml by day 7 ( $p < 0.05$ ). AChE activity in the muscle biopsies decreased by 42% in the steroid treated dogs, from  $1.97 \pm 0.43$  on day 0 to  $1.26 \pm 0.51$  nmol substrate hydrolyzed/gr tissue protein/min on day 7 ( $p < 0.01$ ). There was no change in erythrocyte AChE activity in the control or study groups during the study. There was no change in plasma or muscle AChE activity in the control group during the study. BChE levels remained unchanged in both the plasma and the muscle of treated dogs, attesting to the specificity of the AChE modulations.

These findings demonstrate a complex pattern of multi-tissue responses to steroid hormones in canine AChE activity. It is not yet clear whether this is due to transcriptional, post-transcriptional, translational or functional modifications. In view of the known functions of AChE, these tissue specific changes suggest that AChE may be involved in mediating the actions of steroid hormones.

## 77 CANINE ACETYLCHOLINESTERASE: MOLECULAR AND FUNCTIONAL CHARACTERIZATION. Goldstein RE,<sup>1</sup> Klein O,<sup>1</sup> and Soreq H.<sup>2</sup> 1. Koret School of Veterinary Medicine, Hebrew University of Jerusalem. 2. Institute of Life Sciences, Hebrew University of Jerusalem.

The acetylcholinesterase (AChE) gene has been well characterized in many species including humans, rodents and cats. Three protein products have been shown to be produced from this gene by alternative splicing. Many factors including acute and chronic stress as well as various disease states have been shown to affect gene transcription, splicing and enzyme activity in humans and rodents. Post-transcriptional changes have been associated with a variety of pathological consequences, possibly including the "Gulf War Syndrome" in people. The goals of this study included the cloning and sequencing of the canine AChE gene, the characterization of the canine AChE enzyme and its plasma activity level in normal dogs.

Genomic DNA was isolated from white blood cells of 6 normal mix-breed adult dogs. Polymerase chain reaction (PCR) amplification was performed on the canine genomic DNA using a panel of oligonucleotide primers directed against human and mouse AChE. The gene fragments were then sequenced using an automated sequencer. Cholinesterase (ChE) activity was measured in heparinized plasma obtained from these dogs using the Ellman method. Specific AChE plasma activity was measured by the usage of specific blockers of AChE and Butyryl-cholinesterase (BChE). A sucrose gradient was utilized to identify the quaternary structure (monomer, dimer, or tetramer) of the functional plasma AChE.

The canine AChE sequence revealed marked homology to human AChE (88%) and was closest to the cat gene among known sequences (93%). The canine AChE gene encodes for the 3 protein variants previously recognized in rodents and humans. The major ChE in canine plasma is AChE, which accounts for 75% of the total ChE activity. This differs from the percentage in cats, where just 40% of the total ChE activity is AChE, and the extremely low percentage in people of only 1%-3%. The canine plasma acetylcholine esterase is a tetramer, differing from the human monomer or dimer forms found in the plasma.

We have successfully cloned, sequenced and characterized the canine AChE gene, characterized the active form, and the specific activity levels of canine AChE in the plasma of normal dogs. The significance of the interspecies differences regarding the type of active plasma cholinesterase and the differences in sub-unit structure is unknown.

## 79 ELECTROENCEPHALIC FINDINGS IN TEN DOGS WITH HYDROCEPHALUS D. Faissler, A. Jaggy.<sup>2</sup> Department of Clinical Sciences, Tufts University, North Grafton, MA, USA; <sup>2</sup>Department of Clinical Neurology, University of Bern, Switzerland.

Recent MRI studies reveal that some dogs without obvious clinical deficits have a significantly enlarged ventricular system in the brain. A functional test like electroencephalography (EEG) may be helpful to distinguish potentially hydrocephalic dogs from animals with clinically insignificant ventriculomegalia.

The primary goal of this study was to quantify the EEG recordings in dogs with neurological deficits and confirmed hydrocephalus. In a second step, we compared these values with the recordings of normal dogs of a similar age.

The EEG was recorded under general anesthesia with medetomidine and propofol. The procedure was performed with an 8 channel electroencephalograph. Amplitude and frequency of the basic and its superimposed pattern in all leads were calculated manually. A total of 9 measurements over 30 minutes for each derivation were analysed. The control dogs were a population of 9 healthy Beagles from a previous study (Accatino et al, 1997).

The mean age at presentation was 13.6 months. The reasons for veterinary care were seizures (4/10), vestibular signs (3/10), abnormal vision (3/10), compulsive gait (2/10), circling (2/10) and retarded growth (1/10). CBC, blood chemistry profile and bile acids or NH<sub>3</sub> were within normal limits. Based on cerebrospinal fluid analysis (n = 7), CT-scan (n = 4), ultrasound (n = 3), radiographs (n = 2) and necropsy (n = 6) hydrocephalus was confirmed, but no obvious cause for the condition could be identified. The basic EEG pattern of the hydrocephalic dogs was characterized by a high amplitude of 117.6 ± 36.8 µV and a low frequency of 2.6 ± 0.7 Hz. The mean amplitude of the hydrocephalic dogs differed significantly (p > 0.05) from control dogs (37 ± 4.2 µV). A superior separation between hydrocephalic and control dogs was achieved by comparing the frequency of the superimposed pattern. Dogs with hydrocephalus had a significantly (p > 0.0001) lower mean frequency (5.1 ± 1.4 Hz) than the control dogs (15 ± 1 Hz). To our knowledge, this is the first study proving that quantitative measurement of the superimposed low amplitude fast activity pattern adds important information. A focal paroxysmal spindle activity (73.6 ± 20.1 µV, 12.7 ± 1.8) was noticed only in two dogs suffering from grand mal seizures. In hydrocephalic dogs, there was a significant inverse correlation (r = -0.56, p > 0.05) between the age at presentation and the amplitude of basic pattern. Additionally, a significant correlation (r = 0.66, p > 0.05) was established between age at presentation and frequency of the basic EEG pattern.

## 78 MATRIX METALLOPROTEINASES IN NORMAL CANINE CSF R.L. Bergman, K.D. Inzana, T.J. Inzana, and G.D. Boon. Virginia Tech, Blacksburg, VA.

CSF analysis is a common diagnostic tool for patients with neurologic disease. It can be diagnostic in a few isolated diseases but is generally non-specific. CSF analysis has the potential for providing additional information. Matrix Metalloproteinases (MMPs) are calcium- and zinc-dependent endoproteinases with overlapping substrates that hydrolyze at least one component of the tissue extracellular matrix. MMPs are important in normal physiologic processes such as angiogenesis and wound healing. MMPs play a role in pathological processes that involve unregulated matrix destruction such as arthritis and neoplasia. One class of MMPs, the gelatinases, degrade gelatin and type IV collagen. These include MMP2 and MMP9. Gelatinases have been shown to play a role in multiple CNS diseases. MMP2 is known to be constitutively produced in human CSF while MMP9 is present only in certain pathologic conditions such as multiple sclerosis, neoplasia, and inflammatory diseases. We hypothesize that MMP2 is present in normal canine CSF while MMP9 is absent.

CSF (23) samples were taken from normal dogs. Each CSF sample was evaluated immediately for RBC and WBC numbers, protein, glucose, and cytology. CSF samples were considered normal if the protein was < 25 mg/dl, WBC < 6 cells/mcl, and RBC < 25 cells/mcl. Each dog was euthanized and the brains evaluated by routine histopathology. MMP analysis was done with gelatin zymography and a commercial polyclonal ELISA for proMMP2. For zymography, gelatin was incorporated into a sodium dodecyl sulfate-polyacrylamide gel electrophoresis assay. Bands of enzyme activity were visible following enzyme degradation of the gelatin.

The mean WBC count for CSF was 0.96/mcl (range of 0-3/mcl). The mean protein concentration was 12 mg/dl, with a range of 8-17 mg/dl. The mean RBC count was 3.65/mcl (range of 0-21/mcl). Zymography of all normal samples of CSF revealed a band of clearing that corresponded to the human commercial standard of proMMP2. No other significant bands of clearing were noted and other band results were faint and variable. The mean pro MMP2 levels were 5.61 ng/ml (range of 3.36-10.83 ng/ml).

We conclude that proMMP2 was present in normal canine CSF based on results of gelatin zymography and ELISA. The concentration of proMMP2 was similar to what has been reported in humans (14.6 to 183.2 ng/ml in one study and 0.51 to 1.95 ng/ml in another). MMP9 was not present in normal CSF. Also, the technique for the zymographic analysis of gelatinases in canine CSF was optimized, which will aid in future research. The knowledge of gelatinase composition in normal canine CSF will serve as a baseline for future studies of CNS diseases in dogs.

## 80 EVALUATION OF JITTER BY STIMULATED SINGLE FIBER EMG IN NORMAL DOGS. Sonia Añor,<sup>1</sup> David Lipsitz,<sup>1</sup> Colette Williams,<sup>1</sup> Linda Tripp,<sup>1</sup> Ricardo Maselli,<sup>2</sup> and Richard LeCouteur.<sup>1</sup> <sup>1</sup>School of Veterinary Medicine, <sup>2</sup> School of Medicine, University of California, Davis.

The diagnostic tests currently used to diagnose myasthenia gravis (MG) in dogs include pharmacological testing with edrophonium chloride, repetitive nerve stimulation and detection of serum anti-acetylcholine receptor (AChR) antibodies. The first two tests are relatively nonspecific and insensitive. Detection of AChR antibodies is a sensitive and specific test for most cases of MG but seronegative MG has been described in dogs. In people, single fiber electromyography (SFEMG) is considered to be the most sensitive test for evaluating all categories of MG, being abnormal in 92 to 100% of MG cases. Only one study exists concerning SFEMG in dogs, in which a pelvic limb muscle was studied. In MG, different muscles are involved to different degrees and focal forms also exist. The capability to perform SFEMG in several different muscle groups should increase our ability to diagnose MG in dogs. The purpose of the present study was to develop a technique for *jitter* in a facial muscle and a muscle of the thoracic limb. This study will provide baseline information of *jitter* for use in the diagnosis of MG in dogs.

Ten healthy, neurologically normal, non-conditioned dogs were included in the study. The animals were maintained under general anesthesia for the electrodiagnostic study. SFEMG and determination of jitter were performed in the orbicularis oculi muscle and the extensor carpi radialis muscle. Fifty consecutive single fiber muscle potentials recorded from the same muscle fiber were used to calculate *jitter*, and a minimum of 20 different single muscle fibers was evaluated for each muscle. Mean consecutive differences (MCD) in latency (*jitter*) and standard deviations (SD) were calculated for each single fiber and for each muscle group.

Normal values for individual muscle fiber MCD's and mean *jitter* (MCD) for the fiber pool in each muscle were calculated for the orbicularis oculi and extensor carpi radialis muscles.

SFEMG is a reliable method to study jitter in the orbicularis oculi and the extensor carpi radialis muscles in dogs. Further studies are underway to determine the potential application of SFEMG in the clinical diagnosis of MG in dogs.

- 81** **MALIGNANT CELLS FROM DOGS WITH LYMPHOMA AND LEUKEMIA EXPRESS RECEPTORS FOR INTERLEUKIN-2.** Erin B. Dickerson,<sup>1</sup> Susan Fosmire,<sup>2</sup> Marcia L. Padilla,<sup>1</sup> Jaime F. Modiano,<sup>2,3</sup> Stuart C. Helfand,<sup>1,4</sup> <sup>1</sup>School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, <sup>2</sup>AMC Cancer Research Center, Denver, CO, <sup>3</sup>University of Colorado Cancer Center, Denver, CO, <sup>4</sup>University of Wisconsin Comprehensive Cancer Center, Madison, WI.

Lymphohematopoietic malignancies are common spontaneous diseases of dogs whose clinical presentation and biological behavior closely resemble their human counterparts. The goal of this study was to define the potential to use canine lymphoma and leukemia as suitable models to refine therapeutic approaches targeting the interleukin-2 (IL-2) receptor. We evaluated the patterns of interleukin-2 receptor (IL-2R) expression in thirteen dogs with multicentric non-Hodgkin's lymphoma (NHL) and in six dogs with leukemia (ALL n = 3, CLL in blast crisis, n = 1, AMoL n = 2). We first cloned and sequenced the complete coding domains of the wild type canine IL-2R $\alpha$  chain gene. We next used qualitative RT-PCR to examine expression of the IL-2R  $\alpha$ ,  $\beta$ , and  $\gamma_c$  subunits in the tumors. Messenger RNA for the IL-2R  $\alpha$ ,  $\beta$ , and  $\gamma_c$  subunits that comprise the high affinity receptor were present in samples from all dogs with NHL. Expression of functional surface IL-2R also was demonstrated flow cytometrically in NHL cells from four of four dogs tested. Leukemic cells from one dog with B cell ALL and two dogs with AMoL expressed mRNA for all three subunits, whereas cells from another dog with B cell leukemia and both dogs with T cell ALL expressed only mRNA for the  $\beta$  and  $\gamma_c$  subunits that comprise the intermediate affinity receptor. These results indicate that the IL-2R is commonly expressed in canine lymphohematopoietic malignancies and support the suitability of this large animal model to evaluate targeted IL-2R cancer therapy using approaches of interest in the treatment of humans with hemolymphatic cancers.

- 82** **GENETICALLY MODIFIED SALMONELLA FOR CANINE CANCER: A PHASE I STUDY.** EG MacEwen,<sup>1</sup> D Thamm,<sup>1</sup> ID Kurzman,<sup>1</sup> M Szoln,<sup>2</sup> Z Li,<sup>2</sup> I King,<sup>2,†</sup> <sup>1</sup>University of Wisconsin-Madison, WI, <sup>2</sup>Vion Pharmaceuticals Inc, New Haven, CT.

An attenuated *Salmonella typhimurium* (VNP20009) was generated by deletions in the *msbB* and *purI* genes. VNP20009 is unable to produce wild type lipopolysaccharides or to survive without an external source of purines. VNP20009 has been shown to preferentially accumulate and proliferate in tumors in immunologically intact and immune deficient mice without toxicity. Preclinical studies in murine tumor models show antitumor activity in a variety of tumors. The purpose of this Phase I study was to determine dose-limiting toxicity (DLT), confirm VNP20009 tumor targeting, and potential therapeutic activity in dogs with various measurable tumors.

All dogs were clinically staged using standard methods. Tumor biopsies were obtained prior to and 7 days following the first treatment. VNP20009 was detected using bacterial culture and PCR using day 7 tumor biopsy material. Following treatments, dogs were monitored for clinical, hematological, and biochemical toxicity. Tumor response (CR, PR, SD, PD) was evaluated at each visit. Dogs demonstrating a response were continued on treatment for at least 8 weeks. Treatments were discontinued in dogs showing PD.

Fourteen dogs were entered into this trial. Dogs received VNP20009 IV over 30 mins in doses ranging from  $1.5 \times 10^5$  to  $5 \times 10^6$  cfu/kg. Mild fever occurred 2–4 hours after the infusion. VNP20009 has been detected in 4 of 8 tumor samples tested to date. No significant hematological or biochemical toxicities were encountered. One dog developed a protein-losing nephropathy histologically consistent with membranoproliferative glomerulonephropathy.

A durable CR was detected in one dog with a metastatic melanoma, 3 PRs (local tumor only) were seen in a rhabdomyosarcomas, melanoma, and an anaplastic carcinoma. Four dogs had SD (>8 weeks) and 5 had PD. The results of this early phase I study indicate that VNP20009 is relatively safe at these doses. Furthermore, the antitumor responses noted are significant and additional clinical studies are currently underway.

- 83** **CHARACTERIZATION OF GENETIC ALTERATIONS IN FELINE VACCINE-ASSOCIATED SARCOMA USING WHOLE CHROMOSOME PAINTING PROBES.** E.A. Hoots, E.A. McNiel, S.M. LaRue, Dept. of Radiological Health Sciences, Colorado State University, Fort Collins, Colorado.

Feline vaccine-associated sarcoma poses clinical, ethical, legal, and economic problems for the veterinary profession. Prevention and treatment of this cancer requires an understanding of the underlying causes. Cancer is a genetic disease and chromosomal aberrations have been shown to cause tumors in humans. Characterization of such chromosomal aberrations in feline tumors will allow us to identify the genomic loci important in oncogenesis. Fluorescence in situ hybridization techniques such as fluorescent-labeled painting probes are state-of-the-art, powerful tools for evaluating cytogenetic abnormalities in cancer cells. With painting probes it is possible to directly visualize and identify chromosomal aberrations such as translocations, insertions, amplifications, and deletions more rapidly and reliably than with traditional cytogenetic techniques. These techniques have never been used to evaluate feline tumors. Therefore, we have developed a technique to efficiently generate chromosome specific painting probes for each of the chromosomes of the domestic cat (*Felis catus* n = 38).

Metaphase chromosome spreads on coverslips were made from mitogen-stimulated, cultured lymphocytes. These coverslips were stained with 5% giemsa and viewed under 1000X magnification. A glass needle controlled by a micromanipulator was used to scrape 10 copies of a chromosome from the glass surface. The chromosomes were transferred to a microfuge tube and the DNA is amplified using degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR), a form of PCR that amplifies all genomic elements equally. The amplified DNA fragments are then labeled via a second round of DOP-PCR amplification in the presence of a fluorescent nucleotide, which is incorporated into the newly synthesized DNA. The fluorescent-labeled DNA (painting probe) is then hybridized onto a metaphase spread. In the presence of blocking DNA to eliminate preferential binding to repetitive sequences (Feline Cot-1 DNA), the labeled painting probe will "light up" chromosome specific sequences when viewed with a fluorescent microscope.

Whole chromosome painting probes that specifically identify feline chromosomes were created and can be used to identify chromosomal abnormalities in feline tumors. Our goal is to produce painting probes for each feline chromosome and to screen tumors from clinical cases. Identification and characterization of discrete chromosomal abnormalities in feline tumor cells may be important in understanding the behavior, prognosis, and potential response to therapy of feline cancers.

Supported by grants from the Morris Animal Foundation and the National Cancer Institute.

- 84** **CHARACTERIZATION OF CHROMOSOMAL ABERATIONS IN FELINE VACCINE-ASSOCIATED SARCOMA USING COMPARATIVE GENOMIC HYBRIDIZATION.** E.A. McNiel, E.A. Hoots, S.M. LaRue, Department of Radiological Health Sciences, Colorado State University, Fort Collins, Colorado.

Epidemiologic evidence strongly associates vaccination of cats for rabies and feline leukemia virus with the development of soft tissue sarcomas at the site of administration. These tumors are histologically aggressive, locally invasive, and constitute a difficult legal, ethical, and clinical problem for the veterinary profession. Prevention and definitive treatment of feline vaccine-associated sarcoma requires an understanding of the pathogenesis of this cancer. Cancer is a genetic disease. The transformation of normal cells to tumor cells with increasingly malignant characteristics results from the accumulation of mutations. Causative chromosome rearrangements are recognized in human tumors and many cancer-associated genes have been identified with cytogenetic study. The location of recurrent chromosomal aberrations signals the presence of cancer associated genes at particular genomic locations. Chromosomal studies of human cancers provide an important clinical diagnostic and prognostic tool. We have initiated efforts to characterize the genomic alterations in feline vaccine-associated sarcoma through cytogenetic evaluation of tumors.

Over the last decade, the development of molecular cytogenetic techniques including chromosome and gene specific fluorescence in situ hybridization (FISH) has greatly enhanced the resolution, specificity, and efficiency of tumor cytogenetics. These advancements are particularly important for the study of the complex karyotypic rearrangements found in solid tumors. Comparative genomic hybridization (CGH) is a state-of-the-art technique for detecting numerical chromosome changes, such as amplifications, deletions, aneuploidy, and unbalanced translocations in tumors. CGH involves the simultaneous hybridization of tumor and normal DNA, labeled with 2 different fluorochromes, onto a normal metaphase chromosome spread. Computer image analysis allows for detection of differences in the fluorescent intensity of the two fluorochromes indicating gains or losses in the tumor at particular chromosomal loci.

We have modified CGH for use in the study of feline tumors. So far, five feline vaccine-associated sarcomas have been characterized using CGH and classical banding techniques. These tumors have highly aberrant karyotypes. Further evaluations will be necessary to characterize recurrent mutations. Comparative genomic hybridization provides a rapid method for screening the genome for mutations in cancer related genes.

Supported by grants from the Morris Animal Foundation and the National Cancer Institute.

## 85 ANTIMICROBIAL-INDUCED ENDOTOXIN AND CYTOKINE ACTIVITY IN AN *IN VITRO* MODEL OF FOAL SEPTICEMIA. A.P. Bentley, M.H. Barton, N. Norton, M. Lee. University of Georgia, Athens, GA.

Gram negative bacterial septicemia is the most common cause of death in neonatal foals. The high mortality rate is associated with the proliferation of gram negative bacteria, resulting in endotoxemia. Activation of leukocytes by endotoxin initiates an inflammatory cascade, which may culminate in cardiovascular collapse and death. Although antimicrobials are essential for the treatment of septicemia, bactericidal therapy itself may cause endotoxin release. The purpose of this study was to determine which antimicrobials used to treat *E. coli* septicemia minimize endotoxin release and subsequent inflammatory mediator activity while maintaining bactericidal efficacy.

*E. coli* was isolated from a septicemic foal and minimal inhibitory concentrations (MIC) were determined for 5 antimicrobials: amikacin, ampicillin, amikacin plus ampicillin, ceftiofur, and imipenem. These antimicrobials were then tested in an *in vitro* model at 2 and 20 times the MIC.  $10^6$  mononuclear cells isolated from 6 healthy foals were incubated in tissue culture media with  $10^5$  CFU of the septic foal *E. coli* isolate and each antimicrobial or saline. After a 6 hour incubation, the concentration of viable bacteria remaining was determined using the Most Probable Number scheme. The supernatant was tested for endotoxin and tumor necrosis factor (TNF) activities, using the limulus amebocyte lysate assay and WEHI bioassay, respectively.

Compared to saline, each antimicrobial significantly decreased or reduced to an undetectable level the number of viable bacteria. There was no significant difference between antimicrobials in efficacy of bacterial kill. Treatment using amikacin alone or with ampicillin resulted in significantly less endotoxin activity than did ampicillin, ceftiofur, or imipenem alone. Endotoxin activity was significantly less at 20 times the MIC for amikacin with ampicillin, ampicillin, and imipenem, than at 2 times the MIC. Among the antimicrobials tested, ceftiofur induced the greatest endotoxin activity. There was a correlation between TNF and endotoxin activity, as treatment with amikacin alone or with ampicillin resulted in significantly less TNF activity than ampicillin, ceftiofur, or imipenem, despite similar degrees of bacterial killing.

The results of this study suggest that amikacin alone or in combination with ampicillin would be less likely to induce endotoxemia and TNF synthesis during bactericidal treatment of *E. coli* septicemia than ampicillin, ceftiofur or imipenem alone. Furthermore, endotoxin release and TNF activity induced by treatment with bactericidal antimicrobials may be dose dependent. The use of ampicillin, ceftiofur, or imipenem in the treatment of neonatal septicemia should be accompanied by appropriate therapy for endotoxemia.

## 86 ACTINOBACILLUS SPP. BACTEREMIA IN FOALS: CLINICAL SIGNS AND PROGNOSIS. AJ Stewart, KW Hinchcliff, WJA Saville, CW Kohn, SM Reed, J Hardy and E. Jose-Cunilleras. The Ohio State University, Columbus, OH.

Anecdotal reports suggest that foals with *Actinobacillus spp.* septicemia are affected at an earlier age and are more severely ill than are foals with septicemia or bacteremia caused by other bacteria. However, to our knowledge this association has never been formally investigated. Our hypothesis was that hospitalized foals with *Actinobacillus spp.* bacteremia are affected at a younger age, have more severe signs of disease and have a worse prognosis than do foals with bacteremia of other causes.

Medical records of 96 foals, aged from birth to 113 days, with blood cultures that yielded pathogenic bacteria were reviewed. Data abstracted included signalment, history, duration of illness, physical, hematological and biochemical examination findings, the duration of hospitalization and mortality. Categorical data were analyzed by Chi-square analysis and crude odds ratios (OR) with 95% confidence intervals calculated. Continuous data were analyzed by t-tests. Null hypothesis was rejected at  $P < 0.05$ .

Mixed bacterial infections were present in 31% (30/96) of the foals, with Gram-negative organisms isolated from 87% (85/96) of foals. *Actinobacillus spp.* were cultured from 30% (29/96) of foals and were the sole species isolated in 21% of cases. The overall survival rate for bacteremic foals was 52%. Foals with *Actinobacillus spp.* bacteremia had a slightly, but not statistically significant, greater risk (OR = 1.9 [95% CI: 0.8, 4.5;  $P = 0.45$ ]) of death. Foals with *Actinobacillus spp.* bacteremia were 2.6 (1.0, 6.6;  $P = 0.04$ ) times more likely to have been sick from birth, 3.9 (1.5, 10.0;  $P = 0.004$ ) times more likely to be recumbent on presentation, 4.0 (1.0, 16.2;  $P = 0.04$ ) times more likely to have a sepsis score greater than 11 and 2.8 (1.1, 7.2;  $P = 0.03$ ) times more likely to have pneumonia than other bacteremic foals. Furthermore, *Actinobacillus spp.* bacteremic foals were 4.4 (1.2, 16.6;  $P = 0.02$ ) times more likely to present with a depressed mental status and 8.0 (1.5, 41.6;  $P = 0.008$ ) times more likely to be comatose. Foals with positive blood cultures for *Actinobacillus spp.* had statistically significantly ( $P < 0.001$ ) lower white blood cell, neutrophil and band cell counts and lower blood glucose concentration ( $P = 0.046$ ) on presentation than did foals with bacteremia caused by other etiologic agents. There was no increased risk (0.9 [0.4, 2.3;  $P = 0.48$ ]) of failure of passive transfer (IGG  $> 800$ mg/dl compared to IGG  $< 400$ mg/dl) for foals with *Actinobacillus spp.* bacteremia.

Although foals with *Actinobacillus spp.* bacteremia were more likely to have been sick from birth, be recumbent, have a greater degree of depression, leukopenia and increased risk of pneumonia, their overall survival rate was similar to that of other bacteremic foals.

## 87 CHANGES IN COLLAGEN CONTENT AND MORPHOMETRIC CHARACTERISTICS OF THE EQUINE LUNG FOLLOWING INTRAPULMONARY BLOOD INOCULATION. S.A. McKane and R.F. Slocumbe. Oregon State University, Corvallis, OR. \*University of Melbourne, Australia.

Blood within the equine airways has been shown to reduce exercise performance and induce both acute and chronic changes in alveolar cytology, including prolonged activation of alveolar macrophages. This study examines the effects of intrapulmonary blood on the physical structure of alveoli and its ability to promote pulmonary fibrosis and permanent scarring of the lung.

Seven horses underwent inoculations of paired bronchi with either autologous whole blood or serum, at days 15, 8, 3 and 1, and 30 minutes prior to euthanasia. The lungs were fixed with 10% formalin infused via the airways and sections were cut, from 16 regions selected for morphometric analysis, before staining with either haematoxylin and eosin or Masson's trichrome stains. Measurements made included the numerical density of alveolar macrophages, alveolar size, surface area to volume ratio, septal thickness, volume density of parenchymal tissue, and percentage of parenchyma composed of collagen.

Blood inoculation produced many significant ( $p < 0.05$ ) differences in alveolar morphology, including transient alveolar collapse, increased septal thickness, increased percentage of collagen in alveolar walls, and increased macrophage numbers. Septal thickness increased by approximately 50% to  $6.1 \pm 0.5$  mcm in response to blood but not serum inoculation. This change coincided with initially increased macrophage numbers, from the normal of  $10,688 \pm 1708$  up to  $30,957 \pm 6831$  cells/mcm<sup>2</sup> at day 3, and subsequently an increased alveolar septal collagen content from  $6.6 \pm 0.5\%$  to  $14.1 \pm 1.3\%$  at day 8 and  $27.5 \pm 3.3\%$  at day 15 following blood inoculation. In the first 8 days alveolar collapse was observed in blood but not serum inoculated regions, with estimated alveolar diameters of  $108.0 \pm 6.9$  mcm and  $137.4 \pm 10.5$  mcm respectively.

These findings indicate that intrapulmonary blood induces a macrophage dominated inflammatory response, promotes alveolar collapse and septal thickening, and results in pulmonary fibrosis and permanent changes to alveolar wall structure. If this model accurately mimics exercise-induced pulmonary hemorrhage (EIPH), there are obvious indications for resting horses that have suffered an episode of pulmonary hemorrhage. Compliance differences between collapsed and normal regions of lung could produce shear forces during the forced ventilation of exercise, which may lead to further tissue trauma and hemorrhage. The development of pulmonary fibrosis means full restoration of normal pulmonary mechanical properties is unlikely and this may play a role in the recurrent and progressive nature of EIPH seen in aging performance horses.

## 88 EFFECTS OF INTRAMAMMARY HYPERTONIC SALINE INFUSION IN COWS WITH EXPERIMENTALLY INDUCED COLIFORM MASTITIS. M-F Haddad, D.E. Morin, P.D. Constable, J.B. Messick, and W.L. Hurley. Departments of Veterinary Clinical Medicine, Veterinary Pathobiology, and Animal Sciences, University of Illinois, Urbana, IL.

Intramammary (IMM) infusion of hypertonic saline solution (HSS) has been used to treat clinical mastitis in dairy cows, with little data to support its efficacy or safety. Theoretically, IMM infusion of HSS should induce an osmotically-driven flow of fluid into the mammary gland, thereby facilitating removal of bacteria, endotoxin, and other inflammatory mediators during stripping. The objective of this study was to determine the safety and efficacy of IMM HSS infusion in cows with experimentally induced coliform mastitis.

Coliform mastitis was induced in 6 healthy Holstein cows  $> 1$  month into lactation. Rear glands were inoculated with 50–800 cfu of *E. coli* (MacDonald 487) 2–3 weeks apart, with each cow serving as its own control (randomized crossover design). Treated glands were infused with 1 L HSS (2,400 mosm/L) 14 hours post-inoculation (PI), followed by stripping of the gland 15 minutes later. The same treatment was repeated in 12 hours. Control glands were inoculated with *E. coli*, but received no IMM HSS treatment.

All cows developed clinical (local and systemic) and laboratory signs of acute coliform mastitis within 14 hours PI, and the disease course was not affected by HSS treatment. IMM pressure rose from a mean of 1 cm H<sub>2</sub>O to 55 cm H<sub>2</sub>O immediately after HSS infusion, but then stayed constant until stripping 15 minutes later. IMM pressure remained stable over the 3 time points in control glands. Mean volumes stripped from HSS treated glands were 1,284 (range 500–2,000) and 1,204 (575–1,575) ml after the first and second infusions, compared with 105 (40–150) and 68 (15–110) ml for control glands. After 5 of the 12 treatments with IMM HSS, the volume stripped was less than the 1 L of HSS infused. Osmolality and Na and Cl concentrations of secretions stripped from treated glands were significantly ( $P < 0.05$ ) higher than for control glands. More importantly, serum osmolality and Na and Cl concentrations in the subcutaneous abdominal (milk) veins draining HSS infused glands were increased 15 minutes after infusion, without a concurrent increase in serum albumin or urea nitrogen concentration, indicating movement of infused HSS from treated glands into the blood stream. In conclusion, IMM infusion of HSS did not induce the expected translocation of fluid into mastitic mammary glands or improve the clinical outcome in cows with coliform mastitis. Because some of the HSS was absorbed, the potential exists for concurrent absorption of bacteria, endotoxin, and other inflammatory mediators with IMM HSS treatment.

- 89** EQUINE MUCIN GENES: MUC 5AC BUT NOT MUC 2 IS EXPRESSED IN HORSE AIRWAYS. V. Gerber,<sup>1</sup> N.E. Robinson,<sup>1</sup> J. Rawson,<sup>1</sup> A.M. Jefcoat,<sup>1</sup> J.R. Harkema<sup>2</sup> and J.A. Hotchkiss,<sup>2</sup> <sup>1</sup>Pulmonary Laboratory, Dept. Large Animal Clinical Sciences and <sup>2</sup>Experimental Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824.

We have previously shown that the excessive airway mucus accumulations in equine recurrent airway obstruction (RAO) can only partly be explained by decreased mucus clearability. Up-regulation of specific mucin genes, however, is a primary mechanism of mucin hypersecretion—and resulting mucus accumulation—in other species. No mucin genes have been identified in the horse to date. Our goal was to identify mucin gene(s) expressed in equine airways and investigate mRNA levels in RAO-affected horses and healthy controls. Specific primers were developed for nucleotide sequences coding for two octapeptide domains that are conserved between the human and rat MUC 5 and MUC 2 genes. RT-PCR amplification of total RNA isolated from horse airways, stomach, and colon yielded cDNA (double-stranded complementary DNA) of 500–600 bp. This is in agreement with the size of cDNA fragments of humans and rats. These PCR products were cloned in E.coli and the sequences of the equine homologues of MUC 5AC and MUC 2 were determined. Based on these sequences primers specific for MUC 5AC and for MUC 2 were designed. Semi-quantitative RT-PCR on airways (generations 1, 5, 10, 15; v. small airways and parenchyma) stomach, and colon revealed that MUC 5AC is expressed in equine stomach and in all airway generations. MUC 5AC mRNA levels were also compared to the levels of ZO-1, a tight junction gene representative of the amount of epithelial cells in a tissue sample. MUC5AC/ZO-1 mRNA ratios were higher in RAO-affected (pooled samples from 5 individuals) vs. control horses (pooled samples from 7 individuals) at all airway generations. MUC 2 mRNA levels were detected in colon and very faintly in stomach, but not in airway tissue. We conclude that MUC 5AC but not MUC 2 is expressed in horse airways. MUC 5AC up-regulation may be a primary mechanism responsible for mucus hypersecretion in RAO.

- 90** PHARMACOKINETICS OF AZITHROMYCIN IN FOALS AND CONCENTRATION OF THE DRUG IN SERUM, BODY FLUIDS, AND BRONCHOALVEOLAR CELLS. S. S. Jacks, R. R. Gronwall, M. P. Brown, S. Giguère. Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL.

The pharmacokinetics of azithromycin (Zithromax®) were investigated in six, healthy, 2 to 4 month old foals. Azithromycin was administered to each foal at a dose of 10 mg/kg by both the intravenous (IV) and the intragastric (IG) route using a cross-over design. After the first IG dose, 4 additional doses were administered at 24-hour intervals. During the study, the foals were housed in individual stalls with their dam and had free access to hay and water. A microbiological assay was used to measure azithromycin concentrations in serum, peritoneal fluid, synovial fluid, pulmonary epithelial lining fluid (PELF), and bronchoalveolar cells.

At 3 minutes post IV injection, mean ( $\pm$  SD) serum concentration was  $6.42 \pm 1.57$   $\mu$ g/ml and decreased to  $0.18 \pm 0.04$   $\mu$ g/ml by 24 h. The elimination half-life was 20.25 h, the volume of distribution (area) was  $23.18 \pm 11.35$  L/kg, and the body clearance was  $11.02 \pm 3.38$  ml/min·kg. After the first IG administration, the time to peak serum concentration ( $T_{max}$ ) was  $2.09 \pm 0.73$  h, the peak serum concentration ( $C_{max\ 0-24}$ ) was  $0.57 \pm 0.16$   $\mu$ g/ml, and bioavailability was  $53.4 \pm 22.9\%$ . After repeated IG administration,  $C_{max\ 96-144}$  was  $0.63 \pm 0.10$   $\mu$ g/ml. Peritoneal and synovial fluid concentrations were similar to serum concentrations. Bronchoalveolar cell and PELF concentrations were considerably higher than serum concentrations. No adverse reactions were noted after repeated IG administration. However, rapid IV administration resulted in transient clinical signs ranging from yawning to trembling, weakness, and ataxia.

Based on the pharmacokinetic parameters, MIC of *Rhodococcus equi* isolates, and drug concentrations in PELF and bronchoalveolar cells, a single daily oral dose of 10 mg/kg would likely be appropriate for the treatment of *R. equi* infections in foals. Additional studies are required to confirm the efficacy and safety of this dosage in a clinical setting.

- 91** PHARMACOKINETICS OF AZITHROMYCIN IN FOALS. J.L. Davis, S.Y. Gardner, S.L. Jones, A.B. Schwabenton, M.G. Papich. College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina.

The properties of azithromycin, a macrolide antimicrobial drug, suggest that it may be an alternative to erythromycin for treatment of *Rhodococcus equi* pneumonia in foals. To investigate this possibility, the disposition of azithromycin in plasma, polymorphonuclear leukocytes (PMN), and macrophages was examined after a single dose in foals. Azithromycin suspension was administered orally at a dose of 10 mg/kg body weight to 5 healthy 2–3 month old foals. Two weeks later, azithromycin was administered by intravenous (IV) infusion at a dose of 5 mg/kg to the same foals. Plasma samples were collected prior to and at 15 and 30 minutes and at 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 hours after oral and IV administration. Peripheral blood PMN were collected at 4, 12, 24, 72, and 120 hours after oral administration. Bronchoalveolar lavage fluid and cells were collected 120 hours after oral administration. Azithromycin concentrations in plasma, PMN, and bronchoalveolar lavage fluid and cells were determined by reverse-phase high performance liquid chromatography with coulometric electrochemical detection (HPLC-EC). There were no adverse effects after either drug administration. Azithromycin oral absorption was variable with a mean systemic availability of 33% ( $\pm 16.5\%$ ). The plasma half-life was 15 and 10 hours after IV and oral administration, respectively. As in other species, azithromycin had a very large volume of distribution (VD) of 11.6 L/kg ( $VD_{ss}$ ) and 12.4 ( $VD_{area}$ ), respectively. The large VD can be attributed to high tissue and intracellular concentrations, which was exhibited by the high concentration of azithromycin in PMN. The maximum concentration ( $C_{max}$ ) in PMN was 27.3  $\mu$ g/ml, but only 0.72  $\mu$ g/ml in plasma after oral administration. At 12 hours after drug administration, the concentrations in PMN were greater than 200 times the plasma concentrations. In addition, azithromycin concentrations in PMN persisted for 120 hours after administration (half-life in PMN = 68 hours), even though they were detectable in plasma for only 12–24 hours in most foals after oral administration. Concentrations also were determined in bronchoalveolar cells, but drug was not detected in bronchoalveolar lavage fluid. These pharmacokinetic studies show that despite low plasma concentrations, azithromycin is moderately absorbed orally, is extensively distributed, and persists in high concentrations in leukocytes. These properties indicate that azithromycin is potentially an effective treatment for *Rhodococcus equi* pneumonia in foals.

- 92** EFFECT OF S-ADENOSYL METHIONINE ON BILE INDUCED APOPTOSIS IN MDCK-NTCP CELLS. PA Boria, CRL Webster. Tufts University School of Veterinary Medicine, N. Grafton, MA.

S-adenosyl methionine (SAME) is a natural product that has membrane stabilizing and antioxidant actions. Preliminary studies have shown that SAME can protect against bile-induced apoptosis in hepatocytes. The ability of SAME to protect other epithelial cells from chemically induced apoptosis is not known. SAME may not be transported into other epithelial cells or it may not undergo the same metabolic conversion as seen in hepatocytes. The aim of this study was to determine if SAME has a protective effect on bile acid induced apoptosis in primary cultures of rat hepatocytes and MDCK-Ntcp cells, a canine renal tubule cell line that overexpresses the bile acid transporter. Primary cultures of rat hepatocytes and MDCK-Ntcp cells were grown on cover slips covered with rat-tail collagen and maintained in Minimal Eagles Media. The cells were pretreated with SAME for 2 hrs. MDCK-Ntcp cells were also pretreated overnight with SAME. Apoptosis was induced with 50 $\mu$ M glycochenodeoxycholate (GCDC). After 2 hrs, cells were fixed and stained with the nuclear fluorochrome Hoechst 33258. Under fluorescence microscopy, 500 cells were counted and the number of cells with apoptotic morphology was recorded. The results were expressed as % apoptosis. GCDC induced apoptosis in MDCK-Ntcp cells ( $22.9\% \pm 5.6\%$ ) and in primary rat hepatocytes ( $18.8\% \pm 3.4\%$ ). Pretreatment with SAME for 2 hrs at 50 $\mu$ M or 500 $\mu$ M had no effect on GCDC induced apoptosis in MDCK-Ntcp cells ( $101.4\% \pm 17.8\%$  and  $90.0\% \pm 12.8\%$  of GCDC induced apoptosis, respectively). Pre-incubation of MDCK-Ntcp cells with 500  $\mu$ M SAME for 16 hrs had a modest, but significant, protective effect against GCDC induced apoptosis ( $75.8\% \pm 9.8\%$  of GCDC induced apoptosis) while 16 hr pre-treatment with 50  $\mu$ M was without effect. In primary rat hepatocytes, 2 hr pre-treatment with 50  $\mu$ M or 500  $\mu$ M SAME resulted in a significant reduction in GCDC induced apoptosis ( $77.7\% \pm 4.6\%$  and  $60.5\% \pm 6.5\%$  of GCDC induced apoptosis, respectively). SAME has a modest protective effect against GCDC induced apoptosis in primary rat hepatocytes and MDCK-Ntcp cells. This cytoprotective effect is greater in rat hepatocytes.

SAME supplied as 1,4 butanedisulfonate salt (Denosyl SD4) by Nutramax Laboratories, Inc. Edgewood Md.

93

COMPARISON OF DIFFERENT DOSES OF  $^{13}\text{C}$ -AMINOPYRINE FOR A  $^{13}\text{C}$ -AMINOPYRINE DEMETHYLATION BLOOD TEST IN CLINICALLY HEALTHY CATS. EM Moeller, JM Steiner, SR Gumminger, CG Ruau, JS Suchodolski and DA Williams. GI Laboratory, Texas A&M University, College Station, TX.

We have recently conducted a kinetic study of  $^{13}\text{C}$ -aminopyrine (AP) demethylation in clinically healthy cats. AP was determined to cause an increase in percent dose/min of  $^{13}\text{C}$  administered as AP (PCD) and recovered in gas extracted from blood in all 8 clinically healthy cats. A collection time 90 min after intravenous AP administration was determined to be appropriate for evaluation of AP demethylation. The objective of the current study was to determine an appropriate dose for use in a clinical test.

Four doses, 0.5, 1, 2 and 4 mg/kg, of AP were compared. Eight clinically healthy cats were enrolled in this study. A dose was randomly assigned to each study period, and each cat was given the same dose in each study period. AP was dissolved in deionized water and sterilized by filtration through a 0.1  $\mu\text{m}$  pore-size syringe filter. Cats were sedated and indwelling jugular catheters were placed one day prior to the study. Cats were fasted for at least 12 hours prior to each study. A baseline blood sample was collected and the AP was then administered intravenously to each cat. Blood samples were collected at time points of 60, 75, 90 and 120 min after AP administration during each study period. Gas was extracted from the samples by addition of HCl. The fractional  $\text{CO}_2$  concentration was measured by fractional mass spectrometry and was used to calculate PCD. The mean coefficient of variation for PCDs and cumulative PCDs (CUMPCDs) was compared using a *t*-test. Also, PCDs for the different doses and four sampling times were compared using ANOVA.

Several cats showed mild ptialism at the 2 and 4 mg/kg doses, but no other gross side effects were observed in any of the cats and at any dose. Coefficients of variation (%CV) for PCDs and CUMPCDs were not significantly different ( $p = 0.17$ ). Mean PCDs between 0.5, 1, 2 and 4 mg/kg doses were statistically different for 60, 75 and 90 min ( $p = 0.033$ ,  $0.049$ , and  $0.036$ , respectively), but not for 120 min ( $p = 0.33$ ). Mean PCDs were not significantly different between the 60, 75, 90 and 120 min time points for each dose ( $p = 0.80$ ,  $0.61$ ,  $0.93$  and  $0.10$ , respectively).

We conclude that, as was seen in dogs and in contrast to human beings, PCD is sufficient for measuring AP demethylation in a clinical test. We also conclude that the 1 mg/kg is an appropriate dose and that 90 min is a suitable time point for collection of a post-AP administration blood sample.

94

COPPER, IRON, AND ZINC CONCENTRATIONS IN LIVER TISSUE FROM DOGS WITH VARIOUS LIVER DISORDERS. SA Center, KL Warner. Cornell University, Ithaca, NY.

The transition metals copper (Cu) and iron (Fe) are thought to contribute to cellular injury in necroinflammatory and cholestatic liver disorders. Liver tissue zinc (Zn) is known to be reduced in humans with portosystemic shunting due to complex factors; this may compromise intermediary metabolism, ammonia detoxification and resistance against oxidant damage. We prospectively measured liver Cu, Fe, and Zn in 117 dogs (wedge or laparoscopic biopsies) using atomic absorption spectroscopy ( $\mu\text{g/gm}$  dry weight tissue). Median (range) of values for specific disorders, categorization into necroinflammatory (NI) and non-necroinflammatory (NNI) disorders, and significant differences are shown. Highest tissue Cu and Fe were found in NI disorders and on inspection, lowest tissue Zn was found in dogs with congenital portosystemic shunting.

Disorder or Category	n	Cu (120-400)	Fe (400-1200)	Zn (120-280)
Chronic Hepatitis/Cirrhosis	64	510 (11-4300)	1,710 (134-7,680)	148 (10-694)
Vacuolar Hepatopathy	19	317 (88-1,940)	1,280 (155-4,980)	148 (86-325)
Congenital Portosystemic Shunting	27	286 (3-2,030)	813 (12-10,300)	127 (7-219)
Microvascular Dysplasia	5	291 (109-408)	1,030 (646-2,520)	147 (122-172)
Extrahepatic bile duct occlusion	2	339 (112-566)	2,175 (1,270-3,080)	209 (161-256)
NI Disease	66	510* (11-4,300)	1,710* (134-7,680)	156 (10-694)
NNI Disease	51	291* (3-2,030)	946* (12-10,300)	135 (7-325)

\*  $P < 0.05$  between NI & NNI Categories

95

ALANINE AMINOTRANSFERASE AND ALKALINE PHOSPHATASE ACTIVITY IN LIVER TISSUE FROM PHENOBARBITAL-TREATED EPILEPTIC DOGS. C.L. Gaskill, L.M. Miller, J.S. Mattoon, W.E. Hoffmann,<sup>1</sup> and A.E. Cribb. Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada. <sup>1</sup>College of Veterinary Medicine, University of Illinois, Urbana, Illinois.

Increased serum alanine aminotransferase (ALT) and alkaline phosphatase (AP) activities are commonly found in phenobarbital-treated dogs. The purpose of this study was to determine if increased serum ALT and AP activities in phenobarbital-treated dogs are associated with hepatic enzyme induction.

Liver biopsies were obtained from 12 phenobarbital-treated epileptic dogs with increased serum ALT and/or AP activities but with no clinical signs of liver disease. Liver biopsies were also obtained from 8 healthy control dogs with normal serum biochemical profiles. Biopsies were evaluated histopathologically, and liver homogenates were assayed for ALT and AP activity. As a positive control, cytochrome P4502B (CYP2B), an enzyme known to be induced by phenobarbital, was measured by benzyloxyresorufin-O-dealkylase activity and immunoblotting. Serum AP isoenzyme analyses were also performed.

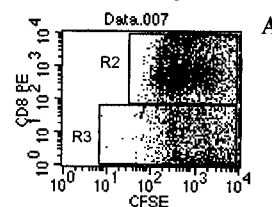
Activities of ALT and AP in liver homogenates were not increased but CYP2B was dramatically increased in the phenobarbital-treated dogs compared with controls. Histopathological examination of liver biopsies revealed more severe and frequent abnormalities in treated dogs compared with controls. Serum AP isoenzyme analyses in treated dogs demonstrated increases in both corticosteroid-induced and liver isoenzymes, but not bone isoenzyme.

We conclude that increased serum ALT activity in phenobarbital-treated dogs likely reflects hepatocellular injury and not induction. Persistently elevated serum ALT activity may help identify dogs that will ultimately develop clinical liver disease. Although no increase in liver homogenate AP activity was seen, induction might have been masked by a simultaneous increase in release of AP from hepatocyte membranes. Further investigation is required.

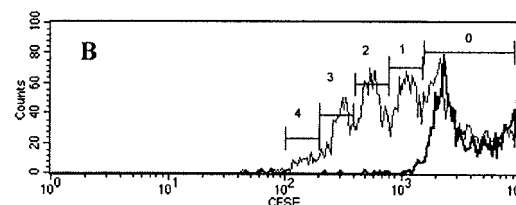
96

THE USE OF CARBOXY FLUORESCIN DIACETATE SUCCINIMIDYL ESTER (CFSE) TO DETERMINE THE PROLIFERATIVE CAPACITY OF LYMPHOCYTE SUBSETS IN THE CAT. N. Mason, L. Aronson. University of Pennsylvania, Philadelphia, PA.

CFSE is a fluorescent dye that becomes stably incorporated into cell membranes. Each subsequent cell division results in the serial halving of the fluorescent intensity of the dye which can be quantified using cytofluorometric analysis. We have utilized this technique in combination with other fluorescently labeled cell surface markers to analyze the proliferative capacity (Cp) of feline lymphocyte subsets in response to a polyclonal stimulus. Mononuclear cells were isolated from the peripheral blood of healthy cats, labeled with CFSE and stimulated with ConA for 72 hours. Cells were then labeled with anti-CD4 or anti-CD8 markers. Cytofluorometric analysis was used to determine the Cp of



lymphocyte subpopulations. The CFSE proliferation profile of CD8+ cells (selected in the R2 gate of Fig. A) is shown in histogram format in Fig. B. Cells that have undergone the same number of divisions fluoresce with the same intensity and appear within the same peak on the histogram. The number of cells undergoing 0, 1, 2 etc. divisions can be determined and the Cp of that subpopulation can be calculated. This technique has numerous advantages over  $^3\text{H}$ -thymidine incorporation: 1) proliferation of specific lymphocyte subpopulations can be determined 2) it is non radioactive 3) small numbers of cells ( $2 \times 10^5$ ) can be evaluated 4) vital dyes can be included so that reduced



proliferation associated with lymphocyte death in culture can be recognized. This tool can be applied to further our understanding of the pathophysiology of disease and effects of immunosuppressants on proliferation of subpopulations e.g. naive vs. memory cells. Selective immunosuppression of specific subsets will be useful and possible.



- 97** **INGESTION OF ZOLPIDEM IN DOGS: 33 CASES (JANUARY 1998–JULY 2000).** Richardson, J.A., Gwaltney-Brant, S.M., Albrechtsen, J.C., Porter, J.A. ASPCA National Animal Poison Control Center, Urbana, Illinois.

Zolpidem is a non-benzodiazepine hypnotic of the imidazopyridine class that is used to treat insomnia in humans. Zolpidem binds selectively to the benzodiazepine omega-1-receptor and causes an increased frequency of chloride channel opening resulting in inhibition of neuronal excitation. A retrospective study was conducted of zolpidem ingestion in dogs that were reported to the ASPCA National Animal Poison Control Center between January 1998 to July 2000. Data analysis included amount ingested, the onset and duration of signs, and the clinical effects. Thirty-three cases of zolpidem ingestion in dogs (ranging in age from 5 months to 16 years) were evaluated. Approximate ingested dosages ranged from 0.24 to 21 mg/kg. In eighty-five percent of cases, clinical signs developed within one hour and usually resolved within 12 hours. Incidence of clinical signs reported included ataxia (18 cases; 54.5%), hyperactivity (10 cases; 30.3%), vomiting (7 cases; 21.2%), depression (5 cases; 15.2%), panting, disorientation, non-specific behavioral changes, and hypersalivation (4 cases each; 12.1%). Other signs reported included tachycardia, tremors, apprehension, vocalization, hypersalivation, weakness, and hyperesthesia. Although CNS depression is reported as a primary effect of zolpidem in humans and would also be expected in dogs, information obtained from this study revealed that some dogs may exhibit a paradoxical excitation reaction. This effect appears to vary between individual dogs and does not appear to be affected by the animal's age or by dosage.

- 98** **OBJECTIVE STRUCTURED CLINICAL EXAMINATION AS A COMPONENT OF THE SMALL ANIMAL GRADUATING EXAMINATION.** H. Bark, R. Shahar, M. Gutnick and R. Cohen. Hebrew University of Jerusalem, Israel.

Objective structured clinical examinations (OSCE) have been used in the medical and dental environment for testing clinical skills in various areas. We adapted the OSCE and used it as a component of the final graduating examination in an attempt to produce an examination that would test the clinical skills and clinical knowledge in a fashion that was more objective, reliable, valid and efficient than the previously used oral examination. The second component of the assessment was a written theoretical examination based on case management questions. Thirty-nine final year veterinary students divided into 2 groups were examined on one day. While 20 students passed consecutively through 20 timed OSCE stations of 8 minutes each, the remaining students sat the theoretical examination. After a 40-minute break the groups switched and took the other examination. The 20 OSCE stations were designed to test student skills in history taking, physical examination, data interpretation, problem solving, and technical skills. At the end of each examination the student completed an evaluation questionnaire.

The average grade for the OSCE was  $80.6 \pm 5.8\%$  and for the written examination  $74.5 \pm 6.3\%$ . In the OSCE the average score for the 20 stations ranged from 68.5 to 89.7%. The reliability co-efficient for the OSCE and written examinations were  $\alpha = 0.48$  and  $0.63$  respectively, less than the desired  $0.80$ . The station total/total score (item validity) correlation ranged from  $0.09$ – $0.66$  and was significant in 14 of 20 stations. Correlation between the OSCE and written examination was  $0.33$  ( $p < 0.05$ ) suggesting that in fact the 2 examinations measured different domains of performance. 84% of students thought that the OSCE was preferable to an oral examination and 60% thought that the written examination was preferable to an oral examination. 82% of the students thought that the level of the OSCE was appropriate while 8% thought it was too difficult and 10% it was too easy. In comparison 21% thought that the level of the written exam was appropriate while 79% thought it was too difficult. 79% of the students thought that the time allocated for the OSCE was adequate, 18% thought it was insufficient and 3% thought it was too long. 97% of the students thought that the time allocated to the written exam was insufficient. The entire faculty participating in the examination expressed uniform satisfaction with the examination and confirmed its superiority to the oral examination.

We conclude that the OSCE combined with a written final examination is a suitable test of knowledge and clinical skills for final year veterinary students. The reliability and validity in our examination needs to be improved.

- 99** **INFLUENCE OF GLUCOSE DOSAGE ON INTERPRETATION OF INTRAVENOUS GLUCOSE TOLERANCE TESTS IN LEAN AND OBESE CATS.** M. Hoening, S. Alexander, J. Holson, D.C. Ferguson; College of Veterinary Medicine, University of Georgia, Athens, GA.

Intravenous glucose tolerance tests (IVGTT) are frequently used in cats and other species to assess insulin (Ins) sensitivity. A variety of glucose (G) dosages have been reported in the literature. The purpose of this study was to establish the dosage maximally stimulating Ins secretion and to compare dosage-dependence in lean and obese animals.

Four lean (Body Fat + SD:  $23.1 \pm 4.2\%$ ) and 4 obese ( $40.7 \pm 4.2\%$ ) spayed female cats were fed a commercial dry diet and weight was strictly maintained. At 3 week intervals, IVGTT were performed with all cats receiving each of the following G dosages in random order: 0.3 (A), 0.5 (B), 0.8 (C), 1.0 (D), and 1.3 (E) g/kg BW. Serum G and Ins were measured at various intervals for 120 min after injection of G.

The glucose disposal rate (K) was significantly lower in obese cats only at the highest G dosage ( $p < 0.04$ ). The area under the curve for insulin (AUCI) increased significantly between dosages A, B, C, and D in lean cats and between A, B, and C in obese cats, plateauing at higher dosages. The most striking difference between lean and obese cats was in the Ins secretion pattern. There were no differences in first phase secretion AUCI between groups or dosages; however, second phase AUCI was significantly higher in obese cats than in lean cats with all but dosage A. Lean cats reached baseline Ins concentrations at all dosages at 120 min; however, obese cats failed to do so at all but the lowest dosage.

We conclude that the G dosage for maximal Ins secretion is 1.0 g/BW in lean and 0.8 g/kg BW in obese cats supporting routine use of 1 g/kg BW to maximally stimulate insulin secretion irrespective of body composition. While lean cats responded to all dosages with a return to baseline at 120 min, all but the lowest dosage of G caused an abnormal Ins secretion pattern in obese cats indicating a secretory defect in insulin secretion with obesity.

- 100** **COMPARISON OF BLOOD GLUCOSE VALUES OBTAINED USING A MARGINAL EAR VEIN LANCE TECHNIQUE VERSUS PERIPHERAL VEIN COLLECTION IN NORMAL CATS AND IN CATS WITH DIABETES MELLITUS.** M. Thompson,<sup>1</sup> S. Taylor,<sup>2</sup> V. Adams,<sup>3</sup> S. Myers,<sup>4</sup> E. Feldman.<sup>5</sup> <sup>1-4</sup>Western College of Veterinary Medicine, Saskatoon, SK. <sup>5</sup>School of Veterinary Medicine, Davis, CA.

Generation of the blood glucose (BG) curve is an integral part of managing cats with diabetes mellitus. Portable blood glucose meters (PBGm) are commonly used as an inexpensive and accurate method of measuring BG in blood collected from peripheral veins or the marginal ear vein (MEV [capillary blood]). The purpose of this study was (1) to compare BG concentrations in blood obtained simultaneously from the MEV and an indwelling peripheral venous catheter (PVC) in healthy and diabetic cats; (2) evaluate BG concentrations (from PVC) 5 minutes after blood collection from the MEV; and, (3) to compare the BG concentrations in blood obtained from the MEV to blood obtained from the medial saphenous vein by direct venipuncture.

10 healthy adult cats and 11 cats with diabetes mellitus were studied. Indwelling PVC's were placed under sedation. The next day, hourly BG samples were obtained from the MEV (Microlet<sup>®</sup> automatic lancing device) and from the PVC. A 5-minute PVC sample was also obtained in diabetic cats. On day 2, hourly blood samples were obtained using the MEV and direct saphenous venipuncture in healthy and diabetic cats. The Bayer Glucometer Elite-XL<sup>®</sup> was used for all measurements.

There was no significant difference in BG values obtained from the MEV versus the PVC in healthy or diabetic cats. Blood taken by the PVC 5 minutes after the ear-nick in diabetic cats did not document "stress hyperglycemia." No clinically important difference in BG was found between blood collected from the MEV and direct venipuncture.

Measurement of BG values using the MEV is comparable to PVC samples and to direct venipuncture making this method a reliable alternative for blood collection for BG curves in diabetic cats.

# 101 COMPARISON OF SERIAL BLOOD GLUCOSE CURVES PERFORMED ON CONSECUTIVE DAYS IN DIABETIC DOGS. Linda M Fleeman, Jacqui S Rand. Companion Animal Sciences, The University of Queensland, Australia.

The purpose of this study was to evaluate the day-to-day variability of serial blood glucose curves in dogs with diabetes mellitus.

Paired 12-h serial blood glucose curves, performed over 2 consecutive days, were obtained on 3 occasions from 10 dogs with spontaneous diabetes. The dogs were admitted into the hospital on the morning of the first day. Dogs received exactly the same dose of porcine lente insulin and the same meal every 12-h on both days. Parameters recorded were morning pre-insulin blood glucose (AM-BG), evening pre-insulin blood glucose (PM-BG), maximum blood glucose (MAX-BG), minimum blood glucose (MIN-BG), time from administration of insulin injection to nadir (t-MIN-BG), difference between AM-BG and MIN-BG (AM-MIN), area under the blood glucose curve (AUC), mean blood glucose (MBG), standard deviation blood glucose (SDBG), and the J-index (J), which arithmetically combines the MBG and the SDBG into a single value. Based on the results of each curve, a theoretical recommendation was recorded for insulin dose adjustment. The three possible recommendations were that the insulin dose should either be increased, decreased, or should remain unchanged.

There was a large degree of variability between the blood glucose curves on days 1 and 2. The mean of the absolute difference between days 1 and 2 for each parameter was significantly greater than zero ( $p < 0.001$ ). The coefficient of variation of the absolute difference between days 1 and 2 for each parameter ranged from 68% for MIN-BG to 103% for AUC.

Evaluation of the 30 sets of paired 12-h curves led to an opposite recommendation for adjustment of the dog's insulin dose on day 2, compared to day 1, on 27% of occasions. For 17% of the curves, a different, but not opposite, recommendation resulted. The same recommendation for dosage adjustment on both days was made in only 57% of the paired curves. The disparity between the dosage recommendations resulting from the paired 12-h curves was more pronounced when the MIN-BG was less than 180 mg/dL (10 mmol/L) on one or both days. In this subset of 20 paired 12-h curves, an opposite recommendation for dosage adjustment was made on 40% of occasions, a different but not opposite recommendation resulted 25% of the time, and the same recommendation for both days occurred in only 35% of the sets of paired curves.

We conclude that: 1) There is large day-to-day variation in the results of 12-h serial blood glucose curves in diabetic dogs. 2) The day-to-day variability of serial blood glucose curves has important clinical implications, particularly in diabetic dogs with good glycemic control.

# 102 ALTERATION IN THE GROWTH HORMONE-INSULIN-LIKE GROWTH FACTOR AXIS IN CATS WITH DIABETES MELLITUS. Claudia E. Reusch,<sup>1</sup> Martina Casella,<sup>1</sup> Juergen Zapf,<sup>2</sup> Jan Mol,<sup>3</sup> <sup>1</sup>Clinic for Small Animal Internal Medicine, University of Zurich, Switzerland; <sup>2</sup>Division of Endocrinology and Diabetology, Department of Medicine, University of Zurich, Switzerland; <sup>3</sup>Department of Clinical Sciences of Companion Animals, Utrecht University, The Netherlands.

The growth hormone (GH)-insulin-like growth factor (IGF I) axis is an integral part of the endocrine system responsible for promoting linear growth. Insulin is a major anabolic effector in the body, and is also an important regulator of the GH-IGF axis. Studies in diabetic humans revealed that insulin deficiency leads to a decrease in IGF I concentrations, although GH levels tend to be high. After starting insulin therapy IGF I concentrations normalize within 1 to 4 weeks. In diabetic cats IGF I and GH measurements are used for the diagnosis of hypersomatotropisms (HS). The objectives of our study were twofold: first, to investigate if there is a difference in IGF I and GH levels in diabetic cats without HS before and after initiating insulin therapy. Second, to investigate if IGF I and GH measurements prior to insulin administrations are reliable to diagnose HS in diabetic cats.

IGF I levels (reference range 223–558 ng/ml) were determined by radioimmunoassay after gel filtration of the serum samples, GH levels were measured using a specific double-antibody radioimmunoassay (reference range 1.5–7.9 ng/ml). In the cats with HS a pituitary tumor was confirmed by computer tomography.

Prior to insulin therapy IGF I levels in cats without HS ranged between 13 and 433 ng/ml (median 163), which was significantly lower than in controls. In 11 of the 15 cats IGF I was below the reference range. GH ranged between 1.6 and 9.0 ng/ml (median 4.3) and was above the reference range in 3 cases. There was an inverse correlation between IGF I and GH. After initiating insulin therapy IGF I levels increased significantly and were not different from control levels after a median of 4 weeks. GH was elevated in all 4 cats with HS, however IGF I was only elevated in those 2 cats which had received insulin therapy for several months (2711 ng/dl, 686 ng/dl). In the other 2 cats IGF I was normal resp. low (293 ng/dl, 42 ng/dl). In both cases IGF I increased above the reference range after initiating insulin therapy (705 ng/dl, 886 ng/dl).

We conclude that in untreated diabetic cats the GH-IGF I axis may be altered due to insulin deficiency. The parameters may be unreliable to diagnose hypersomatotropisms prior to insulin therapy.

# 103 COMPARISON OF A LOW CARBOHYDRATE VERSUS HIGH FIBER DIET IN CATS WITH DIABETES MELLITUS. N Bennett, DS Greco, ME Peterson, Colorado State University, Fort Collins, CO and The Animal Medical Center, New York, NY.

The purpose of this study was to determine whether a low carbohydrate (LC) or a high fiber (HF) diet was more effective in the management of diabetes mellitus in cats. Thirty client-owned cats with naturally-occurring diabetes mellitus were randomly assigned to receive a LC diet (canned Hill's feline growth,  $n = 17$ ) or a HF diet (canned Hill's w/d,  $n = 13$ ) for 4 months. None were ketotic and all were previously treated with insulin (NPH, median dose 5 U). A complete physical examination, history, CBC, chemistry profile, serum fructosamine, TT4 and urinalysis were evaluated on a monthly basis. Mean body weight prior to entrance in the study was 5.85 kg. Mean  $\pm$  SE serum fructosamine ( $487 \pm 45$   $\mu$ mol/L) and fasting blood glucose concentrations ( $343 \pm 39$  mg/dl) were increased prior to treatment in most cats. Four cats were subsequently switched from HF to LC at the owner's request or because of continued clinical signs. Eight cats (4 LC, 4 HF) were eliminated from the study because of concurrent disorders (acromegaly-LC, heart failure-LC, leukemia-HF, pancreatitis-LC) or dietary non-compliance (1LC, 3 HF). Cats were treated with protamine zinc insulin at an initial dosage of 1–3U/cat BID.

All cats in the LC group and 3 cats in the HF group showed improvement in clinical signs (PU/PD, plantigrade stance). Responders tended to lose body weight during the study and non-responders or cats remaining on insulin tended to maintain or increase body weight. Response, defined as a normal serum fructosamine  $< 400$   $\mu$ mol/L and fasting blood glucose  $< 170$  mg/dl respectively, occurred in 12/13 cats in the LC group and in 3/9 cats in the HF group. By the end of the study, 4 cats in the LC group were able to discontinue insulin entirely, 6 cats experienced a reduction in insulin dosage to 1 unit BID and three cats remain on 1.5–3 units BID. None of the cats in the HF group were able to discontinue insulin entirely and median insulin dose for the HF group was 3 units BID.

# 104 ULTRASONOGRAPHIC EVALUATION OF THE THYROID GLAND IN GOLDEN RETRIEVERS. C. Brömel, R. Nelson, V. Samii,\* R. Pollard, A. Davidson, P. Kass. University of California, Davis, CA, \*The Ohio State University, Columbus, OH.

This study assessed ultrasonographic parameters of the thyroid gland in healthy (24 dogs, group 1), hypothyroid (7, group 2) and euthyroid sick (18, group 3) Golden Retriever dogs.

Dogs were categorized as healthy, hypothyroid and euthyroid sick based on clinical signs, physical examination findings, and results of CBC, serum biochemistry panel, thyroid panel (total  $T_4$ , free  $T_4$ , cTSH, thyroglobulin autoantibodies), and response to sodium levothyroxine treatment in group 2 dogs. Ultrasonographic examination of the thyroid gland was performed with dogs positioned in dorsal recumbency, without sedation, and using a multifrequency (5–10 MHz) linear transducer. The following criteria were recorded from both thyroid lobes: shape, size (maximal length, width and height in longitudinal and transverse sections), echogenicity and homogeneity. Measurements were obtained from 3 different longitudinal and transverse images of each thyroid lobe and mean values for length, width and height were determined.

Both thyroid lobes were visualized on ultrasound in all dogs. Thyroid lobes appeared spindle, cigar or elliptical in shape on longitudinal section and triangular, oval or round on transverse section. There was no significant difference in frequency of occurrence of thyroid lobe shape among groups. Left lobe (LL) height in longitudinal section was significantly ( $P < 0.05$ ) smaller in group 2, compared with group 1 and 3 dogs, and LL length and right lobe (RL) height in longitudinal section were significantly ( $P < 0.05$ ) smaller in group 2, compared with group 3 dogs. A significant difference in RL length was not identified among groups. LL height, LL width, RL height and RL width in transverse section were significantly ( $P < 0.01$ ) smaller in group 2, compared with group 1 and 3 dogs. In all groups, thyroid lobes had a homogeneous parenchymal pattern. Thyroid lobes were subjectively less echogenic in group 2, compared with group 1 and 3 dogs.

Results of this study suggest that thyroid lobes are smaller in hypothyroid versus euthyroid Golden Retrievers, based on ultrasonographic measurements of maximum length, width and height.

# 105 A 12-MONTH PROSPECTIVE STUDY OF 234 THYRO-GLOBULIN ANTIBODY POSITIVE DOGS WHICH HAD NO LABORATORY EVIDENCE OF THYROID DYSFUNCTION. PA Graham, RB Lundquist, KR Refsal, RF Nachreiner, AL Provencher. AHDL Endocrinology, Michigan State University, Lansing, MI.

Thyroglobulin antibody (TgAA) has been reported to be a useful serum marker for canine immune mediated thyroiditis. Since the recent availability of a commercial canine TgAA assay, the significance of a positive antibody test result in a dog with no evidence of thyroid dysfunction (normal serum thyroxine (T4) and thyrotropin (TSH)) has been questioned. By following 234 dogs with this combination of test results at 3-month intervals for 12 months we hoped to provide some objective outcome information. The study group included 45 intact females, 82 spayed females, 21 intact males and 86 castrated males. The largest age-group was 2 year-olds. Approximately, 35% of cases were described as healthy and were identified during a screening test. The remainder was initially sampled because of some clinical evidence of disease. One-hundred-and-seventy-one dogs (73%) completed the 12-month follow-up period or had laboratory evidence of thyroid dysfunction prior to that time (defined as TSH > 0.68ng/mL and/or FreeT4 by equilibrium dialysis (FT4d) <5 pmol/L). The remaining dogs were lost to follow up (44), withdrawn without laboratory evidence of thyroid dysfunction (15) or died (4). Thirty-three dogs (19.3%) had laboratory evidence of thyroid dysfunction indicated by either both high TSH and low FT4d (7; 4.1%), high TSH (23; 13.4%), or low FT4d (3; 1.8%). Of those 138 which did not develop evidence of thyroid dysfunction, 98 (57.3%) remained TgAA positive, 14 (8.1%) gave an inconclusive TgAA result and 26 (15%) became TgAA negative. When the data were re-coded to include only those 149 dogs with more than one positive TgAA result the outcome at 12 months was: 22% had laboratory evidence of thyroid dysfunction and of the remainder 65.8% were still TgAA positive, 6% had inconclusive TgAA results and 6% became TgAA negative. Dogs under 5 years and those with thyroid hormone cross-reacting antibodies (T3AA, T4AA) had a slightly increased risk of developing thyroid dysfunction (Odds ratio 95pci 1.08–12.71  $p = 0.019$ ; 1.01–5.38  $p = 0.029$  respectively).

In conclusion, approximately 20% of dogs with laboratory test evidence to suggest subclinical thyroiditis, developed thyroid dysfunction within 1-year. A proportion of TgAA positive dogs became negative over the course of 1-year without any apparent effect on their thyroid function. This proportion was less in dogs that had more than one positive TgAA test result.

# 106 INCREASED PARATHYROID HORMONE CONCENTRATIONS IN DOGS WITH HYPERADRENOCORTICISM. I.K.Ramsey and M.E. Herrtage, Department of Veterinary Clinical Studies, University of Glasgow, Bearsden, Glasgow and Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, UK.

Canine hyperadrenocorticism (HAC) is associated with several clinical problems, such as calcinosis cutis and urolithiasis, that arise as a result of abnormal calcium metabolism. The explanation for these findings has not been fully elucidated although glucocorticoids are known to increase urinary calcium excretion in canine HAC. This study was designed to investigate PTH concentrations in cases of canine HAC.

Parathyroid hormone (PTH) was assayed in 31 dogs in which hyperadrenocorticism had been confirmed using an ACTH stimulation test or a low dose dexamethasone suppression test. PTH concentrations were determined by radioimmunoassay using EDTA plasma that had been stored at  $-20^{\circ}\text{C}$  for not more than 1 month. Cases were classified as adrenal dependent (ADH) or pituitary dependent hyperadrenocorticism (PDH) using a combination of dexamethasone suppression testing, adrenal ultrasonography and endogenous ACTH assays. PTH concentrations were re-assessed at various times following effective treatment with trilostane (1 dog) or mitotane (5 dogs). Dogs with renal failure were withdrawn from this study.

PTH concentrations were found to be increased above the reference range (10–60pg/ml) in 26 dogs (range = 34–>700 pg/ml, mean = 185 pg/ml, s.d. = 153 pg/ml). There was no significant difference between PTH concentrations in ADH cases ( $n = 4$ ) and those with PDH ( $n = 27$ ) when compared using a Mann Whitney U test. Although five cases (one treated with trilostane and four with mitotane) showed a decrease in PTH concentration after 1, 4, 5, 6 and 10 months, in two of these cases post treatment PTH concentrations did not return to the reference range. One case showed a mild increase in PTH concentration after 2 months of mitotane treatment.

It is concluded that PTH concentrations are increased in most dogs with hyperadrenocorticism and take several months to decrease to normal in treated dogs.

# 107 PLASMA 17-HYDROXYPROGESTERONE CONCENTRATIONS IN THE DIAGNOSIS OF CANINE HYPERADRENOCORTICISM. J. M. E. Ristic,<sup>1</sup> H Evans<sup>2</sup> and M. E. Herrtage.<sup>1</sup> <sup>1</sup> Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, UK. <sup>2</sup> CSL Services, Sawston, Cambridge CB2 4TJ, UK.

A number of dogs present with clinical signs and laboratory changes characteristic of hyperadrenocorticism (HAC), but have normal screening test results in response to ACTH stimulation and/or low dose dexamethasone suppression (LDDST). It is possible that these atypical cases of HAC may result from excess production of a hormone other than cortisol. 17-hydroxyprogesterone (OHP) is one of the precursors of cortisol in the glucocorticoid production pathway. The aim of this study was to evaluate plasma OHP concentrations in response to exogenous ACTH in typical and atypical canine HAC.

Twenty three dogs with clinical and laboratory findings suggestive of HAC were included in this study. An ACTH stimulation test was performed on all dogs using intravenous tetracosactide (Synacthen). A LDDST was performed in 12 cases using a standard protocol. A diagnosis of HAC was confirmed by the resolution of clinical signs with treatment using mitotane or trilostane, or by histopathology of the adrenal glands.

Eleven dogs had a positive response with plasma cortisol increasing to above 600 nmol/l (mean 839 nmol/l, range 600–1303 nmol/l) 30 min after ACTH administration. Nine of these dogs had pituitary-dependent HAC and two had adrenal-dependent disease. Plasma OHP concentrations increased in response to exogenous ACTH to abnormal concentrations in all cases (range 6.5–38 nmol/l, mean 15.5 nmol/l, s.d. 8.6 nmol/l). In normal dogs, plasma OHP concentrations do not stimulate above 4.0 nmol/l in response to ACTH administration.

Twelve dogs were classified as atypical HAC in that they had a normal cortisol response to ACTH (mean 361 nmol/l, range 70–590 nmol/l). A LDDST was performed in ten of these cases, four of which showed normal suppression at 8 hours (plasma cortisol <40 nmol/l). Ten of these dogs had pituitary-dependent and two had adrenal-dependent HAC. Plasma OHP concentrations increased in response to exogenous ACTH to abnormal concentrations in all twelve dogs (range 5.4–13.3 nmol/l, mean 9.1 nmol/l, s.d. 3.3 nmol/l).

This study demonstrates that plasma OHP increases to abnormal concentrations in response to exogenous ACTH in all cases of HAC and that abnormal plasma OHP concentrations can be used to identify dogs with atypical HAC that are not detected using conventional screening tests. Following treatment with mitotane, post ACTH plasma OHP concentrations return to normal, however the plasma OHP concentrations increase with trilostane therapy.

# 108 THE INHIBITORY G PROTEIN, $G_{i2}$ , SHOWS DECREASED EXPRESSION IN ADENOMATOUS THYROID TISSUE FROM HYPERTHYROID CATS. C.R. Ward, S.E. Achenbach, M.E. Peterson.<sup>1</sup> University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, <sup>2</sup>Caspary Research Institute of The Animal Medical Center, New York, NY.

Feline hyperthyroidism is a common endocrinopathy in cats that resembles toxic nodular goiter (TNG) in humans. Abnormalities of the TSH receptor—G protein-mediated signal transduction cascade have been shown to be involved in the pathogenesis of human TNG. Previous work from our laboratory has implicated similar pathogenic mechanisms involved in feline hyperthyroidism (Hammer et al., 2000 *AJVR* 61:874–879). We demonstrated that adenomatous thyroid tissue from hyperthyroid cats showed significantly decreased expression of inhibitory G proteins ( $G_i$ ). Three subtypes of  $G_i$  proteins ( $G_{i1}$ ,  $G_{i2}$ ,  $G_{i3}$ ) have been identified that have unique signaling activities in different cell types. The purpose of this study was to identify which  $G_i$  protein subtype(s) showed decreased expression in adenomatous thyroid tissue from hyperthyroid cats.

Adenomatous thyroids were surgically excised from client-owned hyperthyroid cats. The diagnosis of hyperthyroidism was based upon the presence of appropriate clinical signs, elevated serum thyroxine concentrations, and histology of excised tissue. Normal thyroids were removed from healthy, aged-matched cats euthanized in other experimental studies. Serum thyroxine and thyroid histology were normal in the control cats. Enriched membrane preparations were made from thyroid tissue snap frozen in liquid nitrogen. The expression of  $G_{i1}$ ,  $G_{i2}$ , and  $G_{i3}$  were determined at the protein level in normal and hyperthyroid tissue by western blot analysis using specific anti-peptide antibodies. Relative protein amounts were quantified using densitometry and each was compared to its own control on the same blot. As reported previously adenomatous thyroid tissue showed a significant decrease of 53% ( $\pm 14$ ;  $p < 0.001$ ;  $n = 3$ ) in  $G_{i1}$  expression as compared to normal thyroid tissue. Correspondingly,  $G_{i2}$  showed significant decrease of 39% ( $\pm 10$ ;  $p < 0.01$ ;  $n = 3$ ) in adenomatous thyroid tissue as compared to control. In contrast,  $G_{i1}$  and  $G_{i3}$  showed no significant difference in expression from controls ( $-1.8 \pm 1.7\%$  and  $-2.4 \pm 1.3\%$ , respectively;  $p > 0.05$ ;  $n = 3$ ). We conclude that  $G_{i2}$  is the  $G_i$  that shows altered expression in adenomatous tissue and may be involved in the pathogenesis of feline hyperthyroid disease.

# 109 <sup>99m</sup>Tc-PERTECHNETATE SCINTIGRAPHY IN HYPERTHYROID CATS WITH NORMAL SERUM THYROXINE CONCENTRATIONS. K. Tomsa,<sup>1</sup> R. Hardegger,<sup>2</sup> T. Glaus,<sup>1</sup> C. Reusch.<sup>1</sup> <sup>1</sup>Clinic for Small Animal Internal Medicine, and <sup>2</sup>Polyclinic for Nuclear Medicine, University of Zurich, Switzerland.

We previously reported, that hyperthyroid cats suffering from severe non-thyroidal illness (sick hyperthyroid cats) were indistinguishable from sick euthyroid cats based on serum T4 and on thyrotropin-releasing hormone (TRH) stimulation test. Limitation of the TRH stimulation test was mainly poor specificity.

The purpose of this prospective study was to evaluate the usefulness of thyroid pertechnetate scintigraphy for assessment of thyroid function in a population of cats with clinically suspected hyperthyroidism, and normal serum T4 concentration and to evaluate its potential superiority over TRH stimulation test.

Inclusion criteria were: clinical suspicion of hyperthyroidism, serum T4 concentration < 3.5 µg/dl, and available thyroid histology. Complete blood count, serum chemistry, urinalysis and TRH stimulation test were performed in each cat. Qualitative pertechnetate thyroid scan (70 MBq <sup>99m</sup>TcO<sub>4</sub>, imaging after 20 min, 150 000 counts) was acquired using high resolution parallel hole collimator. Scintigraphy was additionally performed in 3 clearly hyperthyroid cats (serum T4 > 3.5 µg/dl) as positive controls.

Up until now a total of 14 cats fulfilled the inclusion criteria (study cats). Serum T4 concentrations ranged from 0.6 to 3.0 µg/dl (median 1.7). Percentage of stimulation after TRH application ranged from -7 to 83% (median 29.5). Eight cats showed stimulation < 50%, 3 cats between 50–60%, and 3 cats > 60%. Thyroid scan was positive in all cats. Thyroid pathology, consistent with hyperthyroidism (nodular hyperplasia or adenoma), was evident on histology in 3 control cats and in 11 of 14 study cats. No thyroid pathology was found in 3 study cats. These were the cats with a stimulation of > 60% after TRH application.

Scintigraphy was a sensitive test for diagnosing hyperthyroidism in sick hyperthyroid cats. However, the positive results in 3 cats with normal thyroid histology raise suspicion about its specificity. Possible explanations for positive scintigraphy in these 3 cats are true false-positives, false-positives associated with iodine depletion, or true-positives but false-negative histopathology. Further studies are needed with larger number of cats, especially euthyroid cats with a lack of stimulation after TRH application.

# 110 EVALUATION OF PROTEINURIA IN HYPERTHYROID CATS. HM Syme, J Elliott. Royal Veterinary College, London, UK.

Many cats are diagnosed with renal failure (RF) following treatment for hyperthyroidism. It is uncertain whether this is due to the deleterious effects of hyperthyroidism *per se* or is a reflection of the high incidence of RF in the geriatric feline population. This study was designed to determine the incidence of proteinuria in cats before and after treatment for hyperthyroidism and to correlate these findings with the development of azotemia. In addition, urinary albumin was measured as a putative marker of structural and hemodynamic glomerular injury.

Urine samples were obtained from cats prior to, and following, treatment for hyperthyroidism. Treatment was with carbimazole therapy alone, or combined with thyroidectomy. Urine protein and creatinine concentrations were measured by standard clinical laboratory methods and urinary albumin was measured by an ELISA validated for use in the cat. Cats were excluded from the study if they were in RF prior to treatment, if a urinary tract infection was diagnosed, or if euthyroidism and plasma creatinine concentrations were not adequately documented during a 6 month follow up period. The plasma creatinine concentration at 6 months was used to define 2 groups, cats in RF (creatinine >1.9 mg/dl) and cats not in RF (NRF). Pre-treatment urine protein:creatinine (UPC) and urine albumin:creatinine (UAC) in these two groups were compared by the Mann-Whitney U test. The Wilcoxon signed rank test was used to compare UPC and UAC pre and post treatment. Data are reported as median values [25<sup>th</sup>, 75<sup>th</sup> percentiles].

Of the 25 cats (12 RF, 13 NRF) included in the study, 13 (52%) had UPC >0.5, and 5 (20%) had UPC >1.0 prior to treatment. The UPC before treatment did not differ between the RF and NRF groups (0.50 [0.27, 1.42] vs. 0.51 [0.26, 0.82]). UPC decreased significantly (P = 0.001, n = 19) from 0.49 [0.26, 0.95] to 0.23 [0.10, 0.33] following treatment. The UAC was not different between the RF and NRF groups before treatment (48 [11, 106] x10<sup>-3</sup> vs. 45 [19, 67] x10<sup>-3</sup>) and decreased significantly (P = 0.03, n = 19) from 42 [13, 64] x10<sup>-3</sup> to 20 [9, 40] x10<sup>-3</sup> following treatment.

In summary, many hyperthyroid cats have significant urinary loss of proteins including albumin. The magnitude of proteinuria can not be used to predict which cats will develop RF when euthyroid. Proteinuria resolves following treatment of hyperthyroidism in most cats including many that develop azotemia.

# 111 EVALUATION OF DOSING REGIMEN IN HYPERTHYROID CATS TREATED WITH METHIMAZOLE. SB Hoffman,\* LA Trepanier,\* M Kroll, I Rodan, L Challoner. \*University of Wisconsin-Madison School of Veterinary Medicine, Madison WI; The CatCare Clinic, Madison WI.

Methimazole (Tapazole®) is the oral antithyroid drug used most commonly in the U.S. to treat cats with hyperthyroidism. Methimazole is usually given twice daily, and has a relatively short plasma half-life in cats. However, because methimazole is actively concentrated in the thyroid gland, its plasma half-life may not reflect its duration of antithyroid activity. Studies in human patients with hyperthyroidism have shown similar remission rates between once daily and more frequent divided daily methimazole dosing regimens. The objective of this study was to determine whether once daily dosing of methimazole is as effective as divided twice daily dosing for the management of hyperthyroidism in cats.

Cats with newly diagnosed, naturally occurring hyperthyroidism were eligible for the study. Cats were initially evaluated with a physical exam, body weight, CBC, biochemical panel, urinalysis, total serum T4, indirect Doppler blood pressure, and owner questionnaire regarding the presence of PU/PD, polyphagia, inappetence, hyperactivity, lethargy, vomiting, diarrhea, or facial pruritus. Cats were then randomized to receive either a single dose of 5 mg methimazole once daily (SID group), or a divided dose of 2.5 mg methimazole twice daily (BID group). Cats were re-evaluated, using the same criteria as for the initial evaluation, 2 and 4 weeks after drug initiation. Data at each evaluation was compared between groups by a repeated measures ANOVA followed by Fisher's LSD test.

To date, 12 cats have completed the study, 6 in the SID group and 6 in the BID group. There was no significant difference between groups at week 0 in clinical status, as measured by serum T4, body weight, heart rate, blood pressure, serum biochemistry, or urine specific gravity. There was also no significant difference between groups in assigned total daily dosage (in mg/kg) of methimazole. However, in the 12 cats evaluated to date, serum T4 was significantly lower after 4 weeks of treatment in the cats treated with 2.5 mg BID (total T4, 1.58 ± 0.37 µg/dl), compared to the cats treated with 5 mg SID (total T4, 4.12 ± 3.87, P = 0.022). Blood pressure, body weight, heart rate, ALT, SAP, bilirubin, BUN, creatinine, and urine specific gravity were not significantly different overall between treatment groups at either 2 or 4 weeks, although one cat in the SID group did develop a hepatopathy (hyperbilirubinemia, 6-fold increase in ALT, 9-fold increase in SAP) at week 4. The preliminary results of this ongoing study suggest that twice daily divided dosing of methimazole may be more effective than the same total daily dose given once daily, in treating cats with newly diagnosed hyperthyroidism.

Normalized Urine Albumin Concentration (# of samples)	Urine Protein Test Strip Result (n = 159)			
	Neg. (112)	Trace (20)	1+ (15)	2-4+ (12)
< 1.0 mg/dL (80)	61 (54%)	12 (60%)	5 (33%)	2 (17%)
> 1.0 and < 30.0 mg/dL (58)	49 (44%)	6 (30%)	2 (13%)	1 (8%)
> 30.0 mg/dL (21)	2 (2%)	2 (10%)	8 (53%)	9 (75%)

# 112 TOPICAL METHIMAZOLE TREATMENT OF CATS WITH HYPERTHYROIDISM. Gaby Hoffmann, Steven L. Marks, Joseph Taboada, Giselle Hosgood-Pagel, Karen J. Wolfshiemer.\* School of Veterinary Medicine, Louisiana State University, and \*Endocrine Diagnostics & Consultation, Baton Rouge, LA.

Transdermal administration of methimazole (Tapazol®) has been advocated as an alternative mode of therapy for feline hyperthyroidism due to ease of administration. Efficacy and side effects of this treatment modality have not been studied. This study evaluated clinical signs and serum thyroxine concentrations before and after topical methimazole in cats with hyperthyroidism.

Thirteen cats that presented with clinical signs and physical examination findings of hyperthyroidism were evaluated. Clinical signs included weight loss (8/13), inappetence (5/13), mental changes (4/13) vomiting (4/13), diarrhea (1/13), and polyphagia (1/13). Additional physical examination findings included a nodule in the region of the thyroid gland (6/13), dehydration (4/13), dry haircoat (2/13), systolic heartmurmur (2/13), tachycardia (2/13), and constipation (1/13). Hyperthyroidism was diagnosed based on increased total thyroxine (TT4: 12/13) or a combination of high-normal TT4, increased free T4 (1/13) concentrations and consistent clinical signs (1/13).

Tapazole was formulated in a pleuronic lecithin organogel (PLO) based vehicle at a concentration of 5 mg/0.1ml gel. This gel was applied to the pinna of the ear at a dosage ranging between 2.5 mg/cat QD to 10 mg/cat BID. After initiation of treatment cats were reevaluated at a mean and median of 4.3 and 4 weeks respectively (recheck-1: 10/13) and after a mean and median of 5.4 and 6 months respectively (recheck-2: 8/13).

According to the owners all cats showed clinical improvement. Resolution of clinical signs was seen as follows: weight loss (5/8), inappetence (4/5), mental changes (3/4) vomiting (3/4), dehydration (3/4), dry haircoat (2/2). Weight gain was documented in 4 of 8 cats with pre-treatment weight loss. None of the cats showed clinical side effects and in 5 patients the dosage of methimazole was reduced after recheck-1. In 9 of 10 cats evaluated at recheck-1 TT4 had decreased, with 7 cats having TT4's in the normal reference range. Three cats showed no or only mild improvement in TT4 concentrations. All three were treated with low dosages of topical methimazole (2.5mg QD, 5mg QD, and 3.75mg BID respectively). At recheck-2 normal TT4 was attained in 7 of 8 cats evaluated.

There was a significant (p < 0.05) decrease in TT4 from pre-treatment concentrations (mean: 97.5 nmol/L, SD: 41.2) at recheck-1 (mean: 39.57 nmol/L, SD: 45.56) and recheck-2 (mean: 36.71 nmol/L, SD: 39.5) but no difference between concentrations at recheck-1 and -2.

# 113 PREVALENCE OF MICROALBUMINURIA IN DOGS. Wayne A. Jensen,<sup>1</sup> Gregory F. Grauer,<sup>2</sup> Janet Andrews,<sup>1</sup> and Dan Simpson.<sup>1</sup> From Heska Corporation,<sup>1</sup> Fort Collins CO, and the College of Veterinary Medicine and Biomedical Sciences, Colorado State University,<sup>2</sup> Fort Collins, CO.

Microalbuminuria has been reported to be an early indicator of progressive renal disease in humans with hypertension and diabetic nephropathy. The purpose of this study was to ascertain the prevalence of microalbuminuria in dogs. Microalbuminuria was defined as urinary excretion of albumin greater than 1.0 mg/dL but less than 30.0 mg/dL.

Two separate populations were studied. One sample population was derived from clinically normal dogs (n = 86) owned by Heska employees. The second sample population was derived from Colorado State University Veterinary Teaching Hospital patients (n = 159) presented for routine health screening, elective procedures, as well as evaluation of health problems. Samples were not excluded on the basis of urine sediment findings. Microalbuminuria was quantitated using an antigen capture ELISA. To account for varying urine concentrations, results were normalized to a specific gravity of 1.010.

Of the 86 clinically normal dogs, 68 (79%) had normalized albumin concentrations <1.0 mg/dL, 16 (19%) had normalized albumin concentrations >1.0 mg/dL and < 30.0 mg/dL, and 2 (2%) had normalized albumin concentrations >30.0 mg/dL. Of the 159 hospital patients, 112 (70%) were urine protein test strip negative and 51 of the 112 (46%) test strip negative samples had normalized albumin concentrations >1.0 mg/dL. Conversely, 19 of 80 (24%) of samples with <1.0 mg/dL albumin were positive on urine protein test strip (see Table).

In the two populations examined, prevalence of microalbuminuria (>1.0 mg/dL and < 30.0 mg/dL) ranged from 19% to 36%. We conclude that microalbuminuria is prevalent in a significant number (P < 0.01) of dogs. Furthermore, use of urine protein test strips for the detection of albuminuria (>30.0 mg/dL) yields a substantial number of false positive results.

# 114 PREVALENCE OF MICROALBUMINURIA IN DOGS EVALUATED AT A REFERRAL VETERINARY HOSPITAL. BM Pressler,<sup>1</sup> SL Vaden,<sup>1</sup> WA Jensen,<sup>2</sup> D Simpson,<sup>2</sup> <sup>1</sup>North Carolina State University, College of Veterinary Medicine, Raleigh, NC; <sup>2</sup>Heska Corporation, Fort Collins, CO.

Microalbuminuria (MA) in humans is defined as urine albumin concentration (UAlb) between 20 and 200 µg/ml at normal diuresis. MA is a positive indicator for development of diabetic nephropathy in humans. MA also occurs with hypertension, neoplasia, cardiovascular disease, and in patients who are critically ill or have severe systemic infections. Recently, an ELISA has been validated for quantitation of canine UAlb. The purpose of this study was to determine the occurrence of MA in a random sampling of dogs evaluated at a referral veterinary hospital.

Urine samples were obtained from 67 dogs evaluated for a variety of clinical conditions. Dipstick urinalyses were performed on all samples; clinician discretion determined method of urine collection and performance of urine sediment examination and/or urine culture. Aliquots of urine were frozen (-80°C) within 15 minutes of collection. UAlb was quantified using an antigen capture ELISA. To account for varying urine concentrations, results were normalized to a specific gravity of 1.010.

UAlb in the study population ranged from 0.1 to >500 µg/ml (median 22.8, Q1 5.6, Q2 175.9). 32 dogs (48%) had negligible UAlb (<20 µg/ml), 20 (30%) had MA (20–200), 15 (22%) had overt albuminuria (>200). When 21 dogs with hematuria (>5 RBC/hpf or >1+ via urine dipstick) and/or urinary tract infections were excluded, 27 of 46 dogs (59%) had negligible UAlb, 12 dogs (26%) had MA, and 7 dogs (15%) had overt albuminuria.

The 46 dogs without hematuria and/or urinary tract infections were categorized based on systemic disease. 3 (100%) dogs with cardiovascular disease, 2 (40%) dogs with urogenital disease, 4 (20%) dogs with neoplasia, and 3 (21%) dogs evaluated for other diseases had MA. 3 (60%) dogs with urogenital disease and 4 (20%) dogs with neoplasia had overt albuminuria. 4 (100%) of dogs without systemic illnesses had negligible UAlb.

This preliminary study shows that MA, defined as UAlb between 20 and 200 µg/ml, occurs in a high percentage of dogs evaluated at a referral veterinary hospital. The incidence of MA in all dogs is unknown because the minimum sensitivity of standard urine dipsticks is 200 to 300 µg/ml. Normal UAlb in dogs has not been established. Many of the diseases associated with MA in people are likewise associated with glomerular disease in dogs. Further studies are indicated to determine the range of UAlb in healthy dogs and in dogs with systemic disease, as well as the predictive value of MA for later onset of renal disease.

# 115 LONGITUDINAL STUDY OF MICROALBUMINURIA IN SOFT-COATED WHEATEN TERRIERS. <sup>1</sup>SL Vaden, <sup>2</sup>W Jensen, <sup>3</sup>S Longhofer, <sup>3</sup>D Simpson. <sup>1</sup>North Carolina State University, College of Veterinary Medicine, Raleigh, NC; <sup>2</sup>Heska Corporation, Fort Collins, CO.

Microalbuminuria (MA) is a predictor of later development of nephropathy in people with diabetes mellitus or essential hypertension. MA is also found in people with systemic diseases that are associated with glomerular disease. MA has been demonstrated to occur in clinically normal dogs and in dogs with medical problems. However, the predictive value of MA for later onset of renal disease in dogs remains to be determined. The purpose of this longitudinal study was to evaluate urine albumin content in a colony of dogs that are genetically predisposed to the development of glomerular disease.

The study population consisted of 9 soft coated wheaten terriers (SCWT) and 8 SCWTxBeagles. Urinalyses and urine protein:creatinine ratios (UP:C) were evaluated in samples from all dogs every 3 months of life. Aliquots of urine were stored at -70°C at irregular intervals from dogs that were between 3 months and 5.7 years of age. The median number of samples stored per dog was 7 (Q1, 5; Q3, 8). Urine albumin concentrations were measured in stored urine samples using an antigen capture ELISA. To account for varying urine concentrations, results were normalized to a specific gravity of 1.010. MA was defined as urine albumin concentration of 1.0–30.0 mg/dL.

13 of the 17 dogs (76%) had MA detected in one or more samples. Of these 13 dogs, the median percent (Q1,Q3) of samples with MA was 50% (9.1, 50). In all dogs, urine albumin concentrations increased with age (p < 0.05). 3 of the 13 dogs with MA developed overt albuminuria (>30.0 mg/dL). Proteinuria (UP:C > 0.5) was detected in 3 of 13 dogs (23%) with MA but only 2 of the 3 dogs with overt albuminuria. In these 2 dogs, the age at onset of MA and proteinuria was the same. In the other dog with proteinuria, MA preceded the onset of proteinuria by 1 year.

This preliminary study shows that the prevalence of MA is high in SCWT and SCWT crosses that are genetically predisposed to develop glomerular disease, when compared to other reports of MA in clinically normal dogs or dogs with other medical problems. In this colony of dogs, MA increased with age. More study is needed to determine if the dogs in this study that have MA but did not have proteinuria will progress to develop overt manifestations of glomerular disease.

# 116 THE EFFECT OF DIETARY SODIUM ON URINE COMPOSITION OF HEALTHY MINIATURE SCHNAUZERS (MS). A.E. Stevenson, W.K. Hynds, P.J. Markwell WALTHAM Centre for Pet Nutrition, Waltham-on-the-wolds, Melton Mowbray, Leicestershire, LE14 4RT, UK.

Urinary supersaturation provides the driving force for crystal formation. The simplest way of reducing supersaturation and thus the risk of crystal formation is to increase urine volume. Increasing sodium (Na) intake has been shown to increase water intake in dogs, a measure that would be expected to reduce calcium oxalate (CaOx) supersaturation, however, Na supplementation as a means of controlling stone formation remains controversial. The aim of this study was to determine the effect of Na supplementation on CaOx relative supersaturation (RSS) in MS.

8 MS were fed either a dry diet containing 0.2g Na per 400 kcal (Diet 1), or the same diet supplemented to achieve 0.8g (Diet 2) and 1.2g Na per 400 kcal (Diet 3) for 3 week periods in a Latin square design. Dogs were housed individually for 48 hour periods, at which times water intake and urine volume were measured daily. During Week 3 of each feeding period, a 48 hour frozen urine collection was conducted and urinary CaOx RSS was measured by previously described methods (Table). Results were compared using ANOVA and multiple range tests.

Parameter	Diet 1	Diet 2	Diet 3
Calcium oxalate RSS	13.87±8.78 <sup>b</sup>	9.13±6.52 <sup>ab</sup>	5.73±2.92 <sup>a</sup>
Urinary calcium concentration (mmol/l)	1.40±0.58 <sup>a</sup>	1.27±0.50 <sup>a</sup>	0.93±0.49 <sup>a</sup>
Urinary oxalate concentration (mmol/l)	1.45±0.75 <sup>a</sup>	0.99±0.60 <sup>a</sup>	0.92±0.53 <sup>a</sup>
Urinary sodium concentration (mmol/l)	69±50 <sup>a</sup>	143±36 <sup>b</sup>	157±65 <sup>b</sup>
Water intake (ml/day)	284±72 <sup>a</sup>	355±95 <sup>ab</sup>	368±67 <sup>ab</sup>
Urine volume (ml/day)	82±70 <sup>a</sup>	116±68 <sup>a</sup>	135±106 <sup>a</sup>

A different superscript within a row indicates a significant difference (p<0.05).

Increasing sodium intake increased water intake and tended to result in an increase in urine volume. Urinary Na concentration increased whereas urinary calcium and oxalate concentrations tended to decrease with increasing dietary Na. CaOx RSS decreased significantly at the highest Na intake from a value close to the formation product to approximately the middle of the metastable region of supersaturation. These observations suggest that increasing Na intake can reduce the risk of CaOx formation in MS.

# 117 INCREASED DIETARY NaCl SIGNIFICANTLY INCREASES URINE VOLUME BUT DOES NOT INCREASE URINARY CALCIUM OXALATE RELATIVE SUPERSATURATION IN HEALTHY CATS. V.C. Biourge, C. Devois, G. Morice and R. Sergheraert. Royal Canin, Research Center, Vannes, France.

Increased frequency of miction and reduced urine saturation can be achieved by stimulating urine output. Preliminary work in our laboratory, comparing commercial diets differing by their NaCl content, showed that higher dietary NaCl intake increases urine output without increased urine CaOx saturation. The purpose of this study was to confirm this observation in a better-controlled setting by comparing 2 experimental diets differing only by their NaCl content.

Five healthy adult cats were fed successively 2 dry expanded acidifying diets formulated with the same ingredients and differing only by their Na and Cl content (0.43 and 1.23 Vs. 0.91 and 2.16 g/100g of diet respectively). Cats were allowed 7 days to adapt to each diet then placed in metabolic cages. All urine were collected 3 times daily over a 5-day period, weighed, and pooled. Urine pH, Ca, Cl, K, Mg, Na, NH<sub>4</sub>Ox, P, Urate, citrate, sulfate concentrations were determined on pooled urine. CaOx Relative Supersaturation (RSS) were calculated using the Equil 89d software package.

Higher NaCl intake was associated with significantly higher 24 hour-urinary water and Ca excretion but Ca and Ox concentrations did not differ significantly between the 2 diets. Higher NaCl intake induced lower CaOx RSS although not significantly.

Those results confirm our previous observations that higher NaCl intake in healthy cats stimulates urine output and does not increase CaOx saturation. Higher dietary NaCl might thus be of benefits in diets formulated to prevent or dissolve uroliths.

# 119 EX VIVO CANINE STRUVITE STONE DISSOLUTION. B.H.E. Smith, W.K. Hynds, P.J. Markwell. Waltham Centre for Pet Nutrition, Waltham-on-the-wolds, Leicestershire, UK.

The aim of this study was to investigate the role of diet in struvite stone dissolution using an *ex vivo* method. The uroliths used in this study were collected from a Miniature Schnauzer with naturally occurring urolithiasis. Composition of 100% struvite was confirmed by quantitative analysis.

Six healthy adult beagles were fed either a diet designed for struvite dissolution<sup>1</sup> (Diet 1) or a commercially available diet (Diet 2) in a cross over design. The dogs were individually housed and 24-hour frozen urine samples were collected using a previously described system. Each 24-hour sample was defrosted under oil and thymol, and incubated with a struvite urolith for the following 24 hours at 38°C. The uroliths were removed from the incubation media and weighed daily. During the periods when the dogs were fed Diet 1, incubation continued for as many days as was required to complete dissolution. Incubation was restricted to 27 days of feeding Diet 2, because of limited dissolution.

The percentage dissolution and rate of dissolution were significantly greater with urine collected during feeding of Diet 1, compared with urine collected during feeding of Diet 2 (Table). The time taken for complete dissolution of the struvite uroliths in urine from the dogs when fed Diet 1 varied from 20 to 26 (mean 22.8 ± 2.8) days, depending on the weight of the urolith.

Diet	% dissolution Mean ± sd	Dissolution rate (g/day) Mean ± sd
1	100*	0.038 ± 0.010*
2	46 ± 44	0.006 ± 0.006

\* Denotes a significant difference within each column

Urine from dogs fed Diet 1 resulted in the rapid total dissolution of canine struvite uroliths. This *ex vivo* technique may provide a useful screen for dietary interventions aimed at controlling urolithiasis.

<sup>1</sup> Waltham Veterinary Diet Canine Low pH Control

# 118 CANINE CALCIUM OXALATE UROLITHIASIS: EPIDEMIOLOGICAL ASSOCIATION BETWEEN DIETARY CALCIUM AND RISK OF UROLITH FORMATION. C. Lekcharoensuk, C.A. Osborne, J.P. Lulich, R. Pusoonthornthum, L.A. Koehler, L.K. Ulich, K.A. Carpenter, L.L. Swanson. Minnesota Urolith Center, University of Minnesota, St. Paul, MN.

Recent studies in humans indicate that dietary restriction of calcium increased the risk of calcium oxalate (CaOx) urolithiasis. We designed a retrospective study of dogs to test the hypothesis that diets lower in calcium were related to increased risk for CaOx uroliths.

Cases consisted of 1,025 dogs with CaOx uroliths analyzed at the Minnesota Urolith Center between 1990 and 1992. Controls consisted of 1,578 dogs without urinary tract diseases (UTD) evaluated by referring veterinarians prior to or after evaluation of dogs with CaOx uroliths. A validated multiple-choice questionnaire was used to collect information from owners about signalment, diet, medical history, and environment. Dogs were excluded if they had a history of UTD, were fed therapeutic diets for UTD, were <1-year-old, or if they consumed their current diets <6 months. The quantity of dietary calcium of 754 dogs with CaOx uroliths and 1,106 control dogs were studied. Univariate and multivariate logistic regression were performed.

Dietary calcium was evaluated as a continuous variable. The mean ± SD quantity of dietary calcium (2.79 ± 1.08 mg/kcal) fed to 754 dogs with CaOx uroliths was lower (p<0.05) than the mean ± SD quantity of dietary calcium (3.14 ± 0.96 mg/kcal) fed to 1,106 control dogs. In context of the diets evaluated in this study, increasing dietary calcium by increments of 1 mg/kcal decreased risk for CaOx urolithiasis (OR = 0.71; 95% CI = 0.65 to 0.78). After adjusting for confounding factors (pure breed, >4 years old, gender, reproductive status, and body condition), the protective effect of increased dietary calcium persisted (OR = 0.72; 95% CI = 0.64 to 0.80).

These results suggest that dietary calcium restriction was associated with increased risk for CaOx uroliths in dogs.

# 120 CHANGES IN BLOOD, ECF & ICF VOLUMES IN RESPONSE TO FLUID AND LASIX ADMINISTRATION TO NORMAL DOGS. LD Cowgill, J Aldrich, D Silverstein, SC Haskins. School of Veterinary Medicine, University of California, Davis, CA.

Interrelated changes in body fluid volumes to agents predicted to induce compartmental fluid alterations have not been well characterized. We used an in-line hematocrit monitor (Crit-Line IIR) to quantitate real-time changes in blood volume (BV) and a multifrequency bioimpedance spectrometer (Xitron 4200) to predict simultaneous changes in extracellular (ECF) and intracellular (ICF) fluid volumes. Change in BV and ECF and ICF volumes were measured every 6 to 20 seconds before and for 240 minutes after no treatment (C) or intravenous administration of: 80 mL/kg of 0.9% NaCl (S), 4 mL/kg of 7.5% NaCl (HS), 20 mL/kg of Dextran-70 (D) and Hetastarch (H), or 4 mg/kg of furosemide (F) to 4 clinically normal, anesthetized dogs.

S and HS caused rapid but transient and nonequivalent expansion of BV and ECF; HS was also associated with a decrease in ICF. D and H caused equal and sustained increases in BV with little influence on ECF or ICF; and F promoted a sustained but nonequivalent contraction of both BV and ECF. These results demonstrate in-line hematocrit monitoring and multifrequency bioimpedance spectroscopy can document simultaneous and real-time changes in compartmental fluid metabolism that may have utility in the assessment and management of disease states.

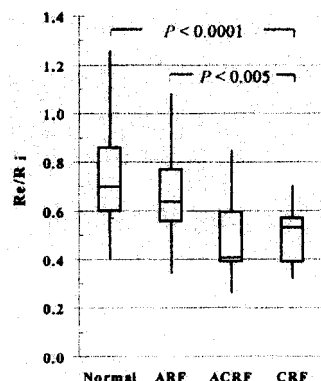
Fluid Volume (% Change from Pre-treatment)

Rx Time	S	D	H	F	C
Post Infusion:	(12 min)	(4 min)	(3 min)	(3 min)	(6 min)
BV	74.6 ± 9.3*	17.1 ± 3.1*	23.9 ± 9.8*	29.4 ± 5.2*	-0.8 ± 1.2
ECF	2.0 ± 4.3	2.2 ± 4.0	0.2 ± 0.4	0.0 ± 0.1	0.1 ± 0.4
ICF	-3.6 ± 8.6	-1.5 ± 2.1	0.8 ± 0.9	0.0 ± 0.1	-0.4 ± 1.5
30 Minutes:					
BV	35.2 ± 9.3*	12.3 ± 0.9*	34.8 ± 9.9*	36.8 ± 6.5*	-7.4 ± 5.1
ECF	14.9 ± 8.0*	10.9 ± 3.2*	0.4 ± 1.4	2.8 ± 0.7*	-1.2 ± 1.1
ICF	-5.1 ± 12.4	-5.4 ± 2.9*	3.1 ± 2.4	1.8 ± 0.8	-1.0 ± 1.8
60 Minutes:					
BV	23.7 ± 9.0*	10.2 ± 1.6*	35.2 ± 6.4*	34.5 ± 7.4*	-10.0 ± 5.9*
ECF	21.4 ± 6.7*	9.2 ± 1.0*	0.8 ± 0.9	3.0 ± 1.7*	-2.5 ± 2.0
ICF	-7.5 ± 11.2	-3.5 ± 0.2*	3.9 ± 1.8	2.2 ± 2.4	-0.4 ± 1.9
240 Minutes:					
BV	18.0 ± 9.7*	2.9 ± 6.1	25.6 ± 16.1*	26.6 ± 8.5*	-11.3 ± 9.8
ECF	17.5 ± 9.7*	7.6 ± 2.7*	-0.2 ± 1.6	2.6 ± 4.4*	-5.7 ± 0.7*
ICF	6.2 ± 16.3	-3.5 ± 1.3	4.5 ± 1.6	5.1 ± 4.8	0.2 ± 3.1

\*, P < 0.05 vs C

## 121 ASSESSMENT OF ELECTRICAL PROPERTIES IN NORMAL AND UREMIC DOGS BY MULTI-FREQUENCY BIOIMPEDANCE SPECTROSCOPY. T Francey, DA Elliot, LD Cowgill. School of Veterinary Medicine, University of California, Davis.

Clinical data suggest subjective alterations in the body composition of uremic dogs, including hydration status (ECF) and lean body mass (ICF). Electrical conductivities of these body compartments correspond to their respective volumes, and can serve as objective predictors of these components of body composition. Correspondingly the ratio of the electrical resistances of the ECF and ICF (Re/Ri) assessed by bioimpedance spectroscopy (BIS) has been used to estimate hydration status. We investigated the use of multi-frequency BIS to document differences in resistance profiles between clinically normal dogs ( $n = 34$ ) and dogs with acute renal failure (ARF,  $n = 42$ ), combined acute and chronic renal failure (ACRF,  $n = 5$ ), and chronic renal failure (CRF,  $n = 10$ ). The electrical conductivity was measured at 50 logarithmically spaced frequencies between 5 kHz and 1 MHz (Xitron 4200) using tetrapolar needle electrodes. From these data, Re and Ri were modeled with algorithms supplied with the device.



In normal dogs there was a wide variation in the Re/Ri, possibly due to differences in body type, gender, and hydration status. Re/Ri was significantly lower in CRF vs. both normal and ARF but indistinguishable from ACRF indicating intrinsic differences in the electrical properties of dogs with CRF. These observations suggest differences in compartmental fluid distribution which could include expansion of the extracellular space (decreased Re), loss of lean body mass due to chronic wasting (increased Ri), or a combination of both.

With further development, including volume modeling from Re and Ri, BIS could be useful as a non-invasive predictor of body composition in uremic dogs.

## 122 EFFECT OF SEDATION WITH KETAMINE/DIAZEPAM OR PROPOFOL ON MEASUREMENT OF GLOMERULAR FILTRATION RATE BY RENAL SCINTIGRAPHY IN CATS. M.E. Kerl, P. Langdon and C.R. Cook. University of Missouri, Columbia, MO.

Determination of glomerular filtration rate (GFR) is the standard parameter used to quantify renal function. Traditional techniques used to determine GFR are cumbersome for clinical use. Renal scintigraphy involves intravenous (IV) administration of a radioisotope freely excreted via glomerular filtration and not reabsorbed by the tubules, followed by quantification of radioactivity in the kidneys to assess uptake. Scintigraphy has been validated in awake cats to accurately determine GFR when compared to inulin and endogenous creatinine clearance. Restraint for renal scintigraphy may be difficult in some awake cats, necessitating sedation. To our knowledge, no study has been published comparing renal scintigraphy values between awake and sedated cats.

We hypothesized that GFR measured by scintigraphy was significantly different in sedated versus awake cats, and that a significant difference existed between GFR measurements between different sedation protocols. To test this hypothesis, we performed renal scintigraphy in 6 healthy, normally hydrated cats using 3 different treatment protocols: 1) Ketamine 5 mg/kg and diazepam 0.25 mg/kg IV (KV), 2) Propofol 6 mg/kg IV (P), and 3) manual restraint only. Treatment order was randomized. On Day 0, cats were sedated (ketamine 5 mg/kg and diazepam 0.25 mg/kg IV) and jugular catheters placed. On Day 1, each cat received one of the 3 treatment protocols. Renal scintigraphy was performed by positioning the cats in left lateral recumbency, with the gamma camera perpendicular to the cranial abdomen. Dynamic nuclear imaging began simultaneously with IV injection of 2 mCi Tc-99mDTPA, followed by a 1–2 ml saline solution flush. Predose and postdose counts of radioactivity in the syringe and IV tubing were obtained. Multiple images of radionuclide distribution were acquired for 3 minutes. Glomerular filtration rate was determined using a computerized image analysis software package (Gamma 600). Data were analyzed for significant differences between the three treatment groups using one way repeated measures ANOVA.

GFR in awake cats was  $2.682 \pm 0.264$  ml/min/kg (mean  $\pm$  SD). Sedation with KV or P did not significantly alter GFR, and GFR for all treatment groups was within normal ranges for cats. Some individual cat variation in GFR was observed between treatment protocols, but did not affect final values.

We concluded that there was no difference in GFR measured by renal scintigraphy between awake cats, and cats sedated with ketamine/diazepam or propofol. Our findings indicate that assessment of GFR by scintigraphy may be accurate with these sedation protocols in cats. Future study of additional sedation protocols is warranted.

## 123 ESTIMATION OF GLOMERULAR FILTRATION RATE (GFR) USING PLASMA CLEARANCE OF IOHEXOL: ESTABLISHING NORMAL VALUES IN DOGS. I. Goy-Thollot, C. Chaffotte, F. Garnier, P. Y. Barthez. École Nationale Vétérinaire de Lyon, 1 avenue Bourgelat 69 280 Marcy l'Étoile. France.

The purpose of this study was to establish normal values of plasma clearance of iohexol in dogs and to determine the optimal method for normalisation.

Thirty one healthy and well-hydrated dogs (ages 1–7 years; weight 8–58 kg) were included. Two IV catheters were placed in 2 different peripheral veins. A bolus of 300 mg/kg of Iohexol was injected using one of the catheters. Blood samples were withdrawn before the injection, and 5, 20, 40, 60, 80, 100, 120, 150, 180, and 240 minutes after injection of tracer. Iohexol plasma concentration was determined using x-ray fluorescence. A plasma tracer elimination curve was generated and the area under the curve was estimated using a 2-compartment pharmacological model. Plasma clearance of iohexol was calculated by dividing the injected dose of iohexol by the area under the curve. Plasma clearance was normalized using the body weight, body surface and extracellular fluid volume (ECV). Mean and standard deviation (SD) of the plasma clearance, before and after normalization, were calculated. The coefficient of variation was determined for the 3 methods of normalization. The method yielding the lowest coefficient of variation was selected as the optimal one.

The plasma clearance of iohexol ranged from 22.77–151.67 mL/min with a mean ( $\pm$  SD) of 69 mL/min ( $\pm$  30). Normalized to body weight, the mean ( $\pm$  SD) plasma clearance of iohexol was 2.91 mL/min/kg ( $\pm$  0.6) and its coefficient of variation was 0.21. Normalized to body surface, the mean ( $\pm$  SD) plasma clearance of iohexol was 80 mL/min/m<sup>2</sup> ( $\pm$  15.5) and its coefficient of variation was 0.19. Normalized to ECV, the mean ( $\pm$  SD) plasma clearance of iohexol was 0.014 /min ( $\pm$  0.003) and its coefficient of variation was 0.23.

It was concluded that the optimal method for normalization of plasma clearance of iohexol in dogs was by using the body surface.

## 124 FELINE PLASMA PRO-ATRIAL NATRIURETIC PEPTIDE MEASUREMENT: CORRELATION WITH SYSTOLIC BLOOD PRESSURE AND RENAL FUNCTION HM Syme,<sup>1</sup> J Elliott,<sup>1</sup> S Attree,<sup>2</sup> Royal Veterinary College, London<sup>2</sup> Guildhay Ltd, Guildford, UK.

Atrial natriuretic peptide (ANP) is a hormone with multiple anti-hypertensive actions. Studies conducted in man and rats have demonstrated that hypertension is associated with a shift of intravascular volume from peripheral to central compartments. The increase in atrial filling pressure that ensues stimulates ANP release from cardiac myocytes. ANP is synthesized as a pro-hormone and is cleaved into ANP(99–126) and proANP(1–98) peptides on release from the myocyte. ProANP(1–98) has a long half-life which facilitates its measurement as the need for sample extraction is obviated and temporal fluctuations in concentration are minimized. The aim of this study was to investigate the influence of blood pressure (BP) on plasma proANP(1–98) concentration in normal cats, and cats with chronic renal failure (CRF).

Heparinized blood samples were collected from cats that were clinically and biochemically normal ( $n = 20$ ), and from cats diagnosed with CRF ( $n = 58$ ) on the basis of their clinical presentation and an elevated plasma creatinine concentration ( $>1.9$  mg/dl). Systolic BP was measured in all the cats using a Doppler technique. ProANP(1–98) concentrations were determined by a sandwich ELISA method using a commercially available test kit. This kit had not been previously validated for use in the cat. The influence of BP and plasma creatinine concentration on the proANP(1–98) concentration was evaluated by linear regression. In addition, in hypertensive cats (BP consistently  $>175$  mmHg), proANP(1–98) concentrations were measured before and after introduction of amlodipine therapy, and compared by a paired Student's *t*-test.

The limit of sensitivity of the ProANP(1–98) assay was 145 fmol/ml. The intra-assay CVs ( $n = 6$ ) were 8.1, 7.5 and 4.9% and the inter-assay CVs ( $n = 6$ ) were 14.4, 9.7 and 20.0% for pooled plasma samples with low, medium and high concentrations. Dilutional parallelism was demonstrated. ProANP(1–98) concentrations increased significantly ( $P < 0.001$ ) with increasing plasma creatinine concentration. However, proANP(1–98) concentrations were not significantly influenced by BP ( $P = 0.073$ ). ProANP(1–98) concentrations decreased in 19 of 25 hypertensive cats following treatment with amlodipine, but this finding did not reach statistical significance ( $P = 0.131$ ).

In conclusion, the proANP(1–98) assay was validated for feline plasma samples. ProANP(1–98) accumulated in cats with CRF in direct proportion to the severity of their excretory failure. The failure of proANP(1–98) concentrations to increase with increasing BP may be relevant to the pathogenesis of systemic hypertension in the cat.

# 125 CLINICAL TRIAL OF AMITRIPTYLINE FOR MANAGEMENT OF ACUTE NONOBSTRUCTIVE FELINE IDIOPATHIC LOWER URINARY TRACT DISEASE. JM Kruger, T Kalkstein, JB Kane-ene, RL Perry, JS Jehl. Michigan State University, East Lansing, MI

Idiopathic lower urinary tract disease (ILUTD) is a common cause of hematuria and pollakiuria in cats. Amitriptyline, a tricyclic antidepressant with anticholinergic, antihistaminic, anti-inflammatory, and analgesic properties, has been advocated for symptomatic therapy of ILUTD. However, the efficacy of amitriptyline for treatment of ILUTD has not been evaluated using a randomized clinical trial. We hypothesized that short-term amitriptyline therapy would be more effective in reducing the severity and duration of clinical signs associated with acute ILUTD than treatment with a placebo.

Thirty-one untreated male and female cats with acute nonobstructive ILUTD were assigned to 2 treatment groups using a block randomization procedure. Group I cats received amitriptyline (5mg/cat/day); group II cats received placebo. A pharmacist directed the distribution of medications. All cats were hospitalized for 7 days and monitored for pollakiuria (>3 urinations/day), hematuria, and adverse reactions. Cats were clinically reevaluated at 1 month and phone interviews were conducted at 6, 12, and 24 months after discharge. Univariable and multivariable statistical analyses were conducted to evaluate the influence of treatment, age, number of prior episodes, and number of days symptomatic prior to treatment on resolution of pollakiuria and hematuria, and on recurrence of clinical signs.

Sixteen cats received amitriptyline; 15 cats received placebo. Two cats receiving amitriptyline were excluded from analysis due to acquired urinary tract infections. All clinical signs resolved completely within 7 days of diagnosis in 8 (57%) amitriptyline treated cats and 10 (67%) placebo treated cats. However, amitriptyline significantly decreased the number of days to recovery from pollakiuria ( $p<0.05$ ), but not hematuria. Increasing age was significantly associated with an increased likelihood ( $p<0.04$ ) and rate ( $p<0.001$ ) of recovery from both pollakiuria and hematuria. In the 6 months following discharge, clinical signs recurred significantly sooner ( $p<0.02$ ) and with greater frequency ( $p<0.03$ ) in amitriptyline treated cats than in cats receiving a placebo. Adverse effects associated with amitriptyline treatment included sedation, urinary tract infection, neutropenia, basophilic, hyperbilirubinemia, and increased serum ALT activity.

Although amitriptyline significantly reduced the duration of pollakiuria in cats with acute ILUTD, increasing age significantly modulated the rate of recovery from both pollakiuria and hematuria. In addition, clinical signs recurred sooner and with higher frequency in amitriptyline treated cats than in cats receiving a placebo.

# 126 EVALUATION OF BLOOD AGAR PLATES AS A TRANSPORT MEDIUM FOR AEROBIC BACTERIAL URINE CULTURES. LJ Blanco, JW Bartges, DA Bemis, JC New, R Duckett, and MJ Bryant. The University of Tennessee, Knoxville, TN.

Bacterial urinary tract infections occur in approximately 10–20% of dogs presented for veterinary care. Quantitative urine cultures are the gold standard in diagnosing bacterial urinary tract infections. However, accurate urine culture results can be difficult to obtain if urine samples cannot be delivered to a laboratory immediately. This situation is particularly frustrating for general practitioners that must send out urine samples for aerobic bacterial culture. The purpose of this study was to evaluate a method of transport by inoculating urine onto a blood agar plate prior to submission of the sample for aerobic bacterial culture.

Forty-four urine samples were collected via cystocentesis from dogs that had not received antibiotics in the 5 days before evaluation. Each sample was processed as follows. One sample was inoculated on a blood agar plate using standard procedures by the principal investigator. After a 24-hour incubation, plates with bacterial growth were placed on cold packs and exposed to the environment for 24 hours. These plates were analyzed qualitatively and quantitatively. A second sample was inoculated on a blood agar plate using standard procedures by a qualified microbiology technician. Plates with bacterial growth were analyzed qualitatively and quantitatively after a 24-hour incubation.

Twenty samples processed through the laboratory were positive and 24 were negative. Of the positive samples, 7 different bacterial species were identified. Nineteen samples processed using the study technique were positive, and 24 were negative. Therefore, 19/20 samples were true positives, and 24/24 were true negatives. Sensitivity for the study technique was 95%, and specificity was 100%. Positive predictive value for the study technique was 100%, and negative predictive value was 99.2%.

Based on the results of this study, inoculating blood agar plates with urine obtained by cystocentesis provides a reliable method for obtaining accurate urine culture results. This technique may be useful for practitioners that must rely on an outside laboratory for performing aerobic bacterial urine cultures.

# 127 CLINICAL PATHOLOGY RESULTS IN DOGS ADMINISTERED CARPROFEN (RIMADYL®) PERIOPERATIVELY. Curto, M., Clark, T.P., Russo, S., Anway, S.D., Smothers, C.D., and Boy, M.G. Pfizer Global Research and Development, Pfizer, Inc., Groton, CT.

Rimadyl® was administered orally or subcutaneously in six controlled studies at a dosage of 2 mg/lb (4.4 mg/kg) approximately 2 hours pre-operatively, and then daily post-operatively as needed. Study subjects were client-owned dogs presenting to veterinary practices for one of the following surgery types: ovariohysterectomy (262 dogs), aural surgery (192 dogs), or cruciate repair (174 dogs). A total of 628 dogs were randomly allocated to treatment with either placebo (312 dogs) or Rimadyl® (316 dogs). The dogs enrolled in the studies were required to have satisfactory clinical pathology results within 7 days prior to enrollment. Clinical pathology variables evaluated included hematology, clinical chemistries, coagulation profile, urinalysis, urinary gamma glutamyl transpeptidase to creatinine ratio and fecal occult blood. The coagulation profile was assessed again the day following surgery, and all clinical pathology variables were evaluated 3 days (ovariohysterectomy and aural) or 4 days (cruciate) post-operatively.

Rimadyl® was administered concurrently with the following medications associated with anesthesia: atropine, glycopyrrolate, acepromazine, thiopental, diazepam, ketamine, tiletamine/zolazepam, propofol, doxapram, isoflurane, halothane, methoxyfluorane, and nitrous oxide.

In addition, Rimadyl® was administered concurrently with various endectocides, heartworm preventatives, antimicrobials, and nutraceuticals.

There were no clinically significant differences in mean clinical pathology variables in dogs administered Rimadyl® compared to dogs administered placebo. Instances of abnormal health were mild and infrequent with similar distributions for the placebo- and Rimadyl®- treated dogs.

# 128 THE STEADY-STATE PHARMACOKINETICS OF CARPROFEN (RIMADYL®) ADMINISTERED ORALLY AND SUBCUTANEOUSLY IN DOGS. Clark, T.P., Nimz, E., Chieffo, C., and Smothers, C.D. Pfizer Global Research and Development, Pfizer, Inc., Groton, CT.

A study was conducted to evaluate the comparative pharmacokinetics of Rimadyl® at steady-state conditions in dogs following oral and subcutaneous administration. Eighteen male beagles were randomized into one of two treatments in a two-sequence, two-period crossover design with a ten-day washout between periods. Twenty-five milligrams of Rimadyl® were administered orally or subcutaneously (50 mg/mL solution) every 12 hours for 7 days. Blood samples were collected from dogs within 1 hour prior to treatment and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 8, and 12 hours following administration of the first dose of each period and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 8, 12, 24, 48, 72, and 96 hours following administration of the last dose of each period. Plasma concentrations of Rimadyl® were determined using a specific, validated, high performance liquid chromatography (HPLC) method with fluorescence detection that was accurate and reproducible in the range of 0.25 to 50 µg/mL. Plasma concentration data were transformed and geometric means were calculated for maximum plasma concentration ( $C_{max}$ ) following the first dose, area under the concentration-time curve after the first dose ( $AUC_{0-12}$ ), and  $AUC_{0-\infty}$  following administration of the last dose. For each variable, the 90% confidence interval was constructed on the difference between the mean of the injectable formulation and the mean of the oral formulation divided by the mean of the oral formulation. Variables were considered statistically similar if the 90% confidence interval of the mean difference was within –20% and 25%. The mean  $C_{max}$  was 16.9 µg/mL following a single oral dose and was 8.0 µg/mL following a single subcutaneous injection. The mean  $AUC_{0-12}$  after a single oral dose was 73.1 µg-hr/mL and was 64.3 µg-hr/mL after a single subcutaneous injection. The 90% confidence interval for  $C_{max}$  was outside of the bioequivalence criteria (–56.8 to –48.7%) whereas the 90% confidence interval for  $AUC_{0-12}$  was within the bioequivalence criteria (–16.3 to –7.5%). At steady-state, the mean  $AUC_{0-\infty}$  was 101.9 µg-hr/mL and 111.0 µg-hr/mL following oral and subcutaneous dosing respectively, and the 90% confidence interval was within the bioequivalence criteria (2.3 to 15.9%). The results of this study indicate that peak plasma concentrations following a single dose of Rimadyl® differ when administered orally or subcutaneously, but total drug exposure at steady-state is bioequivalent.



## 129 VARIABILITY IN AZATHIOPRINE METABOLISM IN DOGS. L. Kidd, L. Trepanier, C. Szumlanski,\* A. Perez,\* C. Yang,\* R. Weinshilboum.\* University of Wisconsin-School of Veterinary Medicine, Madison, WI. \*Mayo Clinic-Mayo Medical School, Rochester, MN.

Azathioprine is a cytotoxic thiopurine drug that is metabolized to 6-mercaptopurine. 6-Mercaptopurine is further converted into active metabolites that exert immunosuppressive and cytotoxic effects, or it is metabolized by the enzyme thiopurine methyltransferase (TPMT). Human RBC TPMT activity is affected by underlying disease (such as renal failure), and certain drugs (such as diuretics). However, the major factor responsible for individual variation in TPMT activity in humans is a genetic polymorphism. Low and intermediate RBC TPMT activity on a genetic basis have been correlated with azathioprine toxicity in human studies. Conversely, relatively high TPMT activity has been associated with poor remission rates and relapse of disease. Therefore, azathioprine dosage regimens in humans are based on TPMT phenotype.

The aims of this study are to validate the TPMT assay for use in dogs, establish the normal range for TPMT activity in dogs, document the presence of individuals with very high or very low TPMT activity in the canine population, and associate the presence of genetic mutations or acquired disease with extremes in TPMT activity.

A radiochemical assay for RBC TPMT activity assay was adapted and optimized for use in dogs. Two to five mls of heparinized whole blood was obtained from dogs in the inpatient and outpatient population at the University of Wisconsin VMTH. Patient signalment, drug history and clinical diagnoses were recorded at the time of phlebotomy or obtained retrospectively. Red blood cell lysates have been prepared and assayed for RBC TPMT activity in 105 dogs to date. The mean RBC TPMT activity was  $22.0 \pm 6.2$  with a range of 7.9–37.1 units of TPMT activity/ml RBCs. In this sample, one dog with very low activity, and four dogs with relatively high activity (each greater than 2 SD from the mean) have been identified. The dog with very low activity had cardiac disease and was receiving multiple cardiac medications including furosemide. Two of the dogs with high activity had concurrent renal failure.

In addition to establishing a validated assay for canine RBC TPMT activity, these results document that there is at least a four-fold difference in TPMT activity in the canine population. Further work will determine whether disease, drugs or genetic mutations affect TPMT activity in dogs, and whether genetic or acquired differences in TPMT activity are associated with clinical outcome of toxicity or poor efficacy.

## 130 PHARMACOKINETICS OF SINGLE-DOSE ORAL AND INTRAVENOUS MYCOPHENOLATE MOFETIL ADMINISTRATION IN NORMAL DOGS. CW Dewey, DM Boothe, WS Wilkie. College of Veterinary Medicine, Texas A&M University, College Station, TX.

Mycophenolate mofetil (CellCept-Roche Pharmaceuticals) is a relatively lymphocyte-specific immunosuppressive drug used primarily in human renal transplant recipients. Prolonged renal allograft survival has also been shown with MMF use (as an adjunct to other drugs) in canine transplant studies. The active metabolite, mycophenolate (MPA), interferes with the *de novo* pathway of GTP synthesis (required by lymphocytes) via inhibition of inosine monophosphate dehydrogenase (IMPDH). In humans and dogs, mycophenolate mofetil (MMF) is rapidly converted to MPA after gastrointestinal absorption. MMF has shown promise as a therapeutic option for several autoimmune disorders in people (e.g. acquired MG, AIHA). The investigators have had encouraging results with MMF treatment of several dogs with severe autoimmune disease. CellCept appears to have a rapid onset of action, and few serious side-effects, compared to more conventional immunosuppressive drugs. The purpose of this investigation was to evaluate the pharmacokinetics of MMF and its metabolites in normal dogs at an approximate dosage of 20 mg/kg.

Six healthy, adult hound dogs were used in a cross-over study design. 500 mg of MMF was administered to each dog, either orally (PO) or via a continuous IV infusion over 2 hours. Each dog received both routes of drug administration, separated by a minimum time interval of 7 days. Blood was drawn at time 0, and 10, 20, 30, 60, 90, 120, 240, 360, 480, 720, 1440, and 2160 minutes following initiation of MMF administration. HPLC was used to measure plasma concentrations of MMF, MPA, and the glucuronidated metabolite of MPA, MPAG. Pharmacokinetic data was analyzed using compartmental and non-compartmental methods.

In all but 1 dog, plasma MMF concentration was below the limit of quantitation following PO administration, at all sample collection times. Mean  $C_{max}$  and  $T_{max}$  values for plasma MPA, after PO and IV administration, were 9.35  $\mu$ g/ml; 62.6 min, and 20.62  $\mu$ g/ml; 63 min, respectively. The calculated mean bioavailability of PO administered MPA was 47.6 %. The elimination half-life for PO and IV administered MPA was 45 minutes and 46 minutes, respectively. The mean residence time of plasma MPA for PO administered drug was 103.6 minutes. 3 dogs developed an apparent allergic reaction during IV MMF administration (facial swelling, urticaria on ventral abdomen) that resolved shortly after diphenhydramine administration (2 mg/kg SQ).

The half-life of PO MPA suggests the need for a frequent dosing interval. However, the duration of biologic effect (e.g. IMPDH suppression) needs to be evaluated before formulating a dosage schedule.

## 131 NOVEL *IN VITRO* ASSESSMENT OF CYCLOOXYGENASE (COX) SELECTIVITY OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID) IN CANINE WHOLE BLOOD. HK Streppa, CJ Jones, SC Budberg. University of Georgia; Athens, Georgia.

NSAID that inhibit COX-2 activity and protect COX-1 should decrease inflammatory symptoms and prevent side effects seen with COX-1 inhibition, such as gastric ulceration. Of the newer, purportedly selective NSAID, there is limited research in dogs, and current data is difficult to assess because it is generated from research with cell lines or isolated cell types from a variety of species. *In vitro* data generated directly from the target species should provide a more accurate assessment of the *in vivo* activity of the COX isoforms. The goal of this study was to document the *in vitro* COX selectivity of several NSAID in canine whole blood.

Ten NSAID were studied at 5 concentrations (100, 10, 1.0, 0.1, 0.01  $\mu$ M). Each drug was tested with whole blood from three healthy, adult canine males, all hound crosses. Thromboxane  $B_2$  was assayed as a measure of COX-1 activity in clotted blood. Prostaglandin  $E_2$  was assayed as a measure of COX-2 activity in heparinized, LPS-stimulated blood. All assays were competitive enzyme-linked immunoassays (ELISAs). COX selectivity was expressed as a ratio of the concentration of a particular NSAID that inhibited 50% of the activity ( $IC_{50}$ ) of COX-1 to the  $IC_{50}$  of COX-2. A ratio of  $<1.0$  indicated selectivity for the COX-1 isozyme, whereas a ratio of  $>1.0$  indicated COX-2 selectivity.

Aspirin was the most COX-1 selective, with a ratio of 0.104. Ketoprofen was also COX-1 selective, with a ratio of 0.45. Etodolac had almost no selectivity with a ratio of 0.89, bordering on COX-1 selectivity. Meloxicam, piroxicam and carprofen demonstrated solid COX-2 selectivity, with ratios of 3.58, 7.87 and 8.06, respectively. These findings illustrate a 95-fold difference in selectivity ratios among the various NSAID.

This methodology provides repeatable data derived from individual dogs, which is comparable to results of previous *in vitro* and *ex vivo* models. The findings are also consistent with *in vivo* canine studies that evaluate efficacy and side effects, suggesting that this is a viable *in vitro* assessment of the COX selectivity of NSAID in dogs. Interestingly, this study confirms the difference between humans and canines in the COX selectivity of etodolac, suggesting that there is species variability in the activity of certain drugs and that target species should be used in their evaluation.

## 132 EVALUATION OF A NOVEL SUSTAINED RELEASE GASTRO-RETENTIVE DOSAGE FORM OF RIBOFLAVIN IN DOGS. E. Lavy,<sup>1</sup> E. Klausner,<sup>2</sup> A. Hoffman<sup>2</sup> and M. Friedman.<sup>2</sup> <sup>1</sup>Koret School of Veterinary Medicine, Hebrew University of Jerusalem. <sup>2</sup>School of Pharmacy Hebrew University of Jerusalem.

A gastroretentive dosage form (GRDF) was developed using a mixture of degradable polymers that are approved for human use. The mechanism of retention in the stomach is due to the size of the dosage form and its mechanical properties. The objective of this work was to assess the effect of the sustained release of riboflavin via GRDF on its bioavailability and pharmacokinetic profile in comparison to standard oral and intravenous modes of administration in dogs.

Six beagles that were deprived of food for at least 18 hrs (with water available *ad libitum*) received 100 mg riboflavin-5-phosphate by four different modes of administration: (1) 5ml of sterile isotonic solution of the drug by intravenous bolus. Concurrent oral administration of 400 ml buffer solution (HCl-KCl, pH = 1.5) was delivered to the stomach by a gastric tube; (2) Per-oral bolus solution of the drug in 400 ml of the same acidic buffer solution; (3) A conventional controlled release tablet of riboflavin in 400 ml of the buffer solution. (4) GRDF, in a size of 5 cm X 2.5 cm containing polymer fragments with high mechanical properties and a drug loaded polymeric matrix that released the drug *in-vitro* in a sustained release manner. The GRDF was folded into a gelatin capsule that was administered into the stomach together with 400 ml of the buffer solution. All of the dogs received the drug by each of these modes, with at least one-week washout period between each phase of the study. Analysis of riboflavin in dog plasma was performed using a HPLC method.

The GRDF administration caused a decrease in the rate of riboflavin absorption when compared with the other oral or intravenous routes. Riboflavin concentrations remained above physiological riboflavin levels for over 48 hours when administered via GRDF, vs. less than 10 hours when given orally via the controlled release form and 6 hours when given orally as a bolus. The absolute bioavailability of riboflavin following oral administration of the drug solution was found to be  $5.8 \pm 2.2\%$ . Mathematical calculations show that the GRDF increases the bioavailability of riboflavin by more than 4 fold.

We conclude that the GRDF mode of administration is possible in dogs, it allows the slow release of riboflavin, increasing bioavailability when compared to other conventional oral methods of delivery. This may be a future method for the enhanced absorption of medications with a narrow absorption window.

### 133 THE EFFECT OF INJECTION VOLUME ON THE PHARMACOKINETICS OF CARPROFEN (RIMADYL®) ADMINISTERED SUBCUTANEOUSLY IN DOGS. Huhn, J.C., Clark, T.P., Nimz, E., and C. Wang. Pfizer Global Research and Development, Pfizer, Inc., Groton, CT.

A comparative study was conducted in dogs to evaluate the effect of subcutaneous injection volume on the disposition of Rimadyl®. Eighteen male beagles were randomized into one of two treatments in a two-sequence, two-period cross-over design with a ten-day washout between periods. Rimadyl® (50 mg/mL solution) was administered as a single subcutaneous injection at a dosage of 2 mg/lb (0.04 mL/lb) in one subcutaneous site or divided equally between two separate subcutaneous sites. Blood samples were collected from dogs within one hour prior to treatment and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 8, 12, 24, and 48 hours after administration of Rimadyl® within each period. Plasma concentrations of Rimadyl® were determined using a specific, validated, high performance liquid chromatography (HPLC) method with fluorescence detection that was accurate and reproducible in the range of 0.25 to 50 µg/mL. Plasma concentration data were transformed to natural logarithms and geometric means were calculated for maximum plasma concentration ( $C_{max}$ ) and area under the concentration-time curve ( $AUC_{0-12h}$  and  $AUC_{0-\infty}$ ). For each variable, the 90% confidence interval was constructed on the difference between the mean of the two-site administration and the one-site administration divided by the mean of the one-site administration. Variables were considered statistically similar if the 90% confidence interval of the mean difference was within -20% and 25%. The mean  $C_{max}$  was 12.4 µg/mL following a one-site subcutaneous injection and 13.4 µg/mL following a two-site subcutaneous injection. The mean  $AUC_{0-12h}$  was 172.0 µg hr/mL and 155.9 µg hr/mL following a one-site and two-site subcutaneous injection, respectively and the mean  $AUC_{0-\infty}$  was 180.1 µg hr/mL and 163.3 µg hr/mL after a one-site and two-site subcutaneous injection, respectively.  $C_{max}$  was statistically similar between one- and two-site subcutaneous injections (-3.7% to 21.4%).  $AUC_{0-12h}$  and  $AUC_{0-\infty}$  fell slightly outside the lower boundary of the confidence interval (-21.0% and -20.1%, respectively), but were essentially equivalent.

This study supports the premise that administering 2 mg/lb (4.4 mg/kg) of Rimadyl® results in similar peak plasma concentrations and drug exposure compared to administering 1 mg/lb (2.2 mg/kg) into each of two subcutaneous sites. It is concluded that subcutaneous absorption of Rimadyl® in dogs is minimally affected by the injection volume deposited into a single site.

### 134 MILK PROTEIN CONCENTRATE FROM HYPERIMMUNIZED COWS EXPRESSES ANTI-INFLAMMATORY ACTIVITY AND CLINICAL UTILITY IN OSTEOARTHRITIS. D.A. Gingerich, J.P. Fuhrer, K.M. Kiser, J.D. Strobel, R.D. Stohrer, C.A. McPhillips, Stolle MBI, Cincinnati, OH, J.L. Zenk, Minnesota Applied Research Center, Chanhassen, MN.

Skim milk from hyperimmunized cows expresses anti-inflammatory activity and exhibits beneficial effects in humans with rheumatoid arthritis. However, intact milk consists of approximately 50% lactose by weight, making it unsuitable for therapeutic use in adults of some common species of domestic animals. We therefore prepared lactose-free milk protein concentrate (MPC) from skim milk of hyperimmunized cows by proprietary ultrafiltration methods and conducted tests to determine whether the resulting MPC expresses the same bioactivity as intact skim milk.

Anti-inflammatory activity of MPC was tested in classic pharmacologic models including topical application of organic extracts of MPC in the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear inflammation assay. Long term effects were tested in autoimmune disease-prone MRL/lpr mice given 0, 0.5, or 5% MPC w/w in the feed. Clinical effects were tested in human patients with osteoarthritis given capsules containing MPC, placebo, or glucosamine and evaluated using the Western Ontario MacMaster University Osteoarthritis Index (WOMAC).

Dose-dependent inhibition of inflammation, which reached more than 60% inhibition at higher doses, was repeatedly demonstrated in the TPA mouse ear edema assay. In the MRL mouse model, MPC feeding resulted in significant inhibition of the increase in rheumatoid factor and delayed the onset of proteinuria and development of lupus-like skin lesions, and increased survival. Results of a dose determination study in human subjects with osteoarthritis showed that 2g of MPC given orally twice daily significantly inhibited symptoms of osteoarthritis. In a six-week, double-blind, placebo-controlled clinical trial in human patients, MPC significantly inhibited symptoms of osteoarthritis relative to placebo and was slightly superior to glucosamine tested as a positive control in the same trial.

These results indicate that the anti-inflammatory bioactivity of intact skim milk from hyperimmunized cows has been captured in MPC. We therefore conclude that evaluation of MPC in inflammatory conditions such as osteoarthritis in dogs, horses, and other species is justified.

### 135 LONGTERM FEEDING OF AN ACIDIFYING DIET TO CATS: EFFECT ON BONE DENSITY. B.H.E. Smith, S. Moodie, P.J. Markwell. Waltham Centre for Pet Nutrition, Waltham-on-the-wolds, Leicestershire, UK.

The aim of this study was to assess whether a diet with a relatively low calcium content, which is designed to acidify urine, can maintain bone density in adult cats.

Twenty-four healthy adult cats were assigned to one of two age and sex matched groups. Group 1 were fed a dry diet designed to result in production of acidic urine; three separate batches with calcium contents of 0.63, 0.51 or 0.41g/400 kcal were fed over the course of the study. Group 2 was fed a dry diet designed to produce a neutral urine pH with a calcium content of 1.14 g/400 kcal. Both diets were fed for 18 months. Initially food allowances were based on estimated maintenance energy requirements (60kcal/kg bwt/d), with subsequent adjustments to control body weight. Whole body composition, compartmentalized to lean tissue, fat and bone expressed as bone mineral content (BMC) and density (BMD), were assessed by dual energy x-ray absorptiometry (DXA) at time 0 and subsequent 6 monthly intervals (Table). Data were analyzed using repeated measures analysis.

The acidifying diet resulted in a mean urine pH of  $6.36 \pm 0.37$  which was significantly lower ( $p < 0.05$ ) than the  $6.98 \pm 0.37$  produced by feeding the control diet.

Time	Group 1		Group 2	
	BMD g/cm <sup>2</sup>	BMC g	BMD g/cm <sup>2</sup>	BMC g
Pre trial	0.295 ± 0.032	126.71 ± 36.54	0.292 ± 0.031	113.96 ± 36.42
6 months	0.309 ± 0.028	138.33 ± 36.49	0.306 ± 0.034	125.68 ± 39.52
12 months	0.307 ± 0.027	140.19 ± 35.06	0.310 ± 0.033	127.18 ± 39.77
18 months	0.315 ± 0.029	143.88 ± 34.14	0.314 ± 0.030	130.41 ± 40.15

BMD and BMC did not differ between the two groups at the start of the study and no significant differences were present either within or between groups at subsequent time.

It was concluded that calcium content of 0.41–0.63g/400 kcal in an acidifying diet was adequate to maintain BMC over an 18-month period for adult cats.

### 136 IN VITRO ALTERATION OF CANINE NEUTROPHIL MEMBRANE FATTY ACID COMPOSITION, FLUIDITY, AND FUNCTION: A MODEL FOR DIETARY INTERVENTION. ML Waldron, SS Hannah,<sup>1</sup> JE Bauer,<sup>2</sup> Ralston-Purina Co., St. Louis, MO, <sup>2</sup>Texas A&M University, College Station, TX.

In vitro experiments were conducted in which the n-3 fatty acid composition of isolated canine neutrophils were modified. Their phagocytic function and membrane fluidity were evaluated and compared pre- and post-modification. Comparison was also made to canine neutrophils that had been modified in a separate study by fish oil feeding for 28 days (1.2 % DM eicosapentaenoic acid, EPA; 0.4% DM docosahexaenoic acid; 40% energy from fat). Neutrophils for the in vitro study were obtained from dogs fed a diet whose primary ingredients were corn, poultry-by-product meal, soybean meal, and animal fat containing small amounts of n-3 fatty acids. The freshly isolated cells ( $5 \times 10^6$  cells) were incubated with 2.4 mg EPA complexed with bovine serum albumin (molar ratio of 4:1) at 37 °C for 40 minutes ( $n = 8$ ). These conditions resulted in increased EPA enrichment compared to the native cells with a corresponding reduction in arachidonic acid (AA) while retaining cell viability. Prior to incubation the AA content of cells ranged from 14.8–19.2%. After incubation AA content ranged from 9.8–14.5% and EPA content ranged from 3.1–5.3%, a 33% increase compared with untreated cells. These amounts closely corresponded to neutrophils from dogs who had been fed the menhaden fish oil diet.

The in vitro modified cells were found to have significantly increased membrane fluidity compared to the native cells as determined by calculated anisotropy using the lipid probe (TMA-DPH) and fluorescence polarization techniques ( $0.2766 \pm 0.006$  native vs  $0.02640 \pm 0.0001$  modified,  $p < 0.001$ ). Significant increases in phagocytosis were also found in the modified cells using fluorescently labelled microspheres/flow cytometry. The percentages of cells that phagocytosed 2, 3, and 4 beads was significantly increased while the percentage of cells taking up 0 bead was significantly reduced ( $p < 0.02$  or better). Also, a linear relationship between anisotropy and phagocytosis was found ( $R^2 > 0.5$ ,  $p < 0.05$ ).

These findings are similar to those observed in neutrophils from 10 dogs fed the fish oil diet for 28 days. It is concluded that this in vitro cellular fatty acid modification technique can serve as a useful alternative to the more tedious and time consuming feeding studies generally used to assess diet fat effects on cell functions.

# 137 EFFECT OF DIETARY PROTEIN ON BODY COMPOSITION AND METABOLIC RESPONSES OF GERIATRIC AND YOUNG-ADULT DOGS. G.M. Davenport,<sup>2</sup> S. Gaasch,<sup>1</sup> M.G. Hayek<sup>2</sup> and K.A. Cummins.<sup>1</sup> <sup>1</sup>Animal & Dairy Sciences, Auburn University, AL 36849 and <sup>2</sup>The Iams Co., Lewisburg, OH 45338.

A study was conducted to evaluate differences in body composition, nitrogen (N) balance, and serum insulin-like growth factor-I (IGF-I) concentrations in young-adult and geriatric dogs fed different levels and/or sources of dietary protein. Female beagles averaging one (n = 12) or twelve (n = 11) years of age were fed nutritionally-adequate diets containing 16 or 32% protein derived from poultry meal, or a 32% protein diet containing one-half the protein from poultry meal and the remainder from corn gluten meal. Diets were fed during a 60-day experimental period. Body composition was assessed using dual energy x-ray absorptiometry (DXA) at the beginning and end of the study. N balance and serum IGF-I concentrations were determined at the end of the study.

Results showed that serum IGF-I was highest (P<.05) in geriatric dogs fed the 32% protein diet containing poultry meal compared with the other diets. Geriatric dogs fed the 32% protein diets retained more N (P<.01) than those fed the 16% protein diet. DXA results showed that increased IGF-I and N balance in geriatric dogs fed 32% protein was accompanied by greater (P<.05) percentages of lean tissue mass and lower (P<.05) percentages of body fat compared with dogs fed 16% protein. Changes in body composition of the young-adult dogs were similar for all diets, with more N retained (P<.01) by the young-adult dogs fed 32% protein diet containing both protein sources.

It can be implied from these results that the high-protein, meat-based diet improved the nutritional status of the geriatric dogs compared with a diet containing similar levels of dietary protein but containing a large portion of vegetable protein. This improvement can be associated with the preservation of lean body mass and loss of body fat as geriatric dogs consume diets with increased levels of animal-based protein.

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# 138 EFFECTS OF DIETARY OXIDIZED LIPIDS ON GROWTH AND IMMUNE FUNCTION OF YOUNG SPECIFIC PATHOGEN-FREE CATS L. Guptill,<sup>1</sup> J. Turek,<sup>1</sup> M. Hayek,<sup>2</sup> J. Christian,<sup>1</sup> B. Watkins,<sup>1</sup> A. Dunham,<sup>1</sup> S. Massimino,<sup>2</sup> I. Schoenlein,<sup>1</sup> CG Aldrich,<sup>2</sup> M. Horgan,<sup>2</sup> J. Stephens,<sup>1</sup> J. Wang.<sup>1</sup> <sup>1</sup>Purdue University, W. Lafayette, IN; <sup>2</sup>The Iams Company, Lewisburg, OH.

In young dogs, dietary oxidized lipids impair weight gain; alter bone metabolism, eicosanoid production, and essential fatty acid concentration; and impair neutrophil oxidative burst and peripheral blood mononuclear cell (pbmc) mitogen response. The feline study reported here was undertaken to test the hypotheses that dietary oxidized lipids negatively affect bone growth and immune function, deplete antioxidants, and increase erythrocyte oxidative stress in cats. Three groups of 8 specific pathogen-free cats aged 4.5 months were fed 1 of 3 diets (non-oxidized diet (control), low-(LO), or high-(HO) oxidized lipid diet) for 16 weeks. Growth, food consumption; pbmc mitogen response; cell membrane lipid peroxidation; blood monocyte and neutrophil oxidative burst; skin eicosanoid production following lipopolysaccharide stimulation; serum biochemistry analysis, complete blood counts (CBC), and blood methemoglobin (Mhb) were measured. Results were evaluated using repeated measures analysis of variance and Newman-Keuls or Tukey multiple comparison tests.

All cats gained the same amount of weight at the same rate. Control cats ate less food than LO or HO cats (P<0.001). Cats had normal CBCs when fed the control diet with no differences among groups. Hematocrit (hct), hemoglobin (hb), erythrocyte count (rbc) and mean corpuscular hemoglobin concentration (mchc) were lower in HO cats than control cats or LO cats after 8 weeks (P<0.001 and P = 0.02). After 16 weeks hct, hb, and rbc of LO and HO cats were lower than control cats (P<0.001); mchc and mean corpuscular volume were lower in HO than in control or LO cats (P = 0.009). No diet-associated compromise in Mhb reduction was noted for resting or oxidant-challenged cells. Serum chemistry values were within reference ranges with no significant differences among groups except for serum albumin and phosphorous. At 8 and 16 weeks, albumin was normal but lower in HO cats than control or LO cats (P = 0.005), and phosphorous was lower in HO cats (P = .01). All cats had normal pbmc responses to mitogens, with no significant differences among groups. There was no significant difference in monocyte or neutrophil oxidative burst or in skin PGE2 or PGE3 production. There was a dose-response difference in skin leukotriene B4 (LTB4) and LTB5 production, with the greatest response in control cats (P = 0.001).

Cats fed dietary oxidized lipid required more food for growth. Dietary oxidized lipids decreased erythrocyte mass, mcv, and mchc. There was no obvious effect of dietary oxidized lipids on phagocyte oxidative burst or lymphocyte blastogenesis. Dietary oxidized lipids decreased leukotriene production in skin.

# 140 EFFECTS OF ACE-INHIBITION ON THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM AND BLOOD PRESSURE IN CATS WITH HYPERTENSION ASSOCIATED WITH CHRONIC RENAL DISEASE. JL Steele, RA Henik, RL Stepien, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI.

Hypertension (indirect systolic arterial blood pressure [BP] > 170 mmHg) associated with chronic renal disease (CRD) is a frequently recognized condition in cats and may be in part due to excessive activation of the renin-angiotensin-aldosterone system (RAAS). Angiotensin-converting enzyme inhibitors (ACE-I) such as enalapril (EN) or benazepril (BEN) are commonly administered to decrease BP, presumably by decreasing aldosterone (ALDO) concentrations and thereby decreasing sodium and water retention. The purpose of this study was to determine the effects of two ACE-I on BP and the RAAS in hypertensive cats with CRD.

In two separate studies, baseline PRA, ALDO, and BP were measured in a total of 16 cats. Six cats received EN (mean dose: 0.36 ± 0.23 mg/kg/d, po) in the first study; 10 cats received BEN (mean dose: 0.64 ± 0.27 mg/kg/d, po) in study 2. After 2 weeks, BP was measured; if BP in individual cats remained ≥ 170 mmHg, ACE-I dose was doubled and BP was re-evaluated 2 weeks later. If BP was still ≥ 170 mmHg, these cats were given additional antihypertensive medication and excluded from further comparisons regarding ACE-I. PRA, ALDO, and BP were measured in 16 cats at week 2, and in the 8 cats receiving only ACE-I at week 8 (time from baseline); mean values of these measurements were compared to mean baseline values. Measurements were also compared to reference ranges for PRA (0.34–5.30 ng Ang I/ml/hr) and ALDO (7.08–104.4 pg/ml) obtained from 10 clinically and biochemically normal cats. Mean PRA values at baseline (1.89 ± 3.30 ng Ang I/ml/hr), week 2 (3.28 ± 5.07 ng Ang I/ml/hr), and week 8 (3.00 ± 1.84 ng Ang I/ml/hr) were within the reference range and the 3 values were not significantly different from each other (P > 0.05). Mean ALDO concentrations at baseline (153.33 ± 132.62 pg/ml) and week 2 (167.29 ± 138.39 pg/ml) were increased compared to the reference range, and the mean ALDO value at week 8 (99.09 ± 74.92 pg/ml) was within the reference range; the 3 values were not significantly different. ALDO values in 11 of 16 cats increased at week 2, and 4 of 8 cats had a further increase in ALDO at week 8. Mean BP measurements at week 2 (192.1 ± 34.0 mmHg; range, 107–240) and week 8 (171.8 ± 40.0 mmHg; range, 140–260) were not significantly different from the mean baseline BP (205.8 ± 27.4 mmHg; range, 178–260). These results suggest that PRA, ALDO concentration, and BP in cats with hypertension associated with CRD do not change significantly with administration of EN or BEN.

# 141 EVALUATION OF DOPPLER ULTRASONIC AND OSCILLOMETRIC ESTIMATES OF BLOOD PRESSURE IN CONSCIOUS DOGS. Scott Brown, Christopher Haberman, and Jefferson Morgan, University of Georgia, Athens, GA.

To evaluate the utility of methods for the indirect measurement of systemic arterial pressure in conscious dogs, results of over 300 measurements by an oscillometric device (Dinamap Model 8300) or a Doppler ultrasonic flowmeter (Parks Model 811) were compared to simultaneously obtained values using radiotelemetry (Model TA11PA-D70 Implants, Data Sciences International) in 12 beagle dogs.

The correlation between indirect estimates and direct values for blood pressure parameters ranged widely for different indirect methods and sites of cuff placement, and the highest correlation between indirect estimates and directly measured values occurred when indirect estimates were obtained as the average of 5 consecutive values. Obtained in this manner, the correlations for the oscillometric device with cuff placement at the coccygeal artery site were: mean arterial pressure,  $R^2 = 0.854$ ; systolic arterial pressure,  $R^2 = 0.886$ ; diastolic arterial pressure,  $R^2 = 0.901$ ,  $P < 0.0001$  for all parameters. For the Doppler device using the average of 5 consecutive measurements at the median artery for systolic arterial pressure, the  $R^2$  value was 0.810 ( $P < 0.0001$ ). Both indirect methods underestimated all blood pressure parameters. The mean difference between the direct and indirect devices ranged from 5 to 20 mmHg for the oscillometric device and from 8–15 mmHg for the Doppler device. As arterial pressure increased, the degree of underestimation increased with both indirect devices. For both devices, the correlation between indirect estimates and direct measurements obtained when 5 consecutive indirect estimates were averaged provided a good correlate of blood pressure. Used in this manner, the devices can provide an reliable approximation of blood pressure in conscious dogs.

# 143 COMPARATIVE SEQUENCE OF FELINE ENDOTHELIN-1 (ET-1). A.W. Biondo, C. E. Wiedmeyer, P.F. Solter, D.D. Sisson. College of Veterinary Medicine, University of Illinois, Urbana-Champaign.

Endothelin-1 is a potent vasoconstrictor peptide that has potential value as prognostic and diagnostic aid in various forms of cardiovascular disease. Our laboratory is investigating the role played by Endothelin-1 in heart disease in cats and searching for potential interspecies crossreactivity of antibodies to these peptides. In this study, we report on the comparative sequence of feline Endothelin-1 (ET-1) and its precursor, big Endothelin-1 (big ET-1). Total RNA was extracted from feline heart tissue to make complementary DNA (cDNA) by reverse transcription using oligo-T primers. Consensus ET-1 gene sequences of known species were identified and used as primers (5'TGCTGTTTGTGGCTTCCAAGG3' and 5'GGCAAAATTCAGCACITCTTG 3') in PCR reaction to amplify a portion of cat ET-1. A cDNA product of approximately the predicted length was obtained (about 320 bp) and subsequently sequenced. The final nucleotide sequence of feline ET-1 and big ET-1 were verified using different primers and sample sources, including genomic DNA (gDNA) from blood. The final sequence was submitted to Genbank (AF320770).

The putative coding sequence of feline big ET-1 is 39 amino acids long and contains 87% to 97% homology with the known sequences from other species (mouse, guinea pig, rat, human, cow, pig and dog). The putative physiologically active portion of feline ET-1 is 21 amino acids long (CSCSSLLDKECVYF-CHLDIIW) and arises from a single exon. Although the ET-1 amino acid sequence is identical among several mammalian species (mouse, guinea pig, rat, human, cow, dog and pig) the cat ET-1 sequence differs at position seven, where it has a leucine residue rather than methionine. These findings suggest that big ET-1 and ET-1 antibodies and immunoassay kits developed for use in other species may need to be validated for crossreactivity with the respective feline ET-1 peptide before use.

# 142 SIGNAL-AVERAGED ECG IN THE ASSESSMENT OF ARRHYTHMOGENIC CARDIOMYOPATHY IN BOXERS. AW Spier, KM Meurs, CG Linn. Ohio State University, Columbus OH.

Arrhythmogenic cardiomyopathy is an inheritable disease of Boxers characterized by the development of ventricular arrhythmias resulting in syncope or sudden death. Identification of animals at risk for developing fatal arrhythmias facilitates early therapeutic intervention and guides appropriate breeding decisions. In people, signal-averaged ECGs (SAECGs) have been used to identify patients at risk for sudden death secondary to ventricular arrhythmias.

A 24 hour ambulatory electrocardiogram (AECGs), echocardiogram, and SAECG were performed on 62 Boxers. Total number of ventricular premature complexes (VPCs) were obtained from AECG, and dogs were grouped into one of three categories: group 1 =  $\leq 10$  VPCs/24 hrs, Group 2 = 11–100 VPCs/24 hrs, and Group 3 =  $> 100$  VPCs/24 hrs. Animals with echocardiographic evidence of myocardial systolic dysfunction were placed into group 3. Patients with acquired valvular or congenital disease were excluded. From SAECG analysis, filtered QRS duration (QRSd), low amplitude signal duration (LAS) and root mean square of the terminal 30 and 40 ms (RMS30 and RMS40, respectively) using a 25Hz and 40Hz high pass filter were obtained for each patient. SAECGs that did not achieve a final noise of  $< 0.75\mu V$  were excluded from the study. A one-way analysis of variance (ANOVA) was performed for each SAECG parameter at both frequencies.

A significant difference was identified for RMS40 and RMS30 at both 25 and 40Hz. Post hoc analysis revealed that RMS40 at 40Hz identified differences between groups 1 and 2 as well as between groups 1 and 3, but not between groups 2 and 3, whereas RMS30 at both 25 and 40Hz, and RMS40 at 25Hz only identified differences between groups 1 and 3.

We conclude that RMS values obtained from SAECG may be useful to identify animals with ventricular arrhythmias that may be at an increased risk for sudden death.

# 144 AORTIC VALVE DISEASE IN BOXERS WITH PHYSICAL AND ECHOCARDIOGRAPHIC FINDINGS OF AORTIC STENOSIS. Jonathan A. Abbott,<sup>1</sup> Robert Duncan,<sup>1</sup> Edward G. Clark,<sup>2</sup> R. Lee Pyle,<sup>1,†</sup> Virginia Tech, Blacksburg, VA. <sup>2</sup>Prairie Diagnostic Services Inc., Western College of Veterinary Medicine, Saskatoon, SK.

Canine aortic stenosis (AS) most commonly results from a fibrous subvalvular ring; valvular aortic stenosis is thought to be uncommon. Boxers are predisposed to the development of subvalvular AS; in this breed, the lesion is believed to be heritable.

Seven cardiac specimens obtained from three western Canadian boxer kennels were collected. Six of these were obtained from dogs identified ante-mortem during screening evaluations for AS; in each case, Doppler echocardiographic examination yielded equivocal findings or substantiated a diagnosis of mild AS. One of the dogs died suddenly and was not examined by the authors ante-mortem. Echocardiographic measurements were obtained retrospectively from VHS videotape recordings of studies performed a variable time prior to sudden death (one dog) or euthanasia (5 dogs) for non-cardiac cause. Measurements from three cardiac cycles were averaged. The following variables were recorded: left ventricular outflow tract (LVOT) velocity, left ventricular diastolic dimension (LVIDd), and aortic diameter at the annulus (Aoa) and sinotubular junction (Aos). The latter two dimensions were normalized to LVIDd (Aoa/LVIDd, Aos/LVIDd).

The age at death or euthanasia ranged from 0.25 to 10 years (mean 5.7). All of the specimens had opaque, irregularly thickened aortic valve leaflets; the valve surfaces were rough and there were ridges on the ventricular surface of one or more valve leaflets. One of the specimens had the additional finding of a subvalvular fibrous ring that extended less than 50% of the outflow tract circumference. Histologically, the valve leaflets had deposition of connective tissue ranging from myxomatous to collagenous with occasional cartilage metaplasia. Interstitial macrophages had cytoplasmic hemosiderin accumulation in three of the cases. Myocardial changes were present in three of the cases and included myocyte hypertrophy, nuclear hypertrophy, nuclear cleavage, multinucleation and perinuclear lipofuscin accumulation. The mean peak LVOT velocities ( $\pm$  standard deviation, range) were: 2.15 ( $\pm 0.28$ , 1.92–2.67) m/s. Based on the Wilcoxon rank sums test, the Aoa/LVIDd and Aos/LVIDd obtained in the boxers were lower than those obtained in a population of 9 healthy mixed-breed dogs ( $p < 0.05$ ).

These findings suggest a morphologic heterogeneity of the LVOT in boxers with systolic ejection murmurs; in some dogs, valvular disease, not subvalvular narrowing, is associated with physical and echocardiographic findings of mild aortic stenosis. In the group of boxers studied, aortic diameters may be abnormally small.

# 145 SEVERE HYPERTROPHIC CARDIOMYOPATHY IN 10 YOUNG RAGDOLL CATS. B. K. Lefbom, S.L. Rosenthal, W. D. Tyrrell Jr., T. G. Saunders, M. J. Ferguson, J. E. Rush,\* M. B. Lesser,\* Chesapeake Veterinary Cardiology Associates, Annapolis, MD. \*Tufts University SVM, N. Grafton, MA. †Advanced Veterinary Care, Lawndale, CA.

Hypertrophic cardiomyopathy (HCM) is the most common heart disease in the cat. It is known to be a heritable disorder in certain cat breeds and in humans. We describe 10 cases of a severe form of HCM in Ragdoll cats.

Retrospectively, data was collected from the medical records of 10 Ragdoll cats that were diagnosed with HCM at 3 different hospitals from May of 1997 to May of 1999. The diagnosis of HCM was made by a board-certified veterinary cardiologist based on physical examination, thoracic radiographs, echocardiographic, and clinical findings consistent with HCM.

Severe concentric left ventricular hypertrophy was evident in every cat. In each case the diagnosis was made at a young age (mean age 15 months; range 5 months – 2 years), with 6 cats presenting at less than 1 year of age. Six of 10 cats presented in congestive heart failure with radiographic findings of pulmonary edema (6 of 6) and/or pleural effusion (2 of 6). The other 4 were asymptomatic and cardiac work-up was performed following identification of a loud systolic heart murmur. Seven of 10 cats were male. Cardiac arrhythmia was not identified in any cat. Eight of 10 cats had a systolic murmur, 4 had a gallop, and 2 had a systolic click. Echocardiographic exam identified concentric left ventricular hypertrophy in each case. 7 of 10 cats had marked interventricular septal (IVS) hypertrophy with diastolic IVS thickness of  $\geq 0.73$  cm (mean 0.88 cm, range 0.73 to 1.14 cm for these 7 cats). Four of 10 cats had systolic anterior motion of the mitral valve. Seven of 10 cats had left atrial to aortic root ratios of over 2.2. The average left atrial dimension in systole of these 7 cats was 1.97 cm. These same seven cats had small aortic root dimensions of less than 0.85 cm.

Ragdoll cats appear to be a breed at risk for hypertrophic cardiomyopathy. Cats in this case series developed severe disease at a young age. Further research is warranted to investigate the potential familial basis for this disease in the Ragdoll cat.

# 147 MEASUREMENT OF CORTICOID CONCENTRATIONS IN NON EXTRACTED CANINE URINE BY CHEMILUMINESCENCE. Gaby Hoffmann, Jason Arble, Joseph Taboada, Giselle Hosgood, Karen J. Wolfsheimer,\* School of Veterinary Medicine, Louisiana State University, \*Endocrine Diagnostics & Consultation, Baton Rouge, LA.

Urinary corticoid:creatinine ratio (UCCR) has previously been reported using a radioimmunoassay (RIA) for measurement of corticoids from non extracted urine in dogs. Chemiluminescence is an alternate automated assay technique that is easy to perform and readily available through current laboratories. This study compared results obtained by chemiluminescence on non-extracted urine to results obtained by RIA. In addition a normal reference range for the UCCR for dogs using the Immulite chemiluminescence assay technique was established.

Morning voided urine samples were collected at home from 19 healthy dogs of either sex, ranging in age from 1–15 years. Samples were kept refrigerated, then aliquots were obtained and frozen at  $-20^{\circ}\text{C}$ . Corticoid concentration was measured by chemiluminescence (Immulite, Diagnostic Products) on unaltered urine using the routine protocol for serum samples and by DPC Coat-A-Count RIA (Diagnostic Products) on both extracted and non extracted urine. Estimates of dilutional parallelism, accuracy and precision were made using pooled samples of canine urine. Urinary creatinine concentrations were determined by use of the Jaffe method.

The data was evaluated for agreement in the absence of a true known value and for correlation using Spearman's rank correlation. There was good agreement between Immulite and RIA on non extracted urine, between Immulite and RIA on extracted, and between RIA on extracted and nonextracted urine. Comparison between Immulite and RIA on non extracted urine revealed significant association ( $p = 0.0002$ ) and moderate correlation ( $r = 0.75$ ). Good precision was achieved with an intra- and inter-assay coefficient of variation of 6.4%, 3.3%, and 4.7%, and 7.5% respectively for a mean concentration of 1214.4, and 129.9 nmol/l. Accuracy was evaluated by adding known amounts of cortisol to urine and revealed 107.1, 105.8, and 108.8% of expected concentrations. Dilutional parallelism for evaluation of specificity measured 97.3, 104 and 105.4% of expected concentrations. Biologic specificity was demonstrated by significantly higher UCCR results of 19 sick dogs compared to 19 healthy dogs. A normal reference range for UCCR of less than 32.2 was established, comparable to ranges reported for RIA on non extracted urine.

cortisol	median	range	urine C:C ratio	median	range
immulite	218 nmol/l	80.2-1094	immulite	14.02	3-33.04
RIA nonextr.	165.5 nmol/l	38.6-1029.4	RIA nonextr.	12.27	2.25-35.35
RIA extracted	29 nmol/l	12.7-235.1	RIA extracted	1.83	0.73-7.54

# 146 CARDIAC HISTOPATHOLOGIC AND ELECTROCARDIOGRAPHIC CHANGES IN CANINE BABESIOSIS E. Dvir, R.G. Lobetti, J. Pearson and L.S. Jacobson. Fac. of Vet. Science, Univ. of Pretoria, South Africa.

Electrocardiographic changes have never been described in canine babesiosis. Based on the metabolic, electrolyte, and myocardial alterations described for the disease, such changes are to be expected. The purpose of this study was to describe ECG changes in canine babesiosis, and to correlate those changes to clinical severity, outcome and cardiac histopathological changes.

Four groups of babesiosis dogs were studied: mild to moderate anemia ( $n = 40$ ), severe anemia ( $n = 35$ ), concurrent immune-mediated hemolytic anemia ( $n = 18$ ) and complicated organ involvement ( $n = 28$ ). Lead II ECG was recorded at admission for 1 minute in all dogs, and repeated after 24 hours in admitted dogs. Six leads were recorded in 88 dogs. Full necropsy was performed between 30–60 minutes after death on 16 dogs (5 died on arrival, 11 had ECG recording). Gross cardiac pathology was recorded and histopathology of myocardial sections from ventricles, atria, apex and interventricular septa was evaluated.

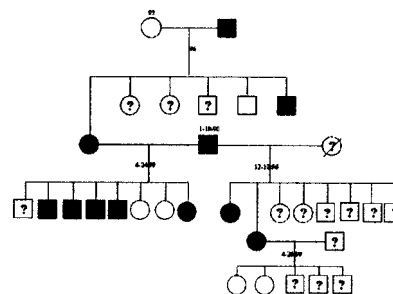
The following ECG changes were recorded: SA (7%) and AV blocks (4%), VPCs (7%), low R-amplitude (23%), prominent Q (33%), axis deviations (40%), prolonged QRS (32%), ST depression and coving (28%), large T (42%), and notched R (28%). Differences between groups were minor and inconsistent. Pathological changes were pericardial effusion (25%) and subpericardial (56%) and subendocardial hemorrhages (63%). Histological changes were hemorrhages (69%), necrosis (50%), inflammation (63%) and fibrin thrombi (75%). The only correlation between pathology and ECG was low R-amplitude and pericardial effusion. There was a significantly higher incidence of sinus bradycardia and sinus irregular rhythm in the non-survivors.

Both ECG and pathological changes were non-specific, but there were similarities to the pattern of changes described for myocarditis and myocardial ischemia. Anti-arrhythmic treatment was only required in 1 dog. Thus, the clinical application of the ECG changes found in this study was limited. However, it was concluded that the heart suffers from the same pathological processes described in other organs in canine babesiosis, namely inflammation and hypoxia. Cardiovascular management, if necessary, should be based on functional monitoring rather than ECG.

# 148 COMPARISON OF BLOOD SPOT AND SERUM SAMPLES TO DETECT TgAA AND ASSESSMENT OF HEREDITY OF THYROIDITIS IN WELSH SPRINGER SPANIELS Nachreiner RF, O'Keefe CM,\* Sislak MD, Graham PA, Refsal KR, and Provencher-Bolliger A. Animal Health Diagnostic Laboratory and Large Animal Clinical Science, Michigan State University, East Lansing MI, and Springfield IL.\*

Blood spots collected on uniform thickness filter paper and sera were analyzed for presence of thyroglobulin autoantibody (TgAA) using a commercially available ELISA (Oxford Laboratories). Two disks of the air-dried blood spots were punched out with a paper punch and incubated overnight in assay buffer at 4C to extract the serum. Then the TgAA assay was performed according to serum specifications. Serum total T4 and TSH were also assayed. Clinically normal Welsh Springer Spaniels (53) were tested. Five dogs tested positive for TgAA. The correlation between the blood spot and serum procedures was 0.99 ( $P < 0.01$ ). Two dogs with positive TgAA were from one breeding line, so additional relatives were tested. A review of the pedigree was performed. Dogs with positive TgAA and/or low T4 and high TSH were considered to be affected. Additionally, 2 of the affected dogs had thyroid biopsies performed and severe lymphocytic thyroiditis was found. The results indicate that thyroiditis is a heritable disease and likely to be autosomal recessive, though a dominant trait cannot be ruled out with the present data.

circle = female, square = male, open = negative, closed = affected  
? = unknown, / = dead.



# 149 COMPARISON OF GLUCOSE MEASURING DEVICES IN CATS. L.A. Cohn, D.L. McCaw, D. Tate. University of Missouri-Columbia, College of Veterinary Medicine.

This study was designed to evaluate the accuracy of available glucose measuring devices as commonly used in cats. Comparisons were made between blood glucose results obtained from each device and those obtained on the same sample using a standard dry chemistry laboratory unit (Ortho-Clinical Diagnostics *vitros* 500) as the gold standard via a simple linear regression. To estimate the difference between glucose values obtained from each device and from dry chemistry, 95% confidence intervals (CIs) for the median difference were calculated.

Venous samples were assayed according to manufacturers' directions using systems including iSTAT (Heska Sensor Devices), Glucometer Encore<sup>®</sup> (Miles Inc; Enc), ExacTech R.S.G<sup>®</sup> (MediSense, Inc; RSG), Glucometer Elite<sup>®</sup> (Miles Inc; Elite), Glucometer<sup>®</sup> 3 (Miles Inc; G3), and Accu-Chek<sup>®</sup> Easy<sup>®</sup> (Boehringer Mannheim Corp; y). Samples (n = 143; glucose range 53 mg/dl – 865 mg/dl) were collected in lithium heparin tubes and assayed within 10 minutes. In each case the device (m) significantly predicted the dry chemistry value (y), but r<sup>2</sup> values varied. Overall r<sup>2</sup> and linear equation for each device were: iStat r<sup>2</sup> = 0.96; y = m1.05–1.52; Enc r<sup>2</sup> = 0.92; y = m1.10 + 22.25; RSG r<sup>2</sup> = 0.81; y = m0.76 + 74.69; Elite r<sup>2</sup> = 0.95; y = m1.07 + 23.92; Gf3 r<sup>2</sup> = 0.80; y = m0.81 + 18.85; Esy r<sup>2</sup> = 0.92; y = m0.83 + 19.41. The samples were divided into 3 subsets based on glucose values. r<sup>2</sup> values were much lower for all devices in the subset where glucose was <100 mg/dl. Individual results often varied markedly from the dry chemistry value. Median differences between dry chemistry and the devices ranged from –26 mg/dl (95% CI:–33,–14.3)) for Esy to 40 mg/dl (95% CI: (29.63,48.69)) for Enc.

Although all devices were able to predict the dry chemistry glucose values, some provided closer estimates than others, with the iSTAT providing the best accuracy. The accuracy of each device was less at lower blood glucose values. All of the devices had occasional readings markedly different than the dry chemistry unit, which could have led to clinically inappropriate treatment choices.

# 150 EFFICACY AND SAFETY OF CANINSULIN<sup>®</sup>, PURIFIED PORCINE INSULIN FOR TREATING DIABETIC DOGS. WE Monroe, \*DJ Laxton, JL Robertson, VA-MD Regional College of Veterinary Medicine, Blacksburg, VA, \*Intervet Inc., Millsboro, DE.

The purpose of this study was to determine the efficacy and safety of CANINSULIN<sup>®</sup>, a purified porcine insulin produced by Intervet Inc. for treating diabetic dogs.

Fifty-three dogs were treated with CANINSULIN<sup>®</sup> for 60 days after an initial dose determination period. To evaluate efficacy, the study population mean blood glucose concentration from 12 hour glucose curves (glucose determined every 2 hours) performed at time 0 (prior to beginning insulin therapy), time 1 (end of dose determination period), time 2 (30 days after time 1) and time 3 (60 days after time 1) were determined. The mean blood glucose nadir at times 0, 1, 2, and 3 was also evaluated. Clinicians judged if a patient's hyperglycemia was under adequate glycemic control based on improvement in clinical signs of diabetes (PU, PD, and ketonuria) and evaluation of 12-hour blood glucose curves determined at times 1, 2, and 3 compared to study time 0. Safety was evaluated based on serial histories, physical examinations CBCs, serum chemistry profiles, and urinalyses.

The mean 12 hour blood glucose concentration at time 0 was 370 mg/dl. This decreased to 151 mg/dl, 181 mg/dl, and 183 mg/dl at times 1, 2, and 3. Mean blood glucose nadir concentration was 307 mg/dl at time 0. This decreased to 92 mg/dl, 120 mg/dl, and 119 mg/dl at times 1, 2, and 3. Compared to the time 0 incidence, polyuria had resolved in 96% (47/49), 82% (40/49), and 94% (46/49) of patients, and polydipsia had resolved in 96% (48/50), 86% (43/50), and 96% (48/50) of patients at times 1, 2, and 3.

Mean 12 hour blood glucose concentration and mean blood glucose nadir were substantially reduced, with most patient's judged to be under adequate clinical control at times 1, 2, and 3 compared to time 0. No unexpected side effects from treatment were observed.

# 151 EVALUATION OF TOTAL AND IONIZED SERUM MAGNESIUM AND CALCIUM CONCENTRATIONS IN ACIDOTIC AND NON-ACIDOTIC DIABETIC DOGS. T.N. Gillespie, K.J. Drobatz, C.R. Ward, R.S. Hess. University of Pennsylvania, Philadelphia, PA.

Electrolyte abnormalities are well-recognized complications of diabetes mellitus (DM) in human patients. In humans, there is an association between hypomagnesemia and diabetic complications including insulin resistance and refractory hypokalemia and hypocalcemia. Ionized and total serum magnesium and calcium concentrations have not been evaluated prospectively in dogs with naturally occurring DM. The purpose of this study was to prospectively assess total and ionized serum magnesium (Mg) and calcium (Ca) concentrations in dogs with uncomplicated non-acidotic DM (NADM) and dogs with acidotic DM (ADM).

Twenty-one dogs with DM were evaluated and total and ionized Mg and Ca were recorded. Total and ionized Mg and Ca were also measured in 9 healthy control dogs and 9 otherwise healthy trauma patients. DM was diagnosed based on appropriate clinical signs and persistent hyperglycemia with glucosuria. Twenty of the 21 dogs were treated with insulin (1 was euthanized) at the time of the study. Mean insulin dose at the time of the study was 0.50 ± 0.24 units/kg (median 0.44 u/kg; range 0.14 to 0.96 u/kg). Diabetic dogs were divided into acidotic (ADM, 7 dogs) and non-acidotic diabetics (NADM, 14 dogs). Mean pH for the ADM and NADM was 7.27 ± 0.04 (median 7.28 pH; range 7.2 to 7.33 pH) and 7.38 ± 0.02 (median 7.38; range 7.34 to 7.42 pH), respectively. Mean ionized Mg in the ADM and NADM was 0.46 ± 0.10 mmol/L (median 0.48mmol/L; range 0.32 to 0.62 mmol/L) and 0.40 ± 0.06 mmol/L (median 0.41 mmol/L; range 0.31 to 0.53 mmol/L), respectively. Mean total serum Mg in ADM and NADM was 2.39 ± 0.53 mg/dL (median 2.20 mg/dL; range 1.8 to 3.1 mg/dL) and 1.85 ± 0.25 mg/dL (median 1.8; range 1.3 to 2.3 mg/dL). Mean ionized Ca for ADM and NADM was 1.25 ± 0.20 mmol/L (median 1.26; range 0.94 to 1.51) and 1.23 ± 0.06 mmol/L (median 1.25; range 1.14 to 1.32 mmol/L), respectively. The mean total serum Ca for ADM was 9.33 ± 1.32 mg/dL (median 9.0 mg/dL; range 7.9 to 11.3 mg/dL) and for NADM was 9.67 ± 0.66 mg/dL (median 9.75 mg/dL; range 8.7 to 10.8mg/dL), respectively. There was no statistical difference between the ADM and NADM dogs and the control dogs with regards to total and ionized Mg and Ca concentrations.

In conclusion, it does not appear that hypomagnesemia or hypocalcemia are a major concern in dogs with naturally occurring DM, with or without acidosis.

# 152 ENDOGENOUS SERUM INSULIN CONCENTRATION IN DOGS WITH DIABETIC KETOACIDOSIS. S.E. Parsons, K.J. Drobatz, C.R. Ward, R. S. Hess. University of Pennsylvania, Philadelphia, PA.

Dogs with diabetic ketoacidosis (DKA) are believed to have low to undetectable endogenous serum insulin concentrations. These dogs are believed to have a decreased serum insulin concentration compared to dogs with uncomplicated diabetes mellitus. The purpose of this study was to measure endogenous serum insulin concentration in dogs with DKA, and to compare it to serum insulin concentration in dogs with uncomplicated diabetes mellitus (DM), diabetic dogs with ketonuria but no acidosis (KDM), and dogs with non-pancreatic (NP) disease.

Diabetic dogs were included in the study if they had no previous exogenous insulin therapy. Dogs were considered to have diabetes mellitus if they exhibited persistent hyperglycemia and glucosuria. Dogs were further divided into those with DKA (ketonuria with acidosis), those with KDM (ketonuria without acidosis), and those with uncomplicated (DM). Dogs in the NP group consisted of patients hospitalized for a minimum of 48 hours whose diagnostic evaluations revealed illness of systems other than the pancreas.

Data was collected on 42 dogs: DKA (9 dogs), DM (8 dogs), KDM (7 dogs), and NP (18 dogs). Blood samples for measurement of endogenous serum insulin concentration were obtained prior to the administration of exogenous insulin. Blood glucose, pH, HCO<sub>3</sub><sup>-</sup>, urinalysis, signalment, clinical signs, physical examination findings, and concurrent illness were recorded to insure proper group assignment.

Endogenous serum insulin concentrations were lowest in the DKA group (mean = 3.96 uIU/ml ± 2.29, median = 4.36 uIU/ml, range of 0.98–7.13 uIU/ml), followed by the DM group (mean = 6.89 uIU/ml ± 2.69, median = 6.90 uIU/ml, range of 3.57–11.27 uIU/ml), and then the KDM group (mean = 8.76 uIU/ml ± 2.73, median = 5.55 uIU/ml, range = 2.18–17.07 uIU/ml). The difference in endogenous serum insulin concentration among the three groups of diabetic dogs was not significantly different. In the NP group, the mean serum insulin concentration was 13.13 uIU/ml ± 5.94, with a median of 10.52 uIU/ml and a range of 6.1–26.64 uIU/ml. Endogenous serum insulin concentration of all diabetic dogs (DKA, DM, and KDM) compared to NP dogs was significantly decreased (P < 0.001). Endogenous serum insulin concentrations of the DKA group only, and the DM group only, compared to the NP group, were significantly decreased (P = 0.003 and p = 0.017, respectively). Endogenous serum insulin concentration of the KDM group compared to the NP group was not significantly different.

We conclude that dogs with DKA have significantly decreased serum insulin concentration when compared to dogs with non-pancreatic disease. However, dogs with DKA do not have serum insulin concentration that is significantly decreased compared to dogs with DM or KDM.

- 153** COMPARISON OF PROTEASE INHIBITORS FOR STABILIZING PARATHYROID HORMONE AND FREE T4 BY DIALYSIS IN SERUM SAMPLES. M. Sislak, RF Nachreiner, KR Refsal, P Graham, A Provencher Animal Health Diagnostic Laboratory and Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI 48824.

This study tested commercially available protease inhibitors to prevent changes in free T4 by dialysis (FT4D) and degradation of Parathormone (PTH) in serum exposed to shipment conditions. Twelve protease inhibitors were evaluated at the manufacturer's recommended concentration. Serum was incubated at 37C for 5 days, the most extreme condition likely to be encountered during shipment. A sub-sample was removed and frozen every 24 hours (H) to determine by immunoassay the amount of FT4D (pmol/L) and PTH (pmol/L) remaining. Control samples of FT4D increased over 500% at 5 days. While this increase was not completely prevented by any of the protease inhibitors, AEBSF and Pefabloc SC had the lowest increases (<200%). Control samples of PTH decreased to 34% remaining after 24H. Aprotinin, AEBSF (4-(2-AminoEthyl)-Benzene Sulfonyl Fluoride), Pefabloc SC (AEBSF 10X), Complete Mini Tabs-EDTA free, and Leupeptin limited degradation of PTH (>60% remaining after 24H). These five inhibitors were then tested with PTH samples at the recommended concentration at 21C and at five times the recommended concentration at 37C and 21C for 48H each, with a sub-sample removed every 24H. Control samples of PTH at 21C had only 37% of the PTH remaining after 48H. Results indicated that the five inhibitors were better able to prevent degradation of PTH at 21C than at 37C and at five times recommended concentration than at recommended concentration. At 21C incubation, 62–88% of PTH remained at 2 days in samples with protease inhibitor. At 5x recommended concentration, stability improved with 78–>100% PTH remaining. Pefabloc SC and AEBSF most effectively prevented degradation, with >100% of the original PTH remaining after 48H at 21C, suggesting that these protease inhibitors may be the best choice for use during shipment of serum samples for PTH analysis.

- 154** DEVELOPMENT AND VALIDATION OF ASSAYS FOR COMPOUNDS IN FELINE SERUM RELEVANT TO SINGLE CARBON METABOLISM. CG Ruaux, JM Steiner and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, Texas.

Cobalamin (Vitamin B<sub>12</sub>) is an essential cofactor for enzymes involved in the transfer methyl (-CH<sub>3</sub>) and formyl (-CHO) and other single carbon functional groups, to proteins, amino acids and nucleic acids. The biochemical and clinical consequences of cobalamin deficiency are not well characterized in feline patients. The purpose of this study was to develop and validate assays for a marker of tissue level cobalamin deficiency (methylmalonic acid (MMA)), the sulfur amino acids methionine, homocysteine, cystathionine and cysteine, and validate an automated immunoassay for cobalamin in samples of feline serum.

MMA and the sulfur amino acids were measured using stable isotope dilution gas chromatography/mass spectrometry. We validated the assays by investigation of precision, accuracy and reproducibility.

Serum cobalamin was measured using a commercially available chemiluminescent immunoassay system. The manufacturer has extensively validated this assay system for human sera. Our validation of this assay involved assessment of precision, accuracy and reproducibility, to assess the possible effects of binding or competitive substances that may be present in feline serum.

The intraassay coefficient of variation (CV) of the MMA was 12.56%. Recovery of spiked MMA, a measure of accuracy, averaged 119, 128 and 92% for three different levels of spiking. The MMA assay interassay CV (reproducibility) was 4.99%. The intraassay CVs in the assay for sulfur amino acids ranged from 1.91% to 9.03%, while interassay CVs were from 1.36% to 3.09%. Spiking recovery of amino acids ranged from 92% at physiological concentrations to 64% at supraphysiological extremes. The cobalamin assay had an intraassay CV of 2.92%, interassay CV of 15.25%, and mean recovery of spiked cobalamin of 86.64, 79.40 and 75.22% at three levels of addition.

We conclude that these assays are sufficiently precise, accurate and reproducible for routine measurement of compounds relevant to single carbon metabolism in feline serum.

- 155** POSTPRANDIAL ALTERATIONS IN SERUM UNCONJUGATED BILE ACID CONCENTRATIONS IN NORMAL DOGS. CG Ruaux, JM Steiner and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, Texas.

The concentration of serum unconjugated bile acids (SUBA) is considered to be an index of bacterial bile acid deconjugase activity in the upper small intestine. Our laboratory has previously described techniques for the measurement of SUBA in canine serum. We have also demonstrated that small intestinal bacterial overgrowth (SIBO) in dogs is associated with an elevated fasting SUBA concentration. The purpose of this study was to evaluate changes in SUBA concentration occurring in normal dogs in the postprandial period.

Seven healthy beagles from a research colony, with resting unconjugated bile acid concentrations in our laboratory control range, were enrolled. Indwelling jugular catheters were placed, and food was withheld overnight. Following collection of a baseline serum sample, the dogs received a standardized meal (Canine Maintenance®, Hills Pet Nutrition). Serum samples were collected at 15, 30, 45, 60, 75, 90, 105, 120, 180, 210, 240, 300, 360, 420 and 480 minutes after feeding. SUBA concentrations at each time point were measured using gas chromatography/mass spectrometry. Data were analyzed with a statistical software package (GraphPad Prism 3.0). Variations in concentrations of five different unconjugated bile acids were analyzed by time-point using one-way, repeated measures ANOVA followed by Dunnett's multiple comparison test, comparing each time-point to the baseline sample. Statistical significance was assigned for values of P<0.05.

Unconjugated bile acids were detected at nanomolar concentrations in all serum samples. Cholic acid (CA) was the most prominent unconjugated bile acid in most samples. Serum concentrations of unconjugated CA and chenodeoxycholic acid were significantly increased at 360, 420 and 480 minutes after feeding. Serum unconjugated CA concentrations exceeded our control range at some time point in all dogs. Unconjugated deoxycholic acid was significantly increased at 360 and 420 minutes, while unconjugated lithocholic acid was significantly increased at 180 minutes. Unconjugated ursodeoxycholic acid showed no significant alteration at any time.

We conclude that SUBA concentrations increase significantly postprandially in normal dogs. Therefore it is crucial that food is withheld before SUBA determination as a diagnostic test for SIBO.

- 156** ORAL BLEEDING DUE TO PANCREATIC EXTRACT IN THREE DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY. GM Rutz, JM Steiner, DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, Texas.

There is anecdotal evidence of rare the occurrence of oral ulceration and bleeding in dogs and human beings with exocrine pancreatic insufficiency (EPI) treated with pancreatic extracts. However, none of these reports have been substantiated in the literature. We report a series of three dogs treated with pancreatic extracts that developed oral bleeding.

Twenty-four dogs with EPI, diagnosed on the basis of a severely decreased serum canine trypsin-like immunoreactivity (cTLI) concentration (cTLI ≤ 2.0 µg/L), were enrolled in a clinical feeding trial. Bleeding in the oral cavity was noted in one dog before enrollment into the feeding trial and in two more dogs during the feeding trial. Bleeding from the oral cavity was reported during or shortly after consumption of meals containing the pancreatic extract. Owners were advised to reduce the amount of the enzyme supplement by 50%, and in all cases oral bleeding stopped and the oral mucosa healed. In two of the cases a decrease of the dose of the pancreatic extract did not affect fecal consistency and the dogs were maintained on the lower dose of pancreatic supplement. In these two dogs no more bleeding episodes from the oral cavity were reported. One of these two dogs was challenged with a higher dose of the pancreatic extract 5 months later, and the bleeding from the oral cavity recurred. The owner of the third dog reported that her dog showed bleeding from the oral cavity on a regular basis. When the dose of pancreatic extract was reduced from ¾ to ½ teaspoon per cup of diet the dog would develop loose stools after approximately 3–4 days. In order to ensure better stool quality the dose would be increased to ¾ teaspoon per cup of diet. After a period of treatment with the higher dose of pancreatic extract this dog's oral bleeding recurred.

In conclusion, high doses of pancreatic extract can cause oral bleeding in dogs with pancreatic insufficiency. Oral bleeding can be managed by pancreatic extract dose reduction.

**157** DEVELOPMENT AND VALIDATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE MEASUREMENT OF CANINE PANCREATIC LIPASE IMMUNOREACTIVITY (cPLI) IN SERUM. JM Steiner, SR Gumminger, and DA Williams, GI Lab, Texas A&M University, College Station, TX.

Serum lipase activity has been used for diagnosis of pancreatitis in human beings and dogs for several decades. However, lipases are secreted by many cell types other than pancreatic acinar cells and thus serum lipase activity is not a specific marker for pancreatic acinar cells. Recently, a radioimmunoassay for measurement of cPLI in serum has been developed and validated. However, radioimmunoassays require frequent radioiodination. Also, because of a perceived risk of use of radioactive material, there are special restrictions and regulations that need to be adhered to. Therefore, the goal of this project was to develop and validate an ELISA for the measurement of cPLI in serum.

A capture sandwich ELISA was developed. Canine pancreatic lipase (cPL) was purified from canine pancreatic tissue, antiserum against cPL was raised in rabbits, and a polyclonal antibody was purified by affinity chromatography. The purified antibody was bound to microtiter plates and used to capture antigen. A portion of the purified antibody was biotinylated and used to identify the captured antigen. A streptavidin horseradish peroxidase preparation and a horseradish peroxidase substrate were used for detection of the secondary antibody. The assay was validated by determination of sensitivity, working range, linearity, accuracy, precision, and reproducibility. A reference range for serum cPLI was determined by the 95<sup>th</sup> percentile in 74 clinically healthy dogs.

Sensitivity and working range were 0.4 µg/L and 0.4 to 999.2 µg/L, respectively. Observed to expected ratios for dilutional parallelism for 4 serum samples and 3 dilutions ranged from 75.0 and 148.8%. Observed to expected ratios for spiking recoveries for 4 serum samples and 6 spiking concentrations ranged from 90.4 to 112.5%, when assuming a 55% recovery. Intra- and inter-assay variabilities for 4 different serum samples were 3.4, 7.4, 2.4, and 5.8% and 13.9, 11.6, 7.7, and 23.5%, respectively. The reference range for serum cPLI was 2.2 to 102.1 µg/L.

We conclude that the ELISA for cPLI described here is sufficiently sensitive, linear, accurate, precise, and reproducible for clinical application. Evaluation of the clinical usefulness of this assay for the diagnosis of exocrine pancreatic disorders in dogs is currently under way.

**158** SERUM CANINE PANCREATIC LIPASE IMMUNOREACTIVITY (cPLI) IN DOGS WITH EXPERIMENTALLY INDUCED CHRONIC RENAL FAILURE. JM Steiner, DR Finco, SR Gumminger, and DA Williams. GI Laboratory, Texas A&M University, College Station, TX.

Serum lipase activity (LA) has been used to diagnose pancreatitis in human beings and dogs for several decades. However, LA is neither sensitive nor specific for pancreatitis in either species and many non-pancreatic factors, including chronic renal failure, increase serum lipase activity in dogs. An enzyme-linked immunosorbent assay for serum canine pancreatic lipase immunoreactivity concentration (cPLI) has recently been developed and validated. A reference range of 2.2 to 102.1 µg/L was established for cPLI. The goal of this project was to examine the influence of experimentally induced chronic renal failure (CRF) on serum cPLI concentrations.

Serum samples were collected from 17 dogs with CRF (15/16 nephrectomy) and were analyzed for creatinine, LA, and cPLI. One of the dogs showed extreme LA and cPLI. The Grubb test for outliers was suggestive of outlier data-points for both LA and cPLI, but not for serum creatinine concentration, suggesting that other factors, such as pancreatitis, may have been causing these data points. This notion was further supported by the fact that the dog died within hours of sample collection. Therefore this dog was removed from further analysis. LA and cPLI of the 16 remaining dogs were statistically compared with those from 74 clinically healthy dogs using a two-sided *t*-test for LA and a Mann-Whitney test for cPLI (data set failed normality).

Mean (±SD) serum creatinine concentration was 4.3 (±1.5) mg/dL with a range of 2.1 to 8.0 mg/dL. LA (mean±SD) was not significantly different between dogs with CRF (283.9±128.7 mg/dL) and clinically healthy dogs (319.1±146.7 mg/dL; *p*-value = 0.370). Median serum cPLI concentration was significantly higher in dogs with CRF (43.4 mg/dL) than in clinically healthy dogs (16.3 mg/dL; *p*-value = 0.0008). However, serum cPLI concentrations did not correlate with serum creatinine concentrations (*r*<sup>2</sup> = 0.02218) and serum cPLI concentration was within the reference range in 14 of 16 dogs with CRF. The highest serum cPLI concentration in any dog with CRF was 132.0 µg/L, which is much less than the currently recommended cut-off value for a diagnosis of pancreatitis of 250.0 µg/L.

In conclusion dogs with experimentally induced chronic renal failure have significantly yet clinically unimportant elevations of serum cPLI concentrations.

**159** DETERMINATION OF A CONTROL RANGE FOR A 5-SUGAR GASTROINTESTINAL PERMEABILITY AND MUCOSAL FUNCTION TEST IN CLINICALLY HEALTHY DOGS. JM Steiner, JR Cardwell, DA Williams. GI Laboratory, Texas A&M University, College Station, TX.

Gastrointestinal permeability and mucosal function testing has been used as a clinical research tool and a clinical tool in human and veterinary gastroenterology. We recently reported about set up and validation of a protocol for concurrent measurement of lactulose (L), rhamnose (R), xylose (X), methylglucose (M), and sucrose (S). We also reported about the kinetics of the urinary recovery of these 5 sugars in clinically healthy dogs suggesting that a 6-hour urine collection is sufficient. Finally, we reported about the comparison of a 2-sugar protocol using L and R, a 4-sugar protocol using L, R, X, and M, and a 5-sugar protocol using L, R, X, M, and S, showing that concurrent administration of these sugars does not alter the urinary recovery of the other sugars. The goal of this study was to determine a control range for a testing protocol utilizing L, R, X, M, and S.

30 clinically healthy Beagle dogs from a research colony were used for this study. After an overnight fast each dog was catheterized to completely empty the urinary bladder. Then each dog received 200 ml of an isotonic sugar solution containing 1 g M, 2 g L, R, and X, and 8 g S. 6 hours later the dogs were again catheterized and the entire volume of urine was collected. One dog only received 175 ml sugar solution, 3 dogs vomited a small amount, and 3 dogs urinated during the 6-hour period. These dogs were excluded from further analyses and data from 23 dogs were analyzed. Control ranges for urinary recovery (%) of each of the sugars and for lactulose/rhamnose recovery ratio (L/R) and xylose/methylglucose recovery ratio (X/M) were calculated by the 90<sup>th</sup> percentile.

The control range was 1.5–5.8% for %L, 17.3–42.6% for %R, 16.0–43.8% for %X, 32.8–81.0% for %M, 0.0–0.6% for %S, 0.05–0.15 for L/R, and 0.40–0.59 for X/M. Coefficients of variation (%CV) were 44.8% for %L, 30.9% for %R, but only 30.0% for L/R. Also, coefficients of variation were 28.8% for %X, 25.5% for %M, but only 11.8% for X/M.

A control range for a protocol using L, R, X, M, and S for gastrointestinal permeability and mucosal function testing was established. Inter-individual variability of recovery ratios were lower than that of urinary recoveries of respective sugars, indicating that, at least in clinically healthy dogs, the use of recovery ratios is preferable over the use of single urinary sugar recoveries.

**160** DEVELOPMENT AND VALIDATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR MEASUREMENT OF  $\alpha_1$ -PROTEINASE INHIBITOR/TRYPsin COMPLEXES IN CANINE SERA. JS Suchodolski, JC Collard, JM Steiner, CG Ruaux, DA Williams. Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX.

Alpha<sub>1</sub>-proteinase inhibitor ( $\alpha_1$ -PI) is a plasma protein that binds reversibly to trypsin (T) during pancreatitis. Studies in human patients suggest that measurement of  $\alpha_1$ -PI complexed with T ( $\alpha_1$ -PI/T) in serum may be a useful tool for the diagnosis and prognosis of pancreatitis. Human beings with severe pancreatitis and rodents with experimentally induced pancreatitis have significantly higher serum concentrations of  $\alpha_1$ -PI/T than subjects with mild disease. The objective of this study was to develop and validate an enzyme-linked immunosorbent assay (ELISA) for the measurement of  $\alpha_1$ -PI complexed with canine cationic trypsin (cCT) in canine serum.

A sandwich ELISA was developed using affinity purified anti-cCT antibody as the capture antibody. An affinity purified, biotinylated anti- $\alpha_1$ -PI antibody was used as reporter antibody. A streptavidin horseradish peroxidase preparation and a horseradish peroxidase substrate were used in the colorimetric development. The assay was validated by determination of sensitivity, working range, linearity, accuracy, precision, and reproducibility.

Sensitivity was 10 µg/L, and the maximum detectable concentration 2,840 µg/L. Observed to expected (O/E) ratios for three serial dilutions of 3 serum samples ranged from 91.3 to 158.1%. O/E ratios for 3 serum samples spiked with 5 different concentrations of  $\alpha_1$ -PI/cCT ranged from 80.1 to 103.6%. Coefficients of variation for intra- and interassay variability of 3 serum samples ranged from 5.0 to 6.9% and from 15.5 to 24.7%, respectively.

Results suggest that the ELISA has acceptable performance indices for measuring  $\alpha_1$ -PI/cCT in canine serum. The assay showed reduced linearity at the lower end and decreased reproducibility at the upper end of the reference range. Previous studies have shown dramatic increases of concentration of  $\alpha_1$ -PI/T in human beings with pancreatitis. If similar results are observed in dogs with pancreatitis, high interassay variability and a reduced linearity, especially at the lower end of the reference range, should not influence the clinical interpretation of the assay results. Further studies to evaluate the sensitivity and specificity of  $\alpha_1$ -PI/cCT for the diagnosis of canine pancreatitis are in progress.



# 161

## ROLE OF APOPTOSIS IN PANCREATIC ACINAR ATROPHY IN DOGS. SR Gumminger,<sup>1</sup> JM Steiner,<sup>1</sup> G Stoica,<sup>1</sup> E Westermarck,<sup>2</sup> ME Wiberg,<sup>2</sup> SL Lovering,<sup>1</sup> J Edwards,<sup>1</sup> DA Williams.<sup>1</sup>

<sup>1</sup>Texas A&M University, College Station, Texas; <sup>2</sup>University of Helsinki, Finland.

Apoptosis is defined as programmed cell death caused by a physiological stimulus and is a part of normal tissue development and remodeling. Pancreatic acinar atrophy (PAA) is an inherited condition, most commonly seen in German Shepherd dogs, that leads to loss of pancreatic acinar tissue. The pathogenic mechanism responsible for acinar cell loss is unknown. Lymphocytic inflammation has been shown to occur during the pathogenesis of the disease, but neither necrosis nor apoptosis has been demonstrated. The goal of this study was to examine the incidence of acinar cell apoptosis in dogs with PAA.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining was performed using a commercially available kit (In Situ Cell Death Detection, POD Kit, Boehringer Mannheim) on the following pancreatic tissue sections from dogs: PAA (n = 12), pancreatitis (n = 6), normal dogs (n = 5) as negative controls, and pancreatic carcinoma (n = 5) as positive controls. Sections were analyzed under a light microscope (400X). An apoptotic index (AI) was calculated from the number of apoptotic nuclei in 100 randomly selected pancreatic acinar cells. Data were analyzed with a statistical software package (GraphPad Prism 3.0) using a one-way ANOVA followed by Dunnett's multiple comparison test to compare AIs to the PAA group. Values of P<0.05 were considered significant.

AI of sections from dogs with PAA did not differ significantly from those for normal dogs and dogs with pancreatitis. AI of sections from dogs with pancreatic carcinoma were significantly greater than those with PAA (P<0.01).

Diagnosis (# of sections examined)	Apoptotic Index (mean ± S.E.)
Normal (5)	1.20 ± 0.20
Pancreatic acinar atrophy (12)	2.33 ± 0.28
Pancreatitis (6)	10.17 ± 6.97
Pancreatic carcinoma (5)	20.80 ± 1.36

In conclusion, dogs with established PAA did not show an increase in apoptotic index in the remaining tissue compared to normal dogs and dogs with pancreatitis. Examination of additional tissue samples obtained from dogs during an earlier progression of the disease is warranted.

# 162

## HELICOBACTER SPP. INFECTION IN CATS: EVALUATION OF THE HUMORAL IMMUNE RESPONSE AND PREVALENCE OF GASTRIC HELICOBACTER SPP. D. Strauss-Ayalí,\*E. Scanziani, D. Deng, K.W. Simpson. Cornell University, Ithaca, NY and \*University of Milan, Italy.

The principal aims of this study were to evaluate the humoral immune response (IgG) of cats with gastric *Helicobacter* spp. infection, and to determine the prevalence of different types of *Helicobacter* spp. in the stomachs of cats.

The *Helicobacter* infection status of 45 cats (12 healthy spay/neuter cats, 9 sick cats, 24 colony cats) was determined by evaluating endoscopic gastric biopsies for urease activity, presence of *Helicobacter*-like organisms (HLO) on histopathology, and genus and species-specific PCR. Serum samples were evaluated with a kinetic enzyme linked immunosorbent assay (ELISA) utilizing the high molecular cell-associated protein (HM-CAP) fraction of *H. felis* ATCC 49179.

Seventeen of 45 cats were infected with *Helicobacter* spp.: "*H. heilmannii*" 9/17, *H. felis* 4/17, mixed "*H. heilmannii*" and *H. felis* 3/17, unclassified-*Helicobacter* spp. 7/17. *H. pylori* was not detected in any cat. Kinetic ELISA results were significantly higher for infected cats, than for uninfected cats. Cats infected with different *Helicobacter* spp. showed similar distribution of OD/min values. There were no effects of age or clinical signs on the results of kinetic ELISA. No correlation between colonization density and seroconversion was observed. There were statistically significant, but weak correlations between the degree of seroconversion and the degree of inflammation, and the number of lymphoid follicles. Infected cats had more severe inflammation in the pylorus and fundus than uninfected cats. Infected sick cats had a higher degree of pyloric, but not fundic inflammation, than healthy infected cats and uninfected sick cats.

This study indicates that naturally acquired infection with gastric *Helicobacter* spp. is associated with seroconversion (IgG) in cats. The similar ELISA values in cats infected with a variety of *Helicobacter* spp. suggests substantial antigenic homology between different *Helicobacter* spp., and the higher degree of inflammation in infected than uninfected cats, supports a role for *Helicobacter* as a cause of gastritis in cats.

# 163

## SYSTEMIC CHANGES DURING CANINE NON-SPECIFIC DIETARY SENSITIVITY. L. McNeill, C.E. Vallance, R.Y. Boddy, R.M. Batt, R.F. Butterwick, V.E. Rolfe. Waltham Centre for Pet Nutrition, Melton Mowbray, Leics, UK.

Fifteen per cent of the dog population produce intermittent loose faeces that respond to dietary change, this mild condition is referred to as non-specific dietary sensitivity (NSDS). Disruption and inflammation of the intestinal epithelial barrier have been demonstrated in NSDS, but it is not known how this could influence systemic health in these dogs. This study was undertaken to determine whether disruption of the intestinal barrier in dogs with NSDS is associated with changes in systemic blood parameters. A group of 12 NSDS dogs and 10 control dogs of various breeds and of mixed age and sex were compared. Routine haematology and standard biochemical parameters were determined. White blood cell (WBC) populations were analysed using flow cytometry (FACScalibur, Becton Dickinson) using directly conjugated anti-canine antibodies (Serotec). C-reactive protein (CRP) and haptoglobin analysis were performed using available assays (Biognosis). Serum Ig levels were analysed by canine specific ELISA (Bethyl). Results were analysed using students T-test with statistical significance assumed at P<0.05. Dogs with NSDS were found to have significantly reduced WBC counts (control 9.9±3.9x10<sup>9</sup>/l, NSDS 5.8±0.8x10<sup>9</sup>/l) due to declines in neutrophil and T cell populations. Analysis highlighted changes in plasma biochemistry, including increased liver enzyme levels (ALT-control 24.5±8.4U/l, NSDS 46.1±12.8U/l). Serum IgG levels were found to be increased (control 14.6±3.9mg/ml, NSDS 29.6±3.1mg/ml) and serum IgA reduced (control 2.6±1.9mg/ml, NSDS 1.2±0.8mg/ml) in the NSDS dogs. Reduced haptoglobin (control 1.49±0.60mg/ml, NSDS 0.19±0.02mg/ml) suggested NSDS RBC may have increased susceptibility to haemolysis. No difference in CRP levels was noted. These findings suggest that NSDS dogs show changes in blood parameters in addition to the intestinal abnormalities already characterised. Further studies are required to understand the importance of these findings in relation to NSDS.

# 164

## ANAL CUTANEOUS LUPUS ERYTHEMATOSUS AS A CAUSE OF CHRONIC SEVERE DYSCHIEZIA IN THE DOG. E. Schrauwen, G. Junius, and T. Maenhout. Small Animal Clinic, Fac. of Veterinary Medicine, Ghent University, Merelbeke, Belgium.

This is to report 8 cases of chronic severe dyschezia, caused by localized anal mucosal lupus erythematosus, with in most cases only very discrete abnormalities on physical examination. Each case was presented with a prolonged history of severe dyschezia, manifested by fear and vocalization on defecation. Signs had been present for 2 months to more than a year. A diagnosis had not been made in any case, and various treatments had been tried, such as antibiotics, NSAIDs, or sulfasalazine, without any effect.

The breeds involved were: Maltese, Tervueren, Groenendaeler, Shi-tsu, German shepherd, Bernese mountain dog, and mixed breed. Age ranged from 3 to 9 years, with 4 females and 5 males.

On physical examination, no abnormalities were noted, except for a very painful perineal region, which necessitated sedation or anesthesia for further evaluation. The anal skin showed in all dogs showed a discrete erosion, easily bleeding on palpation. One dog had also a bilateral perineal hernia. Biopsies were taken with a punch, and showed the following histology: interfacial dermatitis, apoptotic basal cells, focal hydropic degeneration of the basal cells leading to cleft formation and ulceration, focal thickening of the basement membrane zone. Dermal lymphoid infiltrate with round cells was noted: lymphocytes, plasmacells, some histiocytes, and melanophages. These are all important histopathologic features of cutaneous lupus erythematosus.

Since the intense perianal pain precluded topical treatment, all dogs were treated with one of the following: immunosuppressive doses of glucocorticoids, a combination of glucocorticoids and chlorambucil, or tetracycline and niacinamide. Most cases responded very quickly (usually within a few days) and remained without clinical signs during treatment. In several cases treatment could be stopped after a few months, and only some cases relapsed afterwards.

In most of these cases the anal lesions present were very discrete and could easily be overlooked. If only routine diagnostic procedures would have been performed, a diagnosis could not have been made.

# 165 **INTESTINAL INTUSSUSCEPTION IN TWENTY CATS.** Jamie M. Burkitt, Kenneth J. Drobatz, Rebecca S. Hess, Dorothy C. Brown, and Robert J. Washabau. School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Parvoviral enteritis, intestinal helminth, and foreign body ingestion account for most gastrointestinal (G.I.) intussusceptions (INT) in the dog. The incidence, clinical characterization, and pathogenesis of INT in cats are under-reported.

Medical records of cats with a surgical or necropsy diagnosis of intestinal INT were evaluated retrospectively (1987–2000) for signalment, history, physical examination, laboratory, imaging, surgical, and pathologic findings.

Domestic Shorthaired cats were the most frequent (11/20) breed. Ten cats were less than one year of age and nine cats were six years or older. Anorexia (13/17), lethargy (12/17), and vomiting (10/17) were the major presenting clinical signs. Dehydration (14/18), poor body condition (12/18), abdominal pain (7/18), and palpable abdominal mass (7/18) were the major physical examination findings. Mean WBC count was 29,500 cells/microliter with a mild to moderate left shift reported in 7/15 cats. Hypoalbuminemia (1.6–2.2 g/dL) was reported in 5/12 cats. Fecal parasitology performed in one cat was negative for helminth and protozoal parasites. Survey abdominal radiographs revealed abnormalities in 13/13 cats, including dilated loops of bowel (12/13), loss of abdominal detail (5/13), intestinal mass (3/13), and INT (1/13). INT was diagnosed by abdominal ultrasonography in 7/8 cats; additional findings included intestinal mass (4/8) and peritoneal effusion (3/8). Surgery was performed in 13 cats. Eleven cats (85%) required intestinal resection and anastomosis due to a non-reducible INT or bowel compromise. Three out of 13 cats had enteroplication following surgical correction. INT diagnosed at surgery were reported as jejuno-jejunal (7/13), ileocolic (4/13), ileo-cecal (1/13), and duodeno-jejunal (1/13). Intestinal masses were found in association with INT in 3 of these cats, but foreign bodies were not found in any cats. Histologic examination confirmed lymphoma in 3 cats, and marked lymphocytic-plasmacytic inflammation in 1 cat. INT diagnosed at necropsy were reported as ileo-cecal (2/7), ileo-ileal (2/7) jejuno-ileal (2/7), and jejuno-jejunal (1/7). Histologic examination confirmed lymphoma in 4 cats, FIP in one cat, and marked lymphocytic-plasmacytic enteritis in 1 cat. In all cases, INT proximal to the duodenum were not reported and of the cats that were  $\geq$  six years old, 44% (4/9) were associated with lymphoma.

We conclude that G.I. INT in cats: (1) have a bimodal age distribution, (2) are confined to the intestine and colon, (3) occur most commonly as jejuno-jejunal INT, (4) are often associated with lymphoma in older animals, and (5) are more readily diagnosed by abdominal ultrasonography than by survey radiography.

# 166 **ERYTHROMYCIN STIMULATES CONTRACTIONS OF CANINE, BUT NOT FELINE, LONGITUDINAL COLONIC SMOOTH MUSCLE.** Tonatiuh Melgarejo, Daniel Simon, and Robert J. Washabau. School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Erythromycin stimulates sphincteric and propulsive motility in the proximal gastrointestinal tract of several animal species. Erythromycin increases tone in the feline gastroesophageal sphincter, and erythromycin stimulates canine gastric emptying. The effects of erythromycin on the distal gastrointestinal tract have not been characterized in these species.

Longitudinal and circular colonic smooth muscle strips were prepared from ascending and descending colon of healthy dogs and cats. Muscle strips were suspended in a physiologic (HEPES) buffer solution, attached to isometric force transducers, and set at optimal muscle length ( $L_0$ ) following stimulation with acetylcholine (ACh;  $10^{-6}$  M). Muscles were then treated with erythromycin stearate or erythromycin ethyl succinate ( $10^{-6}$  to  $10^{-3}$  M) in a set of dose-response experiments. In a separate set of experiments, the cholinergic- and extracellular calcium-dependence of the erythromycin responses were investigated by pre-treating muscle strips with atropine (1  $\mu$ M) or nifedipine (1  $\mu$ M).

Erythromycin stearate and erythromycin ethyl succinate stimulated isometric stress responses ( $1.35\text{--}4.15 \times 10^4$  N/m<sup>2</sup>) in longitudinal smooth muscle from the ascending and descending canine colon. Maximal responses were attained at  $6.5 \times 10^{-4}$  M erythromycin stearate and  $7.3 \times 10^{-4}$  M erythromycin ethyl succinate concentrations. Maximal erythromycin stearate and erythromycin ethyl succinate responses were 72 and 59% respectively of the maximal ACh response ( $10^{-4}$  M) in ascending or descending canine longitudinal smooth muscle. Erythromycin-induced contractile responses were inhibited 45–50% by atropine (1  $\mu$ M). Nifedipine (1  $\mu$ M) totally inhibited the erythromycin responses. Erythromycin was without effect on circular smooth muscle in the canine colon. Erythromycin also had no effect on longitudinal or circular feline colonic smooth muscle. We conclude that: (1) erythromycin stimulates longitudinal smooth muscle contractions in the canine colon (2) erythromycin-induced contractions in the canine colon are partially cholinergic-dependent and involve influx of extracellular calcium and (3) erythromycin has no effect on feline colonic smooth muscle.

# 167 **KINETICS OF URINARY RECOVERY OF FIVE ORALLY ADMINISTERED SUGARS IN HEALTHY CATS.** M.R. Krecic\* J.M. Steiner<sup>+</sup> M.R. Kern\* J.R. Cardwell<sup>+</sup> and D.A. Williams.<sup>+</sup> \*College of Veterinary Medicine, Mississippi State University, Mississippi State, MS; <sup>+</sup>GI Laboratory, Texas A&M University, College Station TX.

Intestinal permeability and mucosal function testing is a noninvasive and sensitive method for evaluating intestinal integrity and function. Testing consists of oral administration of a sugar solution and determination of the percent urinary recovery of the sugars administered. Lactulose and rhamnose are markers of intestinal barrier function. Methylglucose and xylose are markers of intestinal absorptive capacity. Sucrose is a marker of gastric barrier function. The goal of this project was to describe the kinetics of the urinary recovery of these 5 sugars after oral administration in healthy cats.

Ten clinically healthy male experimental cats were evaluated. An indwelling urethral catheter was placed in a sterile fashion in each cat. One hundred mls of an isotonic solution containing 0.5 g of methylglucose 1.0 g of rhamnose 1.0 g of xylose 4.0 g of sucrose and 1.0 g of lactulose were administered orally via nasoesophageal tube to each cat. Urine samples were collected aseptically from a closed urinary collection system prior to administration of the solution every 2 hours for 12 hours and again 24 hours after administration. Total urine volume was recorded for each time-point. Urinary recoveries of the sugars were determined by high-pressure anion exchange liquid chromatography with pulsed amperometric detection. Mean cumulative urinary recovery for each sugar and mean percent urinary recovery for each sugar and time-point were calculated.

Cumulative 24-hour urinary recoveries (mean  $\pm$  SD) were  $3.0 \pm 1.6\%$  for lactulose  $8.9 \pm 3.6\%$  for rhamnose  $18.8 \pm 7.9\%$  for xylose  $32.0 \pm 11.0\%$  for methylglucose and  $1.2 \pm 0.7\%$  for sucrose. The largest percent urinary recovery of lactulose rhamnose xylose and methylglucose occurred at 4 and 6 hours. The largest percent urinary recovery for sucrose occurred at 4 hours. Greater than 88% of each sugar was recovered by 10 hours and greater than 92% of each sugar was recovered by 12 hours.

We conclude that cumulative urinary recoveries of lactulose rhamnose xylose and methylglucose in healthy cats are less than and cumulative urinary recovery of sucrose is similar to the reported cumulative urinary recoveries of these 5 sugars in healthy dogs. Cumulative urine collection for 10 or 12 hours after an oral dose of a solution containing methylglucose xylose rhamnose lactulose and sucrose is sufficient for estimation of urinary recovery of these 5 sugars in healthy cats.

# 168 **HEMOSTATIC PARAMETERS IN 7 DOGS WITH A PROTEIN LOSING ENTEROPATHY.** A.P. Carr<sup>1</sup> H. Bortnowski<sup>2</sup> <sup>1</sup>Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon Saskatchewan <sup>2</sup>University of Wisconsin-Madison, Madison, Wisconsin.

Certain diseases are associated with severe hypoalbuminemia foremost protein losing nephropathies (PLN) and protein losing enteropathies (PLE). Thrombosis with PLN is thought to partially result from urinary ATIII loss. In contrast thrombosis is rare with PLE though ATIII losses of similar magnitude may occur with PLE as with PLN. This study evaluated hemostatic parameters in dogs with PLE.

PLE was a clinical diagnosis based upon the presence of diarrhea panhypoproteinemia and supporting GI histopathology while ruling out PLN or liver dysfunction. Data collected included signalment albumin concentration total protein concentration cholesterol concentration fibrinogen concentration platelet count APTT OSPT and ATIII levels. An elevation of APTT or OSPT  $>25\%$  of control plasma was considered abnormally prolonged. Hypofibrinogenemia was defined as a fibrinogen concentration  $<50\%$  of control plasma hyperfibrinogenemia as  $>50\%$  of control plasma.

A total of 7 dogs were included in this study. Five dogs had lymphoplasmacytic enteritis with lymphangiectasia 1 dog had lymphangiectasia alone and 1 dog had lymphoplasmacytic enteritis alone. Six different breeds were represented 2 of the dogs were Yorkshire Terriers. Gender distribution was 3 male neutered 1 intact male 2 female spayed and 1 intact female. Median age was  $6 \pm 1$  years with a range of 5 to 10 years albumin was  $1.4 \pm 0.2$  G/dL with a range of 0.9 to 1.7 (reference range 2.5–4.0) total protein was  $3.3 \pm 0.4$  G/dL with a range of 2.6 to 4.0 (reference range 5.4–7.6) and cholesterol was  $116 \pm 32$  mg/dL with a range of 95–187 (reference range 111–290). In four dogs a platelet estimate only was given (adequate in all cases) a numerical platelet count value in 2 of 3 dogs was elevated (603 and 732 thousand/ $\mu$ L). OSPT was normal in all dogs. APTT was prolonged in 3 dogs (43%). Fibrinogen was normal in 4 dogs elevated in 2. Five dogs had normal ATIII activity (90% to 103% of control plasma) two dogs had moderately decreased ATIII activity of 67% and 70%. The dog with 67% ATIII activity also had a prolonged APTT and hyperfibrinogenemia. This dog was suffering from severe enteritis omentitis and fat necrosis.

All dogs studied had severe panhypoproteinemia. Three of the dogs had a prolongation of APTT that could be a sign of a coagulopathy. Hyperfibrinogenemia could result from the severe inflammation present. Interestingly only 2 dogs had moderately decreased ATIII activity. The lack of significant ATIII deficiency and prolongation of APTT may explain why thrombosis is seldom seen with PLE.

## 169 COMPARISON OF DIFFERENT DOSES OF <sup>13</sup>C-AMINOPYRINE FOR A <sup>13</sup>C-AMINOPYRINE DEMETHYLATION BLOOD TEST IN CLINICALLY HEALTHY DOGS. EM Moeller<sup>1</sup> JM Steiner<sup>1</sup> DA Williams<sup>1</sup> M Tetrick<sup>2</sup> and J Burr<sup>2</sup> <sup>1</sup>GI Laboratory Texas A&M University College Station TX; <sup>2</sup>Iams Company Lewisburg OH.

We recently demonstrated the feasibility of a <sup>13</sup>C-aminopyrine (AP) demethylation blood test in clinically healthy dogs. Additionally a pharmacokinetic study of AP demethylation showed that the peak mean percent dose/min of <sup>13</sup>C administered as AP (PCD) and recovered in the gas extracted from blood was determined to be 45 min in clinically healthy dogs. The objective of this study was to determine an appropriate dose of AP for this test.

Nine clinically healthy dogs belonging to a research colony were enrolled into the study. Four doses, 1, 2, 5 and 10 mg/kg body weight, were compared in a randomized order. In each study period, all dogs were given the same dose. Food was withheld from all dogs 12 hours prior to each study and a 2 ml blood sample was collected as a baseline sample. The AP was dissolved in deionized water and sterilized by passage through a 0.1 µm pore-size syringe filter. The AP was then administered to each dog intravenously and additional samples were collected at 30, 45, 60 and 75 min after AP administration. HCl acid was added to each sample in order to extract CO<sub>2</sub>. The extracted gas was analyzed by fractional mass spectrometry to determine the PCD. Mean coefficients of variation for PCD and cumulative PCD (CUMPCD) were compared using a *t*-test. Also, PCDs were compared for the different doses and the four different sampling times using ANOVA.

None of the dogs showed any side effects after administration of AP. The mean coefficient of variation (%CV) for PCD was significantly lower than the mean coefficient of variation for CUMPCD (*p* = 0.0025). Mean PCDs between the 1, 2, 5, and 10 mg/kg doses for each time point were not statistically different (*p* = 0.41, 0.73, 0.58 and 0.70, respectively). Also, mean PCDs were not statistically different between the 30, 45, 60 and 75 min time points for any of the dose groups (*p* = 0.96, 0.67, 0.51 and 0.89, respectively).

We conclude that PCD is superior to CUMPCD for the quantification of AP demethylation in clinically healthy dogs. We also conclude that a 2 mg/kg dose of AP is an appropriate dose and 45 min is a suitable sampling time after intravenous administration of AP in clinically healthy dogs.

## 170 KINETICS OF <sup>13</sup>C-AMINOPYRINE DEMETHYLATION IN CLINICALLY HEALTHY CATS. EM Moeller, JM Steiner, SR Gumminger, JS Suchodolski, CG Ruaux and DA Williams. GI Laboratory, Texas A&M University, College Station, TX.

Breath tests have been developed for non-invasive marker studies of different metabolic functions. One example is a <sup>13</sup>C-aminopyrine (AP) demethylation breath test, which has been used to evaluate hepatic microsomal function in human beings and laboratory animals. However, breath tests are difficult to perform in small animals and a blood test may be preferable. Previous studies have shown that an AP demethylation blood test is feasible in clinically healthy dogs. Also, a 2 mg/kg dose and collection of a 45 min post-AP administration blood sample have been shown to be appropriate in clinically healthy dogs. The goal of this study was to determine the kinetics of AP demethylation in clinically healthy cats by determining the percent dose/min of <sup>13</sup>C administered as AP (PCD) and recovered in gas extracted from blood. We also aimed to determine a sampling time that is most appropriate for a clinical test.

Eight clinically healthy cats were enrolled in this study. Cats were sedated and indwelling jugular catheters were placed the day before the study. Cats were fasted for a period of at least 12 hours prior to each study. A 2 ml baseline sample was collected from each cat. AP was dissolved in deionized water and sterilized by filtration through a 0.1 µm pore-size syringe filter. A 2 mg/kg dose of AP was slowly administered IV to each cat. Additional 2 ml blood samples were collected at time points 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 240 and 300 min after AP administration. HCl was added to each sample to extract CO<sub>2</sub> from blood. Extracted gas samples were analyzed by fractional mass spectrometry and the PCD of each sample was calculated.

Mild ptialism was observed after AP administration in several of the cats. All 8 cats showed an increase in PCD after AP administration. The PCD peaked at 75 min in 2 cats, at 90 min in 3, and at 105 min in the remaining 3. Coefficients of variation (%CV) for PCD were 34.80% at 45 min, 34.80% at 60 min, 32.29% at 75 min, 32.15% at 90 min, 34.90% at 105 min, 33.89% at 120 min, and higher at the other time points. Coefficients of variation of cumulative PCD between the 8 cats were 34.61% at 90 min, 34.26% at 105 min, 34.03 at 120 min, 33.95% at 135 min, 34.31% at 150 min, 34.59% at 180 min, and higher at the other time points.

We conclude that 1) with the exception of mild ptialism, intravenous AP administration caused no apparent side effects in healthy cats, 2) AP administration leads to an increase in PCD of gas extracted from blood samples and 3) 90 min appears to be a suitable time point to collect a blood sample after intravenous AP administration in clinically healthy cats.

## 171 URINE SULFATED BILE ACIDS IN CATS WITH AND WITHOUT LIVER DISEASE. D Trainor, SA Center, JF Randolph, CE Balkman, KL Warner, MA Crawford,\* Cornell University, Ithaca, NY, and Oradell Animal Hospital,\* Oradell, NJ.

Bile acid sulfation occurs when high serum bile acids (SBA) values are sustained. Detection of sulfated bile acids in urine (USBA) may simplify detection of liver disease. We evaluated USBA and urine creatinine (UCr) concentrations, along with SBA and serum biochemistries in 69 cats. A USBA/UCr was calculated in each. The USBA were measured using an enzyme linked colorimetric method.

Fifty cats with liver disease had portosystemic vascular anomaly (*n* = 1), cholangitis/cholangiohepatitis (*n* = 11), extrahepatic bile duct occlusion (*n* = 5), hepatic lipidosis (HL, *n* = 23), hepatic neoplasia (*n* = 3), hepatic necrosis (*n* = 4), and other hepatic disorders (*n* = 3). Eleven cats initially thought to have liver disease were proven to have other disorders; 3 of these were hyperthyroid. Eight healthy cats also were evaluated. Liver diagnoses were based on tissue biopsy except in 12 cats with HL confirmed using ultrasonographic imaging and hepatic aspiration. Liver disorders were subcategorized as necroinflammatory (NI; *n* = 20) or non-necroinflammatory (NNI; *n* = 30). USBA/UCr in cats with liver disease (all cats, NI, NNI) were compared to values in cats lacking liver disease. Correlations between USBA /UCr and liver enzymes, albumin, cholesterol (Chol), total bilirubin (TB) and SBA were evaluated. The high 97.5% CI for USBA/UCr in normal cats and patients lacking liver disease determined a cutoff to identify abnormal values.

Significant differences existed between cats lacking liver disease and all cats with liver disease and cats with NI disease in ALP, ALT, AST, GGT, Chol, TB, SBA and USBA/UCr; and between NNI disease and cats lacking liver disease in ALB, ALP, TB, SBA, and USBA/Cr. A significant positive correlation existed between USBA/UCr and ALP, ALT, AST, GGT, Chol, TB, and SBA (all cats, all with liver disease, NI), and between USBA/UCr and ALP, Chol, TB, and SBA in NNI disease. The 97.5% CI of USBA/Cr in healthy and non-hepatic disease cats was 0.13. With this cutoff, 7/39 cats with liver disease and high SBA concentrations had normal USBA/Cr values.

We conclude that although normal cats may have detectable USBA concentrations, finding a USBA/Cr > 0.14 may simplify detection of important liver disease.

## 172 LEPTIN AS A MARKER FOR THE DEVELOPMENT OF HEPATIC LIPIDOSIS IN CATS. KJ Heuter, J Broussard, J Steiner,\* DA Williams.\* The Animal Medical Center, New York, NY, and \*College of Veterinary Medicine, Texas A&M University, College Station, TX.

We hypothesize that leptin concentrations are significantly elevated in cats with hepatic lipidosis, and represent an important, non-invasive marker for hepatic lipidosis. The purpose of this prospective study was to characterize the leptin concentration of cats with hepatic lipidosis, and to compare those levels to that of cats with other types of liver disease and to control cats. Twenty-three cats with hepatic disease were assigned to 1 of 2 groups on the basis of histopathologic examination of the liver: group 1, hepatic lipidosis (*n* = 12); or group 2, other liver disease (*n* = 6). Five healthy cats undergoing elective procedures were used as controls. Body condition scores were assessed and fasted plasma samples taken from cats at the time of biopsy or elective procedure. Concentrations of leptin were determined in all cats using an RIA validated for use in cats (Linco Research, St. Louis MO).

Leptin levels were not significantly different when compared between all groups. As a whole, leptin levels were found to correlate with body condition score (*P* < .05). However, as individual groups, there was no significant correlation. Body condition score was significantly lower in cats with nonlipidotic liver disease (*P* < .05).

We conclude that leptin concentrations do not differ significantly in cats with liver disease. Larger groups may yield more significant information regarding a marker for hepatic lipidosis. As reported earlier, body condition scores do correlate with leptin concentrations.

**173** *TRITRICHOMONAS FOETUS* AND NOT *PENTATRICHOMONAS HOMINIS* IS THE CAUSATIVE AGENT OF THE RECENTLY DESCRIBED FELINE INTESTINAL TRICHOMONOSIS. MG Levy,<sup>1</sup> JL Gookin,<sup>1</sup> MF Poore,<sup>1</sup> M Dykstra<sup>1</sup> and RW Litaker.<sup>2</sup> <sup>1</sup>North Carolina State University College of Veterinary Medicine, Raleigh, NC and the <sup>2</sup>University of North Carolina at Chapel Hill, Chapel Hill, NC

Recently, several investigators have reported large bowel diarrhea in cats associated with intestinal trichomonad parasites (Romatowski J. *Feline Pract* 1996; 24:10–14 and J Am Vet Med Assoc 2000;216:1270–1272; Gookin JL, et al. *J Am Vet Med Assoc* 1999;215:1450–1455 and *J Vet Intern Med* 2000;14:362). These reports have presumptively identified the flagellates as *Pentatrichomonas hominis*, an organism putatively capable of infecting the intestinal tracts of a number of mammalian hosts including cats, dogs and man. The purpose of the present study was to determine the identity of this recently recognized flagellate by means of SSU rDNA sequence analysis, restriction enzyme digest mapping, and light and transmission electron microscopy.

Isolates of the naturally occurring feline trichomonad and *P. hominis* (ATCC#30098) were axenically cultured in Modified Diamond's medium and ATCC Medium 1404, respectively. DNA was extracted and the SSU rDNA gene amplified using conserved PCR primers. Oligonucleotide primer sets were developed that were capable of distinguishing between *P. hominis* and the feline trichomonad. Restriction enzyme digests (HpaII) of the PCR amplified products were performed. Cultured feline trichomonads were examined using light and transmission electron microscopy.

Examination of the SSU rDNA gene of 3 geographically distinct isolates from cats with chronic large bowel diarrhea revealed 99.9% sequence identity with *Tritrichomonas foetus*, the causative agent of bovine venereal trichomonosis. RFLP results for the feline trichomonad gave the expected pattern for *T. foetus*, whereas *P. hominis* exhibited a distinctive and predicted pattern. Light and transmission electron microscopic analysis of cultured feline trichomonads revealed 3 anterior flagella and axostyle morphology indistinguishable from earlier descriptions of *T. foetus*.

These data identify the causative agent of feline trichomonosis as *T. foetus*. Prior isozymic and genetic analysis of *T. foetus*, *T. suis* and *P. hominis* has revealed the former two to be synonyms while the latter is distinct. Further, experimental infections of various hosts with *T. foetus* or *T. suis* have revealed little host specificity. Thus, the natural history of this organism in the feline host and any relationship to bovine and swine trichomonosis needs further evaluation. In light of the intimate association between infected cats and their human companions, investigation of this organism's zoonotic potential may warrant attention.

**174** PREVALENCE OF SERUM ANTIBODIES TO SIX LEPTOSPIRA INTERROGANS SEROVARS IN ASYMPTOMATIC DOGS FROM THE LOWER PENINSULA OF MICHIGAN. J.E. Stokes, J.M. Kruger, W. Schall, J. B. Kaneene, L. Kaiser, C. Bolin, Michigan State University, College of Veterinary Medicine, East Lansing, MI.

Leptospirosis is a ubiquitous disease that affects many mammalian species. Prior to the introduction of a bivalent canine leptospiral vaccine in the 1960s, serovars icterohaemorrhagiae and canicola were most commonly associated with clinical disease in dogs. Over the past twenty years there appears to have been a shift in the epidemiology of canine leptospirosis, with more clinical cases caused by non-vaccine serovars. The purpose of this study was to prospectively 1) determine the seroprevalence of leptospiral antibodies in asymptomatic dogs, 2) determine whether the epidemiology of canine leptospirosis has changed, and 3) identify risk factors associated with positive leptospiral titers in dogs in Michigan.

Serum was collected from 1,400 healthy dogs from the lower peninsula of Michigan. Antibody titers to six leptospiral serovars (bratislava, canicola, grippotyphosa, hardjo, icterohaemorrhagiae, and pomona) were measured by a microscopic agglutination test. Demographical information including vaccination and travel history, housing, gender, and exposure to animals was collected for each dog to assess risk factors associated with positive leptospiral titers.

Leptospiral antibody titers of  $\geq 1:400$  for canicola and icterohaemorrhagiae and titers  $\geq 1:200$  for the other four serovars were considered indicative of natural exposure. Preliminary results indicate that 82 (37%) of 224 dogs had antibody titers consistent with natural exposure to leptospirosis. The most commonly detected serovar was canicola, followed by grippotyphosa, bratislava, icterohaemorrhagiae, pomona and hardjo. Our initial results suggest that canicola is the most common leptospiral serovar encountered by asymptomatic dogs in the lower peninsula of Michigan.

**175** PRODUCTION OF POLYCLONAL ANTIBODIES FOR THE IMMUNO-HISTOCHEMICAL IDENTIFICATION OF *PYTHIUM INSIDIOSUM*. A.M. Grooters, M.K. Lopez, A.K. Brown, and E.C. Hodgins. Louisiana State University, Baton Rouge, LA.

Pythiosis (caused by the aquatic Oomycete *Pythium insidiosum*) is a devastating disease of dogs, cats, and horses in the southeastern US. Because the clinical and histologic characteristics of pythiosis are similar to those associated with infections caused by *Lagenidium* sp and *Conidiobolus* sp, these diseases are difficult to distinguish from one another. Unfortunately, both isolation and accurate morphologic identification of *P. insidiosum* are difficult; therefore, a definitive diagnosis of pythiosis is rarely made. The goal of this study was to develop and evaluate a polyclonal antibody for the immunohistochemical identification of *P. insidiosum* hyphae in canine tissues.

Polyclonal antibodies were produced in chickens against soluble mycelial antigens extracted from a 5-day old broth culture of *P. insidiosum*. Antigen solution containing 0.2 mg protein was emulsified with Freund's Incomplete Adjuvant and injected intramuscularly into two 16-week old White Leghorn laying hens every 20 days for a total of three injections. Eggs were collected daily for 10 days prior to inoculation (pre-immune eggs), and for 75 days following the first inoculation. Antibodies (IgY) were extracted from egg yolks using a water dilution method followed by ammonium sulfate precipitation, dialyzed against phosphate-buffered saline (PBS), and stored at  $-20^{\circ}\text{C}$ . The polyclonal antibody was rendered specific for *P. insidiosum* by adsorption with sonicated hyphae collected from 5-day old broth cultures of *Lagenidium* sp and *Conidiobolus coronatus*. The antibody was evaluated by using it to stain tissues from one dog with gastrointestinal pythiosis, one with systemic lagenidiosis, and one with pharyngeal conidiobolomycosis (all culture-confirmed). Immunohistochemistry was performed using an avidin-biotin immunoperoxidase system (Vector Labs), with the anti-*Pythium* antibody as the primary antibody and a biotinylated goat anti-chicken IgY as the secondary antibody. A negative control (using pre-immune antibodies as primary), as well as a histologic stain for hyphae (Gomori methenamine silver, GMS) were included for each set of tissues.

Evaluation of staining intensity for each tissue (performed in a non-blinded fashion) showed that the adsorbed anti-*Pythium* antibody was specific for identification of *P. insidiosum* hyphae, but was not as sensitive for the detection of these hyphae as was the GMS stain. We conclude that the adsorbed anti-*Pythium* antibody is a good candidate for further evaluation as a diagnostic tool for pythiosis. These evaluations should include blinded evaluation of a larger number of known positive and negative tissues in order to determine the assay's sensitivity and specificity.

**176** DETECTION OF CANINE DISTEMPER VIRUS BY RT-PCR IN SERUM, LEUKOCYTES, CEREBRO-SPINAL FLUID (CSF), AND URINE IN DOGS WITH DISTEMPER ENCEPHALITIS: 11 CASES. T.B. Saito, A.A. Alfieri, A.F. Alfieri, S.N.E. Beloni, H.S.A. de Moraes, Universidade Estadual de Londrina, Londrina, Paraná, Brazil.

We prospectively compared serum, leukocytes, CSF, and urine for diagnosing distemper encephalitis by RT-PCR in dogs. Poorly vaccinated dogs with progressive multifocal central nervous system disease from our Hospital population were selected for the study. Twenty dogs fulfilled the criteria for inclusion. Eleven of them, including 7 with myoclonus, were diagnosed as having distemper. Distemper encephalitis was confirmed based on a positive RT-PCR in the CSF. When CSF was not available, distemper was diagnosed whenever RT-PCR was positive in serum, leukocytes, and urine. One dog was negative in the CSF, but positive in all other samples. This dog was considered to have distemper. One dog was positive only in the urine and considered negative. The remaining negative dogs were negative in all materials tested. The RT-PCR amplified a fragment from the N gene of the nucleic acid of canine distemper virus (CDV). RNA was extracted using guanidinium thiocyanate. The thermocycle profile consisted of 40 cycles 94 C for 1 minute, 59.5 C for two minutes, and 72 C for 1 minute. The resulting fragment had 287 base pairs. We have previously shown that this RT-PCR is specific for the CDV, but also amplifies the measles virus. CDV and measles virus were differentiated through fragment analysis using a HINF1 enzyme.

RT-PCR for CDV was run in samples of serum, urine, and leukocytes from all dogs. Leukocytes were separated using a percoll gradient of 1.08. The majority of cells obtained were lymphocytes with small amounts of monocytes and neutrophils. RT-PCR was also run in CSF samples from 6 distemper dogs, being positive in 5. The dog considered positive despite the negative RT-PCR in CSF had viral fragment amplified in serum, leukocytes, and urine. Four dogs were positive only in CSF and urine. All distemper dogs had positive results in the urine, but only 7 were positive in leukocytes and serum. In conclusion, serum and leukocytes do not appear to be very sensitive for diagnosing naturally-occurring distemper encephalitis in dogs. Urine was the only fluid found positive in all dogs, suggesting its usefulness as a routine screen for dogs in which infection by CDV is a potential differential for the CNS signs. These results also suggest that elimination of the virus in the urine may play an important role in cross-contamination and maintenance of the virus in the environment.

# 177 A COMPARISON OF URINE COMPOSITION OF LABRADOR RETRIEVERS AND CAIRN TERRIERS. A.E. Stevenson, W.K. Hynds, P.J. Markwell, WALTHAM Centre for Pet Nutrition, Waltham-on-the-wolds, Melton Mowbray, Leicestershire, LE14 4RT, UK.

Data from a number of urolith analysis centres within both North America and Europe show that small and toy breeds of dog present much more commonly with calcium oxalate (CaOx) uroliths than large breeds. The aim of this study was to compare the urine composition of Labrador retrievers (LR) and Cairn terriers (CT) to identify possible predisposing factors.

8 LR and 7CT were fed a dry test diet for three weeks. During Week 3, a frozen urine collection was conducted over a 48 hour period for assessment of urinary CaOx, struvite and brushite (calcium hydrogen phosphate, dihydrate) relative supersaturation (RSS) by previously described methods (Table). Results were compared using an unpaired t-test.

Parameter	Cairn terrier	Labrador retriever
Calcium oxalate RSS	21.5±2.52 <sup>b</sup>	6.55±4.35 <sup>a</sup>
Urinary calcium concentration (mmol/l)	2.06±0.46 <sup>b</sup>	0.54±0.24 <sup>a</sup>
Urinary oxalate concentration (mmol/l)	1.54±0.42 <sup>a</sup>	1.34±0.99 <sup>a</sup>
Struvite RSS	0.27±0.23 <sup>a</sup>	0.51±0.71 <sup>a</sup>
Brushite RSS	1.06±0.46 <sup>b</sup>	0.38±0.31 <sup>a</sup>

A different superscript within a row indicates a significant difference ( $p < 0.05$ ).

CaOx RSS, brushite RSS, and urinary concentrations of calcium were significantly higher in the urine produced by the CT, when compared to the LR urine. CaOx RSS of the CT was above the estimated formation product for this stone type. Struvite RSS and urinary oxalate concentration were unaffected by breed.

The results suggest that the CT are at increased risk of CaOx formation when compared to LR fed the same diet. Differences between breeds should, therefore, be considered when evaluating strategies for managing and preventing CaOx urolith formation.

# 178 DETECTION OF CANINE MICROALBUMINURIA USING SEMI-QUANTITATIVE TEST STRIPS DESIGNED FOR USE IN HUMAN URINE. BM Pressler,<sup>1</sup> SL Vaden,<sup>1</sup> WA Jensen,<sup>2</sup> D Simpson,<sup>2</sup> <sup>1</sup>North Carolina State University, College of Veterinary Medicine, Raleigh, NC; <sup>2</sup>Heska Corporation, Fort Collins, CO.

Commercial testing for microalbuminuria (MA) in people often occurs through point-of-care semi-quantitative test strips, followed by quantitative testing when indicated. An ELISA that quantifies canine MA has been developed, but semi-quantitative test strips for use in the dog are not available. The purpose of this study was to determine the concordance of canine urine albumin concentrations (UAlb) measured by two commercial human test strips and by ELISA.

Urine samples were obtained from 67 dogs evaluated for a variety of clinical conditions. Dipstick urinalyses were performed on all samples; clinician discretion determined method of urine collection and performance of urine sediment examination and/or urine culture. Semi-quantitative test strips [Clinitek Microalbumin (CM) reagent strips, Bayer Corporation, Elkhart, IN; Micral (Mi) test strips, Roche Diagnostics Corporation, Indianapolis, IN] were used in strict accordance with the manufacturers' recommendations. Aliquots of urine were frozen (-80°C) within 15 minutes of collection for subsequent UAlb measurement by an antigen capture ELISA. ELISA results were normalized to a urine specific gravity of 1.010.

Using the ELISA as the gold standard, the CM strips correctly reported UAlb in 41 of 67 (61%) urine samples tested. UAlb was correctly reported in 23 of 35 (66%) dogs with negligible UAlb (<30 µg/ml), 8 of 17 (47%) dogs with MA (30–300), and 10 of 15 (67%) dogs with overt albuminuria (>300). Results obtained by the CM strip and ELISA were in concordance in 19 of 27 (70%) samples obtained by cystocentesis, 2 of 7 (29%) samples obtained by catheterization, and 19 of 32 (59%) free catch samples. Results from the CM strips and ELISA were in concordance in 0 of 2 dogs with bacterial cystitis and 9 of 21 (43%) dogs with hematuria (>5 RBC/hpf or >1+ blood on dipstick).

Urine samples from 12 of 13 (92%) dogs tested with the Mi strips read as negligible UAlb (<20 mg/ml). Concordance between results obtained by the Mi strips and ELISA occurred in only 4 (31%) samples. Further testing with this product was discontinued.

These preliminary results suggest that both the CM and Mi test strips lack sufficient concordance with results obtained by ELISA to be reliable screening tests for MA in the dog. With respect to the CM test strips, catheterization, urinary tract infection and hematuria appeared to negatively impact concordance of results obtained by the CM test strip and ELISA. A reliable, semi-quantitative test strip for canine MA needs to be developed.

# 179 THE POSSIBLE ROLE OF UROLITH-INDUCED FELINE LOWER URINARY TRACT DISEASE IN THAILAND: AN ASIAN POINT OF VIEW. Rosama Pusoonthornthum,\* Pinit Pusoonthornthum,\* and Carl A. Osborne,\*\* \*Chulalongkorn University, Thailand. \*\*University of Minnesota, USA.

Feline lower urinary tract disease (Feline LUTD) or so called FUS characterized by hematuria, dysuria, pollakiuria, and/or urethral obstruction, has been a common veterinary problem. In 1999, lower urinary tract signs were cited as one of the 25 most common problems reported in cats examined at private veterinary practices in the United States. In Thailand, the same trend was also observed. For one and a half year period, we have included 140 cats into our study. Seventy cats with feline LUTD were selected as cases that were followed prospectively. We also have collected data from seventy vaccinated cats as our control cats. The preliminary results of our one year prospective study of 70 cats with Feline LUTD at Chulalongkorn University Veterinary Teaching Hospital (CUVTH) indicated that the proportional morbidity ratio of Feline LUTD in Thai cats was 2.22 %. Most cats with Feline LUTD in our study were mixed breed (81.4%). The mean age was 54.75 ± 45.04 months old. The affected cats were intact male (41.4%), neutered male (30.0%), intact female (17.1%), and neutered female (11.4%). The mean weight for cats with feline LUTD was 4.09 ± 1.10 kilograms. Commercial cat food was consumed by 74.3% of the affected cats. Urine culture was performed in 42 cats and indicated the urinary tract infection with *Pseudomonas aeruginosa* (20.0%), *E. coli* (60%), and mixed infection (20%). Uroliths were observed in 16 cats but was retrieved only in 4 cats and submitted for analysis. The quantitative analysis of the uroliths revealed that they were composed of Magnesium Ammonium Phosphate (struvite)(100%). Urethral Plug were also found in 8 cats. From our prospective study with 70 cats with FLUTD, the causes of feline LUTD were idiopathic (27.1%), calculi (22.9%), urinary tract infection (14.3%), urethral plug (11.4%), anatomical disorders (2.9%), and trauma (2.9%).

# 180 GENETIC CHARACTERIZATION OF A FELINE CALICIVIRUS ISOLATED FROM URINE OBTAINED FROM A CAT WITH OBSTRUCTIVE IDIOPATHIC LOWER URINARY TRACT DISEASE. J.M. Kruger, K.A. Maas, P.J. Venta, A. Gonzales Wise, J. Dulin, R.K. Maes, Michigan State University College of Veterinary Medicine, East Lansing, MI.

Idiopathic lower urinary tract disease (ILUTD) is a common cause of hematuria, and pollakiuria in cats. Urethral obstruction is a potential sequelae of ILUTD. Previous observations of calicivirus-like particles in a large number of urethral plugs obtained from cats with obstructive ILUTD has led us to hypothesize that feline calicivirus (FCV) may have a role in this disease. In 1998 we isolated a FCV (designated FCV-U1) from a cat with nonobstructive ILUTD. Genetic analysis revealed that FCV-U1 was distinct from other FCV strains. We subsequently isolated another FCV (designated FCV-U2) from urine obtained from cat with obstructive ILUTD. It is unknown whether FCV-U2 is a uropathogen or represents innocuous shedding of a respiratory or vaccine strain. Since regions of the capsid protein are unique for each strain, the purpose of this study was to sequence the capsid protein gene of FCV-U2 and compare its nucleotide and amino acid sequences to those of F9 (vaccine), FCV-U1, and other known strains of FCV.

FCV-U2 was propagated and plaque purified in Crandell-Rees feline kidney cells. Viral RNA was extracted from 3 plaques, reverse transcribed into cDNA, and amplified using a one-step RT-PCR and capsid protein gene-specific primers. The 2.3kb RT-PCR products were purified from an agarose gel and sequenced on an automated DNA sequencer. Published FCV capsid protein gene nucleotide and amino acid sequences were obtained from GenBank and compared using a multiple sequence alignment software package.

Overall, the amino acid sequence of the FCV-U2 capsid protein was 89% identical to that of the F9 (vaccine) strain. However, amino acid sequences of hypervariable regions C and E of the FCV-U2 capsid protein were only 60% (region C) and 74% (region E) identical to the corresponding regions of the F9 strain. Identity between the amino acid sequences of the FCV-U2 capsid protein regions A, B, D, and F and those of the F9 strain ranged between 85% and 96%. Identity between the amino acid sequences of the FCV-U2 capsid protein regions A, B, C, D, E, and F and those of urine isolate FCV-U1 were 85%, 92%, 20%, 96%, 65%, and 89% respectively. When compared to other published sequences, FCV-U2 appeared to be genetically distinct from other FCV strains.

In conclusion, our results indicate that the capsid protein gene of the urine isolate FCV-U2 is genetically distinct from those of the F9 (vaccine), FCV-U1, and other known strains of FCV. Further studies are necessary to determine whether FCV-U1 and FCV-U2 have a causative role in ILUTD.

- 181** COMPARISON OF PLASMA CLEARANCES OF CREATININE AND IOHEXOL FOR GLOMERULAR FILTRATION RATE ASSESSMENT IN CATS. A. Le Garrec,<sup>1</sup> V. Laroute,<sup>2</sup> F. de La Farge<sup>3</sup> and H. Lefebvre.<sup>2</sup> <sup>1</sup>Clinique vétérinaire Bellecour, 69002 Lyon, <sup>2</sup>UMR Physiopathologie et Toxicologie Experimentales, Ecole Nationale Vétérinaire de Toulouse, 31076 Toulouse Cedex, <sup>3</sup>Biochimie IV, Hôpital Rangueil, 31054 Toulouse Cedex, France.

Urine clearance procedures for assessment of glomerular filtration rate (GFR) in cats are not feasible in clinical practice. The objective was to compare in healthy and renal-impaired cats plasma clearances of exogenous creatinine (CICr) and iohexol (CII) after bolus intravenous (i.v.) administration.

Six cats (one healthy and 5 with chronic renal failure) aged from 3 to 12 years old and weighing 4.1 to 5.7 kg were studied. The inclusion criteria were to have stable azotemia and no concomitant treatment. Owner's consent was obtained. Cats were withheld from food overnight but water was given ad libitum. 64.7 mg of iohexol (= 30 mg of iodine) and 40 mg of creatinine per kg were simultaneously injected by i.v. route. Samples were obtained before and 5, 15, 30, 60, 120, 180, 360, and 480 minutes after administration. Plasma creatinine concentration was determined by Jaffé's technique, an enzymatic method, and by High-Performance Liquid Chromatography (HPLC). Ioexol concentration was determined by HPLC. Plasma data for ioexol and creatinine were subjected to non-compartmental analysis using a statistical moment approach. Area under the curve, volume of distribution (Vss), CI and mean residence time (MRT) were calculated using the arithmetic trapezoidal rule with extrapolation to infinity. Statistical analyses were carried out using a general linear model.

Basal plasma creatinine concentration was  $1.9 \pm 0.65$  mg/dL (range: 1.1–3.0 mg/dL). Jaffé's method for plasma creatinine assay was less reliable than enzymatic method ( $R^2 = 0.9844$ ) when compared to HPLC assay. Two stereoisomers, exo-iohexol (quantitatively the most important) and endo-iohexol, were identified. CICr ( $2.1 \pm 0.76$  mL/kg/min) was similar to exo-iohexol CI ( $1.6 \pm 0.50$  mL/kg/min), but higher than endo-iohexol CI ( $1.5 \pm 0.49$  mL/kg/min,  $P < 0.01$ ). Exo- and endo-iohexol CI were not statistically different. The Vss ( $P < 0.001$ ) and MRT ( $P < 0.001$ ) values were higher for creatinine ( $477 \pm 107.8$  mL/kg and  $4.3 \pm 2.30$  h) compared to those of endo- ( $130 \pm 20.2$  mL/kg and  $1.6 \pm 0.27$  h) and exo-iohexol ( $156 \pm 24.2$  mL/kg and  $1.7 \pm 0.28$  h).

We conclude CICr may represent an alternative to CII for GFR assessment by single bolus i.v. administration in cats. Enzymatic method for plasma creatinine assay in cats is valid, as shown by comparison with HPLC method. The Vss value of creatinine corresponds moreover to the volume of body water, which could be useful for assessment of hydration state.

- 182** COMPARISON OF SURGICAL METHODS FOR GENE DELIVERY INTO CANINE KIDNEYS. V. Chetboul,<sup>\*</sup> J.L. Pouchelon,<sup>\*</sup> B. Klonjowski,<sup>oo</sup> M. Desvaux,<sup>\*</sup> V. Laroute,<sup>\*\*</sup> M. Eloit,<sup>oo</sup> D. Rosenberg,<sup>o</sup> C. Maurey,<sup>o</sup> F. Crespeau<sup>oo</sup> and H.P. Lefebvre.<sup>\*\*</sup> <sup>\*</sup>Cardiologie, <sup>oo</sup>UMR INRA Genetique moleculaire et cellulaire, Ecole Nationale Veterinaire, 94704 Maisons-Alfort Cedex, <sup>\*\*</sup>Pathologie, CHU Henri-Mondor, 94010 Creteil, <sup>oo</sup>UMR Physiopathologie et Toxicologie Experimentales, Ecole Nationale Veterinaire, 31076 Toulouse Cedex, France.

Renal gene therapy seems promising as a therapeutic strategy for some renal and cardiovascular diseases, but safety and efficiency of such a gene delivery has never been documented in dogs. The purpose of this study was to compare the short-term safety of three different routes of adenoviral administration, i.e. intra-renal-ureteral route (IU) and intra-renal-arterial route with (IAC) or without (IA) clamping of the renal vein, in kidneys of healthy dog puppies ( $n = 9$ ), and to compare their transduction efficiency. Two adenoviral vectors were constructed: AdCMV-betaGal, which contains the E. coli Lac-Z gene with a nuclear localisation signal under the control of the cytomegalovirus (CMV) immediate-early gene promoter, and a control adenovirus vector (AdRSV-gD), which carries the pseudorabies virus glycoprotein gD gene under the control of the Rous sarcoma virus long terminal repeat promoter. The two kidneys of each dog were injected similarly, either with AdCMV-betaGal ( $10^9$  p.f.u./kidney), AdRSV-gD ( $10^9$  p.f.u./kidney) or a Phosphate Buffered Saline solution via one of the three intra-renal routes. Renal function was assessed before and 4 days after surgery. Animals were then euthanized for pathological examination. Renal samples were also monitored for the presence of nuclear beta-galactosidase-expressing cells.

Plasma biochemical parameters (urea, creatinine, protein, sodium and potassium concentrations), glomerular filtration rate and effective renal plasma flow (assessed by plasma exo-iohexol and p-aminohippuric acid clearance, respectively), and sodium and potassium excretion fractions were not altered by surgery, whatever the route of viral administration. No histological lesion was detected in any of the hematoxylin-eosin stained kidney sections, and there was no evidence of ischaemia-reperfusion injury in the kidneys subjected to venous clamping. The IAC injection of AdCMV-betaGal resulted in a strong expression of the reporter gene in the cortex, located mainly in the interstitial cells, and in some endothelial cells, while the IU injection of AdCMV-betaGal showed beta-gal activity in the pyelic epithelial cells and the distal tubular epithelial cells in the outer stripe of the cortex.

These promising results suggest that direct renal gene transfer is feasible and may provide a useful therapeutic approach, especially when secretion of the transgene product is required in the cortical area.

- 183** HYPOPHYSECTOMY IN DOGS USING AN ULTRASONIC ASPIRATOR AND A NEW SURGICAL APPROACH. T. Axlund, E. Behrend, D. Sorjonen, S. Simpson. Auburn University, Auburn, AL.

The purpose of this study was to evaluate the efficacy of hypophysectomy in normal dogs using a novel surgical approach and ultrasonic aspiration as a potential treatment for pituitary-dependent hyperadrenocorticism.

Preoperative localization of the pituitary gland was achieved using contrast-enhanced computed tomographic imaging. The position of the pituitary gland was correlated with the hamular processes of the pterygoid bones, which are used as surgical landmarks. For the surgery, the dogs were placed in dorsal recumbency and a right paramedian incision made to expose the confluence of the digastric and myohyoideus muscles. A dissection plane between the digastric and myohyoideus muscles was developed to expose the medial surface of the palatine tonsillar fold. An incision was made into the tonsillar fold. The resulting orifice allowed access to the pharynx. The tongue was retracted and a 3-cm incision made in the soft palate. A pneumatic drill and burr was used to create a 4 X 4-cm partial-thickness defect on the midline of the sphenoid bone. The remaining inner cortical portion of the sphenoid bone was removed with a 1-mm up-biting rongeur exposing the dura mater underlying the hypophysis. A cruciate incision was made in the dura mater and the hypophysis was extirpated to the level of the diaphragma sellae using an ultrasonic aspirator. The defect in the sphenoid bone was packed with gelfoam and the soft tissues were closed in routine fashion. The animals were maintained on L-thyroxine (0.1 mcg/10# PO bid) and prednisone (0.25 mg/kg PO sid) for the duration of the study. Desmopressin was given for 7 days (1 drop in conjunctiva sid). Endocrinologic testing was performed to assess the pre- and post-surgical status of the anterior pituitary and intermediate lobe. A modified combined anterior pituitary function test was used. Sequential 30-second administrations of thyrotropin-releasing hormone (10 ug/kg IV), corticotropin-releasing hormone (1 ug/kg IV) and gonadotropin-releasing hormone (10 ug/kg IV) were given. Blood samples were collected at 0, 5, 10, 20, 30, and 45 minutes and assayed for ACTH, thyroid-stimulating hormone (TSH), cortisol and thyroid hormone (T4). Haloperidol (1.0 mg/kg IV) was administered and the hormone alpha-melanocyte-stimulating hormone (alpha-MSH) quantitated at 0 and 15 minutes. These tests were performed before surgery, 2 and 10 weeks after surgery. Immunohistochemical analysis of the hypothalamus and sella turcica was performed using labeled antibodies (ACTH, alpha-MSH and TSH) to identify the remaining cells.

Results of this study confirm the efficacy of hypophysectomy in normal animals. Endocrinologic and post-mortem testing suggest that complete hypophysectomies are difficult to perform using this technique.

- 184** AGE, NEUROLOGICAL EXAMINATION, AND CEREBROSPINAL FLUID ANALYSIS AS PREDICTORS OF OUTCOME OF MRI SCANNING IN 115 DOGS WITH SEIZURES. W. Bush, E. Darrin, F. Shofer, C. Vite, S. Steinberg. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

The decision to seek an MRI scan can be difficult since it is expensive and often not readily accessible. The use of CSF analysis in patients with a history of seizures may be helpful in guiding the decision whether or not to perform an MRI scan. The purpose of this retrospective study was to identify which factors might predict an abnormal MRI scan in dogs with seizure.

In this study the neurological examination was considered abnormal when deficits reflected intracranial disease. CSF fluid was considered abnormal if there were more than five nucleated cells per microliter or the protein concentration was 25 mg/dl or greater. MRI was considered abnormal when abnormal anatomy, signal intensity or contrast enhancement was identified.

In dogs with normal neurological examinations who began to seizure after six years of age, the chances of an abnormal MRI was 24% when the CSF was normal and 50% when the CSF was abnormal. Similarly, in dogs with normal neurological examinations who began to seizure before six years of age the chance of a positive scan was 12% when the CSF was normal and 30% when the CSF was abnormal.

In dogs with abnormal neurological examinations who began to seizure after six years of age the CSF findings made a significant difference in the likelihood of an abnormal scan. In these dogs there was a 96% chance of an abnormal MRI when the CSF was abnormal. However, if the CSF was normal the chance of a positive scan fell to 46%. In dogs with abnormal neurological examinations who began to seizure before six years of age, the chances of a positive scan are 93% and 70% with abnormal and normal fluid respectively.

The neurological examination was more reliable predictor of an abnormal scan than the CSF analysis. The sensitivity and specificity of the neurological examination in predicting an abnormal scan were 82% and 80% respectively, versus 72% and 68% with CSF analysis alone. Lastly, if a Receiver Operator Characteristic curve is established for all dogs, a CSF protein of 30 mg/dl has an 80% sensitivity and a 60% specificity for identifying an abnormal scan. The positive predictive value of a protein concentration greater than 30 mg/dl was 80%.

- 185** COMPUTED TOMOGRAPHIC IMAGING GUIDED BRAIN BIOPSY USING A DISPOSABLE REAL-TIME STEREOTACTIC SYSTEM IN THE DOG. Thomas Flegel,<sup>1</sup> Michael Podell,<sup>1,3</sup> Philip March,<sup>1</sup> Donald Chakeres.<sup>2</sup> <sup>1</sup>Department of Veterinary Clinical Sciences, College of Veterinary Medicine, and Departments of <sup>2</sup>Radiology and <sup>3</sup>Neuroscience, College of Medicine, The Ohio State University, Columbus, OH.

Brain biopsies are often required for a definitive diagnosis to establish an appropriate treatment plan in dogs with intracranial lesions. Current methodology is limited to complex, expensive, time-consuming, stereotactic techniques that operate without the advantage of real-time imaging. The objectives of this study were to assess the feasibility, accuracy, and safety of securing site-selective brain biopsies using a real-time computed tomographic (CT) guided Utopix® stereotactic navigator system from the dog.

Brain biopsies were first performed on 2 canine cadavers, followed by 2 male intact Beagle dogs under general anesthesia. Serial scored neurologic examinations were performed prior to and after brain biopsy. A predetermined target and vector were selected prior to biopsy. This system consists of a lower and upper device with specific V-shaped coordinate image conspicuous patterns (Chakeres pattern). The pattern encodes the location of two points on the device to the mm along the chosen vector towards the target. The lower device is applied to the skin and defines the entry point. The upper device is supported several centimeters above the head, which has a remote control needle support that is used to define the second point. A 3-point system including the target and both devices are used to establish a trajectory for biopsy needle placement. Two cerebrocortical biopsies were performed via "mini" burr hole craniectomy (3 mm) created with a handheld drill. Both anesthetized dogs were biopsied twice, 10 and 3 weeks apart, respectively. A routine flap-craniectomy was not made. Diagnostic quality brain tissue was obtained each time precisely at the pre-chosen location. Mild lateralizing forebrain deficits were present in one dog after 1 biopsy, which resolved within one week. Pathologic evaluation of the brains demonstrated only mild hemorrhage along the biopsy tract in 1 dog.

These results indicate that the Utopix® navigator system is an accurate, safe, fast, and reliable method for real-time acquired brain biopsy in the dog without the need or risk of a standard flap-craniectomy.

- 186** INTERVERTEBRAL DISC EXTRUSION IN SIX CATS. M.F. Knipe, K.M. Vernau, and R.A. LeCouteur, University of California, Davis, CA.

Existing reports concerning intervertebral disc disease (IVDD) have focused almost exclusively on dogs, although a small number of individual reports of IVDD in cats have been published. Spontaneous disc extrusion with clinical signs of myelopathy was diagnosed in six cats at the VMTH between September 1995 and July 2000. Hospital records and radiographs were retrospectively reviewed. The purpose of this report is to describe the history, neurologic signs, myelographic findings, surgical therapy, pathology, and neurologic recovery in six cats with intervertebral disc extrusion.

Each cat was evaluated by physical and neurologic examinations. Cerebrospinal fluid (CSF) analysis, vertebral column radiographs, and myelography were completed under general anesthesia in all cats. Surgical decompression by means of hemilaminectomy and removal of the compressive mass was done in all cats, and extradural material recovered at surgery was examined histopathologically. All cats were re-examined by the authors at the VMTH from several weeks to years post-operatively.

A single extradural compressive lesion was identified on myelography of three cats, two compressive lesions in one cat, and three compressive lesions in one cat. A single significant compressive lesion and multiple chronic disc protrusions were seen in one cat. Surgical decompression was done in all cats, compressive material was removed, and histopathology confirmed the presence of degenerating disc material in all cases.

All cats had residual neurologic deficits when re-examined. Both the clients and the authors assessed five of the six cats to have a good to excellent outcome. One cat was assessed to have a poor outcome, but in contrast to the other cats, an additional progressive disease process was suspected.

From the analysis of the six cats in this report, it is concluded that disc extrusion resulting in clinical signs of a myelopathy in cats is an infrequent problem, but does occur. Surgical removal of extruded disc material in healthy cats may result in good neurologic and functional recovery.

- 187** STATUS EPILEPTICUS IN DOGS WITH IDIOPATHIC EPILEPSY: A LONG-TERM EVALUATION OF 32 CASES. M. Saito, KR. Muñana, N.J.H. Sharp, N.J. Olby. North Carolina State University (NCSU), Raleigh, NC.

Despite the high prevalence of idiopathic epilepsy (IE) in dogs, there are few reports documenting the course of disease over an affected animal's lifetime. Within the population of dogs with IE, there is a group of animals that develop status epilepticus (SE). SE refers to repeated seizure activity with which there is no intermission. Untreated seizure activity can lead to irreversible neuronal injury in addition to systemic complications and as such is considered a medical emergency. It is not known why certain individuals with seizure disorders develop SE; however, pathophysiological studies suggest that recurrent uncontrolled seizures on their own can predispose to future episodes of SE and poor long-term seizure control. Currently, little information is available on the population of dogs with IE that have episodes of SE during the course of their disease. The purpose of this retrospective study is to identify risk factors that predict the occurrence of SE among dogs diagnosed with IE and determine the influence of SE on long-term outcome and survival of epileptic dogs.

Medical records of dogs born before 1990 (such that they would be at least 10 years of age if still alive at the time of this study) that were referred to NCSU between 1990 and 1996 in which idiopathic epilepsy was diagnosed were reviewed. Thirty-two dogs met these criteria. Information on signalment, seizure onset, initiation of therapy, anticonvulsants administered, presence of SE, overall seizure control, long-term outcome and final disposition was obtained from the medical records and through telephone interviews. Differences between dogs with SE and dogs without SE were statistically evaluated.

Fifty-nine percent of dogs in the study had one or more episodes of SE. Body weight was the only variable determined to significantly differ between the group of dogs with SE and those without SE. Thirteen dogs (9 with SE, 4 without SE) were still alive at the time of the study at 10 years of age or older. Six deaths in dogs with SE were directly attributed to the seizure disorder, resulting in a long-term mortality rate of 31.6%. The average lifespan for dogs with SE and without SE was 8.3 years and 11.3 years, respectively. A difference in survival was demonstrated between the two groups.

A large percentage of dogs with idiopathic epilepsy develop SE. Dogs with greater body weight are more likely to develop SE. Early, appropriate seizure treatment does not appear to decrease the risk for developing SE. Most dogs with idiopathic epilepsy have a normal expected lifespan, although the presence of SE increases the mortality associated with the disease.

- 188** A COMPARISON OF THE NUTRITION OF HOME-PREPARED AND COMMERCIAL DIETS FOR DOGS. EL Streiff,<sup>1</sup> RF Butterwick,<sup>2</sup> JE Bauer,<sup>1</sup> <sup>1</sup>Texas A&M University, College Station, TX, USA, <sup>2</sup>Waltham Centre for Pet Nutrition, Melton-Mowbray, UK.

This study was undertaken to determine whether home-prepared diets for dogs were nutritionally adequate. The study was an analysis of data gathered from a population of pet owners in Vienna, Austria. The control group consisted of dogs fed complete and balanced commercial diets, and the experimental group consisted of dogs fed home-prepared diets. Data sources included a seven-day food diary, as well as laboratory analysis of food and serum samples.

The mean energy and fat contents of both the home-prepared and control diets were higher than American Association of Feed Control Officials (AAFCO) recommendations, and the trend was that the home-prepared diets were higher than the control diets. However, the home-prepared diets were significantly lower in calcium and phosphorus than the control diets and AAFCO recommendations. Also, for the fat-soluble vitamins A, D, and E the home-prepared diets' mean values tended to be below AAFCO recommendations and the control diets.

In addition the home-prepared diets were higher in total saturated fat and lower in total polyunsaturated fat compared to a selected group of American commercial diets analyzed in our Texas A&M (TAMU) laboratory. Serum phospholipid relative fatty acid relative concentrations of the dogs fed home-prepared diets were significantly lower in 18:2n-6 and 20:4n-6 than serum of dogs fed the American commercial diets in a colony at TAMU.

The home-prepared diets were energy dense, but without an increased nutrient density. However, no clinical signs of deficiency were noted. Reasons for these findings include that the AAFCO recommendations have a sufficient margin of safety above absolute physiological requirements and that the analyzed diets may not have been representative of long-term intake of the animals. The fatty acid analyses of the food and serum provided evidence that different fat sources were used between European home-prepared and American commercial canine diets.

# 189 CHARACTERIZATION OF $\Delta$ -6-DESATURASE ACTIVITY IN DOG LIVER MICROSOMES. BL Dunbar, JE Bauer. Comparative Nutrition Research Laboratory, Texas A&M Univ., College Station, TX.

Long Chain Polyunsaturated Fatty Acids (LCPUFA) are physiologically important precursors for eicosanoids, leukotrienes, and prostanoids. Desaturation of essential fatty acids (EFAs) by  $\Delta$ 6-desaturase is considered the rate-limiting step in conversion of EFAs to LCPUFA. This study was designed to characterize dog liver  $\Delta$ 6-desaturase activity using both n-3 and n-6 fatty acid substrates.

Liver microsomes were prepared using fresh liver from healthy dogs. Microsomes were incubated with  $^{14}$ C labeled 18 carbon EFA substrates. Following incubation, lipids were extracted, saponified and phenacylated. The resulting fatty acid phenacyl esters (FAPES) were separated by HPLC. Radioactivity in the FAPES was measured with a liquid scintillation counter. Accumulation of radioactive product was converted to enzymatic activity and expressed as pmol/min/mg protein.

Using 18:3n-3 as substrate,  $\Delta$ 6-desaturase maximal velocity was 42.2 pmol/min/mg protein and using 18:2n-6 substrate, the maximal velocity was 3.3 pmol/min/mg protein. Apparent  $K_m$  values were 14.2  $\mu$ M for 18:3n-3 and 15.6  $\mu$ M for 18:2n-6. Maximal velocities were lower than those previously reported in dogs and other species. Possible explanations for the low values include the presence of high endogenous fatty acid concentration inherent in the dog liver microsome preparations providing high competitive amounts of non-radioactively labeled substrate, or methodological differences used in other studies.

These data show that dog liver microsomes have the ability to desaturate EFAs. Also, the maximal velocity of  $\Delta$ 6-desaturase of 18:3n-3 is considerably higher than that of 18:2n-6 *in vitro*, while  $K_m$  values are similar. Physiologically, 18:3n-3 concentration in liver (2.4  $\mu$ M) may never exceed the  $K_m$  for desaturation in the absence of high dietary amounts while 18:2n-6 amounts are readily converted because its concentration (64.4  $\mu$ M) easily exceeds the  $K_m$ . These phenomena may explain the low *in vivo* conversion rate of 18:3n-3 in dogs and other species. These findings suggest that high levels of 18:3n-3 supplementation may be necessary to exceed the  $\Delta$ 6-desaturase  $K_m$  and significantly affect physiological levels of n-3 LCPUFA in the dog.

# 191

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# 190 THE EFFECT OF DIETARY MEDIUM CHAIN TRIGLYCERIDES ON DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY. GM Rutz,<sup>1</sup> JM Steiner,<sup>1</sup> JE Bauer,<sup>2</sup> and DA Williams.<sup>1</sup> <sup>1</sup>Gastrointestinal Laboratory, Texas A&M University, College Station, Texas, <sup>2</sup>Comparative Nutrition Research Laboratory, Texas A&M University.

Based on studies of digestion and absorption of medium chain triglycerides (MCTs) it has been suggested that replacing long chain triglycerides with MCTs in the diet of dogs with exocrine pancreatic insufficiency may be beneficial. The goal of this project was to prove or disprove this hypothesis.

The effect of three diets containing either 0, 15, or 35% of the fat content as MCTs on dogs with EPI was studied. MCTs were mainly C<sub>12:0</sub> triglycerides. The diets were fed to 24 dogs with spontaneous exocrine pancreatic insufficiency (EPI) without complicating diseases, and to 6 healthy control dogs. The effect of the diet was evaluated by subjective measures as assessed by the owners and by measurement of objective parameters. Dogs served as their own controls and were randomized into six feeding order groups. All dogs received each of the diets for 12 weeks.

In dogs with EPI mean serum cholesterol ( $p < 0.0001$ ), vitamin E ( $p = 0.001$ ), retinyl stearate ( $p = 0.049$ ), and retinyl palmitate ( $p = 0.001$ ) concentrations were all significantly higher when fed the 35% MCT compared to the 0% MCT diet. Also, serum retinyl stearate ( $p = 0.019$ ) and retinyl palmitate ( $p = 0.011$ ) concentrations were significantly higher after feeding the 35% MCT compared to the 15% MCT diet. Mean serum vitamin E concentration was significantly higher in healthy control dogs on the 35% MCT diet compared with those on the 15% MCT diet ( $p = 0.014$ ). MCT content of the diets did not have any effect on any of the other parameters measured (CBC, serum chemistry profile, retinol, retinol oleate, vitamin D, cTLL, cobalamin, folate, digestibility of protein, fat, ash, moisture, caloric digestibility, and metabolizable energy). Analysis of the subjective data revealed no significant difference between the three experimental diets for appetite, attitude, drinking behavior, volume of feces, defecation frequency, color of feces, consistency of feces, flatulence, or borborygmus.

We conclude that a higher MCT content in the diet led to increases in vitamin E, cholesterol, triglyceride, retinyl stearate, and retinyl palmitate concentrations in serum from dogs with EPI, and vitamin E concentration in serum from healthy control dogs. However, there was no effect on subjective parameters evaluated by owners.

# 192 PHARMACOKINETICS OF ENROFLOXACIN, A FLUOROQUINOLONE ANTIBIOTIC, IN NEONATAL KITTENS. M.A. Seguin,<sup>1</sup> M.G. Papich,<sup>2</sup> and J.K. Levy.<sup>1</sup> <sup>1</sup>Univ. of Florida, Gainesville, FL; <sup>2</sup>North Carolina State Univ., Raleigh, NC.

Neonatal sepsis is a leading cause of fading kitten syndrome. Effective treatment of septicemia in kittens is hampered by a lack of information on appropriate dosing in neonates. In particular, neonates of all species may have different rates of hepatic drug metabolism and renal clearance, which can markedly affect serum concentrations and drug half-lives. Enrofloxacin is a fluoroquinolone antibiotic with an excellent spectrum of activity against most of the common bacterial species in feline sepsis. The purpose of this study was to provide pharmacokinetic data for enrofloxacin in kittens aged 2–8 weeks in order to establish treatment guidelines for neonatal kittens with bacterial sepsis.

Following a single IV, oral, or SQ dose of enrofloxacin at 5 mg/kg, samples were collected at 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h in kittens aged 2, 4, 6, and 8 weeks. Samples from 6–8 healthy kittens were taken for each timepoint. For purposes of comparison, time concentration curves were established from 7 adult cats following IV administration of 5 mg/kg enrofloxacin. Plasma concentrations of enrofloxacin and its active metabolite, ciprofloxacin, were determined using reverse-phase HPLC with UV detection. The method of extraction was modified by the use of solid-phase-extraction cartridges to allow for small sample sizes. Data from adult and 6–8 wk kittens was analyzed by two-stage population pharmacokinetic methods using compartmental modeling and least-squares. Data from 2–4 wk kittens was analyzed by the naïve pooling method. Statistical comparisons were made by ANOVA.

Serum half-lives in 2–8 wk kittens following IV administration were comparable (4–4.6 h) but were less than that in adult cats (6.7 h). In addition, the volume of distribution was greater in 6–8 wk kittens as compared to adults, resulting in decreased maximum serum concentrations (C<sub>max</sub>). SQ injection resulted in similar C<sub>max</sub> and half lives in same-aged kittens as those by IV injection. Oral administration, however, resulted in lower serum concentrations ( $\leq 0.5$  mcg/ml at all timepoints) and longer half lives as compared to age-matched IV controls, reflecting decreased bioavailability of oral administration in nursing (unfasted) 2–6 wk kittens. Oral, SQ, and IV administration were equivalent in 8 wk kittens. Metabolism of enrofloxacin to ciprofloxacin contributed approximately 10% to the total C<sub>max</sub>.

SQ administration of enrofloxacin to neonatal kitten provides an equally effective and more convenient route of administration as compared to IV dosing. Oral administration in an unfasted kitten may not result in sufficient serum levels at standard therapeutic dosing.



- 193** FELINE NASOPHARYNGEAL POLYPS: HISTORICAL, CLINICAL, AND PCR FINDINGS FOR FELINE CALICI VIRUS AND FELINE HERPES VIRUS-1 IN 21 CASES. JK Veir,<sup>1</sup> MR Lappin,<sup>1</sup> and JE Foley.<sup>2</sup> From the College of Veterinary Medicine and Biomedical Sciences, Colorado State University,<sup>1</sup> Fort Collins, CO and the School of Veterinary Medicine, University of California,<sup>2</sup> Davis, CA.

The objectives of this study were to describe the historical and clinical findings of cats with histopathologically confirmed nasopharyngeal polyps and to determine whether feline herpes virus-1 (FHV-1) or feline calici virus (FCV) could be detected in tissue by use of polymerase chain reaction (PCR) and reverse transcriptase-PCR (RT-PCR), respectively.

Records were reviewed for 21 cats (23 polyps) with histopathologically confirmed nasopharyngeal polyps diagnosed at the CSU-VTH between January 1, 1987 and December 31, 1999. Signalment, age at onset of clinical signs, major presenting complaint (otic or nasal), history of upper respiratory tract infection (URTI) within the last 3 months, radiographic evidence of bulla involvement, method of polyp removal (traction avulsion or ventral bulla osteotomy- VBO), post-treatment complications and duration, and recurrence of signs were recorded. Five sequential sections from formalin fixed polyps embedded in paraffin blocks were obtained and processed as follows: section 1 was stained with standard H&E for histopathologic review, section 2 was assessed by FHV-1 PCR, section 4 was assessed by FCV RT-PCR, and sections 3 and 5 were reserved for later analysis. In addition, 2 of the 23 polyps were preserved in fresh saline at  $-70^{\circ}\text{C}$  prior to FHV-1 PCR and FCV RT-PCR.

The median age at onset of signs was 5 years, the average age was 6.1 years, previous URTI was reported for 3 cats, the chief complaint was otic for 16 cats, and the chief complaint was nasal/respiratory for 5 cats. Radiographic evidence of bulla involvement was present for 11 cats. Traction/avulsion alone was used to treat 6 cats, VBO alone was used to treat 10 cats, both treatments were used to treat 4 cats, and 1 cat was not treated. Of the 10 cats treated with traction/avulsion alone, 6 were radiographically normal and 4 had abnormal bulla; recurrence of the polyp occurred in 1 cat with normal radiographs and all 4 cats with abnormal bulla. All samples were negative for FHV-1 and FCV.

We conclude that the traction/avulsion technique is a reasonable primary treatment for nasopharyngeal polyps if the bullae are radiographically normal at the time of initial diagnosis. Failure to detect FHV-1 or FCV by the techniques utilized suggest that tissue persistence of these viruses is not associated with the development of nasopharyngeal polyps.

- 194** BACTERIAL CULTURE RESULTS IN CATS WITH UPPER AND LOWER AIRWAY DISEASE: 255 CASES (1995–1999). JE Stein, MR Lappin. Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, Colorado.

The purpose of this study is to report the prevalence of bacteria isolated from the upper and lower airways of client-owned cats with respiratory disease.

The microbiology database of the Veterinary Diagnostic Laboratory at Colorado State University was searched for bacterial culture results from cats from 1995–1999. The respiratory tract of 255 cats was cultured for aerobic bacteria during the time period. For 122 cats, nasal biopsies or swabs were cultured. Cultures of lower airways were performed on 75 samples obtained by transtracheal/transoral washings (TTW) and 58 samples obtained by bronchoalveolar lavage (BAL). *Mycoplasma* sp. culture was performed on 51 TTW or BAL samples.

Of the nasal cultures, 87 of 122 (71%) were positive for bacterial growth. *Pasteurella* sp. (31%), *Staphylococcus* sp. (23%), and *Pseudomonas* sp. (13%) were the most common isolates. *Bordetella bronchiseptica* was grown from 5% of the cats. A single bacterium was isolated from 69% of the cultures and multiple bacteria were isolated from 31% of the cultures.

Overall, 68 of 133 (51%) lower airway cultures were positive for bacterial growth. These included 43 of 75 (57%) TTW samples and 25 of 58 (43%) BAL samples. *Pasteurella* sp. was the most common isolate in both groups (47%) followed by *Streptococcus* sp. (18%), and *Escherichia coli* (9%). *Bordetella bronchiseptica* was isolated from one TTW sample (1.5%) but no BAL samples. A single bacterium was isolated from 79% of the cultures and multiple bacteria were isolated from 21% of the cultures. *Mycoplasma* sp. were isolated from 8 of 51 (16%) lower airway samples.

Bacteria were commonly isolated from the respiratory passages of cats with respiratory disease. Since *B. bronchiseptica* was rarely cultured, the organism appeared to an insignificant cause of respiratory disease in this group of client-owned cats.

- 195** A MODEL FOR NON-INVASIVE MONITORING OF OVARIAN FUNCTION IN THE DOMESTIC CAT. B. Griffin,<sup>1,2</sup> L. H. Graham,<sup>3</sup> A.M. Heath,<sup>2</sup> J. C. Wright,<sup>2</sup> D. W. Young,<sup>2</sup> H. J. Baker,<sup>1,2</sup> Scott-Ritchey Research Center,<sup>1</sup> College of Veterinary Medicine,<sup>2</sup> Auburn University, Auburn, AL, Disney's Animal Kingdom,<sup>3</sup> Bay Lake, FL.

Euthanasia of healthy, unwanted domestic cats remains the most common cause of death in this species; therefore, methods to study and control their reproduction are needed. The purpose of this study was to develop a model for noninvasive monitoring of ovarian function for studying feline reproductive physiology and effects of potential nonsurgical contraceptives. Monitoring is challenging because cats are polyestrous, lack physical changes at estrus and estradiol (E2) concentrations may rise sharply and return to baseline within as few as 2–3 days. Obtaining vaginal cytology can induce ovulation. Fortunately in the cat, E2 is eliminated through the feces, where it may be measured.

Two colonies of 6 queens each were group housed in an enriched environment on a stimulatory light cycle (12D:12L). All cats were observed daily for behavioral estrus. All stool samples were collected for fecal E2 concentrations. Blood was collected biweekly for serum progesterone (P4) concentrations. Queens readily consumed 0.5 ml of bakers' paste food coloring once daily in canned cat food. Colorings served as fecal markers by imparting a distinct coloration to each queens' feces, allowing identification in the litter box. E2 was extracted from fecal samples with ethanol at  $90^{\circ}\text{C}$ . Fecal extracts and serum samples were assayed for 17- $\beta$  E2 and P4, respectively using solid phase radioimmunoassay kits (Coat-a-Count, Diagnostic Products Corp., CA). Assay validity was confirmed by recovery of added hormone and dilutional parallelism.

To optimize detection of behavioral estrus, three observation methods were employed at two times of day during the initial 66 days of the study. Cats were observed both in a group and individually each morning and evening. To avoid induction of ovulation, physical stimulation of the dorsal rump/genital area was avoided. For all cats, estrous behavior was best detected by group observation ( $p = 0.001$ ). More behaviors were detected in evenings than in mornings ( $p = 0.02$ ). Two/12 queens exhibited silent estrus. A separate group of 12 queens were monitored identically except vasectomized tom cats were used for behavioral estrus detection. Data indicated silent estrus (3/12), behavioral nymphomania (1/12), and preferences/aversions to particular tom cats, thus the use of tom cats did not improve behavioral estrus detection. Fecal E2  $> 75\text{ ng/g}$  confirmed estrus.

Components of a reliable system of non-invasive monitoring of feline ovarian function include: environmentally enriched social housing with a stimulatory light cycle, group behavioral observation (daily in the evening), fecal estradiol concentrations (once every 24–48 hours), and serum progesterone concentrations (biweekly).

- 196** LESSONS IN REPRODUCTIVE PHYSIOLOGY AND BEHAVIOR IN DOMESTIC QUEENS FROM NON-INVASIVE MONITORING. B. Griffin, Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, Auburn, AL.

The purpose of this study was to utilize a model for non-invasive monitoring of ovarian function to study the normal reproductive physiology and behavior of domestic cats under laboratory conditions. Information gained from this study will be used to optimize evaluation of potential nonsurgical contraceptives/sterilants in cats.

Colonies of 7–8 DSH queens each were group housed in an enriched environment on a stimulatory light cycle (12D:12L). A total of 18 queens ranging in age from 5 months to 5 years were monitored for 12 months. All cats were observed each evening for behavioral estrus. To avoid induction of ovulation, physical stimulation of the dorsal rump/genital area was avoided. Stool samples were collected once every 48 hours for fecal estradiol (E2) concentrations. Blood was collected biweekly for serum progesterone (P4) concentrations. Hormonal estrus was defined as fecal E2 concentrations  $> 3$  times baseline and  $> 75\text{ ng/g}$  dry weight feces. Behavioral estrus was defined as lordosis and/or treading and/or tail deflection. Ovulation was defined as serum P4 concentration  $> 2\text{ ng/ml}$ . Social order of cats in established colonies was independently ranked.

Duration of behavioral estrus ranged from 1–10 days. Homosexual behavior was frequently observed in some queens (6/18). Interestrous intervals ranged from 2–51 days, with 2–3 weeks typical. Eleven/18 queens ovulated and 6 ovulated following every estrus. Pyometra occurred in one queen after ovulation. Following ovulation, interestrous intervals (including diestrus) ranged from 36 to 103 days (mean 51 days). Comparison of data from a separate group of 12 queens identically monitored except using vasectomized tom cats to detect estrus (copulation was not permitted) revealed no significant difference ( $p > 0.05$ ) in the rate of ovulation. Queens  $< 1\frac{1}{2}$  years of age tended to cycle irregularly, while those  $\geq 1\frac{1}{2}$  years of age maintained unique, but consistent patterns of estrus/interestrous. Three/18 queens exhibited silent heat. These were ranked lowest in social order in their colonies and relocation to rooms with fewer or juvenile queens resulted in displays of estrus behavior. Two queens exhibited behavioral estrus at the end of diestrus. In all other queens, excellent correlation between behavioral and hormonal estrus was present. In some colonies, synchronization with the dominant queen was observed. Late onset of puberty ( $> 14$  mos. of age) was seen in 2/3 queens that were introduced into one colony at 5 months of age.

These results illustrate the importance of establishing baseline data in individual queens prior to contraceptive testing. In addition, caution must be used in interpretation of estrus suppression in juvenile and low social order queens.

# 197 ENDOGENOUS MELATONIN CONCENTRATIONS IN THE DOMESTIC CAT. D. W. Young,<sup>2</sup> B. Griffin,<sup>1,2</sup> Scott-Ritchey Research Center,<sup>1</sup> College of Veterinary Medicine,<sup>2</sup> Auburn University, Auburn, AL.

The purpose of this study was to validate an assay for measuring plasma melatonin concentrations in domestic cats and to measure the normal endogenous plasma melatonin concentrations in queens maintained on long and short photoperiods.

Plasma samples were assayed for melatonin using solid phase radioimmunoassay kits (Buhlman, American Laboratory Products Co., Windham, NH). To evaluate extraction efficiency, 1 ml plasma from 10 cats was spiked with <sup>3</sup>H melatonin and allowed to equilibrate overnight at 5° C prior to extraction using solid phase columns provided with the kit. Mean extraction efficiency was 88.9 % with a coefficient of variation of 3.4 %. A linear relationship was found between extraction volume and counts recovered. Sensitivity of the assay is 0.3 pg/ml according to the manufacturer. The assay was highly specific for melatonin as indicated by low cross-reactivity (≤0.003%) to related compounds. Assay validity was further confirmed by high recovery of added hormone (103% and 96.4% at 10 pg/ml and 20 pg/ml, respectively). Intraassay and interassay coefficients of variation were 2.22% and 16.0 %, respectively.

Plasma melatonin concentrations were measured in 3 healthy 2-year-old, DSH queens group housed in an enriched environment under full spectrum natural lights (Vita-lites™, Duro-test Corp., Fairfield, NJ). The queens were entrained to a long photoperiod (14L:10D) for 1 month. They were sedated with ketamine (10 mg/kg) and diazepam (0.5 mg/kg) IV for insertion of jugular catheters. Following recovery from sedation, plasma was collected every 2 hours for 24 hours. A red light (25 watt bulb, red Wratten filter, Eastman Kodak, Rochester, NY) remained on during the entire study period facilitating collection of nighttime samples with 0 lumens of white light measured. The same cats were entrained to a short photoperiod (6 L:18 D) and the procedure was repeated.

Data revealed that endogenous plasma melatonin concentrations in cats are similar to those of other species and not significantly higher as reported by others. Concentrations did increase during darkness and decrease during light periods. In the long photoperiod, cats remained at basal levels (≤0.53 pg/ml) until within 2 hours of darkness at which time they began to rise. Peak night time concentrations were 4.3–9.33 pg/ml. All cats returned to basal concentrations within 2 hours after the onset of illumination. On the short photoperiod, cats began to rise between 1–3 hours after the onset of darkness, peaking at 7–9 hours after the onset at 5.2–14.14 pg/ml, and remaining elevated throughout the dark period. All cats abruptly returned to basal concentrations within 1 hour prior to illumination and remained ≤0.65 pg/ml during illumination.

# 198 RELATIONSHIP BETWEEN RACING PERFORMANCE AND ECHOCARDIOGRAPHIC MEASUREMENTS BEFORE AND AFTER TRAINING IN THOROUGHBRED HORSES. Porciello F, Rueca F, Fruganti A, Pieramati C, Macolino GP. Center for Athletic Horse Studies, University of Perugia, Perugia, Italy.

Each individual has an innate athletic ability that can be enhanced with training. Athletic training is known to induce changes in heart rate, structure, and function. In the racing horse recognition of potential before and after training would allow ideal selection of animals. Echocardiography provides a means to evaluate structure and function of the heart. The purpose of this study in thoroughbred horses was to investigate the relationship between the echocardiographic (echo) measurements of the LV obtained before and after training and the racing success of each horse as judged by their earnings obtained during their first racing season.

Nine healthy yearlings 15 to 18 months of age were studied. Each horse was examined by echo before (T1) and after (T2) training. T2 examination was performed just before the starting of the 2-year-old racing season. The heart was imaged from the right parasternal window using a mobile machine (Scanner 200 Vet-Pie Medical Equipment B.V. Maastricht, Holland) and a 3.5MHz sector transducer. The heart rate was recorded during the examination, and from the long axis image of the LV the following parameters were measured both in diastole (D) and in systole (S): LV length (LVLD and LVLS), LV diameter (LVD) and LV internal perimeter. The following variables were also calculated on the basis of the formula of the "ellipsoid by area": LV volume at end diastole (LVVED) and end systole (LVVES), stroke volume (SV), ejection fraction (EF), cardiac output (CO); finally, dividing LVVED and SV by the body weight the diastolic index (DI) and stroke index (SI) were calculated. We used total earnings (E) and earnings per start (E/S) to evaluate the performance of each horse. Echo values at T1 and at T2 were compared with the performance variables using the stepwise multiple linear regression and Spearman rank correlation coefficients.

The echo measurements obtained at T1 were not correlated with performance. However, at T2, E was correlated with LVLD and LVVD, ( $r^2 = 0.72$ ;  $P < 0.05$ ); E/S was correlated with SI ( $P < 0.01$ ), LVLD ( $P < 0.01$ ), and LVVD ( $P < 0.001$ ) ( $r^2 = 0.96$ ). Additionally, the Spearman rank correlation coefficients showed that DI, SI, LVLD and LVVD at T2 were correlated to both E and E/S.

We conclude that resting 2D-echo LV evaluation in yearling thoroughbred horses after race training may be predictive of subsequent athletic performance.

# 199 CHRONIC CLENBUTEROL ADMINISTRATION NEGATIVELY ALTERS CARDIAC FUNCTION IN HORSES. M.M. Sleeper,<sup>1</sup> Charles F. Kearns,<sup>2</sup> Kenneth H. McKeever.<sup>1</sup> 1Cardiopet-Veterinary Referral Center, Little Falls, NJ; 2Rutgers University, New Brunswick, NJ.

The beta 2 sympathomimetic agent, clenbuterol is a potent repartitioning agent that has been used to promote growth in cattle and sheep by increasing protein accretion and fat removal with little or no change in total body weight. In the horse, clenbuterol is typically administered in much lower doses (mcg/kg) than other species to achieve bronchodilatory effects. Clinical improvement has been shown in horses with chronic obstructive pulmonary disease (COPD), bronchitis and pneumonia, however, previous studies have only assessed short term dosing of no more than five days. In fact, the only chronic administration study performed in the horse evaluated clinical signs of COPD after 30 days of clenbuterol administration without assessment of cardiac function. Chronic administration of pharmacological levels of beta 2-agonists have been shown to have toxic effects on the heart; however, no data exist on cardiac function in the horse after chronic clenbuterol administration. The purpose of this study was to examine the effect of therapeutic levels of clenbuterol on cardiac performance.

Twenty unfit Standardbred mares were divided into four experimental groups: clenbuterol (2.4 mcg/kg twice daily, 5days/week) plus exercise (20 minutes at 50%  $VO_{2max}$ ) (CLENEX; n = 6), clenbuterol (CLEN; n = 6), exercise (EX; n = 4) and control (CON; n = 4). M-mode and 2-dimensional echocardiography were used to measure cardiac size and function before and immediately after an incremental exercise test, before and after eight weeks of drug and/or exercise treatments. After treatment, CLENEX and CLEN demonstrated significantly higher post exercise % difference ( $[(\text{post-treatment immediately after exercise} - \text{pre-treatment immediately after exercise}) / \text{pre-treatment immediately after exercise}] \times 100$ ) of left ventricular internal dimension (LVD) at end diastole ( $23.7 \pm 4.8\%$ ;  $25.6 \pm 4.1\%$ ), LVD at end systole ( $29.2 \pm 8.7\%$ ;  $40.1 \pm 7.9\%$ ), interventricular septal wall thickness at end diastole ( $28.9 \pm 11.0\%$ ;  $30.7 \pm 7.0\%$ ), interventricular septal wall thickness at end systole ( $29.2 \pm 8.7\%$ ;  $40.1 \pm 7.9\%$ ) and left ventricular posterior wall systolic thickness ( $43.1 \pm 14.4\%$ ;  $45.8 \pm 14.1\%$ ). CLENEX and CLEN had significantly increased aortic root dimensions ( $29.9 \pm 6.1\%$ ;  $24.0 \pm 1.7\%$ ), suggesting increased risk of aortic rupture. Taken together, these data indicate that chronic clenbuterol administration may negatively alter cardiac function.

# 200 MYOCARDIAL DISEASE IN HORSES: RETROSPECTIVE STUDY IN 25 CASES (1998–2000). D. Jean, UP Clinique Equine, DEPEC, Ecole Nationale Vétérinaire d'Alfort, Paris (France).

Myocardial disease can affect cardiac function in horses. The incidence of myocardial disease in horses is probably underestimated. Lower grade myocardial disease leading to the development of arrhythmias or to sufficient poor myocardial function resulting in poor athletic performance may be more common than has been recognised previously.

The purpose of this study was to describe the clinical, medical imaging, laboratory features and outcome of 25 horses with suspected myocardial disease (myocarditis/cardiomyopathy) between 1998 and 2000. Horses were selected for inclusion based on the following criteria: history and physical examination, electrocardiographic and echocardiographic findings, clinical laboratory values (LDH and isoenzymes) and/or post-mortem findings as well as the absence of other established causes for poor performance. All procedures were not performed in all horses.

The mean age was 8.6 years old (range; 3–16 years old). Although not significant, there was a trend for gelding (64%) to be more frequently represented in horses with myocardial disease. Presenting complaints included poor performance (72%), cardiac murmurs (52%), atrial fibrillation (12%), seizures (8%) and acute pulmonary edema (8%). Myocardial disease was also detected in horses with other problems (4 cases). Atrial fibrillation (3 horses) and premature ventricular beats (2 horses) were detected by electrocardiogram. Echocardiographic findings revealed; reduced fractional shortening in 59%, left ventricular enlargement in 36% and dyskinesia in 24%. The serum concentrations of LDH-1 (41%) and LDH-2 (56%) were significantly increased.

Two horses were euthanized because of their severe myocardial disease and one horse died rapidly after sinus surgery. Two others horses were euthanized for other problems than cardiac disease. Five horses were retired of activity and 2 horses were reformed. One horse returned to normal activity after rest and another showed recurrence of poor performance after transient improvement. Follow-up stay incomplete in 10 horses.

In conclusion, myocardial disease can pose a diagnostic challenge in horses and should be always considered in horses with poor performance.

## 201 EFFECT OF CARDIAC CATHETERIZATION ON cTNI AND CK-MB IN EXERCISING HORSES. MM Durando, K Kline, VB Reef, and EK Birks. University of Pennsylvania, Kennett Square, PA.

This study evaluated the effect of left and right ventricular catheterization in exercising horses on cardiac troponin I (cTNI) and CK-MB concentrations, in order to evaluate potential myocardial damage from heart catheterization. Left and right ventricles were catheterized to measure dp/dt maximums and minimums as part of another study evaluating cardiac function in exercising horses. We wished to verify that these invasive techniques did not cause damage to the heart.

Six Thoroughbred horses, with catheters passed into the LV and 5 with catheters in the RV, ran on a high-speed treadmill at speeds eliciting VO<sub>2</sub> max. Heparinized blood was collected pre-exercise, at maximal exercise, and 1, 3, 6, 9, 12, and 24 hours post-exercise. cTNI and CK-MB concentrations were measured with a commercially available enzyme immunoassay system (Stratus CS, Dade Behring, Inc.) that uses antibodies against human cTNI and CK-MB. Samples were compared with those obtained from uncatheterized horses exercising under identical conditions. Results were analyzed by a multivariate ANOVA with post hoc analysis done by Tukey-Kramer HSD. Statistical significance was placed at  $p < 0.05$ .

There were no significant differences in cTNI or CK-MB between uncatheterized horses and horses with either LV or RV catheters at any given time point. Values for cTNI were significantly higher at 3 and 6 hours post-exercise versus pre-exercise samples for LV catheterization ( $0.022 \pm 0.003$  ng/ml, LV pre vs.  $0.062 \pm 0.012$  ng/ml, LV 3 hr; and  $0.022 \pm 0.003$  ng/ml, LV pre vs.  $0.048 \pm 0.005$  ng/ml, LV 6 hr). Although not significantly different, cTNI concentrations in uncatheterized horses tended to be greater than in LV and RV horses at all time points. All values were within the reported normal range for healthy humans. The pre-exercise CK-MB concentrations were significantly less than those observed at all other time points except the 1 and 24 hour samples. CK-MB concentrations were significantly greater in the 12 and 24-hour samples than in the pre-exercise samples in LV horses. There was no effect of time on CK-MB in the RV horses. As with cTNI, the uncatheterized horses tended to have higher CK-MB values than the RV or LV horses.

Compared to uncatheterized horses, there was no effect of either LV or RV catheterization on cTNI or CK-MB concentrations in exercising horses. These horses also had a full stress-test, including stress echocardiograms and exercising ECGs, performed 5–7 days post catheterization. These tests were similar to the pre-catheterization stress tests. These results suggest that LV and RV catheterizations do not cause lasting myocardial damage, as assessed by circulating cTNI and CK-MB concentrations and post catheterization stress-tests.

## 202 EFFICACY OF TRILOSTANE IN THE MANAGEMENT OF EQUINE CUSHING'S DISEASE. C.M. McGowan and R. Neiger. The Royal Veterinary College, London, United Kingdom.

Trilostane (Modrenal, Stegram Pharmaceuticals, UK), a 3 $\beta$ -hydroxysteroid dehydrogenase inhibitor, acts to block adrenal steroidogenesis. The aim of this study was to assess the efficacy of trilostane in twenty horses (mean age 21, SD 5.7 years) diagnosed with equine Cushing's disease using the combined dexamethasone suppression thyrotropin releasing hormone (TRH) stimulation test (17 horses) or TRH stimulation test (3 horses). Serum samples for cortisol analysis were collected via indwelling catheter at baseline, at 210 minutes (30 min after 1 mg i.v. TRH) and 22 hours after 40 mcg/kg dexamethasone i.m. Affected horses were treated with 0.4 to 1 mg/kg (mean 0.5 mg/kg) trilostane administered once daily in feed for a period of 30 days. After 30 days endocrine testing was repeated. Serum cortisol before and after treatment was compared by paired t-test.

Polyuria and/or polydipsia, present in 11 horses, was reduced in all after treatment. Lethargy was present to some degree in 19 horses, but improvement in demeanour was evident in all horses after therapy. Recurrent, chronic or persistent laminitis, present in 17 horses, improved or showed no recurrence in 14 cases. Six horses had phenylbutazone therapy discontinued during the trial due to improvement in lameness attributed to laminitis and 1 horse had phenylbutazone therapy reduced from 2 g to 1 g daily. Three horses showed severe, acute laminitis on presentation, with rotation and sinking evident on radiographs, which did not respond to trilostane. One of these horses was euthanised 14 days into the trial. One horse developed a mild bout of laminitis during the trial (Obel grade 1), but was sound by the time he was re-presented at 30 days.

While baseline cortisol (mean: 141, SD 54 nmol/l) and 22 hours post dexamethasone cortisol (mean: 109, SD 34 nmol/l) in horses before treatment were not significantly different to post treatment (baseline (mean: 159, SD 64 nmol/l), post dexamethasone (mean: 104, SD 48 nmol/l)), there was a significant reduction ( $p = 0.023$ ) of cortisol following TRH administration before (mean: 176, SD 52 nmol/l) and after (mean: 147, SD 61 nmol/l) trilostane.

In conclusion, trilostane caused an improvement in clinical signs in all horses, the most consistent being an improvement in demeanour and a corresponding decrease in cortisol response to TRH administration. Thus trilostane is a useful therapy for the treatment of equine Cushing's disease, particularly with respect to improving the quality of life of affected animals. Safety and long term effect is being investigated for a further period of 12 months.

## 203 THE ROLE OF NSAIDS IN RECOVERY OF BILE-INJURED EQUINE COLON. N. B. Campbell, S. L. Jones, A.T. Blikslager. Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Colitis is a life-threatening condition in the horse triggered by a variety of infectious agents and non-steroidal anti-inflammatory drugs (NSAIDs). Clinical signs and mortality are attributable to disruption of intestinal barrier function. We have shown a critical role of cyclooxygenase (COX) elaborated prostanoids in recovery of intestinal barrier function. For example, the non-specific COX inhibitor flunixin prevented recovery of intestinal barrier function of equine ischemic-injured jejunum, whereas the selective COX-2 inhibitor etodolac permitted full recovery. In this study, the effects of flunixin and etodolac were compared on the healing of bile salt-injured colonic mucosa. The pelvic flexure of 7 horses was isolated, perfused with 1.5mM deoxycholate for 30 minutes, then the mucosa was mounted in Ussing chambers. Mucosal barrier function was assessed by monitoring transepithelial electrical resistance (TER) and mucosal-to-serosal fluxes of <sup>3</sup>H-mannitol over 4 hours.

Deoxycholate induced significant reductions in TER ( $35 \pm 1.8 \Omega \cdot \text{cm}^2$ ) compared to control tissues ( $104 \pm 14.7 \Omega \cdot \text{cm}^2$ ;  $p < 0.05$ ), but TER recovered to levels approximately 70% of control within 4 hours, associated with histologic evidence of epithelial restitution. There was no significant effect of either flunixin or etodolac on mucosal recovery. However, there was a marked and significant reduction in TER in control tissues treated with flunixin compared to those treated with etodolac and untreated tissues ( $67 \pm 4.8 \Omega \cdot \text{cm}^2$ ,  $101 \pm 10 \Omega \cdot \text{cm}^2$ ,  $134 \pm 14.7 \Omega \cdot \text{cm}^2$  respectively;  $p < 0.05$ ). Similarly, there were significant increases in mucosal-to-serosal fluxes of mannitol in tissues treated with flunixin and etodolac compared to untreated tissues over the 4 hour recovery period. Despite these changes, there was no histologic evidence of damage, suggesting an effect on paracellular pathways.

These studies indicate exposure to 1.5mM deoxycholate injured equine colonic mucosa, but mucosal recovery was independent of COX-elaborated prostanoids. However, flunixin caused marked increases in permeability of control colonic mucosa. Etodolac also triggered increases in permeability, but they were far less than those of flunixin, suggesting etodolac may lessen detrimental side-effects in horses requiring NSAID therapy.

## 204 SERUM IgM LEVELS IN HEALTHY AND SICK ADULT HORSES. G Perkins, D Nydam, MJ Flaminio, DM Ainsworth. Cornell University, Ithaca, New York.

IgM deficiency ( $\leq 60$  mg/dl) has been reported in adult horses with lymphoma, as well as in very young horses with chronic infections. The purpose of this study was (1) to characterize the conditions under which IgM deficiencies occur in the adult horse and (2) to determine if the deficiency is transient or persistent. The manufacturer of the single radial immuno-diffusion assay (SRID) (VMRD, Inc.) provides normal ranges of IgM levels based on adult shetland ponies (which may not be applicable to all equids). Therefore, we also sought (3) to establish IgM levels in normal adult horses and (4) to determine if healthy horses have fluctuations in IgM levels over time.

The case records of adult horses presenting to the Cornell Hospital for Animals (1994–1998) that were diagnosed with an IgM deficiency ( $n = 32$ ) were analyzed. Owners of surviving horses were contacted, horses were re-examined and a serum IgM level was re-measured. Serial serum IgM was also measured in healthy adult Thoroughbred horses ( $n = 25$ ) every 6-weeks over a 4-month period to establish normal IgM ranges and repeatability of measurements. A one-way analysis of variance with repeated measures on time and a compound symmetry covariance structure were used to analyze the data.

An IgM deficiency was found in 23 horses that were discharged from the hospital and in 9 horses that were euthanized. Of these 9 horses, lymphoma was confirmed in 5. Of the 23 survivors, 2 had confirmed neoplastic conditions (lymphoma and seminoma) and 1 was suspected of having lymphoma. In the remaining 20 horses, inflammatory conditions involving the respiratory (2/20), gastrointestinal (6/20), neuromuscular (1/20), hematologic (1/20), ophthalmologic (3/20), dermatologic (4/20) and endocrine (1/20) systems were diagnosed. A definitive diagnosis was not found in 2/20 horses; 1 with a complaint of decreased performance and 1 with paraphimosis. Nine survivors were re-tested; 7 were clinically healthy and 2 had persistent chronic inflammatory conditions. Nearly 60% (5/9) including both (2/5) horses with chronic inflammation remained IgM deficient. The mean IgM levels were  $106 \pm 46$ ,  $97 \pm 37$ ,  $104 \pm 36$ , and  $106 \pm 40$  mg/dl at each time point in healthy horses. There was no significant difference in the IgM measurements over the 4-months ( $p = 0.62$ ).

In summary, the normal range of serum IgM is 63–143 mg/dl using the SRID. Serum IgM levels remain constant in healthy adult horses. Lymphoma was diagnosed in  $< 20\%$  of the horses with an IgM deficiency indicating that an IgM deficiency is not specific for equine lymphoma but also occurs with chronic inflammatory conditions of multiple organ systems in the adult horse.

**205** FLOW CYTOMETRIC DETECTION OF INTRACELLULAR INTERFERON-GAMMA IN NORMAL HORSES AND IN HORSES CHALLENGED WITH *RHODOCOCCLUS EQUI*. M. Hines, D. Stone, T. McGuire, L. Norton, and S. Hines. Washington State University, Pullman, WA.

The purpose of this study was to develop and validate a flow cytometric method for measuring intracellular IFN- $\gamma$  in the horse, and to apply the technique to the study of equine rhodococcal pneumonia. Monoclonal antibodies (Mab) were raised against a synthetic peptide corresponding to the predicted amino terminus of equine IFN- $\gamma$  (EqIFN- $\gamma$ ) and were shown by immunoblotting to react with a full-length recombinant EqIFN- $\gamma$  molecule. One of these antibodies was further shown to detect intracellular IFN- $\gamma$  in individual lymphocytes following ex vivo stimulation with ionomycin and phorbol 12-myristate 13-acetate (PMA). Importantly, the specificity of the assay was demonstrated on each horse using synthetic peptide to competitively inhibit binding of the anti-IFN- $\gamma$  Mab to cells. Staining with Mab to CD2, CD8 and/or CD4 prior to fixation allowed for identification of the cell types producing IFN- $\gamma$ .

The production of IFN- $\gamma$  by peripheral blood mononuclear cells (PBMC) from normal horses ( $n = 5$ ) was characterized. As described in other species, no significant production of IFN- $\gamma$  was detected in unstimulated cells. However, following stimulation with ionomycin/PMA the percentage of CD2+ T lymphocytes producing IFN- $\gamma$  ranged from 13.5% to 45.7%. This variation is similar to that described in humans and other species. The percentage of CD8+ lymphocytes producing IFN- $\gamma$  was 17.6–31.5%, whereas 18.1–47.2% CD4+ lymphocytes were positive for IFN- $\gamma$ .

Previous work has shown that adult horses efficiently clear *R. equi* from the lung in association with an influx of CD4+ and CD8+ T lymphocytes. Using tricolor fluorescence we examined cells in bronchoalveolar fluid (BALF) for IFN- $\gamma$  production both before and after intrabronchial challenge with virulent *R. equi* ( $n = 2$ ). Prior to challenge with *R. equi*, approximately 10% of CD2+, CD4+ T lymphocytes and 10% of CD2+, CD8+ T lymphocytes in BALF produced IFN- $\gamma$  when stimulated with ionomycin/PMA ex vivo. Following challenge, there was a dramatic increase in BALF in the numbers of CD2+, CD4+ T lymphocytes that produced IFN- $\gamma$  upon stimulation and a small increase in the numbers of CD2+, CD8+ T lymphocytes that produced IFN- $\gamma$  (examined at Days 7 and 14 post challenge). On a percentage basis, approximately 30% of both CD2+, CD4+ T lymphocytes and 30% of CD2+, CD8+ T lymphocytes in BALF obtained following challenge produced IFN- $\gamma$  when stimulated ex vivo. These results suggest that clearance of a pulmonary challenge is associated with increased IFN- $\gamma$  production by T lymphocytes. Future work will include examination of intracellular IFN- $\gamma$  following stimulation with *R. equi* antigen.

**206** METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* OUTBREAK IN A VETERINARY TEACHING HOSPITAL. DC Ruffin, QC Moore, JC Wright, KV Brock, JS Stringfellow, TL Hattcock, J Barbaree. Auburn University, Auburn AL.

Methicillin-resistant *Staphylococcus aureus* (MRSA) are strains of *S. aureus* resistant to multiple antibiotics. MRSA colonize the skin and nasal passages of humans and are commonly associated with nosocomial infections acquired from human hospitals. In order to investigate pathogen transmission and prevent further infection of patients, MRSA isolates from two distinct outbreaks in a veterinary teaching hospital were characterized. The 2 outbreaks occurred 10 months apart and involved different age groups of horses and different clinical presentations.

Isolates from hospital staff, students and horses were compared by pulsed-field gel electrophoresis (PFGE). DNA extracted from MRSA was digested with *Spe* I and subjected to PFGE. Phylogenetic analysis of data was performed with Diversity Database (Biorad).

Genotypic identity was found among human MRSA isolates obtained during the first outbreak and equine MRSA isolates from the first and second outbreaks. Our results indicate that equine patients are at risk from nosocomial MRSA infections as the result of MRSA colonized veterinary personnel. This is the first report of two distinct veterinary outbreaks with different clinical presentations associated with the same MRSA isolate.

**207** CARDIOVASCULAR DYSFUNCTION IS PRESENT IN HORSES WITH EXPERIMENTALLY INDUCED LEUKO-ENCEPHALOMALACIA. G.W. Smith, P.D. Constable, J.H. Foreman, R.M. Eppley, A.L. Waggoner, M.E. Tumbleson, and W.M. Haschek. College of Veterinary Medicine, University of Illinois, Urbana, IL and U.S.F.D.A., Washington DC.

Fumonisin are a group of mycotoxins produced primarily by *Fusarium verticillioides*, a fungus that commonly contaminates corn. Fumonisin ingestion has been associated with field cases of equine leukoencephalomalacia (ELEM; moldy corn disease, corn staggers) and porcine pulmonary edema. Experimental administration of fumonisin induces acute hepatotoxicity in all species, nephrotoxicity in rats, rabbits, sheep, goats and calves, pulmonary edema in pigs, and neurological disease in the horse (ELEM). The pathophysiology of ELEM is currently unknown. Because research in our laboratory suggested that porcine pulmonary edema was due to a fumonisin-induced decrease in cardiac contractility and heart rate, we hypothesized that cardiovascular dysfunction was also present in horses with ELEM.

Eleven horses were randomly allocated to receive purified fumonisin B<sub>1</sub> at 0.20 mg/kg (high dose, HD;  $n = 4$ ) or 0.01 mg/kg (low dose, LD;  $n = 3$ ) IV daily, or 10 ml saline IV daily (control, C;  $n = 4$ ). Clinical signs of neurological disease were observed only in the HD group. Horses were anesthetized (xylazine/ketamine) and instrumented for cardiovascular studies on day 7–8 (HD and C), when all 4 HD horses had moderate to severe signs of neurological disease consistent with ELEM (study terminated in control horses at same time as a paired HD horse), and on day 28 (LD, study terminated). Hemodynamic measurements were obtained 90 minutes after recovery from anesthesia.

Compared to control horses, HD horses had significantly ( $P < 0.05$ ) decreased heart rate (HD,  $36 \pm 6$ ; C,  $52 \pm 9$  bpm; mean  $\pm$  SD), cardiac output (HD,  $18.1 \pm 3.2$ ; C,  $28.0 \pm 2.7$  L/min), cardiac contractility (assessed by maximal rate of change of right ventricular pressure; HD,  $356 \pm 72$ ; C,  $525 \pm 113$  mmHg/s), and coccygeal artery pulse pressure (HD,  $29 \pm 14$ ; C,  $48 \pm 5$  mmHg). There were no differences in mean coccygeal artery pressure, mean pulmonary arterial pressure, mean right atrial pressure, and rate of right ventricular relaxation between HD and control horses. All cardiovascular values for LD horses were similar to controls. This is the first report of cardiovascular dysfunction in horses with neurological signs of ELEM. The results suggest that fumonisin-induced cardiovascular dysfunction may play a role in the pathophysiology of ELEM.

**208** CARDIORESPIRATORY AND THERMOREGULATORY EFFECTS OF ENDOPHYTE-INFECTED FESCUE IN EXERCISING HORSES. Vivrette SL, ME Stebbins, (North Carolina State University, Raleigh, NC), K Dooley, D Cross (Clemson University, Clemson, SC).

Ingestion of fescue grass infected with the endophytic fungus *Neotyphodium coenophialum* is known to cause reproductive problems in horses, including prolonged gestation, placental thickening, dystocia, delivery of weak or stillborn foals, and agalactia. In cattle, consumption of endophyte-infected fescue can result in a condition known as "fescue toxicosis" characterized by decreased weight gain, milk production, and conception rates, and a reduced ability to dissipate body heat. This study was designed to investigate if horses grazing endophyte-infected fescue pastures have a decreased ability to dissipate heat and have prolonged recovery from exercise in high ambient temperatures. Fourteen horses of light-horse breeding were maintained on fescue pasture that was free of endophyte (Group E-,  $n = 7$ ) or infected with endophyte (Group E+,  $n = 7$ ). The horses were exercised at a speed of 9 miles/hr (brisk trot) for 30 min (4.5 miles) over a mildly hilly course. The ambient temperatures and relative humidity were 32–34 °C (90–93 °F) and 30%, respectively.

Respiratory rates during the 210 minutes post-exercise period were higher ( $p = 0.0567$ ) in the horses fed endophyte-infected fescue (E+) compared to control horses (E-). The mean post-exercise heart rate for horses in Group E+ remained significantly above pre-exercise values for 150 minutes, compared to 90 minutes for horses in Group E- ( $p < 0.05$ ). The mean skin temperature for horses in Group E+ remained significantly above pre-exercise values for 180 minutes, compared to 90 minutes for horses in Group E- ( $p < 0.05$ ; Figure 2). During the 210 minutes that the horses were observed post-exercise, horses in Group E+ drank significantly more water ( $9.45 \pm 3.85$  gallons) than horses in Group E- ( $5.59 \pm 3.57$  gallons;  $p < 0.03$ ). No other examination parameters were significantly different between groups.

The results of this study demonstrate that exposure to endophyte-infected fescue pasture has an adverse effect on recovery following exercise during hot weather in horses. These findings may have significant implications for exercise and post-exercise recovery in horses, especially those performing in competitions such as endurance racing and eventing, where heart rate recovery and other parameters are used to determine whether the horse is fit to continue.

- 209** ASSESSMENT OF CHANGES IN BODY FLUID VOLUMES DURING DEHYDRATION AND REHYDRATION USING BIOELECTRICAL IMPEDANCE MEASURES. H.C. Schott II, P. Budom, S.M. Axiak, and S.W. Eberhart, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI 48824-1314.

To investigate fluid shifts during dehydration and rehydration with a noninvasive, commercially available device measuring bioelectrical impedance (Eqsitat® 2005), 4 horses were studied during a 48 hr period of dehydration (induced by furosemide administration [0.5 mg/kg IM, q 6 h over the initial 24 h] and withholding of feed and water [entire 48 h]) and a subsequent 36 h rehydration period (providing horses free access to hay and water). Body weight, (BW) bioelectrical impedance (BI) at 5 and 200 Hz, plasma osmolality (Posm), protein concentration (TP), and [Na<sup>+</sup>] were measured every 12 h as well as 30 min after initial access to water. BW decreased by  $7.4 \pm 0.2\%$  and  $10.4 \pm 0.2\%$  after 12 and 48 h of dehydration, respectively, and was accompanied by increases in Posm (by 7.3 and 17.4 mOsm/kg), TP (by 1.4 and 2.1 g/dl), and Na<sup>+</sup> (by 2.9 and 3.2 mEq/l). Plasma volume (estimated by changes in TP) decreased by  $19.6 \pm 2.0\%$  and  $25.7 \pm 1.6\%$  after 12 and 48 h of dehydration, respectively, suggesting a greater loss of extracellular fluid (ECF; ~20%) than intracellular fluid (ICF; ~10%). Although BI predicted a mean decrease in total body fluid volume (TFV) of 46.9 l (compared to a mean BW loss of 41.2 kg) over the initial 12 h of dehydration, variability between horses was considerable and changes in estimates of ECF and ICF as percentages of TFV, were not detected. Further, despite persisting BW loss, estimated TFV returned to near pre-dehydration values after 36 and 48 h of the dehydration period. When water was provided, horses drank  $18.6 \pm 2.4$  l during the initial 30 min of the rehydration period. Despite rapid decreases in Posm (8.4 mOsm/kg), TP (0.2 g/dl), and plasma Na<sup>+</sup> (3.8 mEq/l) with initial drinking, neither an increase in TFV nor ECF expansion was demonstrated via BI. These data indicate that BI measures did not consistently detect expected changes in body fluid volumes and, thus, may have limited value for field assessment of hydration in equine athletes.

- 210** VASODILATOR RESPONSES OF EQUINE DIGITAL BLOOD VESSELS TO PEPTIDE NEUROTRANSMITTERS. Katz LM, Berhane Y, Marr CM, Elliott J, Royal Veterinary College, London, UK.

The factors that regulate blood flow through the normal equine digital vasculature are of importance if we are to understand what predisposes animals to digital ischemia and laminitis. Endogenous vasodilator mediators are elaborated by the endothelium and sensory-motor nerves innervating the vasculature. Substance P (SP) and calcitonin gene-related peptide (CGRP), both potent vasodilatory mediators, are contained in nerves that have been detected in the equine digit. The aim of this study was to determine the potency and efficacy of these peptides as vasodilators of equine digital veins (EDVs) and arteries (EDAs) and to examine the effect of the endothelium on these responses.

Rings of EDA and EDV were obtained from adult horses for isometric tension recording. Some segments were denuded of their endothelium by intimal abrasion. Endothelium-intact (e+) and endothelium-denuded (e-) EDAs and EDVs from the same horse (n = 2 to 6) were examined within a given experiment. All vessels were constricted initially with depolarizing Krebs solution (DKS; 118mM K<sup>+</sup>) to evaluate smooth muscle function. Baseline tension was re-established and vessels were then constricted with U44069 (9, 11-dideoxy-9 $\alpha$ , 11 $\alpha$ -epoxymethano-prostaglandin F2 $\alpha$ ; 30nM). The relaxant responses of these vessels to carbachol (1 $\mu$ M) were then used to confirm the presence or absence of the endothelium. After washing, vessels were contracted with U44069 again and cumulative concentration relaxant response curves (CRCs) to CGRP ( $10^{-10}$ – $10^{-7}$ M) and SP ( $10^{-10}$ – $3 \times 10^{-5}$ M) were obtained. Maximal relaxation ( $E_{max}$ ) was expressed as a percentage relaxation of the U44069 response  $\pm$  sem and the EC<sub>50</sub> values were expressed as geometric means with 95% confidence limits. The CRC data were fitted using computerized non-linear curve fitting and EC<sub>50</sub> and  $E_{max}$  values for SP compared using a paired Students *t*-test.

Both CGRP and SP were potent vasodilators. CGRP vasodilation was non-endothelium dependent and more efficacious in EDAs ( $E_{max}$   $104 \pm 7.7\%$ ) than EDVs ( $E_{max}$   $52.8 \pm 12.2\%$ ). SP mediated vasodilation was totally dependent on the presence of the endothelium. Two phases of the CRC to SP could be discerned with a plateau (or increase in vessel tone) occurring between  $3 \times 10^{-8}$ M and  $3 \times 10^{-7}$ M. EC<sub>50</sub> and  $E_{max}$  values were obtained for the first phase and revealed that EDV(e+) were significantly more sensitive (EC<sub>50</sub>  $0.38[0.2-0.95] \times 10^{-9}$ M;  $p = 0.01$ ) to SP than EDA(e+) (EC<sub>50</sub>  $1.29[0.69-1.7] \times 10^{-9}$ M). The  $E_{max}$  did not differ significantly between the two vessel types. These results indicate that a dysfunctional endothelium may impair the vasodilatory action of SP on EDAs and EDVs. Further studies are needed to fully characterize these endogenous vasodilator systems and their importance in regulating equine digital blood flow.

- 211** CHARACTERIZATION OF THE PHARMACODYNAMIC PROPERTIES OF THE ANGIOTENSIN-CONVERTING ENZYME INHIBITOR, ENALAPRIL, IN HORSES. S.Y. Gardner, C.E. Atkins, R.A. Sams,\* A.B. Schwabenton, M.G. Papich, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, \*College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.

Angiotensin-converting enzyme (ACE) inhibitors have become the cornerstone for therapy of heart failure and hypertension in both human and small animal veterinary cardiology and have similar potential in the horse. The pharmacodynamics of enalapril after a single oral dose in mares was investigated. Enalapril was administered by nasogastric tube at a dose of 0.5 mg/kg body weight to 5 healthy mares. Serum samples were collected prior to and at 15, 30, and 45 minutes and at 1, 2, 3, 4, 6, 8, 12, 24, and 48 hours after drug administration for measurement of ACE activity, potassium concentrations, and enalapril and its active metabolite, enalaprilat, concentrations. In addition, at these same time points, the heart rate and systolic, diastolic, and mean arterial blood pressures (MAP) were measured indirectly by oscillometric technique. Digital venous blood samples were obtained prior to and at 4 hours after drug administration for measurement of blood gases and lactate. Serum creatinine and blood urea nitrogen (BUN) were measured prior to and at 24 hours after drug administration. Two weeks later, enalapril was administered by nasogastric tube at a dose of 0.5 mg/kg body weight to the same 5 mares. To mimic activation of the renin-angiotensin-aldosterone system, angiotensin I was administered at a dose of 0.5  $\mu$ g/kg, followed by blood pressure and heart rate measurement, prior to and 30 minutes and 1, 2, 3, 4, 6, 8, 12, and 24 hours after enalapril administration. There was a trend ( $p = .0625$ ) towards a decrease in ACE activity at 45 minutes and 1 and 2 hours after enalapril administration, but ACE activity was never suppressed below 84% (SD 11%). There was a trend ( $p = .0625$ ) towards a decrease in MAP at 6 and 8 hours after enalapril administration. Serum concentrations of potassium, creatinine, and BUN and digital venous blood gases and lactate levels did not change during the study. In response to angiotensin I administration, there was a trend ( $p = .0625$ ) towards a decrease in the MAP response from 4–24 hours after enalapril administration. Serum concentrations of enalapril and enalaprilat are pending. Single dose enalapril at 0.5 mg/kg did not demonstrate significant pharmacodynamic effects, nor was there substantial suppression of ACE activity. Chronic dosing studies are needed to determine if enalapril is efficacious under steady-state conditions.

- 212** AMINES FROM THE EQUINE HINDGUT MAY CAUSE DIGITAL VASOCONSTRICTION BY DIRECT OR INDIRECT MECHANISMS. S.R. Bailey, Y. Berhane, C.M. Marr, J. Elliott, Royal Veterinary College, London, UK.

Acute laminitis has been associated with overgrowth of bacteria in the caecum. It is hypothesized that the release of trigger factor(s) from the gut may result in digital vasoconstriction. We have recently shown that a number of amines are formed in the equine caecum and the rate of formation increases with fermentation. If released into the circulation, many monoamines may have direct or indirect vasoactive effects, due to their structural similarities with 5-hydroxytryptamine (5-HT; serotonin) and catecholamines. The aim of the present study was to examine the ability of monoamines shown to be formed in the equine caecum to displace 5-HT from equine platelets and to cause constriction of isolated digital blood vessels.

Washed equine platelets were loaded with [<sup>3</sup>H]5-HT (10 microM; 2 microCi/microM) for 20 min. The platelets were then washed again, and incubated in the presence of either tryptamine, tyramine, phenylethylamine, isobutylamine or isoamylamine (0.1 microM – 10 mM) for 30 min. The displaced [<sup>3</sup>H]5-HT was measured by liquid scintillation counting. Concentration displacement curves were constructed and the threshold concentration of amine causing significant displacement above background release was determined. Rings of equine digital vein (EDV) were obtained from horses killed at an abattoir and prepared for isometric tension recording. Cumulative concentration response curves to all five monoamines were constructed and the vasoconstrictor potency calculated.

All of the monoamines tested displaced 5-HT from platelets, with the aromatic amines being more potent than the aliphatic amines. Tryptamine and tyramine caused 5-HT release from platelets at concentrations as low as 2 and 3 microM respectively, and the thresholds for phenylethylamine, isoamylamine and isobutylamine were 150, 500 and 300 microM respectively (n = 4). Only the aromatic amines caused concentration dependent vasoconstriction of rings of EDV, with EC<sub>50</sub> values (mean with 95% CI; n = 3) of 1.3 (1.0–1.7) and 64.8 (52.6–79.9) microM for tryptamine and tyramine respectively and 0.15 (0.1–0.2) mM for phenylethylamine.

These data show that various monoamines formed in the equine caecum caused displacement of 5-HT from platelets. 5-HT is a potent and selective vasoconstrictor of the equine digital vasculature, capable of exerting its effects even at normal plasma concentrations. Small rises in circulating concentrations could cause the digital vasoconstriction which has been associated with the developmental phase of acute laminitis. In addition, the aromatic amines have vasoconstrictor actions (directly or via an indirect sympathomimetic effect) and so could contribute to digital vasoconstriction in this way.

## 213 CLINICAL EFFICACY OF A LEUKOTRIENE RECEPTOR ANTAGONIST (MONTELUKAST) IN EQUINE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD): A PILOT STUDY. G. Stark, R. Schmid, K. Riedelberger, and H. Zappe, Equine Medical Clinic, Veterinary University of Vienna, Austria.

Equine COPD is characterized by recurrent airway obstruction, airway hyperresponsiveness, and chronic airway inflammation. Bronchoconstriction and hyperreactivity have been shown to be associated with the presence of cysteinyl leukotriene (LT) D<sub>4</sub> within the equine airways. This pilot study was to determine the effects of oral LT receptor antagonist therapy, at a dosage of approximately 0.11 mg/kg BW s.i.d. for a period of 26 days. The efficacy was determined by changes in clinical signs, endoscopy, arterioalveolar pO<sub>2</sub> difference and lung function testing in five clinic-owned horses with moderate to severe COPD. Montelukast plasma levels were determined by means of high performance liquid chromatography.

We did not observe significant differences in clinical signs, endoscopic evaluation or arterioalveolar pO<sub>2</sub> difference pre and post treatment in any horse. However, pulmonary resistance decreased in two horses from 0.59±0.07 cmH<sub>2</sub>O/l/s and 1.00±0.08 cmH<sub>2</sub>O/l/s to 0.34±0.40 cmH<sub>2</sub>O/l/s and 0.74±0.32 cmH<sub>2</sub>O/l/s, resp. (p<0.05). As opposed to this, it increased in two horses from 0.92±0.06 cmH<sub>2</sub>O/l/s and 0.57±0.05 cmH<sub>2</sub>O/l/s to 1.03±0.03 cmH<sub>2</sub>O/l/s and 0.62±0.04 cmH<sub>2</sub>O/l/s, resp., as well (p<0.05). Dynamic compliance increased in three horses from 2.05±0.90 l/cmH<sub>2</sub>O, 0.95±0.05 l/cmH<sub>2</sub>O and 0.96±0.08 l/cmH<sub>2</sub>O to 2.83±0.57 l/cmH<sub>2</sub>O, 1.23±0.06 l/cmH<sub>2</sub>O and 2.43±1.26 l/cmH<sub>2</sub>O, resp. (p<0.05), and decreased in one horse from 3.41±0.36 l/cmH<sub>2</sub>O to 2.80±0.18 l/cmH<sub>2</sub>O, (p<0.05). The maximal changes in pleural pressure (dPmax) declined in two horses from 48±1 cmH<sub>2</sub>O and 18±2 cmH<sub>2</sub>O to 39±0.3 cmH<sub>2</sub>O and 13±2 cmH<sub>2</sub>O. dPmax values in the other horses remained constant between 7 cmH<sub>2</sub>O and 9 cmH<sub>2</sub>O.

Serum kinetics of oral montelukast revealed a peak concentration (C<sub>max</sub>) of 12±4 ng/ml at 46±15 minutes (t<sub>max</sub>) after oral therapy. C<sub>max</sub> was highly correlated with kg BW (r = 0.99; p = 0.002).

Oral montelukast at 0.11 mg/kg s.i.d. was not effective in the treatment of moderate to severe equine COPD. Despite the fact that the montelukast dose per kg BW was roughly the same as that for humans, the C<sub>max</sub> in equine serum was 28 times less. t<sub>max</sub> in the studied horses was also markedly different than t<sub>max</sub> in humans, which is 3.9±1.4 hours. The results can therefore be explained by the relative low montelukast plasma levels. Oral bio-availability of montelukast in horses seems far lower than that in man.

## 214 ATYPICAL PARAPOXVIRUS INFECTIONS IN SHEEP. G.W. Smith, G. Scherba, P.D. Constable, V. Hsiao, M.J. Behr, and D.E. Morin. College of Veterinary Medicine, University of Illinois, Urbana, IL.

This report describes clinical and laboratory findings for five sheep with extensive proliferative skin lesions grossly resembling warts on the distal limbs. The 4 Suffolks and 1 Hampshire ranged from 4 months to 3 years of age. In all sheep, the lesions affected the front and rear extremities and in 2 sheep, the lesions were also present around the head. The lesions were painful to the touch and most sheep were reluctant to move. Three of the sheep were from a single farm and had been housed with 30 other sheep that remained unaffected. The other 2 sheep were from different farms, and had been housed closely with unaffected herdmates. Various treatments, including systemic antibiotics, topical antibiotics, and antifungal ointments were administered; however, clinical improvement was not seen in any case. A variety of diagnostic tests were done on the lesions. In each case, histopathologic examination indicated proliferative cutaneous papillomatosis and in 4 cases intranuclear inclusion bodies were seen. Skin scrapings performed on 2 sheep were negative for ectoparasites. Fungal culture was attempted in 3 sheep and no isolates were identified. Bacteriology was done on samples of lesions from 4 sheep and a heavy growth of a *Staphylococcus* species was isolated from 2 of these animals. Electron microscopic examination of lesions from 3 sheep revealed numerous parapoxvirus particles. Immunohistochemical analysis of biopsy samples from 2 sheep was negative for papillomavirus. All 5 animals were ultimately euthanized. These cases represent an atypical presentation of parapoxvirus infection in sheep (orf, contagious ecthyma, scabby mouth). The infection appears to be minimally contagious, however the lesions do not self-resolve as does typical orf. This appears to be the first report of these lesions in multiple sheep in North America, although similar lesions have been reported in Israel.

## 215 PHARMACOLOGICAL INHIBITION OF INFLAMMATORY CYTOKINE PRODUCTION BY BOVINE ALVEOLAR MACROPHAGES. C. Malazdrewich, T. R. Ames, and S. K. Maheswaran. College of Veterinary Medicine, University of Minnesota, St. Paul, MN.

The inflammatory cytokines tumor necrosis factor-alpha (TNF), interleukin-1 beta (IL-1), and interleukin-8 (IL-8), secreted by alveolar macrophages in response to *Mannheimia (Pasteurella) haemolytica* virulence factors, are suspected to contribute to the pathogenesis of bovine pneumonic pasteurellosis (BPP). These mediators may therefore represent important therapeutic targets that could be pharmacologically modulated for purposes of disease treatment and prevention. The objective of this study was to evaluate the ability of six pharmacological inhibitors of cytokine production to suppress the expression of TNF, IL-1, and IL-8 genes and proteins by bovine alveolar macrophages in vitro. The compounds tested were pentoxifylline (PTX), rolipram (ROL), tetrahydropapaveroline (THP), thalidomide (THA), SB 203580 (SB), and dexamethasone (DEX). Compounds were selected on the basis of their ability to suppress the production of one or more of these cytokines in other experimental systems.

Alveolar macrophages (AM) were collected from Holstein calves by bronchoalveolar lavage. Cell cultures containing 1×10<sup>7</sup> AM were treated with cytokine inhibitors 30 min prior to the addition of purified *M. haemolytica* lipopolysaccharide (LPS; 100 ng/mL) and leukotoxin (LKT; 2 LU/mL). AM stimulated with LPS/LKT in the absence of inhibitors served as positive controls, while untreated AM served as negative controls. Secretion of TNF, IL-1, and IL-8 into the culture supernatant was quantitated by enzyme-linked immunosorbent assays (ELISA). Steady-state accumulation of cytokine-specific mRNA was quantitated by northern blot analysis of total RNA purified from AM lysates.

Significant (P < 0.05) dose-dependent inhibition of cytokine secretion occurred in response to pretreatment with PTX (TNF, IL-1), ROL (TNF, IL-1), THP (TNF, IL-1, IL-8), SB (TNF, IL-8), and DEX (TNF, IL-1, IL-8). DEX was the most effective inhibitor; pretreatment with this compound yielded > 95% inhibition of all 3 cytokines over a broad range of concentrations (10 nM – 100 μM). Significant (P < 0.05) dose-dependent inhibition of cytokine mRNA occurred in response to pretreatment with DEX and THP only.

The findings of this study demonstrate the ability of PTX, ROL, THP, SB, and DEX to suppress the secretion of inflammatory cytokines by bovine AM in vitro. THP and DEX appear to regulate cytokine production at the transcriptional level, while PTX, ROL, and SB exert post-transcriptional effects. If pulmonary cytokine production may be similarly inhibited in vivo, anti-cytokine therapy may represent an important new strategy for the management of BPP, and possibly other diseases characterized by overproduction of inflammatory cytokines.

## 216 PULMONARY EXPRESSION OF INFLAMMATORY CYTOKINES IN EXPERIMENTAL BOVINE PNEUMONIC PASTEURELLOSIS. C. Malazdrewich, T. R. Ames, M. S. Abrahamsen, and S. K. Maheswaran. College of Veterinary Medicine, University of Minnesota, St. Paul, MN.

Inflammatory cytokines are suspected to contribute to the pathogenesis of bovine pneumonic pasteurellosis (BPP) through neutrophil recruitment, leukocyte activation, and the induction of a broad array of soluble inflammatory mediators. The objectives of this study were to characterize the pulmonary expression kinetics of tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1 beta), and interleukin-8 (IL-8) genes and proteins during the acute phase of BPP, and to identify major cellular sources of these cytokines within affected lung.

Experimental BPP was induced in 15 male Holstein calves by endobronchial inoculation of *Mannheimia (Pasteurella) haemolytica* A1, and groups of 3 calves were euthanized at 2, 4, 8, 16, and 24 hours post-infection (PI). Three control calves received mock infections and were euthanized at 24 hours PI. Expression of cytokine mRNA and proteins in bronchoalveolar lavage (BAL) fluid, BAL cells, and pneumonic lung parenchyma was quantitated and localized using northern blot analysis, enzyme-linked immunosorbent assays (ELISA), and in situ hybridization.

Expression of TNF-alpha, IL-1 beta, and IL-8 genes and proteins was significantly (P < 0.05) increased in the airways and lung lesions of infected calves as compared to mock-infected controls. Although specific kinetic patterns varied between cytokines, in all cases peak levels of cytokine mRNA were achieved within 8 hours PI and peak cytokine concentrations occurred within 16 hours PI. In all samples, IL-8 was expressed to the greatest extent and TNF-alpha was least expressed. In situ hybridization studies demonstrated that alveolar and interstitial macrophages were the major source of IL-1 beta and IL-8 in the first 4 hours of disease, and that bronchial and bronchiolar epithelial cells were also significant sources of IL-8 during this period. By 8 hours PI, however, neutrophils within pulmonary alveoli and airways were the dominant source of both IL-1 beta and IL-8. TNF-alpha expression was restricted to alveolar macrophages throughout the study period.

These findings establish a correlation between early pulmonary expression of inflammatory cytokines and the development of acute BPP, further substantiating a role for these mediators in BPP pathogenesis. Inflammatory cytokines may therefore represent important therapeutic targets that could be pharmacologically modulated for purposes of disease treatment or prevention. Since pulmonary expression of IL-8 was much greater than that of TNF-alpha or IL-1 beta at all time points studied, anti-cytokine agents targeting this mediator may prove to be most useful in disease management.