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ACVIM American College of Veterinary Internal Medicine | Forum

2016 ACVIM Forum Research Abstract Program

2016 ACVIM Forum Research Abstract Program
Denver, Colorado, June 9–10, 2016
Index of Abstracts

ORAL PRESENTATIONS – Thursday, June 9

Time	#	Presenting Author	Abstract Title
CARDIOLOGY			
9:00 am	C01	Bryan Eason	Sinus Rate Approximation with VVI, VVIR, and VDD in Dogs with Third Degree Atrioventricular Block (*ACVIM Resident Research Award Eligible*)
9:15 am	C02	Janne Lyngby	Validation of a Method for Quantitation of Clopidogrel and Clopidogrel Active Metabolite in Feline Plasma (*ACVIM Resident Research Award Eligible*)
9:30 am	C03	Dar Ozer	Efficacy of Bronchial Stenting in Dogs With Myxomatous Mitral Valve Disease and Bronchial Collapse (*ACVIM Resident Research Award Eligible*)
9:45 am	C04	J.D. Rhinehart	Study of Echocardiographic Variability in Estimating Pulmonary Artery Pressure and Pulmonary Vascular Resistance in Dogs (*ACVIM Resident Research Award Eligible*)
10:30 am	C05	Kursten Roderick	Changes in NT-PROBNP Associated with Treatment and Survival Time in Cats with Congestive Heart Failure (*ACVIM Resident Research Award Eligible*)
10:45 am	C06	Courtney Smith	Right Ventricular Outflow Tract Obstruction and Coronary Anatomy in French and English Bulldogs with PS (*ACVIM Resident Research Award Eligible*)
11:00 am	C07	Melissa Tropf	Cardiac Function and Metabolic Parameters in Obese Dogs (*ACVIM Resident Research Award Eligible*)
11:15 am	C08	Darcy Adin	Comparison of Furosemide Infusion Diluted with 2.4% Hypertonic Saline Vs. Dextrose 5% in Water (D5W)
11:30 am	C09	Amelie Beaumier	Medical Management and Survival Time Associated with Congestive Heart Failure Stage D: A Retrospective Study
11:45 am	C10	A. Ray Dillon	MRI 7T Resonance Spectroscopy (MRS) Predicts Cardiac Energetic Reserves in Dogs with Preclinical Mitral Insufficiency
12:00 pm	C11	Chris Lam	Immunohistochemical Study of the Pro-Natriuretic Peptide Convertase Corin in Severe Canine Myxomatous Mitral Valve Disease
12:15 pm	C12	Giulio Menciotti	Anatomic Regurgitant Orifice Area Using 3D-Echocardiography in Dogs with Myxomatous Mitral Valve Disease
2:15 pm	C13	Takeshi Mizuno	Analysis of Mitral Valve Morphology with Real-Time 3-Dimensional Echocardiography in Dogs Undergoing Mitral Valve Repair

2:30 pm	C14	Lisbeth Olsen	Low-Density Lipoprotein Oxidation Is Breed and Gender Dependent in Dogs with Myxomatous Mitral Valve Disease
2:45 pm	C15	Nicole LeBlanc	Cardiac Output Measured By Echocardiography and Cardiac-Gated Computed Tomography Compared to Thermodilution
3:00 pm	C16	Kathryn Meurs	Angiotensin Converting Enzyme Activity and Response to Enalapril in Dogs with an Ace Gene Polymorphism
3:15 pm	C17	Kathryn Meurs	Angiotensin Converting Enzyme Activity in Cavalier King Charles Spaniels with an Ace Gene Polymorphism
3:30 pm	C18	Laura Nafe	Prevalence of *Dirofilaria immitis* Antigen in Client-Owned Pet Dogs Before and After Serum Heat Treatment
3:45 pm	C19	Lena Pelander	Cardiac Biomarkers Troponin I and N-Terminal Pro-B-Type Natriuretic Peptide in Canine Chronic Kidney Disease Patients
4:30 pm	C20	Rebecca Tracey	Establishing Normal 24 Hour Holter Monitor Values in Healthy Puppies
4:45 pm	C21	Jessica Ward	Distribution of Alveolar-Interstitial Syndrome in Dyspneic Veterinary Patients Assessed By Lung Ultrasound Versus Thoracic Radiographs

ONCOLOGY

9:00 am	O01	Zachary Neuman	The Association of Endothelin-1 Signaling with Bone Alkaline Phosphatase Expression and Pro-tumorigenic Activities in Canine Osteosarcoma (*VCS Award Winner*)
9:15 am	O02	MacKenzie Pellin	Safety Evaluation of Combination Doxorubicin and Toceranib Phosphate (Palladia) in Tumor Bearing Dogs: a Phase I Dose Finding Study (*VCS Award Winner*)
9:30 am	O03	Jerome Calvalido	Comparison of Serum Cytokine Levels Between Dogs with Multicentric Lymphoma and Healthy Dogs (*ACVIM Resident Research Award Eligible*)
9:45 am	O04	Zachary Crouse	Post-Chemotherapy Perforation in Cats with a Diagnosis of Intermediate or High Grade Gastrointestinal Lymphoma (*ACVIM Resident Research Award Eligible*)
10:30 am	O05	Sindy Piscoya	A Retrospective Study on the Incidence of Proteinuria Associated with the Use of Toceranib Phosphate (*ACVIM Resident Research Award Eligible*)
10:45 am	O06	Krystal Harris	Growth Pathways in Feline Oral Squamous Cell Carcinoma
11:00 am	O07	Michelle Turek	A Retrospective Analysis of Multimodality Treatment for Canine Oral Melanoma: 126 Cases
11:15 am	O08	Lucia Borlle	Enhancement of Doxorubicin Effectiveness When Combined with Salinomycin in FISS Cell Lines
11:30 am	O09	Martha MaloneyHuss	Clinical Advancement of a RNA-Transfected CD40-B Cell Vaccine for the Treatment of Canine Non-Hodgkin's Lymphoma
11:45 am	O10	Nicole Weidner	Vitamin D Status and Acute Phase Protein Concentrations in Canine Cancer Patients
12:00 pm	O11	Jenny Ellis	Retrospective Evaluation of Metronomic Cyclophosphamide in Epithelial and Mesenchymal Malignant Tumours

NEUROLOGY

9:00 am	N01	Michele Provencher	Kinematic Magnetic Resonance Imaging for Evaluation of Disc-Associated Cervical Spondylomyelopathy in Doberman Pinschers (*ACVIM Resident Research Award Eligible*)
9:15 am	N02	Melissa Carpentier-Anderson	3D Magnetic Resonance Imaging of the Dog Spine in Hansen Type I Intervertebral Disk Disease (*ACVIM Resident Research Award Eligible*)

9:30 am	N03	Aude Castel	Clinical Characteristics of Dogs with Progressive Myelomalacia Following Acute Intervertebral Disc Herniation (*ACVIM Resident Research Award Eligible*)
9:45 am	N04	Serene Lai	*In Vitro* Anti-Tubulin Effects of Benzimidazole Anthelmintics Mebendazole and Fenbendazole on Canine Glioblastoma Cells (*ACVIM Resident Research Award Eligible*)
10:30 am	N05	Ilyssa Meren	Subarachnoid-Subarachnoid Shunting for Treatment of Subarachnoid Cerebrospinal Fluid Flow Obstruction in 9 Dogs (*ACVIM Resident Research Award Eligible*)
10:45 am	N06	Jessica Rivera	11-Dehydro Thromboxane B2 As a Biomarker for Intracranial Neoplasia in Dogs (*ACVIM Resident Research Award Eligible*)
11:00 am	N07	Christine Sibigtroth	Lumbar Fractalkine and M2 Microglia Increase Throughout Disease Progresssion in Canine Degenerative Myelopathy (*ACVIM Resident Research Award Eligible*)
11:15 am	N08	Christine Sibigtroth	Increased Phosphorylated Neurofilament Heavy in Cerebrospinal Fluid As a Disease Marker of Canine Degenerative Myelopathy (*ACVIM Resident Research Award Eligible*)
11:30 am	N09	Muna Qahwash	Etiology of Feline Juvenile Onset Seizures (*ACVIM Resident Research Award Eligible*)
11:45 am	N10	Samantha Vitale	Comparison of Serum Trace Nutrient Concentration in Dogs with Primary Genetic Epilepsy Versus Healthy Dogs
12:00 pm	N11	Devon Hague	Lactate As a Prognostic Factor in Dogs and Cats with Head Trauma: 93 Cases (2003–2014)
12:15 pm	N12	Nick Jeffery	Comparison of Fecal Microbiomes Between Dogs with Meningoencephalomyelitis of Unknown Origin and Controls

SMALL ANIMAL INTERNAL MEDICINE – ENDOCRINOLGY**

2:15 pm	EN01	Nubia Lopes	Glucose Homeostasis Deteriorates More Rapidly with Age in Burmese Cats Compared to Non-Burmese
2:30 pm	EN02	Arnon Gal	Perturbations in Serum Fructosamine Level in Diabetic Hyperthyroid Cats – A Retrospective Study
2:45 pm	EN03	Hannah Pipe-Martin	Pharmacodynamic and Pharmacokinetic Properties of Insulin Aspart Following Subcutaneous and Intramuscular Injection in Cats (*ACVIM Resident Research Award Eligible*)
3:00 pm	EN04	Isabelle Rast	The Effect of Tetra-Hydroxylated Bile Acid on Adipocyte Size and Insulin Sensitivity in Healthy Cats (*ACVIM Resident Research Award Eligible*)
3:15 pm	EN05	Chen Gilor	The Effect of Adiposity and Diet on Secretion of Incretin Hormones in Cats
3:30 pm	EN06	Victoria Crossley	Breed, Coat Colour and Hair Length As Risk Factors for Feline Hyperthyroidism
3:45 pm	EN07	Allison Rowland	Does a Limited Iodine Diet Affect the Response to Radioactive Iodine Therapy in Hyperthyroid Cats?
4:30 pm	EN08	Vincent Ziglioli	Effects of Levothyroxine Adminstration and withdrawal on the Hypothalamic-Pituitary-Thyroid Axis in Euthyroid Dogs (*ACVIM Resident Research Award Eligible*)
4:45 pm	EN09	Alisdair Boag	Variability of P450scc Autoantibody Persistence in Dogs Affected with Hypoadrenocorticism
5:00 pm	EN10	Emily Brown	Juvenile Hypoadrenocorticism in the Nova Scotia Duck Tolling Retriever: A Recessive Monogenic Autoimmune Disease
5:15 pm	EN11	Arnon Gal	Variability in Post ACTH Stimulation Serum Cortisol Following Administration of Cortisone Acetate in Healthy Dogs

5:30 pm	EN12	Muzzammil Sayyid	Ionized Hypercalcemia in Cats: Etiologies and Associated Clinical Signs

**Also See Small Animal Internal Medicine – Endocrinology abstracts, Friday, June 10.*

SMALL ANIMAL INTERNAL MEDICINE – GASTROENTEROLOGY**

2:15 pm	GI01	Lauren Cochran	Serum Pancreatic Lipase Immunoreactivity Concentrations in Dogs with Gastrointestinal Foreign Bodies (*ACVIM Resident Research Award Eligible*)
2:30 pm	GI02	Sarah Cocker	Serum Pancreatic Lipase Immunoreactivity Concentrations After Chronic Administration of Supraphysiologic Doses of Glucocorticoids to Dogs (*ACVIM Resident Research Award Eligible*)
2:45 pm	GI03	Agostino Buono	Serum Il-2, Il-6, Il-8, and TNF-α Concentrations in Dogs with Increased Serum Spec CPL Sup® Concentrations
3:00 pm	GI04	Micah Bishop	Fishhook Foreign Bodies in Dogs and Cats: 107 Cases (2004–2015)
3:15 pm	GI05	Alana Redfern-Allen	Perturbations of the Intestinal Microbiota and Bile Acid Metabolism in Dogs with Diabetes Mellitus (*ACVIM Resident Research Award Eligible*)
3:30 pm	GI06	J.B. Honneffer	Variation of the Microbiome and Metabolome Along the Canine Gastrointestinal Tract
3:45 pm	GI07	Jan Suchodolski	Effects of Hydrolyzed Protein and Metronidazole on the Fecal Microbiome and Metabolome in Healthy Dogs
4:30 pm	GI08	Blake Guard	Altered Fecal Bile Acid Metabolism in Dogs with Chronic Enteropathy
4:45 pm	GI09	J.B. Honneffer	Altered Fecal Sterol Profiles in Dogs with Chronic Inflammatory Enteropathy
5:00 pm	GI10	Jan Suchodolski	The Fecal Microbiome of Dogs with Exocrine Pancreatic Insufficiency
5:15 pm	GI11	Adam Rudinsky	Effect of Weight Loss and Diet on Fecal Microbiota and Fecal Metabolomics in Cats
5:30 pm	GI12	Albert Jergens	Probiotic Mixture Vsl#3 Increases Beneficial Fecal and Mucosal Microbiota in Canine Inflammatory Bowel Disease
5:45 pm	GI13	Julien Dandrieux	Changes in Intestinal Macrophage Populations Following Clinical Resolution in Dogs with Chronic Enteropathy
6:00 pm	GI14	Sara Wennogle	Histopathologic Characteristics of Intestinal Biopsies From Dogs with Chronic Enteropathy with and without Hypoalbuminemia (*ACVIM Resident Research Award Eligible*)

**Also See Small Animal Internal Medicine – Gastroenerology abstracts, Friday, June 10.*

SMALL ANIMAL INTERNAL MEDICINE – HEMATOLOGY

9:00 am	HM01	Benoît Cuq	Reproducibility, Stability and Biological Variability of Thrombin Generation Using Calibrated Automated Thrombography in Healthy Dogs (*ACVIM Resident Research Award Eligible*)
9:15 am	HM02	Kelly Makielski	Development and Validation of a Novel Canine Immune Thrombocytopenia Bleeding Score (*ACVIM Resident Research Award Eligible*)
9:30 am	HM03	Natalie McLewee	Effects of Aspirin Dose Escalation on Canine Platelet Function and Urinary Thromboxane and Prostacyclin Levels (*ACVIM Resident Research Award Eligible*)
9:45 am	HM04	Jessica Pritchard	B Cell Activating Factor As a Biomarker in Dogs with Primary Immune Mediated Thrombocytopenia
10:30 am	HM05	Sophie Saati	Comparison of Multiplate, Platelet Function Analyzer-200, and Plateletworks in Dogs Treated with Aspirin and Clopidogrel (*ACVIM Resident Research Award Eligible*)

10:45 am	HM06	Jenny Ellis	Evaluation of the Risk of Relapse of Canine Immune-Mediated Thrombocytopenia After Routine Vaccination
11:00 am	HM07	Rachel Hegedus	Predicting *In Vivo* Response to Low-Dose Aspirin in Healthy Dogs Using *In Vitro* Platelet Aggregometry
11:15 am	HM08	Cyril Parachini-Winter	Retrospective Evaluation of Anemia and Erythrocyte Morphology in Dogs with Lymphoma and Inflammatory Bowel Disease
11:30 am	HM09	Michele Wilkinson	An In-Vitro Assessment of Canine to Feline Red Blood Cell Xenotransfusion
11:45 am	HM10	James Swann	Characterization of the Immunophenotype of Dogs with Immune-Mediated Hemolytic Anemia (*ACVIM Resident Research Award Eligible*)
12:00 pm	HM11	Masahiko Sato	A Retrospective Study on Use of Leflunomide in Dogs with Immune Mediated Diseases (*ACVIM Resident Research Award Eligible*)
12:15 pm	HM12	Samantha Muro	Effects of Leukoreduction and Storage on Eicosanoid Concentrations in Units of Canine Packed Red Cells (*ACVIM Resident Research Award Eligible*)

SMALL ANIMAL INTERNAL MEDICINE – INFECTIOUS DISEASE

2:15 pm	ID01	Elena Contreras	Evidence for Genetic Predispositon to *Borrelia burgdorferi* Infection in Purpose Bred Beagles
2:30 pm	ID02	Kirsten Cooke	Rapid Diagnosis of *Babesia gibsoni* Using Point-of-Care Insulated Isothermic Polymerase Chain Reaction Assay
2:45 pm	ID03	Robert Six	Prevention of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* Transmission From *Ixodes scapularis* to Dogs By Sarolaner
3:00 pm	ID04	Milica Kovacevic Filipovic	Molecular and Serological Analysis of Canine Vector Borne Disease Prevalence in Most Populated Serbian Area
3:15 pm	ID05	Diana Scorpio	Enhanced Serologic Surveillance to Detect Prevalence of Canine Vector-Borne Infections on St. Kitts, West Indies
3:30 pm	ID06	Julie Levy	Performance of Point-of-Care Assays for FELV and FIV
3:45 pm	ID07	Cynda Crawford	Does a Diva Test Exist for Differentiating FIV Infection From FIV Vaccination?
4:30 pm	ID08	Morihiro Tateno	An Epidemiological Study of Gammaherpesviruses in Domestic Cats in Japan
4:45 pm	ID09	Nyssa Reine-Salz	Canine Influenza H3N2 Infection in Four Dogs
5:00 pm	ID10	Rhonda LaFleur	Demonstration of Protection Against Canine Influenza Virus H3N2 Infection Following Vaccination with Inactivated CIV H3N2
5:15 pm	ID11	Nyssa Reine-Salz	Prevalence of Canine Infectious Respiratory Disease Complex Pathogens in Dogs in Georgia and North Carolina
5:30 pm	ID12	Jason Stull	Frequency, Benefits and Health Risks of Animals in Nursing Homes: Cross-Sectional Study of Ohio Facilities
5:45 pm	ID13	Modest Vengust	Effect of Quorum Quenching with Azithromycin on *Pseudomonas aeruginosa* Associated Otitis Externa/Media in Dogs

SMALL ANIMAL INTERNAL MEDICINE – NUTRITION/METABOLISM

9:00 am	NM01	Hanna Mila	Effect of the Hyper-Immune Egg Yolk Supplementation on Weight Gain in Neonate Puppies
9:15 am	NM02	Yuanlong Pan	Effects of Dietary Medium Chain Triglycerides on Voluntary Activity in Dogs and Cats

9:30 am	NM03	Dagmar Tarkosova	Effects of Dietary Macronutrient Content and Feeding Pattern on Leptin Concentrations in Lean Healthy Cats
9:45 am	NM04	Hui Xu	Effect of High Sodium Diet on Blood Pressure and Cardiac Function in Healthy Adult Dogs.

SMALL ANIMAL INTERNAL MEDICINE – NEPHROLOGY/UROLOGY

9:00 am	NU01	Sarah Guess	Longitudinal Evaluation of Serum Symmetric Dimethylarginine (SDMA) and Creatinine (SCR) in Dogs with Early CKD (*ACVIM Resident Research Award Eligible*)
9:15 am	NU02	Julie Cross	SDMA Correlates Better with Creatinine Than High Throughput Immunoturbidometric Cystatin C Assay in Feline Serum
9:30 am	NU03	Laura Harjes	Fibroblast Growth Factor-23 in Canine Chronic Kidney Disease (*ACVIM Resident Research Award Eligible*)
9:45 am	NU04	E. Hathaway Fiocchi	The Use of Darbepoetin Alfa to Stimulate Erythropoiesis in Dogs with Chronic Kidney Disease (*ACVIM Resident Research Award Eligible*)
10:30 am	NU05	Fernanda Chacar	Vitamin D-Binding Protein – Early Marker of Tubular Injury in Dogs with Chronic Kidney Disease
10:45 am	NU06	Stacie Summers	Assessment of Repeated Administration of a Feline FVRCP Vaccine As a Model for Interstitial Nephritis (*ACVIM Resident Research Award Eligible*)
11:00 am	NU07	Ellen Behrend	Comparison of Visual and Automated Interpretation of Urinary Dipsticks with Glucose:Creatinine Ratio and Glucose Concentration
11:15 am	NU08	Kanae Takada	Impact of Canine Pancreas-Specific Lipase on the Outcome of Dogs with Hemodialysis-Dependent Acute Kidney Injury
11:30 am	NU09	Jonathan Foster	Characterization of Subclinical Bacteriuria, Urinary Tract Infection, and Pyelonephritis in Dogs with Chronic Kidney Disease
11:45 am	NU10	Ewan Wolff	Initial Outcomes and Complications of the Subcutaneous Ureteral Bypass Procedure at Two University Hospitals (2012–2015) (*ACVIM Resident Research Award Eligible*)
12:00 pm	NU11	Melanie Puchot	Occult Urinary Tract Infection in Cats: Prevalence and Findings on Contemporaneous Urinalysis (*ACVIM Resident Research Award Eligible*)
12:15 pm	NU12	Romy Heilmann	Diagnostic Performance of Urinary Canine Calgranulins in Dogs with Lower Urinary Tract Carcinoma

SMALL ANIMAL INTERNAL MEDICINE – PHARMACOLOGY

10:30 am	P01	Leanne Fowler	Itraconazole Absorption From Proprietary and Compounded Formulations in Healthy Cats
10:45 am	P02	David Griffin	Bioavailability of a Novel Formulation of S-Adenosylmethioine Given with Food in Beagle Dogs
11:00 am	P03	Dianne Mawby	Posaconazole Pharmacokinetics in Cats After oral and IV Administration
11:15 am	P04	J.E. Slovak	Evaluation of Intravenous Mycophenolate Mofetil Use in Healthy Cats

SMALL ANIMAL INTERNAL MEDICINE – RESPIRATORY

11:30 am	R01	Kimberly Hooi	Comparison of Bronchoalveolar Lavage Techniques for Sampling Lower Airways in Cats (*ACVIM Resident Research Award Eligible*)
11:45 am	R02	Aida Vientos-Plotts	Characterization of the Feline Respiratory Microbiome (*ACVIM Resident Research Award Eligible*)

EQUINE**

9:00 am	E01	Kate Echeverria	Pulmonary Disposition and Pharmacokinetics of Oral Minocycline in the Adult Horse (*ACVIM Resident Research Award Eligible*)
9:15 am	E02	Teresa Burns	Intravenous Administration of Cobalt Chloride Is Associated with Hemodynamic Alterations in Horses
9:30 am	E03	Andrew Gestrich	The Pharmacokinetics of Intravenous Gentamicin in Healthy Young-Adult Versus Geriatric Horses
9:45 am	E04	Nicholas Parkinson	Endotoxin-Induced Microrna Expression in Equine Peripheral Blood Mononuclear Cells (*ACVIM Resident Research Award Eligible*)
10:30 am	E05	Nicolas Herteman	Severe Equine Asthma (Heaves) Is Associated with an Increased Number of Circulating Low-Density Granulocytes
10:45 am	E06	Amy Santonastaso	Can Levamisole Upregulate the Equine Cell-Mediated Immune Response *In Vitro*?
11:00 am	E07	Helena Carstensen	Novel Pharmacological Treatment Regimes in Equine Atrial Fibrillation
11:15 am	E08	Linda Frellstedt	Can Exercising Electrocardiography Predict Performance in Young Standardbred Horses at the Start of Training?
11:30 am	E09	Eva Hesselkilde	Is ECG in Horses Only for Dysrhythmia Diagnosis? Introducing a New Method for 12-Lead ECG
11:45 am	E10	Kate Hepworth-Warren	Humoral Hypercalcemia of Malignancy in Horses: A Retrospective Study (2010–2015)
12:00 pm	E11	Gonçalo Silva	Development of a Technique for Determination of Pulmonary Artery Pulse Wave Velocity in Horses (*ACVIM Resident Research Award Eligible*)
12:15 pm	E12	Olivia Lorello	Repeated Measurements of Autonomic Tone Markers Over a Training Season in Eventing and Endurance Horses
12:30 pm	E35	Breanna Sheahan	Normal Ultrasonographic Pleural Thickness in Clinically Healthy Adult Horses (*ACVIM Resident Research Award Eligible*)
2:15 pm	E13	Steven Grubbs	Epidemiological Characteristics of Horses with Hyperinsulinemia in a Large Population of Horses
2:30 pm	E14	Steven Grubbs	Management of Early PPID in Horses
2:45 pm	E15	Nicholas Frank	Development of an Octreotide Response Test for Detection of Insulin Dysregulation in Horses
3:00 pm	E16	Nicholas Frank	Insulin and Incretin Hormone Concentrations in Horses During an Oral Sugar Test and Pasture Challenge
3:15 pm	E17	Katarzyna Dembek	Association of Androgens and Pregnanes Response to ACTH Stimulation with Adrenal Dysfunction in Hospitalized Foals
3:30 pm	E18	Katarzyna Dembek	Association of Oxytocin and Neurosteroids with Neonatal Maladjustment Syndrome (NMS) in Hospitalized Foals
3:45 pm	E19	Elaine Norton	Identification of Genetic Loci Underlying Equine Metabolic Syndrome and Laminitis Risk in Welsh Ponies
4:30 pm	E20	Ramiro Toribio	The Fibroblast Growth Factor-23/Klotho Axis in Healthy and Hospitalized Foals
4:45 pm	E21	Jennifer Brown	Effect of RRR-Alpha-Tocopherol Formulation on Serum and CSF Alpha-Tocopherol Concentrations in Vitamin E Deficient Horses (*ACVIM Resident Research Award Eligible*)

5:00 pm	E22	Ann Kemper	Differential Gene Expression in Equine Subcutaneous and Internal Adipose Depots (*ACVIM Resident Research Award Eligible*)
5:15 pm	E23	Elizabeth Nelson	Effects of a Commercial Anionic Supplement on Urinary Acidification in Horses (*ACVIM Resident Research Award Eligible*)
5:30 pm	E24	Katherine Williamson	Effects of Abrupt Concentrate Increase and Prebiotic Supplementation on Equine Cecal pH and Lactate
5:45 pm	E25	Frank Andrews	Effects of a Supplement (Alfa-Lox Forage®) on Equine Gastric Ulcer Scores and Gastric Juice pH
6:00 pm	E26	Angelika Schoster	Changes of the Equine Neonatal Intestinal Microbiota Associated with Age and Diarrhea
6:15 pm	E27	Daniela Luethy	Comparison of Tube, Gel, and Immunochromatographic Strip Methods for Evaluation of Equine Blood Transfusion Compatibility (*ACVIM Resident Research Award Eligible*)

***Also See Equine abstracts, Friday, June 10.*

FOOD ANIMAL

9:00 am	F01	Véronique Bernier Gosselin	Prevalence of Coagulase-Negative *Staphylococci* Species in Intramammary Infection in Dairy Goats (*ACVIM Resident Research Award Eligible*)
9:15 am	F02	Diego Gomez	Assessment of an Antimicrobial-Use Algorithm for Treatment of Diarrhea in Dairy Calves
9:30 am	F03	Diego Gomez	Prevalence of Bovine Coronavirus in Feces of Healthy and Diarrheic Calves
9:45 am	F04	Joe Smith	Use of an Alivecor Heart Monitor for Heart Rate and Rhythm Evaluation in Domestic Goats
10:30 am	F05	Rachel Oman	Left Displacement of te Abomasum in Four Beef Calves (*ACVIM Resident Research Award Eligible*)
10:45 am	F06	Jennifer Halleran	Apparent Efficiency of Colostral Immunoglobulin Absorption in Holstein Heifers (*ACVIM Resident Research Award Eligible*)
11:00 am	F07	Kevin Washburn	Concentrations of Chlortetracycline in Fetal Tissues Following Oral Administration in the Ewe
11:15 am	F08	Vincent Dore	Hyperketonemia As a Tool to Predict Mortality in Dairy Goat During Last Month of Pregnancy (*ACVIM Resident Research Award Eligible*)
11:30 am	F09	Christie Balcomb	Efficacy and Pharmacokinetics of Intravenous Famotidine in Adult Cattle
11:45 am	F10	Andrew Gestrich	The Pharmacokinetics of Intravenous Gentamicin in Healthy Young-Adult Versus Aged Alpacas
12:00 pm	F11	Marie-Ève Bilodeau	Prognosis Associated with Cerebrospinal Fluid Analysis Results in Recumbent Dairy Cattle: Retrospective Study (2006–2014) (*ACVIM Resident Research Award Eligible*)

ORAL PRESENTATIONS – Friday, June 10

Time	#	Presenting Author	Abstract Title
EQUINE			
2:15 pm	E28	Amanda Adams	Influenza-Specific Immune Responses to a Combination Vaccine in Naïve Ponies
2:30 pm	E29	Amanda Adams	WNV-Specific Immune Responses to a Combination Vaccine in Naïve Ponies
2:45 pm	E30	Brandy Burgess	Characteristics of Infection Control Practices at North American Veterinary Teaching Hospitals
3:00 pm	E31	Sandra Taylor	Anti-Endotoxic Properties of Ketorolac Tromethamine and Flunixin Meglumine in Horses
3:15 pm	E32	Amy Stieler	Effects on Sweating of Chloramphenicol and the Macrolide Gamithromycin: Comparison with Erythromycin
3:30 pm	E33	Arlie Manship	Comparison of the Clinicopathologic Signatures of Equine Coronavirus and *Salmonella enterocolitis*
3:45 pm	E34	JunJie Liu	Phenotypic Characterization of *Sarcocystis neurona* Lesions in Gravely Affected Horses
4:30 pm	E36	Jacquelyn Bowser	Syringe Versus Mechanical Suction with N-Butylscopolammonium Effects on BAL Parameters in Horses with Pasture RAO
4:45 pm	E37	Julie Dauvillier	Prevalence of Fungi in Respiratory Samples of Horses with Inflammatory Airway Disease
5:00 pm	E38	Cyprianna Swiderski	Intravenous Magnesium Sulfate As a Rescue Therapeutic for Bronchoconstriction in Horses
5:15 pm	E39	Emily Medlin Martin	Investigation of Misoprostol As a Novel Anti-Inflammatory in Equine Leukocytes
5:30 pm	E40	Sian Durward-Akhurst	Do Endocrine Disrupting Chemicals Play a Role in Horses with Equine Metabolic Syndrome?
5:45 pm	E41	Jane Manfredi	Evaluation of an Oral Sugar Test for Dynamic Assessment of Five Equine Breeds' Insulin Response/Sensitivity
SMALL ANIMAL INTERNAL MEDICINE – GASTROENTEROLOGY			
9:00 am	GI15	Karin Allenspach	Human Granulocyte Immunofluorescence Assay for Anti-Neutrophil Antibodies Shows Strong Association with Canine Food Responsive Diarrhea
9:15 am	GI16	Jonathan Lidbury	Serum Citrulline Concentrations in Dogs with Chronic Enteropathy
9:30 am	GI17	Linda Toresson	Oral Versus Parenteral Cobalamin Supplementation in Dogs with Chronic Enteropathies and Hypocobalaminemia
9:45 am	GI18	Nozomu Yokoyama	Plasma Essential Trace Element Concentrations in Dogs with Chronic Enteropathy
2:15 pm	GI19	Roman Husnik	Validation of Ultrasonographic Measurement of Gastric Emptying Time in Healthy Cats Using Radionuclide Scintigraphy
2:30 pm	GI20	Toshiaki Kakimoto	Effect of Mosapride on Postprandial Gallbladder Motility and Plasma Levels of Motilin in Dogs
2:45 pm	GI21	M. Katherine Tolbert	Evaluation of the Presence and Role of Cysteine Protease 30 in Feline *T. foetus*

3:00 pm	GI22	Emily Gould	Evaluation of the Effect of Omeprazole on Serum Calcium, Magnesium, Gastrin and Bone in Cats
3:15 pm	GI23	Jillian Haines	Gravity-Assisted Esophageal Transit Characteristics in Dogs with Megaesophagus
3:30 pm	GI24	Jonathan Lidbury	Feasibility of Measuring Gastrointestinal Transit Time in Healthy Dogs Using ALICAM
3:45 pm	GI25	Jill Pomrantz	Comparison of Gastric Transit Time in Healthy Dogs and Dogs with Signs of Gastric Hypomotility
4:00 pm	GI26	Jill Pomrantz	Feasibility of a Novel Gastrointestinal Imaging Device for Use in Dogs

SMALL ANIMAL INTERNAL MEDICINE – HEPATOLOGY

8:00 am	HP01	Sara Wennogle	Fluorescence in Situ Hybridization Identifies Occult Bacterial Infection in Gallbladder Mucoceles (*ACVIM Resident Research Award Eligible*)
8:15 am	HP02	Kathleen Aicher	Association of Gallbladder Mucocele Formation with Occult Hypothyroidism in Dogs: A Matched Case-Controlled Study (*ACVIM Resident Research Award Eligible*)
8:30 am	HP03	Jonathan Lidbury	Interobserver Agreement for Histological Scoring of Canine Hepatic Fibrosis
8:45 am	HP04	Matthias Schneider	Embolization of Intratrahepatic Portosystemic Shunts in Dogs with a Prototype Coil

SMALL ANIMAL INTERNAL MEDICINE – OTHER

8:00 am	OT01	Alison Khoo	Effect of Three Resuscitative Fluid Protocols on N-Terminal Prohormone Brain Natriuretic Peptide in Healthy Dogs (*ACVIM Resident Research Award Eligible*)
8:15 am	OT02	Mira Korpivaara	Dexmedetomidine Oromucosal Gel for Alleviation of Acute Anxiety and Fear Associated with Noise in Dogs
8:30 am	OT03	Katherine Scotti	Prognostic Indicators in Cats with Septic Peritonitis: 55 Cases (2002–2015)
8:45 am	OT04	Franck Jolivet	Reliability of Thermometer Protective Sheaths for Measurement of Rectal Temperature in Dogs

POSTER PRESENTATIONS

On Display: Thursday, June 9, 9:30 am–5:00 pm Friday, June 10, 9:30 am–5:00 pm

Attended by ALL Authors

Thursday, June 9, 9:50 am–10:30 am Friday, June 10, 9:50 am–10:30 am

Attended by ALL Authors – Wine & Cheese Reception:

Thursday, June 9, 5:45 pm–7:30 pm

#	Presenting Author	Abstract Title
CARDIOLOGY		
C22	SeungWoo Jung	Circulating Plasma Mirna As Novel Molecular Biomarkers in Congestive Heart Failure
C23	Randolph Winter	A Multicenter Evaluation of Signalment and Comorbid Conditions Associated with Aortic Thrombosis in 291 Dogs
C24	Marlos Goncalves Sousa	Right Ventricular Systolic Function in Dogs with Post-Capillary Pulmonary Hypertension
C25	Marlos Goncalves Sousa	Vasovagal Tonus Index in Dogs with Myxomatous Mitral Valve Disease
C26	Maria Helena Matiko Akao Larsson	Sick Sinus Syndrome and Sinus Node Dysfunction: Case Series Reports of FMVZ-USP Cardiology Service (2007-2015)
C27	Kensuke Nakamura	The Utility of Spectral Doppler of the Hepatic Veins in Dogs with Tricuspid Regurgitation
C28	Emily Menzen	Cutaneous Use of an Implantable Loop Recorder (Reveal LINQ^{TM}) for Cardiac Event Monitoring in Dogs
C29	Lilian Shen	Aortoseptal Angle and Response to Balloon Valvuloplasty in Dogs Affected with Severe Subaortic Stenosis
C30	Melissa Wilson	Evaluation of Pulmonary Hypertension in Cats with Cardiac Disease and Pleural Effusion Versus Pulmonary Edema
C31	Junseok Lee	Post-Operative Dysrhythmias After Cardiac Surgery Under Cardiopulmonary Bypass
NEUROLOGY		
N13	Jourdan Brune	Characterization of Iatrogenic Blood Contamination on Lactate Dehydrogenase and Creatine Kinase in Canine Cerebrospinal Fluid
N14	Lorenzo Mari	Outcome Comparison in Dogs with Thoracolumbar Acute Non-Compressive Nucleus Pulposus Extrusion or Fibrocartilaginous Embolic Myelopathy
N15	Katia Marioni-Henry	Morphology of the Caudal Fossa in Mesaticephalic and Brachycephalic Cats and Associated Clinical Signs
N16	Pablo Amengual	Diagnostic Investigation in 13 Cats with Suspected Feline Hyperesthesia Syndrome
N17	Hilary Hu	Cystometric Characterization of Urinary Bladder Dysfunction in Chronically Paralyzed Dogs (*ACVIM Resident Research Award Eligible*)

N18	Michele Provencher	Evaluation of Kinematic Magnetic Resonance Imaging in Dogs with Osseous-Associated Cervical Spondylomyelopathy (*ACVIM Resident Research Award Eligible*)
N19	Lisa Bartner	*Bartonella* Spp. PCR Assay Results Using Cerebrospinal Fluid of Dogs with Central Nervous System Disease
N20	Hilary Levitin	Preictal, Postictal and Interictal Behavioral Changes in Dogs with Genetic Epilepsy Compared to Control Dogs

ONCOLOGY

O12	Erika Berger	Evaluation of Toceranib Phosphate (Palladia®) in the Treatment of Feline Mast Cell Neoplasia: 53 Cases
O13	Valter de Medeiros Winkel	Expression of P Glycoprotein (ABCB1) in Cats with T-Cell Lymphocytic Gastrointestinal Lmyphoma
O14	Tracy Gieger	Early Experiences with Stereotactic Radiation Therapy for the Treatment of Canine Non-Lymphomatous Nasal Tumors
O15	Tracy Gieger	Treatment of Canine Appendicular Osteosarcoma with Amputation, Carboplatin, and Toceranib Phosphate
O16	J. Paul Woods	Safety Assessment of a Novel Oncolytic Maraba Virus in Cats
O17	Daniel Regan	Role of Monocyte Recruitment in Hemangiosarcoma Metastasis in Dogs (*VCS Award Winner*)
O18	Nicholas Szigetvari	Phase I Clinical Trial of the Targeted Chemotherapeutic Drug, Folate-Tubulysin, in Dogs with Urinary Tract Transitional Cell Carcinoma (*VCS Award Winner*)

SMALL ANIMAL INTERNAL MEDICINE – ENDOCRINOLOGY

EN13	Ashley Gold	Evaluation of Basal Cortisol Concentrations for the Diagnosis of Hypoadrenocorticism in Dogs (*ACVIM Resident Research Award Eligible*)
EN14	Christina Marino	Thyroid Profiles in Healthy Kittens Aged Two to Sixteen Weeks of Age (*ACVIM Resident Research Award Eligible*)
EN15	Eileen Seage	Spectrophotometry and Ultracentrifugation for Measurement of Plasma Lipids in Dogs with Diabetes Mellitus (*ACVIM Resident Research Award Eligible*)
EN16	Hye-Ryung Choo	Effect of Hydrocortisone Administration on Leptin and Adiponectin Synthesis in Healthy Dogs
EN17	Jon Fletcher	Using Purified Feline Insulin to Evaluate Cross-Reactivity with a Human Insulin Analog Elisa
EN18	Maria Jericó	Steroid Hormone Panel Test and Trilostane and Melatonin Therapy in Pomeranian Dogs with Alopecia X
EN19	Mia Reeve-Johnson	Metabolite Differences Between Senior Burmese and Non-Burmese Cats, and Associations with Measures of Glucose Metabolism
EN20	Yaiza Forcada	A Genome-wide Association Study Identifies Novel Candidate Genes for Susceptibility to Diabetes Mellitus in DSH Cats (*ESVE Award Winner*)

SMALL ANIMAL INTERNAL MEDICINE – GASTROENTEROLOGY

GI27	Alison Manchester	Correlation Between Novel Immunohistochemical Markers, Histopathology and Clinical Scores in Cats with Chronic Enteropathy (*ACVIM Resident Research Award Eligible*)

GI28	Amanda Blake	Fecal D-/L-Lactate Concentrations and Abundance of Lactic Acid Bacteria in Dogs with Exocrine Pancreatic Insufficiency
GI29	Gena Esposito	Evaluation of Serum Beta-Hydroxybutyrate Concentrations in Dogs with Chronic Enteropathies
GI30	Ellen Everson	Effect of Serum Creatinine on Feline Serum DGGR-Lipase and Serum Pancreatic Lipase Immunoreactivity
GI31	Roman Husnik	Prevalence of *Helicobacter* Species and Their Association with Gastric Pathology in Dogs with Gastrointestinal Disease
GI32	Jill Pomrantz	Normal and Abnormal Findings in the Canine Gastrointestinal Tract Using Ambulatory Light-Based Imaging
GI33	Joerg Steiner	Fecal α1-Proteinase Inhibitor Concentrations in Dogs with Cardiac Disease
GI34	Rebecca Timmons	Evaluation of the Effects of Pre-Conditioning on Female Canine Adipose-Derived Mesenchymal Stem Cell Cytokine Production

SMALL ANIMAL INTERNAL MEDICINE – HEMATOLOGY

HM13	Ian McClure	Incidence of DEA 5 in Canine Population Using Novel Canine Antisera
HM14	Keitaro Morishita	Prospective Study in the Treatment of Nonregenerative Immune-Mediated Anemia in 8 Dogs
HM15	Hyeri Shin	Validation of Rapid Thromboelastographic Analysis on Citrated and Native Whole Blood From Healthy Dogs
HM16	Sarah Shropshire	Serial Evaluation of Thromboelastography and Platelet Aggregometry in Healthy Dogs

SMALL ANIMAL INTERNAL MEDICINE – HEPATOLOGY

HP05	Daniel Langlois	Investigation of Hepatic Copper Accumulation in Dogs From Two Time Periods (1982-1988 and 2009-2015)

SMALL ANIMAL INTERNAL MEDICINE – INFECTIOUS DISEASE

ID14	Melissa Beall	Comparative Evaluation of Five In-Clinic Rapid Tests for Feline Leukemia Virus Infection
ID15	Ramaswamy Chandrashekar	Detection of Giardiasis in Dogs: Comparison of Three Rapid Diagnostic Tests
ID16	Jesse Buch	Seroprevalence of Antibodies to *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in Domestic Cats
ID17	Jesse Buch	Validation of a High-Throughput Serological Elisa Method for FELV P27 Antigen Detection
ID18	Amber Caress	Investigation of Whether *Leptospira* Vaccinal Antibodies React with *Borrelia* Peptides Used in a Commercial Assay
ID19	Elena Contreras	Clinical and Laboratory Findings in Dogs with *Ixodes*-Induced Chronic *Anaplasma phagocytophilum* Infection After Prednisolone Administration
ID20	Michael Lappin	Serum Neutralization of Feline Caliviviruses Using Sera From Kittens Administered a Vaccine Containing Two Strains
ID21	Michael Lappin	Risk Factors Associated with Giardia and *Cryptosporidium* in Pet Dogs and Cats in the USA

ID22	Jiayou Liu	Significant Differences in Sensitivity Levels of Rapid Tests for Antibodies to *Anaplasma* Spp. in Dogs
ID23	Katie MacMillan	Prevalence of Select Infectious Disease Agents in Client Owned Cats in Moscow, Russia
ID24	Alison Manchester	*Coxiella burnetii* DNA Not Identified in Fleas From Domestic Cats in Australia and the USA
ID25	Kirk Miller	Risk of Heartworm Infection in Domestic Canines of Northwestern oregon
ID26	Cody Minor	Ectoparasites and Vector-Borne Pathogens of Dogs in Baja California Sur
ID27	Jason Stull	Identifying Agreement and Barriers to Proposed Canine Infectious Disease Guidelines for Dog Group Settings
ID28	Julia Veir	Correlation of *Mycoplasma* Quantitative PCR to Severity of Conjunctivitis in Cats

SMALL ANIMAL INTERNAL MEDICINE – NUTRITION/METABOLISM

NM05	Aparecido Camacho	Determining the Lactate and Glucose Thresholds and the Acid-Base Imbalances in Beagles Dogs
NM06	Dennis Jewell	When Fed Foods with Similar Palatability, Cats Choose 30%, Dogs 23% of Calories As Protein
NM07	Jennifer MacLeay	A Double Masked Clinical Trial of a Therapeutic Food in the Management of Canine Atopy

SMALL ANIMAL INTERNAL MEDICINE – NEPHROLOGY/UROLOGY

NU13	Crystal Cooley	Survey of Subcutaneous Fluid Practices in Cats with Chronic Kidney Disease (*ACVIM Resident Research Award Eligible*)
NU14	Ryan Dulaney	Quantifying Urine Elimination Behaviors in Cats Using a Video Recording System (*ACVIM Resident Research Award Eligible*)
NU15	Alisdair Boag	Evaluation of the Effect of Urine Dip Versus Urine Drip on Multitest Strip Results
NU16	Jean Hall	Positive Impact of Nutritional Interventions in Client-Owned Dogs with Iris Stage-1 Chronic Kidney Disease
NU17	Marcia Kogika	Fibroblast Growth Factor 23 (FGF-23) in Dogs with Naturally Occurring Chronic Kidney Disease
NU18	Maciej Parys	Serum Cytokine Profiles of Cats with Idiopathic Cystitis
NU19	Camille Torres-Henderson	Cystolith Dissolution in Cats Using a Commercially Available Diet

SMALL ANIMAL INTERNAL MEDICINE – OTHER

OT05	Katrina Stewart	Isolation and Identification of Microrna From the Mature Feline Erythrocyte: A Pilot Study (*ACVIM Resident Research Award Eligible*)
OT06	Laura McPhee	Effects of Sedation and Anesthesia on Canine Hematologic and Serum Biochemical Analyses During Preventive Healthcare
OT07	Igor Yankin	Does Fine Needle Aspiration Affect Management of Dogs with Incidental Splenic Nodules or Heterogeneous Parenchyma?

SMALL ANIMAL INTERNAL MEDICINE – PHARMACOLOGY

P05	Darren Berger	The Accuracy, Precision and Stability of Compounded Milbemycin Oxime in Aqueous Suspension
P06	Andrea Herndon	Pharmacokinetics of Intravenous and Subcutaneous Dolasetron and Pharmacodynamics of Subcutaneous Dolasetron in Purpose-Bred Cats
P07	Lara Zajic	Investigation of the Pharmacokinetics of Transdermal Ondansetron in Normal Purpose-Bred Cats

EQUINE

E42	Harold Schott	Basal Insulin Concentratrations in Competition Draft Horses
E43	Kamila Gravena	Matrix Metalloproteinase-2 and -9 Levels in Horses with Experimental Small Colon Intraluminal Obstruction
E44	Michael Keowen	Effects of Collagen Hydrolysates on Equine Gastric Ulcer Scores and Gastric Juice Ph in Horses
E45	James Prutton	Safety, Humoral Immune Response and Fecal Shedding of Modified-Live Bovine Coronavirus Vaccines Given to Horses
E46	Sarah Elzinga	Do Horses with Equine Metabolic Syndrome Have Reduced Immune Responses to Vaccination?
E47	Melissa Siard	Immunological Comparisons of Aged Horses with Vs. without Pituitary Pars Intermedia Dysfunction
E48	Marta Barba	*Corynebacterium pseudotuberculosis* Antibody Detection in Horses: Synergistic Hemolysis Inhibition Test and Small Ruminant ELISA Comparison
E49	Martha Mallicote	Effects of Erythromycin on Responses Induced in Foals By Intravenous Epinephrine
E50	Barbara Qurollo	Equine Vector-Borne Diseases Determined By Serological and Molecular Methods
E51	Andrew Allen	Selenium Deficiency Associated with the Deaths of Fifteen Adult Horses
E52	Frank Garza, Jr.	Effects of Alfa-Lox Forage® on Blood Glucose and Insulin Activity After Grain Feeding in Horses
E53	Breanna Sheahan	Venous Blood Gas, Electrolyte and Metabolite Findings in Healthy Neonatal Foals Receiving Sodium Lactate Infusions (*ACVIM Resident Research Award Eligible*)
E54	Randolph Winter	Growth and Function of Equine Endothelial Progenitor Cells Labeled with Semiconductor Quantum Dots

C01

SINUS RATE APPROXIMATION WITH VVI, VVIR, AND VDD IN DOGS WITH THIRD DEGREE ATRIOVENTRICULAR BLOCK. Bryan Eason[1], Dan Hogan[1], Ryan Baumwart[2]. [1]Purdue University, West Lafayette, IN, USA, [2]Oklahoma State University, Stillwater, OK, USA

The purpose of this study was to investigate which pacing modality (VVI, VVIR, or VDD) most closely approximates the intrinsic sinus rate in dogs with third degree AV block (3AVB) over a range of typical daily activities. A secondary aim of the study was to determine if echocardiographic parameters were different between pacing modalities. We hypothesized that VDD would most closely track the intrinsic sinus rate for all activities and that echocardiographic dimensions would be different between AV asynchronous (VVI, VVIR) and AV synchronous (VDD) pacing modalities.

Dogs were prospectively recruited following implantation of a St. Jude dual-chamber pacemaker system for 3AVB; dogs with evidence of sinus node dysfunction or clinically important arrhythmias other than 3AVB were excluded. All dogs were programmed to VDD at study entry. The average, lowest and highest heart rates during VDD pacing were determined; the average heart rate was programmed as the base rate for VVI and VVIR. The highest heart rate was programmed as the upper tracking rate for VVIR with a threshold of 2.5, slope of 16, and fast reaction and recovery times. The rest function was used for all pacing modalities and was programmed to the lowest heart rate during VDD pacing; if the lowest heart rate was at the lower rate limit, then the rest rate was programmed 20% slower. Dogs were randomly assigned to VVI or VVIR after initial VDD interrogation and echocardiography (2D, Doppler, TDI). Ambulatory ECGs were recorded for 36 hours and the owners were instructed to record the time and duration of 5 specific activities: (1) urination/defecation, (2) eating, (3) sleeping, (4) walking, and (5) play. After three months, echocardiography was repeated, the dog was crossed over to VVI or VVIR pacing, and ambulatory ECG monitoring was repeated. After three additional months, echocardiography was repeated, the pacing modality was programmed to VDD and ambulatory ECG monitoring was repeated. Atrial rate, ventricular rate, and atrial-to-ventricular ratio were determined for each of the 5 activities after an initial 12 hours acclimatization period.

This is an ongoing study and currently 6 dogs are enrolled where 5 dogs have completed the VVI phase, 4 dogs have completed the VVIR phase, 4 dogs have completed both the VVI and VVIR phases, and 1 dog has completed all VVI, VVIR, and VDD phases. Atrial rates and atrial-to-ventricular ratios for VVI, VVIR, and VDD at each activity are as follows: urination/defecation (156.0 ± 42.9, 2.01 ± 0.39),(134.4 ± 24.0, 0.95 ± 0.14), (105.7, 1.0); eating (116.4 ± 20.8, 1.51 ± 0.23),(126.2 ± 11.7, 0.97 ± 0.22), (95.7, 1.0); sleeping (99.1 ± 38.3, 1.32 ± 0.62),(79.8 ± 15.7, 0.88 ± 0.30), (51.5, 0.76); walking (183.2 ± 24.7, 2.4 ± 0.41), (157.4 ± 38.9, 1.09 ± 0.12), (91.4, 1.0); play (125.4 ± 38.3, 1.66 ± 0.61),(105.8 ± 17.0, 0.83 ± 0.07), (108.9, 1.0). Echocardiographic data is available for all 6 dogs in VDD, 4 dogs in VVIR, and 2 dogs in VVI. The left ventricle appears to be larger in diastole and systole for VVI (LVAd/Ao = 6.85 ± 0.76, EDV/Ao = 26.08 ± 2.80, LVAs/Ao = 2.74 ± 0.19, ESV/Ao = 9.23 ± 0.08) and VVIR (LVAd/Ao = 5.24 ± 0.57, EDV/Ao = 24.78 ± 3.04, LVAs/Ao = 2.38 ± 0.47, ESV/Ao = 10.65 ± 3.21) than VDD pacing (LVAd/Ao = 5.14 ± 1.38, EDV/Ao = 23.13 ± 7.46, LVAs/Ao = 2.30 ± 0.84, ESV/Ao = 7.43 ± 4.27).

As the study is ongoing no final conclusions can be made although synchronous VDD pacing appears to more closely approximate sinus rate and have slower atrial rates than asynchronous VVI or VVIR pacing. All dogs will have completed the study within the next two months at which time final conclusions can be made.

C02

VALIDATION OF A METHOD FOR QUANTITATION OF CLOPIDOGREL AND CLOPIDOGREL ACTIVE METABOLITE IN FELINE PLASMA. Janne G. Lyngby, Michael H. Court, Pamela M. Lee. Program in Individualized Medicine (PrIMe), Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA

Clopidogrel is considered mainstay therapy for the prevention of cardiogenic thromboemboli in cats and has been shown to be superior to aspirin. Clopidogrel is a pro-drug and requires hepatic bioactivation through cytochrome p450 for formation of the clopidogrel active metabolite (CAM), which is unstable and challenging to quantitate. After bioactivation, CAM binds to the ADP-receptor on platelets and inhibits platelet aggregation. Recently, a method to stabilize CAM in human plasma has been developed by adding 2-bromo-3'methoxyacetophenone (BMAP) to blood tubes. Once stabilized, the derivatized CAM (CAM-D) can be quantitated using high-performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS).

Our aim is to validate HPLC-MS/MS for the detection of clopidogrel, CAM-D, and other metabolites in feline plasma. We hypothesized that HPLC-MS/MS is both accurate and precise. The quantitation of CAM is the first step in determining if cats can consistently metabolize clopidogrel or if significant interindividual variation exists as in humans with genetic polymorphisms of hepatic cytochrome P450. Furthermore, having an accurate and precise method for measuring the plasma concentration of CAM will allow safer use of clopidogrel by potentially establishing a therapeutic index and by evaluating whether coadministration of drugs like omeprazole inhibit CAM formation in cats.

FDA guidelines to assess accuracy and precision were followed. The *in vitro* stability of clopidogrel, CAM-D, and clopidogrel acid in both citrate and EDTA feline plasma was evaluated at room temperature, 4°C, −20°C and −80°C at 0, 6, 12, 24, 48, 72 hours, and 7 days. Linear regression was performed on an 8 point standard curve (clopidogrel and CAM-D at 1–100 ng/mL, clopidogrel acid at 0.2–20 μg/mL). To evaluate clinical application, two healthy cats were given clopidogrel (Plavix) at the clinically used dose of 18.75 mg orally once daily. Blood was taken prior to clopidogrel administration and at 20, 40, 60, 90 minutes, 4 hours, 6 hours, 8 hours, and 12 hours post clopidogrel administration. All blood samples were collected aseptically into 2 mL cryotubes containing 10 uL of 500 mM BMAP and 20 uL of 500 mM EDTA and then centrifuged. The supernatant was then transferred to new cryotubes and stored at −80°C. All samples were analyzed using HPLC-MS/MS in duplicate with a standard curve.

In vitro samples were stable at all temperatures for a minimum of 72 hours. No difference was appreciated in the stability of clopidogrel and its metabolites between EDTA and citrated plasma. Standard curves showed linearity for clopidogrel, CAM-D, and clopidogrel acid (r = 0.99). The assay measured clopidogrel, CAM-D, and clopidogrel acid with an accuracy of <±18.7% and a precision within runs of <±7%. Plasma concentrations of clopidogrel, CAM-D, and clopidogrel acid were readily detected in plasma from healthy cats receiving clopidogrel.

This is the first simultaneous quantitation of clopidogrel, CAM-D, and clopidogrel acid in feline plasma. HPLC-MS/MS is an accurate and precise method for simultaneous quantitation of clopidogrel and metabolites in feline plasma both *in vitro* and from healthy cats receiving oral clopidogrel.

C03

EFFICACY OF BRONCHIAL STENTING IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE AND BRONCHIAL COLLAPSE. Dar Ozer[1], Samantha Siess[2], Brienne Williams[1], Nikki Gaudette[1], George Kramer[1]. [1]Atlantic Coast Veterinary Specialists, Bohemia, NY, USA, [2]Stony Brook University, Stony Brook, NY, USA

Chronic airway disease and myxomatous mitral valve disease (MMVD) are frequent comorbidities in small breed dogs. Bronchial collapse can cause coughing, tachypnea and hypoxemia. Pulmonary hypertension can be seen with both MMVD and chronic

airway disease. The purpose of this study was to review outcomes in dogs that had bronchial stents placed due to bronchial collapse. It was hypothesized that moderate to severe MMVD or the presence of pulmonary hypertension would not have a negative effect on lifespan after stent placement.

Medical records of 18 small breed dogs that had bronchial stent placement for chronic coughing secondary to bronchial collapse were reviewed. A hierarchical multiple linear regression analysis was conducted to predict lifespan after bronchial stent placement based on age at time of stent placement, severity of MMVD and the presence of pulmonary hypertension.

Eighteen dogs had bronchial stents placed over a period of 7 years. Age at the time of stent placement ranged from 6.5 years to 14 years of age (M = 10.47 ± 1.85). Breeds represented included Cavalier King Charles spaniel (2), beagle (2), Chihuahua (4), Pomeranian (3), toy poodle (2), Yorkshire terrier (2), Maltese (1), Coton de Tulear (1) and shih tzu (1). There were 11 males and 7 females. Twelve dogs (66.67%) had evidence of moderate to severe MMVD and 4 (22.22%) had evidence of pulmonary hypertension. Six dogs (33.33%) had CHF prior to stent placement and 6 dogs (33.33%) had CHF after stent placement. Syncope was reported in 6 dogs prior to stent placement and 5 dogs after stent placement. The average lifespan after stent placement was 203.56 ± 250.72 days. Three dogs are currently alive post-stent placement (1013, 559 and 411 days).

A hierarchical multiple linear regression was calculated to predict lifespan after placement of a bronchial stent based on age at the time of stent placement, the presence of pulmonary hypertension, and severity of MMVD. In stage one, age at the time of stent placement significantly predicted lifespan after placement of a bronchial stent ($\beta = -7.10$, $P < .02$); with lifespan decreasing by 7.10 days for each additional month of age at the time of stent placement. The presence of pulmonary hypertension did not significantly predict lifespan ($\beta = 235.32$, $P = .10$). Severity of MMVD did not contribute significantly to the model in stage two ($\beta = 101.38$, $P = 0.43$).

Results from this study indicate that the severity of MMVD or the presence of pulmonary hypertension did not negatively affect lifespan after bronchial stent placement. As such, moderate to severe MMVD and the presence of pulmonary hypertension should not be viewed as exclusion criteria when assessing candidates for possible bronchial stenting. Prospective studies should be conducted to further investigate the clinical benefit of bronchial stenting in dogs with severe bronchial collapse.

C04
STUDY OF ECHOCARDIOGRAPHIC VARIABILITY IN ESTIMATING PULMONARY ARTERY PRESSURE AND PULMONARY VASCULAR RESISTANCE IN DOGS.

JD Rhinehart, JD Bonagura, BA Scansen, KE Schober. College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

Pulmonary hypertension (PH) is important in clinical practice and is related to clinical symptoms and prognosis. We hypothesized that Doppler echocardiographic (DE) indices of PH and pulmonary vascular resistance (PVR) are influenced by a variety of independent factors leading to clinically important variability of DE-based pressure estimates in dogs.

Dogs with naturally acquired tricuspid regurgitation (TR) were studied prospectively. All dogs had degenerative valve disease. Target variables were acquired during 4 study periods (dogs in lateral recumbency or standing, after a 6-minute walk test [6-MWT] and after sedation [0.25 mg/kg butorphanol, IM]), and by two different observers. Heart rate (HR), TR flow velocity (TRFV), PVR, estimates of right atrial pressure, stroke volume, cardiac output, and 23 other variables were quantified. Statistical methods included repeated-measures ANOVA and mixed model analysis. $P < 0.05$ was considered significant.

Thirty-two dogs of 10 small breeds with varying TRFVs (1.9–5.28 m/s) and PVR (2.9–33.0 WU) were studied. There was a significant effect of observer ($P < 0.05$), respiration ($P < 0.01$), and image quality ($P < 0.05$) on outcome variables whereas imaging position (dogs lying versus standing) did not cause differences ($P > 0.20$). Sedation significantly increased mean TRFV ($P < 0.05$, from 3.15 ± 0.71 m/s to 3.51 ± 0.83 m/s; increase observed in 55% dogs) whereas 6-MWT had no effect (3.16 ± 0.75 m/s, $P > 0.05$). HR declined after sedation (mean reduction 13 ± 19 bpm; $P < 0.05$) and increased after 6MWT (mean increase 30 ± 28 bpm; $P < 0.05$) but had no correlation to TVFV or PVR. 6-MWT did not influence TRFV and PVR.

These data document relevant variability of DE estimates of PH that deserve clinical consideration.

C05
CHANGES IN NT-PROBNP ASSOCIATED WITH TREATMENT AND SURVIVAL TIME IN CATS WITH CONGESTIVE HEART FAILURE.

Kursten Roderick, John Rush, Lisa Freeman, Vicky Yang, Suzanne Cunningham. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA

Survival time in cats with congestive heart failure (CHF) is variable, and predictors of outcome would be useful to help guide treatment and patient assessment. In people with CHF, the change in NT-proBNP concentrations after initiating treatment and/or targeting NT-proBNP concentrations below an absolute level provide valuable information that can be used to help guide treatment. The change in NT-proBNP concentrations in cats with CHF after treatment and their association with survival have not been reported. Therefore, the purpose of this study was to measure the change in NT-proBNP in cats with CHF after treatment and to determine whether serial NT-proBNP measurements provide prognostic information in this population.

Client-owned cats presenting with new onset CHF secondary to cardiomyopathy were eligible for enrollment. Blood was collected for NT-proBNP analysis within 4 hours of admission, on the day of discharge, and at the re-evaluation 7–10 days later.

Thirty-two cats were enrolled (25 male, 7 female; all neutered), with a median age of 9.6 years (range 1.1 to 18.0 years). Thirty-one (97%) cats survived to discharge and 28 cats (88%) survived to the 7–10 day re-evaluation. Median NT-proBNP concentrations at admission and hospital discharge were 1,713 pmol/L (range, 160 to 3,784 pmol/L) and 902 pmol/L (range, 147 to 3,223 pmol/L), respectively. Median NT-proBNP concentration at re-evaluation was 1,124 pmol/L (range, 111 to 2,727 pmol/L). Median NT-proBNP concentrations decreased significantly from admission to discharge ($P < 0.001$) and from admission to re-evaluation ($P < 0.001$), but not from discharge to re-evaluation ($P = 0.28$). Median survival time was 109 days (range, 1 to 606 days), with 6 cats (19%) still alive at the time of analysis. Cats that received pimobendan had a significantly greater decrease in NT-proBNP concentrations [−821 pmol/L (range, −2,163 to −267 pmol/L) from admission to discharge compared to cats that did not receive pimobendan [−561 pmol/L (range, −2137 to 889 pmol/L); $P = 0.045$]. Cats whose owners had difficulty administering medications (n = 11) had a shorter survival time [median 60 days (range, 2 to 234 days)] compared to owners who did not report difficulty [n = 21, median 115 days (range, 1 to 606 days)]; however this was not statistically significant ($P = 0.12$).

The association between pimobendan and a larger decrease in NT-proBNP concentration warrants additional research. Larger studies are needed to determine whether NT-proBNP can be used to help guide treatment in cats with CHF.

C06
RIGHT VENTRICULAR OUTFLOW TRACT OBSTRUCTION AND CORONARY ANATOMY IN FRENCH AND ENGLISH BULLDOGS WITH PULMONIC STENOSIS.

Courtney Smith, Katherine Scollan, David Sisson, Nicole LeBlanc. Oregon State University, Corvallis, OR, USA

Pulmonic stenosis (PS) is one of the most common congenital cardiac defects in dogs. Stenotic lesions described range from a normal pulmonic annulus with domed leaflets to a hypoplastic

pulmonary annulus with thickened leaflets. Additionally, anomalous coronary artery anatomy (commonly described as an R2A) has been reported impinging on the right ventricular outflow tract (RVOT) in English Bulldogs and a Boxer dog with PS. The aim of this study was to investigate the stenotic lesion morphology and coronary artery anatomy in French Bulldogs in comparison to English Bulldogs to assess similarities and/or differences between the breeds.

Medical records were reviewed for French and English Bulldogs presented to Oregon State University for heart murmur evaluation or imaging prior to balloon valvuloplasty (BVP). Dogs were included if they had severe PS (pressure gradient >80 mmHg estimated via echocardiography) and confirmation of their coronary artery anatomy by either angiography or computed tomography (CT). Echocardiographic, angiographic, and CT images were reviewed. The pulmonic annulus (PA), aortic annulus (Ao), PA:Ao ratio, valvular anatomy, and coronary anatomy were assessed on each imaging modality by three observers (CS, KS, DDS) and averaged. Tricuspid regurgitation (TR) was assessed on echocardiographic and angiographic studies. Breed medians or means were compared by Mann-Whitney U test or unpaired t-tests as indicated. In the dogs that had both CT and angiographic studies, values were compared by paired t-tests.

Twenty-six dogs were reviewed and 6 dogs (all English Bulldogs) were excluded due to lack of definitive coronary artery anatomy imaging. Of the 20 included dogs, 7 were English (7 male) and 13 were French Bulldogs (4 female and 9 male). Mean weight was 13.8 ± 4.5 kg in the English and 8.9 ± 3.9 kg in the French Bulldogs ($P = 0.02$). CT and angiographic imaging was performed on 6/7 and 2/7 English, and 9/13 and 12/13 French Bulldogs, respectively. An R2A coronary artery anomaly was definitively identified in 6/7 English Bulldogs and 0/13 French Bulldogs. Median PA:Ao annulus ratio was not statistically different between the English (0.88, range 0.64–1.0) and French Bulldogs (0.89, range 0.85–1.1; $P = 0.37$). In addition, no difference was found between CT and angiographic measurements of the PA:Ao ratio ($P = 0.38$). The mean PA annulus indexed to body weight to the 1/3 power was not different between English (0.54 ± 0.07) and French Bulldogs (0.53 ± 0.04; $P = 0.73$). TR was noted in 9/13 French Bulldogs (4 mild, 2 moderate, 3 severe) while 3/7 English Bulldogs had TR (1 mild, 1 moderate, 1 severe).

Anomalous development of the coronary arteries was not observed in any of the French Bulldogs evaluated in this study, whereas the R2A coronary anomaly was identified in 6/7 English Bulldogs with a RVOT obstruction. Marked thickening of the pulmonic valve leaflets with markedly reduced leaflet mobility was observed in all of the French Bulldogs and annular hypoplasia was not present or was very modest in both breeds. In the dogs with R2A anomalies, the curvature and degree of encroachment from the left coronary artery on the RVOT was markedly variable. A combination of valvular and supravalvular obstruction was identified in the only English Bulldog with normal coronary anatomy.

C07

CARDIAC FUNCTION AND METABOLIC PARAMETERS IN OBESE DOGS.

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In people, obesity is an independent risk factor for the development of obesity related cardiac dysfunction (ORCD) characterized by systolic dysfunction, diastolic dysfunction, and/or vascular endothelial dysfunction. Components of metabolic syndrome including dyslipidemias, elevated inflammatory markers, and insulin resistance are believed to play an important role in the pathophysiology of ORCD. Obesity is reaching epidemic proportions in dogs and it is estimated that approximately 34% of dogs are overweight and 5% are obese in the United States. Recent studies performed in dogs suggest that metabolic syndrome also exists in obese dogs, warranting further investigation into the risk for ORCD.

We hypothesized that obese dogs have cardiac dysfunction and have elevations in inflammatory markers, altered insulin sensitivity, and dyslipidemia consistent with metabolic syndrome when compared with non-obese dogs. The aims of this study were first, to assess the presence of cardiac dysfunction in obese dogs compared to dogs with ideal body composition and second, to compare metabolic parameters including lipid analysis and inflammatory markers in obese versus normal dogs.

Healthy dogs weighing less than 25 pounds with ideal body condition (n = 17) and obese body condition (n = 29) were enrolled. All dogs had normal physical exam and thoracic auscultation with no evidence of systemic illness on routine laboratory diagnostics. A body composition using a validated 9 point scale, systolic arterial Doppler blood pressure, a 6-lead electrocardiogram, and an echocardiogram (M-mode, B-mode, Doppler and strain imaging) were obtained. Finally, metabolic parameters including a lipid profile (cholesterol, triglyceride, HDL, LDL), insulin, adiponectin, and a canine inflammatory marker ELISA panel (GM-CSF, IL2-18, TNF-alpha, INF-gamma, TNF-gamma CRP, MCP-1, IP-10, KC-like) were evaluated. Paired T tests were performed to compare the study groups and a *P*-value of less than 0.05 considered statistically significant.

Compared to dogs with ideal body condition scores, obese dogs had significantly increased septal wall thickness, increased systolic function measures, and significantly reduced diastolic function measures. Obese dogs had significantly increased insulin, insulin: glucose ratio, cholesterol, triglyceride, and HDL levels. The remaining metabolic parameters including adiponectin were not significantly different between the groups. IL-8 and KC-like inflammatory markers were significantly higher in the obese group. While the mean blood pressure was higher in obese patients, the difference was not statistically significant. A number of obese dogs (n = 6) were confirmed to have systemic hypertension on subsequent examinations.

In conclusion, obese dogs do have alterations in cardiac structure and function as well as dyslipidaemia, altered insulin sensitivity, and elevated inflammatory markers. These findings warrant additional studies to investigate inflammation, dyslipidaemia, or possibly systemic hypertension as potential etiologies of altered cardiac function.

C08

COMPARISON OF FUROSEMIDE INFUSION DILUTED WITH 2.4% HYPERTONIC SALINE VERSUS DEXTROSE 5% IN WATER (D5W).

Darcy Adin[1], Teresa DeFrancesco[1], Clarke Atkins[1], Kari Kurtz[1], Manoela Penteado[2]. [1]North Carolina State University, Raleigh, NC, USA, [2]2University of Sao Paulo, Sao Paulo, Brazil

Continuous rate infusion (CRI) of furosemide causes more diuresis than intermittent bolus administration, but can exacerbate dehydration and renal dysfunction. The co-administration of hypertonic saline (HS) may mitigate this, however, volume expansion could be detrimental in heart failure.

Furosemide CRI with D5W (FCRI-D5W) was compared to furosemide CRI with 2.4% hypertonic saline (FCRI-HS) in 6 normal dogs, in a randomized, blinded, crossover study. Furosemide was diluted to 2.2% with either 1.5 mL/kg D5W for the FCRI-D5W treatment or with 1.0 mL/kg D5W and 0.5 mL/kg of 7.2% hypertonic saline for the FCRI-HS treatment. The furosemide CRI rate was 0.66 mg/kg/hr for 5 hours in equal volume for both treatments and was preceded by a 0.66 mg/kg furosemide IV bolus.

Mean PCV and %increase in BUN were less for FCRI-HS versus FCRI-D5W (P = 0.046 both). Mean CVP decreased over time ($P < 0.001$), with no treatment difference ($P = 0.4$). Urine output, water intake, and creatinine increased significantly, while serum osmolality, serum/urine electrolytes, urine furosemide, and body weight decreased significantly, without differences between treatments. The %decrease in serum potassium ($P = 0.09$) and %increase in urinary sodium ($P = 0.08$) trended higher in FCRI-HS. Plasma furosemide was stable and not different between treatments. Urinary neutrophil gelatinase-associated lipocalin (NGAL) was unchanged, while both treatments doubled urinary aldosterone excretion suggesting similar activation of RAAS.

Diuresis was similar for both treatments however, FCRI-HS appeared to produce less dehydration (lower PCV, smaller %increase in BUN). Absence of intravascular volume expansion, based on CVP, suggests FCRI-HS may be safe in heart failure.

C09

MEDICAL MANAGEMENT AND SURVIVAL TIME ASSOCIATED WITH CONGESTIVE HEART FAILURE STAGE D: A RETROSPECTIVE STUDY. Amelie Beaumier, John E. Rush, Lisa M. Freeman. Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA

Degenerative mitral valve disease (DMVD) is the most common acquired heart disease in dogs. The ACVIM consensus statement for dogs with DMVD provided recommendations for diagnosis and management of Stages A through D. Recommendations for Stage D were limited by the paucity of published information for dogs with advanced congestive heart failure (CHF).

The objective of this retrospective study was to investigate the survival and treatment of dogs diagnosed with stage D CHF. For the purpose of this study, we defined stage D as the persistence of CHF despite receiving the label-approved dose of pimobendan, an angiotensin-converting-enzyme inhibitor (ACEI), and furosemide ≥4 mg/kg/day. Dogs receiving a dosage of furosemide <4 mg/kg/day were enrolled only if the dose was increased to ≥4 mg/kg/day and if a new medication to control CHF was introduced at the same visit.

Forty-three dogs (27 male and 16 female, all neutered) met eligibility criteria and were included in the study. Mean age at the time of diagnosis of Stage D CHF was 10.1 ± 1.8 years. The most common breeds represented were Cavalier King Charles spaniel (n = 9), Chihuahua (n = 4), Dachshund (n = 4), and Shih Tzu (n = 3). Twelve dogs were hospitalized on the day of diagnosis of stage D CHF [median duration of hospitalization = 1 day (range, 1–3 days)]. The median duration between the first diagnosis of CHF and diagnosis of stage D was 157 days (range, 11–743 days). At the time of diagnosis of stage D, 29 dogs had medications added [1 medication added (n = 26), >1 medication added (n = 3)], the diuretic dose was escalated in 27 dogs, and the pimobendan dose was increased to >0.6 mg/kg/day in 20 dogs.

Thirty-two (74%) dogs had subsequent additional changes in medications during the course of stage D CHF. Most dogs (n = 31; 72%) dogs were ultimately receiving at least 5 cardiac medications for the management of stage D CHF. Ultimate medication dosages during Stage D were: Furosemide (7.4 ± 2.7 mg/kg/day), pimobendan (1.0 ± 0.3 mg/kg/day), and ACEI (0.9 ± 0.2 mg/kg/day). Additional medications administered to these dogs included spironolactone (n = 31), sildenafil (n = 21), torsemide (n = 16), digoxin (n = 8), and hydrochlorothiazide with spironolactone (n = 8). Median survival time after onset of Stage D CHF was 311 days (range, 9–658 days), with 14 dogs still alive at the time of analysis.

Multiple medication adjustments were necessary to manage dogs with stage D CHF. However, the survival time may be encouraging to the dedicated owner.

C10

MRI 7T RESONANCE SPECTROSCOPY (MRS) PREDICTS CARDIAC ENERGETIC RESERVES IN DOGS WITH PRECLINICAL MITRAL INSUFFICIENCY. A Ray Dillon[1], Tom Denney[2], Seungwoo Jung[1], Randolph Winter[1], Juming Zhong[1], Michael Tillson[1], Xiaoxia Zhang[2], Nouha Salibi[3], Sharron Barney[1]. [1]College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [2]College of Engineering, MRI Center, Auburn University, Auburn, AL, USA, [3]Siemens Healthcare, MR Research and Development, MRI Center, Auburn University, Auburn, AL, USA

Early stages of heart disease are characterized by reduction in energy reserves with impairment of myocardial contractility and relaxation. However, these alterations are difficult to measure with existing clinical imaging modalities such as echocardiography. Recent advances in phosphorous (^{31}P) magnetic resonance spectroscopy (MRS) enable changes in energy reserves to be measured non-invasively.

The creatine kinase (CK) reaction is the primary energy reservoir of the heart. The CK reaction reversibly converts adenosine diphosphate (ADP) and phosphocreatine (PCr) to ATP and creatine. In heart failure, the flux through the CK reaction decreases as both creatine concentration (Cr) and CK activity decreases. Unlike ATP, which can be generated by de novo synthesis pathways in the myocyte, Cr is not produced in excitable tissues. Therefore, in heart failure the decrease in Cr occurs earlier and is faster than the decrease in ATP. In this study, the PCr/ATP ratio was used as a marker of cardiac energetics. The hypothesis was that changes in cardiac energetic reserves measured by MRS precede global MRI, echocardiographic, and biochemical markers in volume overload heart failure.

This hypothesis was evaluated in a canine model of preclinical isolated mitral regurgitation (MR) via chordal rupture. In each group, 2 dogs had minimal and 2 dogs had more severe MR at induction. One group of dogs (4) was evaluated at baseline and data collected at 2, 6, 12, 16 and 18 weeks after induction. A second group of dogs (4) was evaluated at baseline and 2, 4, and 6 weeks after induction. At these parallel time points, cardiac evaluation, thoracic radiographs, echocardiography, and MRS and standard 7T MRI were collected. In addition, peripheral blood samples were collected for microRNA and biomarker evaluation (NTproBNP). Baseline and endpoint cardiac catheterization were recorded for cardiac output, right and left ventricular function and vascular resistance. At the conclusion of the study, myocardial function was assessed by measuring isolated myocyte morphology, size, and myocyte contractility and Ca flux. Left ventricle and mitral valve tissues were collected for molecular studies of mRNA and miRNA.

Utilizing a 7 Tesla (T) MRI scanner, ^{31}P magnetic resonance spectra were collected with an ungated, free breathing three-dimensional chemical shift imaging sequence. In each dog, three 2.8 cc voxels on the septal wall near midventricle were selected for analysis. The PCr/ATP ratio was computed without saturation or blood correction in each voxel and averaged.

Although there was a range in severity of MR in each group, all dogs were asymptomatic, untreated and stage B1 or B2 during the observation period. All data were collected at each time point. Using each dog as its own control for echocardiographic evaluation, the end-point LVDd increased in diameter (35.1% in 18 weeks dogs, 19.9% in 6 weeks dogs) and left atria dimensions increased (27.3% in 18 weeks dogs and 31.2% in 6 weeks dogs) and the fractional shortening increased (22.6% in 18 weeks dogs, 24.3% in 6 weeks dogs). Compared to baseline, the LVDs dimension increased (18.7% at 18 wks in the 18 weeks dogs, and 8.6% in the 6 weeks dogs). At endpoint catheterization, the forward LV stoke volume decreased (35.2% in 18 weeks dogs, 22.2% in 6 weeks dogs) in all dogs.

The normal PCr/ATP ratio without saturation or blood correction was determined at baseline for normal dogs (1.455 mean, 0.097 SEM). In the unloaded LV at 2 weeks, the PCr/ATP ratio was increased in 6 of the 8 dogs. Over time with dysfunctional remodelling in MR dogs, the PCr/ATP ratio decreased as myocytes energy reserves were depleted especially in the dogs with systolic dysfunction.

PCr/ATP ratio would appear to be a measure of cardiac energetics early in the remodelling phase of volume overload MR and allows for the linear measure of the same dog over time to determine disease progression. MRS may provide a useful tool in the evaluation of drug therapy on the cardiac energetics of MR in dogs.

C11

IMMUNOHISTOCHEMICAL STUDY OF THE PRO-NATRIURETIC PEPTIDE CONVERTASE CORIN IN SEVERE CANINE MYXOMATOUS MITRAL VALVE DISEASE. Alex Sahagian[1], Chris Lam[2], Bob Bao[1], Sanaz Maleki[1], Brett Hambly[1], Niek Beijerink[2]. [1]Discipline of Pathology, Bosch Institute and School of Medical Sciences, Sydney University, Sydney, NSW, Australia, [2]University Veterinary Teaching Hospital, Faculty of Veterinary Science, Sydney University, Sydney, NSW, Australia

Corin is expressed by cardiomyocytes and converts pro-natriuretic peptides to their mature forms, which play a key role in fluid homeostasis and cardiac remodelling. In mice and human with failing hearts, myocardial corin protein levels have been reported to be increased, however without a concomitant increase in corin activity. These findings can partly explain the clear paradox in congestive heart failure (CHF) in that the natriuretic peptides are

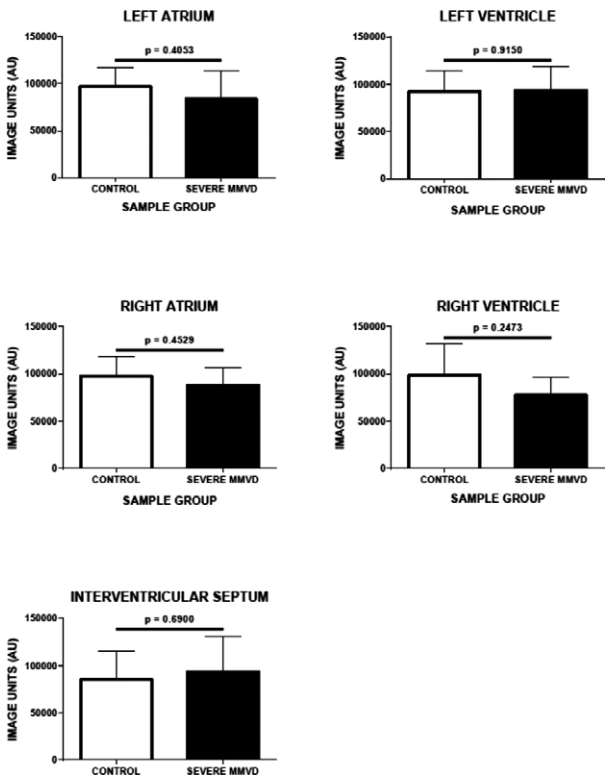
elevated, yet the body is fluid retaining (ultimately resulting in CHF). Interestingly in the Cavalier King Charles Spaniel, 2 loci associated with development of myxomatous mitral valve disease (MMVD) have been identified. Corin is on one of these loci on the latest dog assembly. This further raises the question whether deficiencies in myocardial corin expression or activity could be a mechanism explaining the progression of heart disease and CHF in dogs as well. The purpose of this study therefore was to characterize the immunohistochemical expression of corin in myocardial tissue in normal dogs and in dogs with severe MMVD.

Two groups of dogs of a variety of breeds were recruited including 5 control dogs (euthanized for severe non-cardiac disease; age range 4 months-12 years), and 6 dogs with severe MMVD (euthanized for refractory CHF; age range 9-14 years). Samples were collected from the right atrium (RA), right ventricle (RV), left atrium (LA), left ventricle (LV) and interventricular septum (IVS). Immunohistochemical staining of all tissues with a polyclonal rabbit anti-human antibody against corin was carried out, and computational quantification was used to compare the staining density between the two groups and between cardiac regions.

Positive transmembrane and intracellular immunostaining for corin was widely observed in all parts of the myocardium in both groups. Corin expression was not up- or downregulated in any of the cardiac chambers of the dogs with severe MMVD (figure 1), nor was any difference seen between the chambers within each group.

Our results indicate that corin expression might be a rate-limiting step in dogs with CHF due to MMVD. A lack of an increase in corin expression or activation can be postulated to be a possible important pathophysiological mechanism responsible for the natriuretic peptide paradox in dogs with CHF, and warrants further investigation.

Figure 1: Quantitative corin expression in myocardial tissue in control dogs, and in dogs with congestive heart failure secondary to myxomatous mitral valve disease.



C12

ANATOMIC REGURGITANT ORIFICE AREA USING 3D-ECHOCARDIOGRAPHY IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE (MMVD). Giulio Menciotti[1], Michele Borgarelli[1], Sandra Müller[2], Jonathan Abbott[1]. [1]VA-MD College of Veterinary Medicine, Blacksburg, VA, USA, [2]Utrecht University, Utrecht, The Netherlands

Aims of this study were to determine feasibility and repeatability of measuring anatomical regurgitant orifice area (AROA) using real-time 3D transthoracic echocardiography (RT3DE) in dogs affected by MMVD, and to determine if AROA obtained using RT3DE relates to the severity of MMVD assessed both with an echocardiographic scoring system (MRSS) and the American College of Veterinary Medicine (ACVIM) staging system.

RT3DE datasets of the mitral valve were acquired in 81 privately owned dogs diagnosed with MMVD and AROA was traced and measured using dedicated software. Dogs were classified as mild, moderate or severe according to an echocardiographic MRSS, and as ACVIM Stage B1, B2 or C. Technique feasibility (as % of dataset measurable) and inter- and intra-operator repeatability were assessed. Differences in AROA between dogs in different MRSS and ACVIM stages were investigated.

AROA was measurable in 60 (74.1%) dogs. The inter- and intra-operator coefficients of variation were 26% and 21%, respectively. AROA was significantly greater in dogs with severe MRSS compared to dogs with mild MRSS ($P = 0.04$). There were no differences in AROA between dogs with mild versus moderate, and moderate versus severe MRSS ($P = 0.84$ and $P = 0.28$, respectively). There was no difference between AROA of dogs in different ACVIM clinical stages ($P = 0.17$)

In conclusion obtaining AROA using RT3DE is feasible and offers an additional, non-invasive technique to stratify MR severity in dogs with MMVD. The relatively low repeatability suggests standardization of the technique is desirable. Diagnostic and prognostic utility of AROA deserve further investigation.

C13

ANALYSIS OF MITRAL VALVE MORPHOLOGY WITH REAL-TIME 3-DIMENSIONAL ECHOCARDIOGRAPHY IN DOGS UNDERGOING MITRAL VALVE REPAIR. Takeshi Mizuno, Masashi Mizuno, Kayoko Harada, Hiroshi Takano, Arane Takahashi, Kazuya Mamada, Junsoek Lee, Sayaka Takeuchi, Ayaka Chen, Tamotsu Sawada, Takahiro Mizukoshi, Asako Shinoda, Shuhei Uchida, Takurou Mori, Kazuki Takamura, Junichirou Takeuchi, Masami Uechi. JASMINE Veterinary Cardiovascular Medical Center, Yokohama, Kanagawa, Japan

Three-dimensional (3D) saddle shape of the mitral valve annulus was first reported in 1987 by Levine and colleagues. Previous studies showed annulus height to commissural width ratio (AHCWR), an index parameter for saddle-shape of mitral annulus, is preserved across mammalian species (15–20%). This suggests that nature conserves the saddle-shaped annulus for mechanical benefit. Menciotti et al described 3D transthoracic echocardiography (TTE) of normal dogs and AHCWR was $21 \pm 0.05\%$. There are no reports on 3D transesophageal echocardiography (TEE) studies on dogs with myxomatous mitral valve disease (MMVD). This study aims to evaluate mitral valve annulus morphology using 3D TEE in dogs with MMVD.

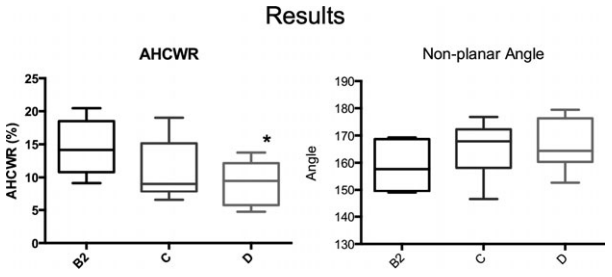
3D TEE was performed in dogs weighting more than 5 kg during mitral valve repair surgery. The 3D geometry of mitral valve apparatus was measured with dedicated quantification software. The classification of MMVD dogs used in this study was the basis of ACVIM staging. The 3D TEE measurements were compared between each group of dogs.

Twenty-six dogs were included in the study, 2 dogs were excluded from the study due to the uninterruptable echocardiographic images. Thus, a total of 24 dogs were evaluated in this study. The study group included 7 dogs with ACVIM Stage B2, 10 dogs with Stage C, and 7 dogs with Stage D. Characteristics of each group were summarized below:

	B2 (n=7)	C (n=10)	D (n=7)
Age (month)	101 ± 16	131 ± 14*	123 ± 21
Body Weight (kg)	6.84 ± 2.64	7.61 ± 1.33	7.6 ± 2.43
NT-proBNP (pg/ml)	2448 ± 1016	4786 ± 2893	3650 ± 2277
VHS (v)	12.1 ± 0.85	12.9 ± 1.3	13.2 ± 2.43
LA/Ao	1.85 ± 0.24	2.45 ± 0.34*	2.81 ± 0.25*
LVEDDN (cm/kg$^{0.294}$)	2.29 ± 0.22	2.32 ± 0.27	2.53 ± 0.30

Significant difference compared with B2 group, P < 0.05 (Tukey's multiple comparison test)

AHCWR decreased as the disease progressed (Stage B2; 14.2 ± 4.2%, Stage C; 9.0 ± 4.2 5, Stage D; 9.5 ± 3.3%, Figure). Stage D dogs showed significantly lower values ($P < 0.05$) compared to Stage B2 dogs. Non-planarity angle measurement tends to increase in value as the severity of MMVD worsened, however, there were no significant differences between the groups (B2; 157.7 ± 8.1°, C; 167.9 ± 9.2°, D; 164.4 ± 8.5°, Figure). There were no significant differences between the groups for annular circumference and annular area values.



In conclusions, the mitral valve of dogs with MMVD loses the saddle shape as the mitral valve disease progresses. The results of this study suggested that as the severity of MMDV worsened, more mechanical stress would be placed on the mitral valve, which could increase the progression of mitral regurgitation. To the best of our knowledge, this is the first report about the morphological changes of mitral valve annulus according to disease severity in dogs with MMVD.

C14

LOW-DENSITY LIPOPROTEIN OXIDATION IS BREED AND GENDER DEPENDENT IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE. Maria Josefine Reimann[1], Jens Häggström[2], Jacob Eifer Møller[3], Jens Lykkesfeldt[1], Torkel Falk[4], Lisbeth Høier Olsen[1]. [1]University of Copenhagen, Frederiksberg C, Denmark, [2]Swedish University of Agricultural Sciences, Uppsala, Sweden, [3]Odense University Hospital, Odense, Denmark, [4]Din Veterinär, Helsingborg, Sweden

Oxidative stress has been associated cardiovascular disease and suggested to contribute to cardiac remodeling in human patients with mitral regurgitation. Few previous studies have evaluated the relationship between oxidative stress and myxomatous mitral valve disease (MMVD) in dogs. The objective of this study was to determine plasma concentrations of certain markers of oxidative stress and investigate if they were associated with MMVD severity, selected clinical variables and disease progression in dogs with no or different severities of MMVD.

The study included 89 privately-owned dogs: 14 healthy control Beagles, 59 cavalier King Charles spaniels (CKCS) with different severities of MMVD, and 16 dogs of different breeds with clinical signs of congestive heart failure due to MMVD.

Markers of oxidative stress including malondialdehyde (MDA), oxidized low-density lipoprotein (oxLDL) and vitamin E (α-tocopherol and γ-tocopherol) were measured in plasma and their influence on 2-year progression of MMVD was determined.

Plasma oxLDL concentration was significantly lower in control Beagles compared to CKCS ($P = 0.0005$) and females had a significantly lower oxLDL plasma concentration compared to males ($P = 0.007$). Plasma vitamin E concentrations were associated with body condition score (BCS) (α-tocopherol, $P = 0.002$; γ-tocopherol, $P = 0.01$). All the markers of oxidative stress (MDA, oxLDL, vitamin E) were positively associated with serum cholesterol ($P \leq 0.02$) but none of the markers were associated with severity or progression of MMVD. The effect of BCS disappeared when adjusting for serum cholesterol concentration.

In conclusion, Beagles and female dogs appear to have lower plasma concentrations of oxLDL. The results cannot confirm a role of oxidative stress in dogs with MMVD.

C15

CARDIAC OUTPUT MEASURED BY ECHOCARDIOGRAPHY AND CARDIAC-GATED COMPUTED TOMOGRAPHY COMPARED TO THERMODILUTION. Nicole LeBlanc, Katherine Scollan. Oregon State University, College of Veterinary Medicine, Corvallis, OR, USA

The accurate measurement of stroke volume and cardiac output (CO) has important applications in both clinical and research arenas. Invasively measured CO by thermodilution (TD) remains the gold standard method. To date, there are very few comparative studies evaluating echocardiographic CO in dogs using noninvasive imaging modalities. There are no direct comparisons between noninvasive CO estimation using 64-slice multidetector computed tomography (MDCT) and TD in dogs. The aim of this study was to evaluate the accuracy of CO estimation by noninvasive methods (MDCT, 1-, 2-, and 3-dimensional echocardiography) compared to invasively measured CO obtained by TD.

Six intact hound cross dogs weighing between 19.5–23.8 kg were anesthetized using a standardized protocol and spontaneous ventilation. Each dog underwent MDCT, echocardiography, and TD. Dogs were randomized to the order of MDCT and echocardiography, and TD was performed last. CO using TD was measured in triplicate with <10% variation and minimal changes in heart rate. For imaging methods, CO was calculated by volumetric measurements of SV multiplied by the average heart rate. Echocardiographic left ventricular (LV) end-diastolic volumes (EDV) and end-systolic volumes (ESV) were measured on right long axis 1D images (Teichholz) and right long axis and left apical 2D images (single plane Simpson's method of disks, MOD) on 3 averaged consecutive beats. Real-time 3-dimensional echocardiographic (RT3DE) LV volumes were measured using 3D functional analysis software from both right long axis and left apical imaging planes. MDCT EDV and ESV were measured using specific LV functional analysis software on ECG-gated studies.

Excellent correlation was found between CO values measured by TD and 2D MOD ($r = 0.98$) from the left apical window, as well as 2D MOD ($r = 0.90$) and RT3DE ($r = 0.92$) from the right long axis window. A less robust correlation was found between RT3DE values obtained from the left apical window ($r = 0.68$) and TD. MDCT-derived CO were also strongly correlated ($r = 0.80$) with TD. CO estimation by Teichholz formula ($r = -0.05$) had no significant correlation with TD. Bland-Altman analysis showed a systemic underestimation of CO values derived both from echocardiographic and MDCT measurements. The bias for RT3DE CO obtained from the right long axis window was −0.23 L/min (95% confidence interval (CI) −0.56 to 0.09 L/min), and for 2D from the left apical window was −0.66 L/min (95% CI −0.91 to −0.42 L/min). The bias for CO values obtained from MDCT was −0.57 L/min (95% CI −0.99 to −0.15 L/min).

These results suggest certain echocardiographic techniques available for CO measurement are comparable to invasively measured CO. Specifically, 2D MOD using the right long axis and left apical 4-chamber views, or RT3DE from the right long axis view, had excellent agreement with TD. The excellent correlation between methods indicates a close relationship between select techniques, although they are not interchangeable. CO is underestimated by the noninvasive techniques studied here compared to TD. The differences between TD and noninvasive measures may be due to errors of the method, patient stroke volume variability, heart rate

fluctuation, or inaccuracies of TD. One of the major limitations of this study is the small number of patients, which allows preliminary conclusions only. A larger prospective study is needed to delineate the benefits and constraints of these methods.

C16

ANGIOTENSIN CONVERTING ENZYME ACTIVITY AND RESPONSE TO ENALAPRIL IN DOGS WITH AN ACE GENE POLYMORPHISM. Kathryn Meurs[1], Darcy Adin[1], Brent Aona[1], Teresa DeFrancesco[1], Bruce Keene[1], Yamir Reina[2], Josh Stern[1], Sandy Tou[3], Jess Ward[1], Kate Woodruff[1], Clarke Atkins[1]. [1]North Carolina State University, Raleigh, NC, USA, [2]University of California, Davis, Davis, CA, USA, [3]Iowa State University, Ames, IA, USA

Myxomatous mitral valve disease (MMVD) is the most common heart disease in the dog. Angiotensin converting enzyme (ACE) inhibitors are frequently recommended for management of dogs with MMVD and asymptomatic cardiac enlargement. However, the benefit of ACE inhibition in dogs before the onset of congestive heart failure (CHF) is controversial, with different studies showing conflicting results. A variable response to ACE inhibitor therapy has also been observed in human beings with heart disease and in some cases this has been attributed to a polymorphism in the ACE gene. We have previously demonstrated a polymorphism in the canine ACE gene, although the clinical significance of this finding is unknown.

We hypothesized that dogs with MMVD, cardiac enlargement and the ACE gene polymorphism would be less responsive to ACE inhibition with enalapril, as measured by plasma ACE activity, than dogs without the polymorphism.

We evaluated dogs with pre-CHF MMVD and a vertebral heart score of ≥ 11, presenting to the NCSU CVM in the last 18 months. Dogs were genotyped for the ACE polymorphism. Serum samples were collected and analyzed for ACE activity measurement by radioimmunoassay. Dogs were prescribed enalapril, 0.5 mg/kg orally twice a day, and revaluated in 14–21 days with a second measurement of ACE activity. A t test was used to compare ACE activity in the wildtype (normal canine sequence) and DNA variant groups at baseline and after therapy. A paired t test was used to compare each genotype's ACE activity before and after therapy.

Thirty-one dogs were evaluated. Genotypes included 12 homozygous for the DNA variant and 19 homozygous for the wild type. Median baseline ACE activity was significantly lower for dogs homozygous for the DNA variant (18.05 U/L) than for dogs with the wildtype sequence (27.4 U/L) ($P = 0.01$). Twenty-seven dogs returned for re-evaluation in 14–21 days. Both genotypes showed a significant suppression of ACE activity on enalapril, ($P = 0.0065$ DNA variant dogs; $P < 0.0001$ wildtype dogs). Median post therapy ACE Activity was not different for the two genotypes (DNA variant, < 5.0 U/L; wildtype dogs <5.0 U/L) ($P = 0.24$)).

We conclude that dogs that are homozygous for the ACE variant have lower baseline levels of ACE activity but still demonstrate ACE inhibition in response to enalapril. Further study is warranted to evaluate the clinical importance of these findings.

C17

ANGIOTENSIN CONVERTING ENZYME ACTIVITY IN CAVALIER KING CHARLES SPANIELS WITH AN ACE GENE POLYMORPHISM. Kathryn Meurs[1], Maria Josefine Reimann[2], Lisbeth Heier Olsen[2]. [1]North Carolina State University, Raleigh, NC, USA, [2]University of Copenhagen, Copenhagen, Denmark

The Cavalier King Charles Spaniel is one of the most commonly reported breeds of dogs affected with myxomatous mitral valve disease (MMVD). Angiotensin converting enzyme (ACE) inhibitors are frequently recommended for management of dogs with MMVD. However, the benefit of ACE inhibition in MMVD before congestive heart failure has developed is controversial with different studies showing conflicting results. A variable response to ACE inhibitor therapy has also been observed in human beings with heart disease and has been attributed to a polymorphism in the ACE gene. We have previously demonstrated a polymorphism in the canine ACE gene in small breed dogs.

We hypothesized that the polymorphism would be common in CKCS dog and that this would impact ACE activity.

We collected DNA and plasma samples from CKCS with MMVD. All dogs were genotyped for the known ACE polymorphism. Plasma samples were collected and analyzed for ACE activity measurement with a radioimmunoassay. A t test was used to compare ACE activity in the wildtype (control sequence) and DNA variant groups.

Sixty-two dogs were evaluated. Genotypes of 38 (61%) dogs were homozygous for the DNA variant, 3 (5%) were heterozygous and 21 (34%) were homozygous for the wild type (normal canine sequence). Samples from 40 dogs were submitted for ACE analysis including 20 homozygous for the wild type and 20 homozygous for the variant. Median baseline ACE activity was significantly lower for dogs homozygous for the DNA variant (25.0 U/L) than for dogs with the wildtype sequence (31.0 U/L) ($P = 0.02$).

We conclude that the ACE polymorphism appears to be common in the CKCS and dogs that are homozygous for the ACE variant have lower baseline levels of ACE activity. Further study is warranted to evaluate the clinical importance of these findings.

C18

PREVALENCE OF *DIROFILARIA IMMITIS* ANTIGEN IN CLIENT-OWNED PET DOGS BEFORE AND AFTER SERUM HEAT TREATMENT. Laura Nafe, Susan Little, Paul DeMars, Ryan Baumwart, Nalani Yamada, Eileen Johnson. Oklahoma State University, Stillwater, OK, USA

Pet dogs are routinely screened for heartworm disease as a component of annual wellness evaluation. Recent evidence suggests that antigen-antibody complexes in canine serum may preclude accurate diagnosis of heartworm disease with commercially available *Dirofilaria immitis* antigen assays. The objective of this study was to determine whether normal dogs presenting to the Oklahoma State Veterinary Teaching Hospital Community Practice Service for annual veterinary care warrant heat treatment of serum to confirm a negative heartworm antigen test by 4DX Snap (Idexx). Serum and EDTA samples were collected from 201 client-owned pet dogs between December 2014 and October 2015. Dogs ranged in age from 10 months to 15 years (median 4 yrs) and included 108 males and 93 females. Complete survey results (available for 194/201 dogs) revealed 139 dogs (72%) received heartworm preventative regularly over the past year. All EDTA samples were evaluated for microfilaria and serum samples for *D. immitis* antigen before and after heat treatment using a commericial assay (DiroCHEK, Zoetis). Four dogs (2%) tested positive for *D. immitis* antigen on 4DX Snap Test (Idexx) and 2 were microfilaremic. On DiroCHEK antigen testing 2 dogs were positive before and after heat treatment, 1 dog was positive after heat treatment only, and 1 dog was negative before and after heat treatment despite being microfilaremic. One dog received heartworm preventative (Heartgard; Merial) regularly (2/12 doses missed). Heat treatment does not appear to improve detection of *D. immitis* antigen in asymptomatic pet dogs undergoing routine heartworm disease testing with 4DX Snap Test (Idexx).

C19

CARDIAC BIOMARKERS TROPONIN I AND N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE IN CANINE CHRONIC KIDNEY DISEASE PATIENTS. Lena Pelander, Jens Häggström, Ingrid Ljungvall. Department of Small Animal Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Increased concentrations of N-terminal pro brain natriuretic peptide (NT-proBNP) and cardiac Troponin I (cTnI) in azotemic dogs, cats and people have been repeatedly reported. Knowledge

of the mechanisms behind increased concentrations of these cardiac biomarkers in azotemic dogs is warranted for correct interpretation of test results. The aim of this study was to investigate the association between measured glomerular filtration rate (GFR) and plasma concentrations of cTnI and NT-proBNP in stable canine CKD patients.

Fifty client-owned dogs were prospectively included into the study: Twenty-seven dogs with a diagnosis of CKD (structural and/or functional abnormalities of one or both kidneys that had persisted for at least 3 months) and without signs of other significant disease and twenty-three healthy dogs were included as controls. All dogs were investigated by a physical examination, repeated blood pressure measurements, a complete urinalysis, a full hematology and biochemistry panel, an echocardiographic examination, an ultrasound examination of the entire urinary tract, and a scintigraphic examination for estimation of kidney glomerular filtration rate (GFR). Plasma from each dog was stored in −70°C and assayed in batch.

Median age of included dogs was 6 years (range 0.5–14 years). Median bodyweight was 19.5 kg (range 2–42 kg). There was no difference in age or weight between healthy and CKD dogs. Among dogs with CKD, 16 had a decreased GFR and 11 had a GFR within the normal range. Dogs with CKD and a GFR in the normal range had other evidence of kidney disease such as persistent renal proteinuria or morphological abnormalities.

Univariate analyses were performed to evaluate associations between dog characteristics and cTnI and NT-proBNP, respectively. Age ($r = 0.59$; $P < 0.0001$), body weight (BW; $r = 0.29$; $P = 0.04$), creatinine ($r = 0.29$; $P = 0.04$) and systolic blood pressure (SBP; $r = 0.42$; $P = 0.06$) were associated with cTnI concentration. Creatinine ($r = 0.46$; $P = 0.001$), GFR ($r = -0.39$; $P = 0.0063$), albumin ($r = -0.35$; $P = 0.0141$), and erythrocyte volume fraction (EVF; $r = -0.35$; $P = 0.0072$) were associated with NT-proBNP concentration.

All variables that were associated with cTnI or NT-proBNP concentration, respectively, with a P-value of <0.2 were included in the multiple regression analysis (except creatinine because of covariance with GFR). Age, BW and SBP were the variables remaining in the final model for cTnI (adjusted R-square 0.50, $P < 0.0001$). GFR, BW and serum albumin concentration were the variables remaining in the final model for NT-proBNP (adjusted R-square 0.29, $P < 0.0001$).

In conclusion, plasma concentration of cTnI in dogs with stable CKD was not influenced by a decreased GFR. Plasma concentration of NT-proBNP in stable canine CKD patients on the other hand, appeared to be influenced by GFR.

C20

ESTABLISHING NORMAL 24 HOUR HOLTER MONITOR VALUES IN HEALTHY PUPPIES.

Rebecca Tracey[1], Dongyun Wang[2], Pamela Lee[1]. [1]Washington State University, Pullman, WA, USA, [2]University of Idaho, Moscow, ID, USA

Currently, published literature focuses on the normal 24 hours Holter monitor values of adult dogs greater than 1 year of age. Studies have shown that normal healthy adult dogs may have occasional ventricular premature complexes (VPCs) and that more frequent or sequential VPCs on Holter monitoring may indicate cardiac or clinical disease. This poses a problem for cardiac evaluation of puppies for veterinarians. This lack of information inhibits the ability to screen puppies for indicators of cardiac disease. This is especially important in breeds that tend to have inherited cardiac arrhythmias such as German Shepherd puppies. The purpose of this study is to establish normal ambulatory ECG values using Holter monitors in puppies in an effort to enhance the ability to screen puppies for cardiac disease.

The primary objective was to determine the minimum, average, and maximum heart rate and incidence of arrhythmias for healthy puppies. The secondary objective was to evaluate the correlation between Holter heart rate and age, weight or breed. We hypothesized that healthy puppies will have less arrhythmias and higher Holter minimum, average, and maximum heart rates than healthy adult dogs.

Eligible puppies had to be healthy and between the ages of 12–51 weeks. History, physical exam, and thorough auscultation were performed. An echocardiogram was performed on any puppy with a heart murmur on auscultation. Puppies with physiologic heart murmurs were included in the study while puppies with structural heart disease were excluded. A 24 hours 3-channel Holter monitor was placed on all puppies included in the study. Holter placement followed established methods from the WSU Veterinary Holter Service.

A total of 44 puppies representing 20 breeds were included in this study. Minimum, average, and maximum Holter heart rates were higher in puppies compared to healthy adult dogs. The number of supraventricular complexes seen in puppies were less than in healthy adult dogs. The range of ventricular complexes in puppies were higher than in healthy adult dogs. Minimum and average Holter heart rate decreased with increasing age while maximum Holter heart rate was not correlated with age. No correlation between minimum, average, and maximum Holter heart rates and weight was appreciated. There was a statistically significant difference between maximum Holter heart rate and different breed classifications by size (small versus medium versus large versus giant breed). No correlation between minimum and average Holter heart rate and breed classifications was identified in healthy puppies.

This study is the first study to evaluate normal ambulatory electrocardiographic values in healthy puppies. This information can be used to potentially screen puppies at an earlier age for cardiac disease. The number of ventricular arrhythmias considered normal for healthy adult dogs is thought to vary with dog breed. Whether or not this breed variation is true in puppies is unknown. Now that a normal baseline has been established for puppies, further studies can be conducted to evaluate for breed variation.

C21

DISTRIBUTION OF ALVEOLAR-INTERSTITIAL SYNDROME IN DYSPNEIC VETERINARY PATIENTS ASSESSED BY LUNG ULTRASOUND VERSUS THORACIC RADIOGRAPHS.

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Lung ultrasound (LUS) is a point-of-care imaging technique that identifies alveolar-interstitial syndrome (AIS) through the observation of ultrasound artifacts called B-lines. This study assessed distribution of AIS detected by LUS compared to thoracic radiographs (TXR) in a population of 100 acutely dyspneic veterinary patients (76 dogs and 24 cats).

Patients underwent LUS and TXR within 6 hours. LUS images were scored for presence of B-lines at 4 sites on each hemithorax. An individual LUS site was scored positive if >3 B-lines were observed within a single intercostal space at that site. TXR were scored for presence of alveolar-interstitial infiltrates at 4 sites on each hemithorax, analogous to LUS. An individual TXR site was scored positive if infiltrate was present in least 25% of the site. Medical records were evaluated for final diagnosis. Agreement in distribution of positive sites between LUS and TXR was compared using a Cohen's Kappa coefficient. Patterns of distribution of AIS among different final diagnoses were compared using Fisher's exact tests.

When considered site-by-site, agreement in distribution of AIS between LUS and TXR was poor to fair ($K = 0.25–0.5$). However, when considering larger spatial quadrants, agreement between LUS and TXR was good ($K = 0.45–0.65$). Distribution of AIS differed significantly based on final diagnosis for both cardiogenic and noncardiogenic causes of dyspnea. Dogs with mitral valve disease were more likely to have a caudal distribution of AIS ($P = 0.0031$), while dogs with dilated cardiomyopathy were more likely to have diffuse AIS ($P = 0.0008$). Patients with airway disease were more likely to have absence of AIS in all sites ($P = 0.0006$), while patients with pneumonia were more likely to have unilateral AIS ($P = 0.0055$).

LUS was useful for detecting AIS apparent on TXR. Agreement between the two modalities improved when considering larger spatial quadrants rather than smaller focal sites. Distribution of AIS differed based on final diagnosis, suggesting that a pattern-based LUS approach may prove diagnostically useful.

C22
CIRCULATING PLASMA MIRNA AS NOVEL MOLECULAR BIOMERKERS IN CONGESTIVE HEART FAILURE.

SeungWoo Jung, Amy Bohan. Auburn University, Auburn, AL, USA

Congestive heart failure (CHF) carries poor prognosis in dogs with myxomatous mitral valve disease (MMVD). Early identification of dogs predisposed to developing CHF is critical to improve long term survival outcome. The aim of this study is to characterize plasma molecular profiles in dogs with CHF secondary to MMVD and to develop novel molecular biomarkers via liquid biopsy. MicroRNAs (miRNAs) are small, non-coding RNAs and have been known to regulate expression levels of target genes involved in human CHF. Regulatory networks of miRNAs in dogs with MMVD need to be established to further enhance our understanding of CHF pathogenesis. The hypothesis tested in the study was that plasma miRNAs may be differentially expressed in dogs with CHF secondary to MMVD.

A minimum of seven dogs in each group (normal versus CHF) were needed to demonstrate the statistical power with a probability of 0.9 and type one error of $P < 0.05$ based on previous publications. Blood was collected in EDTA tubes from 9 normal geriatric dogs (free of heart murmur and absent history of CHF) and 8 dogs with CHF secondary to MMVD (confirmed by clinical signs, chest x-ray and echocardiography). Plasma samples were subjected to isolation of miRNAs with a miRNeasy Plasma Kit (Qiagen) and subsequently reverse-transcription reaction with a miScript II RT Kit (Qiagen). Validated canine primers of 12 candidate miRNAs and miR-39 (spike-in control) were employed to quantify relative expression levels of each miRNA between normal and CHF dogs via real time PCR (miScript SYBR Green PCR Kit, Qiagen).

Canine specific miRNAs were successfully isolated from plasma and amplified. Plasma miR-21 ($P = 0.015$), miR-133 ($P = 0.01$), and miR-1 ($P = 0.01$) were differentially expressed in CHF dogs with statistical significance when compared to normal dogs. These miRNAs are known to be involved in extracellular matrix remodeling and the regulation of fibrotic genes such as TGF-β and Smad. The results suggest that alterations in the interaction between miRNAs expression profiles and their downstream target genes may play a role in cardiac progression to CHF in dogs with MMVD. Further investigation remains warranted to determine their diagnostic potential as molecular biomarkers for early detection of CHF in dogs with MMVD.

C23
A MULTICENTER EVALUATION OF SIGNALMENT AND COMORBID CONDITIONS ASSOCIATED WITH AORTIC THROMBOSIS IN 291 DOGS.

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Aortic thrombotic disease is a primary disease or secondary complication of a systemic disease state with devastating effects to both dogs and cats. Cats with aortic thromboembolism often have significant concurrent cardiac disease. While there is a substantial amount of information available about cats with this disease process, aortic thrombosis (AT) is not well characterized in dogs.

In order to assess signalment and concurrent disease processes in dogs diagnosed with aortic thrombotic disease, a retrospective review was performed of records available through the Veterinary Medical Database of dogs examined between 1985 and 2011. Information on age, sex, breed, weight, and concurrent disease processes in dogs reported with AT were reviewed. Five control dogs without a diagnosis of AT were selected for each AT case.

AT was diagnosed in 291 of 984,973 dogs included in the VMDB from 1985 to 2011. The odds of a dog having AT did not differ significantly by sex ($P = 0.0593$), age ($P = 0.9833$), or weight ($P = 0.7766$). Compared to mixed breed dogs, Shetland sheepdogs had a significantly higher odds (OR=2.44, $P = 0.0027$) of being diagnosed with AT. Protein-losing nephropathy was the most commonly reported disease occurring concurrently in dogs with AT (64/291).

Signalment data including age, sex, and weight were not significantly different in dogs diagnosed with AT compared to dogs not diagnosed with AT. Dogs with AT were commonly diagnosed concurrently with PLN. Contrary to previous reports, cardiac disease was not a common concurrent diagnosis in the dogs with AT included in this study.

C24
RIGHT VENTRICULAR SYSTOLIC FUNCTION IN DOGS WITH POST-CAPILLARY PULMONARY HYPERTENSION.

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The function of right ventricle (RV) has gained increased recognition over the past years. In pulmonary hypertension (PH) patients, this led to the reconceptualization of the RV as part of right ventricular pulmonic circulation unit and the acknowledgement that RV function is a major determinant of prognosis in pulmonary arterial hypertension. With this in mind, we sought to investigate the RV systolic function in dogs with post-capillary PH ascribed to mitral valve disease (MVD). Twenty nine dogs with PH (2.5–17.2 kg; 7–15 years) and 51 dogs without PH (1.5–32.0 kg; 2–16 years) were recruited into this prospective cross-sectional observational study. The PH group was subdivided into mild PH (n = 19), moderate PA (n = 6), and severe PH (n = 4). Several breeds were represented. All animals underwent a complete echocardiogram, which included the measurement of RV fractional area change (RV FAC); tricuspid annular plane systolic excursion (TAPSE); RV free-wall longitudinal systolic velocity (tricuspid annular S′ wave), and RV fractional shortening (RV $FS_{\%}$). For improved statistical analysis and normalization of data according to animal size, a body weight-indexed TAPSE ($TAPSE_{BW\text{-}indexed}$) was created. Some standard echocardiographic indices of congestion and LV function, including early-to-late LV filling velocities ratio (E/A), isovolumic relaxation time (IVRT), E-to-IVRT ratio (E/IVRT), left atrium-to-aorta ratio (LA/Ao), and LV fractional shortening (LV $FS_{\%}$) were recorded as well. The data was not normally distributed. The Mann-Whitney test showed no differences ($P > 0.05$) to exist for RV FAC, RV $FS_{\%}$, TAPSE, $TAPSE_{BW\text{-}index}$, and tricuspid annular S′ wave between dogs with PH and those without PH. Also, the analysis of the subdivided PH group showed no statistical difference between the three degrees of PH. Spearman correlation coefficients between RV systolic data and LV indices of congestion and function showed a significant positive correlation between TAPSE and E/IVRT ($R = 0.3917$, $P = 0.0003$), TAPSE and LA/Ao ($R = 0.3298$, $P = 0.0029$), and $TAPSE_{BW\text{-}indexed}$ and LV FS% ($R = 0.2757$, $P = 0.0166$). Finally, a significant positive correlation was documented between RV FAC and RV $FS_{\%}$ ($R = 0.3507$, $P = 0.0100$), as well as between TAPSE and tricuspid annular S′ wave ($R = 0.4575$, $P < 0.0001$). Because only a few animals with severe PH were recruited into this study, the absence of association between the severity of PH and the identification of RV systolic impairment needs further investigation. Nevertheless, we observed a downward trend for RV FS %, TAPSE, $TAPSE_{BW\text{-}indexed}$, and tricuspid annular S′ wave along with the aggravation of PH. Our study found correlations between a few congestion and LV function parameters, which may prove useful in monitoring RV function in dogs with severe MVD and overt signs of post-capillary PH. Since the majority of PH cases were mild, it is likely that only a few had reactive PH, which may explain why RV function of either group behaved very similarly. Also, it is not clear whether RV remodeling and impairment would be stronger if pre-capillary PH dogs had been included in this investigation.

C25

VASOVAGAL TONUS INDEX IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE. Bruna Cristina Bruler[1], Gustavo Dittrich[1], Amalia Turner Giannico[1], Tilde Rodrigues Froes[1], Aparecido Antonio Camacho[2], Marlos Goncalves Sousa[1]. [1]Federal University of Parana, Curitiba, PR, Brazil, [2]Sao Paulo State University, Jaboticabal, SP, Brazil

Decreased heart rate variability (HRV) has been found in dogs in congestive heart failure (CHF) and previous studies have acknowledged the use of HRV indices as prognostic indicators in patients with mitral valve disease (MVD). The vasovagal tonus index (VVTI) is an unconventional time domain indicator of HRV, which is mainly influenced by cardiac parasympathetic tone. In this cross-sectional observational study, we sought to investigate the VVTI in dogs with MVD. Electrocardiographic recordings of 30 dogs (7–16 years, 3.5–15.5 kg) previously classified into ACVIM stages A (controls), B, or C (10 dogs each group) were used to calculate the VVTI. For this, 20 consecutive R-R intervals were measured from each ECG recording (R-R1 to R-R20), and the index was obtained from the formula VVTI = LN [VAR (R-R1 – R-R20)], where LN: natural logarithm and VAR: variance. Bad quality ECG tracings and recordings from dogs with non-sinus rhythms or animals undergoing anti-arrhythmic treatment were not included in this investigation. Also, we recorded the BW-indexed LV in diastole and systole, wall stress index in diastole and systole, fractional shortening, left atrium-to-aorta ratio, mitral E wave, mitral E/A, isovolumetric relaxation time, and the E-to-IVRT ratio. All data underwent the Shapiro-Wilk test to check for normal distribution, while ANOVA, followed by Tukey's test, were used to compare the VVTI between groups. Pearson's test was used to search for linear correlations between the VVTI and the echocardiographic data. The mean values (with lower and upper 95% CI of mean) of VVTI for dogs in stages A, B, and C were, respectively: 8.45 (7.36 – 9.54), 6.09 (4.47 – 7.71) and 6.34 (5.01 – 7.68). A significant difference was found between groups ($P = 0.0189$), with the mean VVTI being significantly higher in control animals as compared to dogs with stage B MVD ($P < 0.05$). When it comes to the relationship between VVTI and cardiac rhythms, a significant difference existed between animals in sinus rhythm (SR), sinus arrhythmia (SA), and sinus tachycardia (ST) ($P = 0.0083$). The lowest VVTI was documented for dogs in ST (5.82; 95% CI 4.14 – 7.49), while the higher was found for animals presenting SR (8.27; 95% CI 7.40 – 9.15). Significant negative correlations were found between VVTI and LA/Ao ($R = -0.3699$; $P = 0.0443$), as well as between VVTI and heart rate ($R = -0.4864$; $P = 0.0064$). Although no correlation existed between body weight and the VVTI, age and VVTI attained a significant negative correlation ($R = -0.3827$; $P = 0.0369$). The negative correlation between VVTI and heart rate is likely ascribed to the role played by the parasympathetic tone in VVTI, therefore producing higher values when slower rates and irregular rhythms are present. Even though uncontrolled conditions during ECG recording, including stress and agitation, may increase HR, the lower VVTI found in animals exhibiting sinus tachycardia may suggest a sustained sympathetic activation. Although further investigation is warranted, the confidence intervals of this study point to the VVTI <5 being a potential prognostic indicator for CHF, whereas a VVTI >7.7 is likely suggestive of reduced risk for congestion. This is supported by the correlation between VVTI and LA/Ao, as well as the difference between the means of control dogs (stage A) and stage B MVD animals.

C26

SICK SINUS SYNDROME AND SINUS NODE DYSFUNCTION: CASE SERIES REPORTS OF FMVZ-USP CARDIOLOGY SERVICE (2007–2015). Rebecca Bastos Pessoa[1], Guilherme Teixeira Goldfeder[1], Patricia Pereira Costa Chamas[2], Denise Saretta Schwartz[1], Paula Staudacher Leal de Carvalho[1], Maria Helena Matiko Akao Larsson[1]. [1]School of Veterinary Medicine and Animal Science – University of Sao Paulo (FMVZ-USP), São Paulo, SP, Brazil, [2]Paulista University, São Paulo, SP, Brazil

Sick Sinus Syndrome is a complex disease characterized by an abnormal cardiac impulse formation on the sinus node. The term Sinus Node Dysfunction refers to the variety of electrocardiographic features involved in this condition. When Sinus Node Dysfunction is associated with clinical manifestations it is referred to as the Sick Sinus Syndrome. A survey of affected dogs attended at the Cardiology Service in the Department of Internal Medicine of the Veterinary Teaching Hospital (HOVET) of the School of Veterinary Medicine and Animal Science - University of Sao Paulo (FMVZ-USP) from 2007 to 2015 was performed. Seventeen cases were reviewed. Breeds involved were Schnauzer (71%), Cocker Spaniel (6%), Poodle (6%) and Dachshund (6%), besides a mongrel dog (6%). There was a predominance of female dogs (71%). The most common clinical manifestations observed were syncope (71%), presyncope (24%), dyspnea (18%) and exercise intolerance (12%). Twelve percent of the dogs were asymptomatic. The most frequent electrocardiographic changes found were sinus arrest (75%), junctional escape rhythm (73%), supraventricular tachycardia (53%) and sinus arrhytmia (53%). Forty-seven percent of the dogs presented tachycardia-bradycardia syndrome. Only 24% of the animals received pacemaker implants, given the high percentage of animals unable to go through chirurgical/anesthetic procedures (29%), and the presence of asymptomatic animals. The dogs that received pacemaker implants lived for 20.4 months (mean value), whereas the ones that were treated conservatively lived for 10.8 months (mean value). These values showed benefit to those patients who received pacemaker implants, but was hindered by the high percentage of comorbidities in older animals, besides the small number of patients enrolled in this retrospective study.

C27

THE UTILITY OF SPECTRAL DOPPLER OF THE HEPATIC VEINS IN DOGS WITH TRICUSPID REGURGITATION. Kensuke Nakamura, Tatsuyuki Osuga, Tomoya Morita, Keitaro Morishita, Noboru Sasaki, Hiroshi Ohta, Mitsuyoshi Takiguchi. Hokkaido University, Sapporo, Japan

Echocardiographic assessment of the severity of tricuspid regurgitation (TR) is less reliable compared to mitral regurgitation due to the complicated geometry and function of right ventricle. Spectral Doppler of hepatic veins (SDHV) has emerged as a useful examination to evaluate the severity of TR in humans. The hepatic vein blood flow waveform is multiphasic and typically consisted with four peaks. A small retrograde a-wave occurring after the P-wave of the electrocardiographic is created by retrograde blood flow during atrial contraction. A large anterograde S-wave occurring immediately after the QRX complex is created by blood flow toward the heart during ventricular systole. A small retrograde v-wave occurs after ventricular systole. A large anterograde D-wave occurring after T-wave is created by ventricular diastole. The objective of the present study was to investigate the correlation between the presence of ascites and the findings of SDHV in dogs with TR. This is a clinical cohort study including twenty-seven client-owned dogs with TR. Dogs were divided into two groups based on the presence of ascites. Physical examination, SDHV and echocardiographic variables were compared between the groups. For the assessment of the comparative accuracy in identifying patients with ascites, receiver operating characteristic (ROC) curves was used. Peak velocity of v-wave and D-wave in dogs with ascites (n = 11) was significantly higher than dogs without ascites (n = 16). The highest accuracy was obtained for v-wave, with an area under the ROC curve (AUC) of 0.99, followed by D-wave, with an AUC of 0.95; late diastolic velocity of the septal mitral annulus (Am), with an AUC of 0.907. TR peak velocity had no significant difference between two groups. In conclusion, v-wave and D-wave obtained by SDHV has strong correlation with the presence of ascites in dogs with TR.

C28

CUTANEOUS USE OF AN IMPLANTABLE LOOP RECORDER (REVEAL LINQTM) FOR CARDIAC EVENT MONITORING IN DOGS. Emily Menzen, Amara Estrada, Brandy Winter, Melanie Powell. University of Florida, College of Veterinary Medicine, Gainesville, FL, USA

Event monitors are a useful diagnostic tool for evaluating patients with episodic cardiac events. The ideal event monitor for use in veterinary practice would be non-invasive, affordable, accurate and reusable. Such devices are becoming increasing difficult to obtain. This study aimed to prospectively evaluate the feasibility of using the Medtronic Reveal LINQTM human implantable cardiac monitor (ICM) for cutaneous event monitoring for up to 5 days in 14 clinically healthy canines (21.5 kg, range 13.4–31.2 kg; 4.5 years range 1.5–13 years old) and two canines with reported collapse episodes (4.7 and 6.4 kg, 2.5 and 11 years old). The study consisted of developing a method to secure the low-profile device without causing discomfort at the location generating the strongest signal, adjusting the auto activation parameters, activating the device manually, and assessing the recordings for accuracy.

Reveal LINQTM wireless cardiac device were obtained via donation following explantation from human patients and the battery life assessed prior to use in each canine patient using a Medtronic Carelink® programmer with appropriate software loaded. An area on the left lateral thorax was clipped and alcohol was used to remove oils. Adhesive electro-conductive gel pads were applied underneath the device and it was placed within the 4th intercostal space parallel to the ribs and then slowly moved in a grid pattern to find the most consistent electrocardiogram (ECG) on the programmer screen. The device was then secured to the thorax using TegadermTM film, gauze, and Elastikon®. It was then taped around the thorax with 2 inch white tape and VetraptM and the patients were fitted with a Holter monitor vest to reduce likelihood of device tampering or consumption of bandage materials and device. The device was programmed with the patient's information and the pre-programmed manufacturer heart rate parameters Patients wore the ICM for 4 to 5 days and owners were asked to activate the monitor manually for 4 total recordings during different activities and to keep a diary. Accuracy was assessed based on quality of ECG tracings (consistent ability to measure heart rate, visualization of entire p-QRS-T waves) and presence of a recording during manual activation. Two dogs were fitted with 24 hour Holter monitors as a control.

Of the sixteen patients, two were unable to be fitted with a device due to a weak signal, one client terminated the study after 1 day, and the device moved out of place and stopped recording on one dog. The remaining twelve dogs had at least 3 out of 4 interpretable tracings from manually activated recordings. The auto-activated recordings triggered by pre-set parameters were of good quality. After each set of auto-activated recordings were reviewed, the parameters were adjusted to define the outer range of normal and recorded rhythms were reclassified. Over the course of the study patient heart rates did not exceed 222 bpm for 32 beats or reach 30 bpm for 12 consecutive beats. Fewer auto-activations occurred per dog when the pause parameter was set at 4.5 seconds than 3 seconds. One symptomatic patient had three episodes while wearing the device however there was no indication of a rhythm abnormality in the manually activated recordings. The other symptomatic patient did not have an episode while wearing the monitor and the device incorrectly logged an atrial fibrillation episode. During this time period, the ECG from the ICM was not of sufficient quality to identify p waves but the control Holter recording clearly showed a sinus arrhythmia was present. The device was still recording at time of removal in all but one dog. Five dogs had self-limiting, mild skin irritation after removal of the device.

The Medtronic Reveal LINQTM ICM is a viable option for cutaneous cardiac monitoring in dogs for up to 5 days. Veterinary parameters for auto-activation of the device should be programmed at the upper and lower limits of the auto-activated parameters to reduce the number of incorrectly labelled recordings and all recordings should be reviewed by a veterinarian familiar with normal cardiac rhythms.

C29

AORTOSEPTAL ANGLE AND RESPONSE TO BALLOON VALVULOPLASTY IN DOGS AFFECTED WITH SEVERE SUBAORTIC STENOSIS. Lilian Shen[1], Amara Estrada[1], Etienne Côté[2], Brandy Winter[1], Kenneth Lamb[3]. [1]University of Florida, Gainesville, Florida, USA, [2]University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, [3]Lamb Consulting, West St. Paul, Minnesota, USA

Subaortic stenosis (SAS) is the most common congenital cardiac defect identified in dogs. While it is not difficult to diagnose severe forms of this disease, treatment and decisions on when to consider interventional therapy remain a challenge. It has been suggested that SAS, in some breeds, may be related to an abnormally steep aortoseptal angle (AoSA) - a quality which may limit efficacy of ballooning methods. If an abnormally steep AoSA contributes to SAS and is not remedied by ballooning, it is possible that the magnitude of the AoSA may serve as a useful criterion for determining which dogs will respond better to balloon valvuloplasty and are therefore better candidates for the procedure.

This study investigated the relationship between the measured AoSA and response to combined cutting and high pressure balloon valvuloplasty (CB/HPBV), in dogs with severe SAS. Retrospective evaluation of angiographic and echocardiographic video loops of 22 client-owned dogs of various breeds affected with severe SAS (PG mean 143.36 mmHg; range 80.49–322.5 mmHg) who underwent CB/HPBV were evaluated at the baseline time point immediately prior to the procedure. Two board-certified veterinary cardiologists and 1 novice evaluated these video loops to identify still frame images to be used to measure the AoSA. Each evaluator was blinded to the other 2 evaluators' selected images and subsequent measurements of the AoSA. All evaluators were blinded to initial PG and patient outcomes following the procedure. The percentage decrease in PG at 24 hours, 6 months, and 12 months after ballooning was then calculated and correlated to the measured AoSA at each recheck and for each imaging plane.

The inter-observer variability analysis suggested significant mean differences for the novice observer when compared to the 2 cardiologists for all angle types. Significant differences were observed between the cardiologists for left apical echocardiographic ($P = 0.0163$) AoSA measurements, while no significant differences were observed for angiographic ($P = 0.1042$) or right sided long axis LVOT echocardiographic AoSA ($P = 0.7058$) measurements or for all measurements of AoSA combined combined ($P = 0.1937$).

Based on right sided long axis LVOT echocardiographic AoSA measurements made by the cardiologists, greater mean differences of PG reduction ratio were observed for angles >160° compared to angles <160°. At 24 hours, significantly greater ($P = 0.003$) mean differences of PG ratio were observed in the >160° (54.45 SE ± 3.8) compared to the mean in the <160° (39.88 SE ± 2.09). This difference persisted at 6 months (>160° mean 57.73 SE ± 10.9; <160° mean 28.22 SE ± 3.42) and at 12 months (>160° mean 76.11 SE ± 17.5; <160° mean 27.61 SE ± 6.44).

The findings of this study suggest that dogs with severe SAS with an AoSA >160° as measured on right sided long axis LVOT echocardiographic imaging respond to CB/HPBV better than dogs with an AoSA <160°. The difference in response to the same procedure by the two sets of dogs suggests that the magnitude of the AoSA does play a role in SAS and its treatment, and it is possible that measurement of the AoSA may be helpful in the screening process and determination of prognosis following CB/HPBV. However, further investigation of the effect of LVOT PG reduction on survival times and quality of life is warranted in order to determine if AoSA is truly a suitable criterion for selecting candidates for CB/HPBV.

C30

ECHOCARDIOGRAPHIC EVALUATION OF PULMONARY HYPERTENSION IN CATS WITH CARDIAC DISEASE AND PLEURAL EFFUSION VERSUS PULMONARY EDEMA. Melissa Wilson, Katherine Scollan, Nicole LeBlanc. Oregon State University, Corvallis, OR, USA

Cardiomyopathies, including hypertrophic and restrictive, are the most common acquired cardiac diseases in cats. Many affected cats develop pulmonary edema secondary to left-sided congestive heart failure (L-CHF). Alternatively, cats with primarily left-sided cardiomyopathy can develop pleural effusion without the presence of pulmonary edema. It is well known, but not well understood, why cats with left-sided CHF can develop both pulmonary edema and pleural effusion. The development of pulmonary hypertension could lead to right-sided heart failure and potentially cause pleural effusion in cats with predominately left-sided cardiac disease. The aim of this study was to evaluate for echocardiographic evidence of pulmonary hypertension in cats in L-CHF with pleural effusion in comparison to cats with pulmonary edema, other pulmonary disease, and healthy cats.

Medical records and echocardiographic studies from 2008–2015 were reviewed and measured. Cats were categorized into four groups: 1) L-CHF cats with pleural effusion, with or without pulmonary edema, 2) L-CHF cats with pulmonary edema only, 3) cats with pulmonary disease without evidence of cardiomyopathy, and 4) healthy cats. Pulsed wave Doppler recordings of pulmonic outflow were used to measure acceleration time (AT), ejection time (ET), AT:ET ratio, and maximum velocity through the pulmonic valve (PA Vmax). The main pulmonary artery diameter (MPA), aortic diameter (Ao), MPA:Ao ratio, left atrial diameter (LA), and LA:Ao ratio were measured from right parasternal short axis images, while right atrial (RA) maximum diameter, left atrial maximal diameter (LA_{Long}), RA:LA_{Long} ratio, and RA:Ao ratio were measured from right parasternal long axis images. Studies were reviewed for the presence of tricuspid regurgitation (TR) and the maximum TR velocity (TR Vmax) was measured when available. Groups were compared by one-way ANOVA or Kruskal Wallis tests where appropriate.

Ninety-three cats were included in the study, 31 cats with L-CHF and pleural effusion, 25 cats with L-CHF and pulmonary edema, 15 cats with pulmonary disease without evidence of cardiomyopathy, and 21 healthy cats. The median RA:Ao of the pleural effusion group (1.54, range 0.85–2.70) was significantly larger ($P = 0.0004$) than all other groups. The mean RA:LA ratio of the pleural effusion (0.73 ± 0.2) and pulmonary disease (0.75 ± 0.1) groups were different from the pulmonary edema (0.56 ± 0.2) and healthy (0.95 ± 0.1) groups ($P < 0.0001$), though not different from each other. Comparison of pulsed wave Doppler measurements of the pulmonic outflow revealed no significant difference in AT, ET, AT:ET, or PA Vmax between any groups. TR Vmax was measured in 7 pleural effusion cats (median 2.2 m/s, range 1.2–3.2) and 1 healthy cat (1.7 m/s).

The results of our study indicate that cats that developed pleural effusion had significantly more right atrial enlargement in addition to left atrial enlargement than cats that developed only pulmonary edema. No echocardiographic evidence of pulmonary hypertension was found in any of the four groups of cats. The potential causes for right atrial enlargement in the cats with pleural effusion include concurrent right-sided cardiac disease or pulmonary hypertension that was not detected by these echocardiographic parameters. Additional studies determining the degree of RA enlargement associated with the development of pleural effusion could be useful in predicting CHF manifestation in cats with cardiomyopathy.

C31

POST-OPERATIVE DYSRHYTHMIAS AFTER CARDIAC SURGERY UNDER CARDIOPULMONARY BYPASS. Junseok Lee, Takeshi Mizuno, Masashi Mizuno, Kayoko Harada, Hiroshi Takano, Arane Takahashi, Kazushi Mamada, Sayaka Takeuchi, Ayaka Chen, Tamotsu Sawada, Takahiro Mizukoshi, Aasako Shinoda, Shuhei Uchida, Takurou Mori, Kazuki Takamura, Junichirou Takeuchi, Masami Uechi. Japan Animal Specialty Medical Institute, JASMINE Veterinary Cardiovascular Medical Center, Yokohama, Kanagawa, Japan

In human, post-operative dysrhythmia is an important complication after cardiac surgery, a major cause of increasing mortality and lengthening hospitalization. In veterinary field, however, there is little knowledge on rhythmic disturbances complicated by cardiac surgery. The aim of this study is to characterize post-operative arrhythmias and to determine peri-operative predisposing factors to arrhythmias in dogs with open-heart surgery under cardiopulmonary bypass (CPB).

This is a prospective and observational study of 70 consecutive dogs undergoing mitral valve repair surgery. The incidence and the types of post-operative arrhythmias were determined by continuously monitoring electrocardiogram for the first 7 days during hospitalization. Occasional premature atrial or ventricular beats were considered insignificant. Serologic and blood-gas parameters measured at serial time points during CPB were normalized and compared between two groups with non-arrhythmic and arrhythmic episodes. All patients showed normal sinus rhythm before surgery.

32 patients (45.7%) had a total of 55 episodes of arrhythmia after cardiac surgery, where ventricular arrhythmias were the most frequently identified (26 of 55; 47.3%). Supraventricular, nodal arrhythmias and conduction blocks accounted for 23.6%, 14.5% and 14.5%, respectively. 53.2% of patients with arrhythmic episodes were recovered to normal sinus rhythm within the first 2 days after surgery. Patients with persistent arrhythmia until discharge were 18.8% (6 of 32). There was no case that permanent pacemaker should be implanted. Although intravenous bolus injection of lidocaine (2 mg/kg) was applied to 2 dogs with polymorphic ventricular tachycardia lasted until 7 days, no improvement was found. No hospital deaths were caused by post-operative arrhythmias (deaths in hospital; 1 respiratory failure, 2 embolic events). Furthermore, the arrhythmic group showed longer duration of CPB (107.2 ± 33.6 minutes, $P < 0.05$) and aortic cross-clamping time (59.9 ± 13.3 minutes, $P < 0.01$), and lower glucose level normalized by CPB duration (82.6 ± 38.9 mg/dL/CPB time, $P < 0.01$), compared to the non-arrhythmic group (CPB time, 90.3 ± 25.7 minutes; cross-clamping time, 50.1 ± 20.6 minutes; normalized glucose level, 126.8 ± 66.7 mg/dL/CPB time). Also, logistic regression model for the probability of post-operative arrhythmias revealed significant effects of the aortic cross-clamping time and the normalized glucose level (adjusted R^2=0.30, $P < 0.001$).

The incidence of cardiac arrhythmias is quite high after open-heart surgery under CPB in dogs. However, their clinical significance as a complication of cardiac surgery may be minimal. In addition, hypoglycemic or myocardial ischemic environment during CPB may predispose to post-operative cardiac dysrhythmias.

N01

KINEMATIC MAGNETIC RESONANCE IMAGING FOR EVALUATION OF DISC-ASSOCIATED CERVICAL SPONDYLOMYELOPATHY IN DOBERMAN PINSCHERS. Michele Provencher, Amy Habing, Laurie Cook, Sarah Moore, Gary Phillips, Ronaldo da Costa. The Ohio State University, Columbus, OH, USA

Disc-associated cervical spondylomyelopathy (DA-CSM) is a common condition in Doberman Pinschers (DPs) that is similar in pathogenesis to cervical spondylotic myelopathy in humans (hCSM). In patients with hCSM, kinematic MRI (kMRI) is used to evaluate the cervical vertebral column in flexion and extension. The purpose of this study was to evaluate kMRI in DPs with DA-CSM using a novel positioning device that allowed controlled flexion and extension of the cervical vertebral column. We hypothesized that kMRI would cause worsening of compressive lesions and the development of new compressions, especially in extension.

Nine client-owned DPs that had a neurologic examination consistent with a cervical myelopathy were prospectively evaluated. All dogs underwent standard MRI in a neutral position using a 3.0 T magnet. Following standard MRI, the patients were placed in right lateral recumbency on a positioning device and additional MRI sequences were obtained with the cervical vertebral column positioned in flexion and extension. Morphologic and morphometric assessments were performed using the neutral, traction, flexion and extension images. Morphologic analysis included a modified spinal cord compression score (previously published), direction of spinal cord compression, signal intensity changes within the spinal cord, and worst site of spinal cord compression. Morphometric assessment included spinal cord height, intervertebral disc width,

spinal cord width, spinal cord area, and vertebral canal height. A Fischer's exact test was used to evaluate the morphologic data and mixed-effects linear regression was used to analyse the morphometric data. Interobserver and intraobserver analyses were performed.

Due to space constraints, only part of the results is presented. With neutral positioning, 14 total compressions were noted; 5/9 patients had 1 compression, 3 patients had 2 compressions, and 1 patient had 3 compressions. With flexion, 12 total compressions were noted; 1 patient had no compressions, 5 patients had 1 compression, 2 patients had 2 compressions, and 1 patient had 3 compressions. With extension, 26 total compressions were noted; 1 patient had 1 compression, 6 patients had 2 compressions, 3 patients had 3 compressions, and 1 patient had 4 compressions. In neutral, the majority of lesions were mild (11/14) and located ventrally (9/14). Extension was associated with worsening of compressions in 7 out of 14 sites and the presence of severe compressions (4/26). It was also associated with multiple directions of compression; there was ventral and dorsal compression at 12/26 sites with extension. Morphometric results revealed a decrease in spinal cord height at C6–C7 (midsagittal images) from neutral to extension ($P = 0.003$) and from flexion to extension ($P = 0.001$).

This study supports the use of kMRI to evaluate the dynamic component of DA-CSM. Results suggest that extension is most useful; not only did it demonstrate worsening of multiple compressions but it was also able to detect new compressions not visualized in a neutral position.

N02

3D SOLITARY SEQUENCE MAGNETIC RESONANCE IMAGING OF THE DOG SPINE IN HANSEN TYPE I INTERVERTEBRAL DISK DISEASE. Melissa Carpentier-Anderson[1], Fred Wininger[2], Shannon Holmes[3], Daniela Mauler[2]. [1]Veterinary Specialty Servies, Manchester, MO, USA, [2]University of Missouri Veterinary Health Center, Columbia, MO, USA, [3]University of Georgia College of Veterinary Medicine, Athens, GA, USA

SPACE (Sampling Perfection with Application optimized Contrasts using different flip angle Evolutions) is a T2-weighted three-dimensional magnetic resonance imaging sequence. Currently, 2 dimensional T2-weighted spin echo imaging is the gold standard diagnostic imaging for thoracolumbar intervertebral disk disease. SPACE is potentially advantageous to standard MRI in that it is a single acquisition, faster, technically easier modality with higher resolution and the potential to reconstruct the image set in any plane of section. The purpose of this study is to determine the agreement between SPACE and T2-weighted standard spin echo imaging sequences in the presence of Hansen Type I intervertebral disk disease in the thoracolumbar spine of dogs to determine if SPACE is a viable imaging sequence in the canine patient. Our hypothesis was that the MRI sequence SPACE is able to positively identify Hansen Type 1 intervertebral disk disease with equal accuracy to standard T2-weighted spin echo imaging.

This was a double blinded controlled study of dogs with an acute onset T3-L3 compressive myelopathy secondary to Hansen Type I intervertebral disk disease. All patients had standard preanesthetic screening and underwent magnetic resonance imaging of the T3-L3 spine using a Siemens 1.5T scanner. Images were obtained, in at least, the following sequences: standard T2-W sagittal and transverse, STIR dorsal, and SPACE. All dogs underwent a decompressive hemilaminectomy based on the imaging findings. The MRI sequences were randomized and three blinded individuals interpreted the MR images a total of three times. The evaluators were of different skill levels, including one ECVN diplomate, one ACVR diplomate, and a third year neurology resident. The standard T2-weighted and SPACE sequences were individually evaluated for site, side, and degree of compression as well as surgical recommendation and subjective confidence of imaging findings.

23 dogs met the inclusion criteria. There was statistically significant agreement for both site and side (P-Value <0.001) between the standard T2-weighted spin echo imaging and SPACE. There was complete agreement by all three researchers concerning both the site and side of disk herniation (comparing standard T2-weighted standard spin echo imaging and SPACE) for 20 of the 23 dogs, and partial agreement for the other 3 dogs. Compressive Length Ratio (CLR) estimates by the two methods were similar, with more variability due to evaluator variability than to differences between the two systems, and similar internal (intra-rater) variabilities between the two systems. The degree of compression, as measured by the standard mild/medium/severe rating protocol shows significantly positive association between the two methods, although not as strong as that between the two methods for the CLR measurements.

The MRI sequence SPACE was able to positively identify Hansen Type I Intervertebral disk disease in the canine thoracolumbar spine as confirmed via surgery with equal accuracy to standard T2-weighted imaging.

N03

CLINICAL CHARACTERISTICS OF DOGS WITH PROGRESSIVE MYELOMALACIA FOLLOWING ACUTE INTERVERTEBRAL DISC HERNIATION. Aude Castel, Natasha Olby. North Carolina State University, College of Veterinary School, Raleigh, NC, USA

Progressive myelomalacia (PMM) is a catastrophic disease associated with acute intervertebral disc herniation (IVDH). Published data on the clinical characteristics of this disease are limited. The aim of this retrospective study was to describe the onset and progression of clinical signs of PMM in a large case cohort.

Dogs with confirmed IVDH and either a histopathologic diagnosis of PMM or a high clinical suspicion (acute paraplegia, loss of nociception, loss of patellar reflex and cranial advancement of the cutaneous trunci muscle reflex inconsistent with location of disc herniation) were identified by medical record search.

Forty-eight cases were identified, 16 confirmed via histopathology, and 32 suspected based on clinical signs and imaging. The majority of dogs were chondrodystrophic, including 24 Dachshunds. Onset of PMM signs ranged from present at first evaluation (n = 16) to 5 days after presentation, with 43/48 developing signs within 3 days. Progression of signs from onset to euthanasia, excluding 10 cases euthanized at presentation, ranged from 1 to 13 days with 32 being euthanized within 72 hours. Systemic signs (depression, anorexia, abnormal temperature and diffuse pain) were documented in 34 dogs. Focal disc herniation was present in 35 dogs, while 7 dogs had extensive herniations.

In summary, the majority of dogs that develop PMM do so within 3 days of presentation and progress to euthanasia within another 3 days. However, onset can be delayed up to 5 days after presentation with progression to death taking as long as 2 weeks. Non-specific systemic signs are commonly reported.

N04

***IN VITRO* ANTI-TUBULIN EFFECTS OF BENZIMIDAZOLE ANTHELMINTICS MEBENDAZOLE AND FENBENDAZOLE ON CANINE GLIOBLASTOMA CELLS.** Serene Lai[1], Jennifer Koehler[2]. [1]Department of Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn, AL, USA, [2]Department of Pathobiology, Auburn University College of Veterinary Medicine, Auburn, AL, USA

Benzimidazole anthelmintic drugs have been reported to have antiproliferative effects on several cancers both *in vitro* and *in vivo*, with reduced off-target toxicity as compared to other microtubule-disrupting drugs. In addition, these small, lipophilic drugs readily cross the blood-brain barrier. The purpose of this study was to evaluate the *in vitro* chemosensitivity of canine glioblastoma J3T cells to mebendazole (MBZ) and fenbendazole (FBZ).

Cells were exposed to drugs for 72 hours, and cell viability was evaluated using the MTT assay. The half-maximal inhibitory concentration (IC_{50}) of MBZ and FBZ was calculated using a four-parameter variable slope curve fit nonlinear regression analysis. The IC_{50} was compared between the two drugs. Western blot was used to compare the ratio of polymerized to depolymerized tubulin between drug-treated cells and untreated controls. Immunocytochemistry was performed on treated and untreated cells to visualize effects of treatment on tubulin.

The mean IC_{50} of MBZ and FBZ were 0.026 μM ± 0.03 and 0.55 μM ± 0.02 respectively. Treatment with MBZ and FBZ resulted in increased depolymerization of tubulin compared to the untreated control. Immunocytochemical studies showed disruption of tubulin and evidence of apoptotic bodies after only 2 hours of treatment.

Our *in vitro* data suggest that MBZ and FBZ may be good candidates for treatment of canine glioblastomas. Further *in vivo* studies are required.

N05

SUBARACHNOID-SUBARACHNOID SHUNTING FOR TREATMENT OF SUBARACHNOID CEREBROSPINAL FLUID FLOW OBSTRUCTION IN 9 DOGS. Ilyssa Meren, Jessica Chavera, Nick Jeffery. Iowa State University, Ames, IA, USA

Cerebrospinal fluid (CSF) flow in the subarachnoid space can be impeded by developmental anomalies of the subarachnoid space (i.e. diverticula) or acquired adhesions in older animals. Developmental lesions can occur in any type of dog and clinical signs typically present in animals less than one year old; acquired lesions appear to be most common in middle-aged and older small dogs, notably pugs. Clinical signs include progressive proprioceptive ataxia and, at later stages, fecal and urinary incontinence. Traditional surgical treatment of acquired subarachnoid adhesions (constrictive myelopathy) is associated with unreliable outcomes. Here we report on the surgical procedure and outcome using subarachnoid-subarachnoid shunting as a means of bridging the area of adhesion.

Nine dogs were treated by this technique at Iowa State University between November 2013 and August 2015. The spinal cord was exposed via routine dorsal laminectomy in 8 cases (thoracolumbar spinal cord) and via C2 laminotomy in one. A durotomy was made over the lesion site using a hypodermic needle bevel and expanded laterally to disrupt adhesions between the pia mater and arachnoid mater. A shunt tube consisting of the ventricular catheter from a ventriculoperitoneal shunt kit or a red rubber catheter of appropriate diameter was trimmed to span the lesion plus ~5 mm on either side of the adhesion and secured within the subarachnoid space using 5/0 non-absorbable (polypropylene [Prolene]) sutures. The dura was then closed over the shunt.

One patient had a developmental subarachnoid diverticulum (the patient with a C2 laminotomy), and the remaining 8 had an acquired adhesion (constrictive myelopathy) documented at surgery. Four patients showed improvement of clinical signs, of which three showed complete recovery. The one patient that has not shown a complete recovery in this group was the young patient with a developmental subarachnoid diverticulum, but is continuing to improve at 3 months post-operatively. One patient had mixed success: improvement of fecal control, but deterioration in gait (although with subsequent gradual improvement). Three patients did not show major improvement after surgery. Of these, one showed mild improvement after surgery but acute progression of paraparesis at 10 months prompted euthanasia (cause of acute paraparesis was not ascertained because of lack of post mortem examination). The other two cases showed unchanged clinical signs at 7 and 24 months post operatively. The last remaining case improved immediately post-operatively, but was lost to long-term follow-up.

Obstruction of CSF flow in the subarachnoid space can be associated with severe and life-threatening (fecal incontinence is a common reason for euthanasia) neurologic deficits. Our results suggest that subarachnoid-subarachnoid shunting can be an effective surgical technique to ameliorate or prevent progression of associated clinical signs.

N06

11-DEHYDRO THROMBOXANE B2 AS A BIOMARKER FOR INTRACRANIAL NEOPLASIA IN DOGS. Jessica A. Rivera[1], Elizabeth Hurst[2], James L. Mobley[2], Peter J. Dickinson[1]. [1]University of California, Davis, Davis, CA, USA, [2]Cayman Chemical Company, Ann Arbor, MI, USA

Meningiomas and gliomas are the most common intracranial neoplasms in dogs, and definitive diagnosis is often problematic due to the cost and invasiveness of an intracranial biopsy. Biomarkers in serum, urine, and/or CSF can provide adjunctive diagnostic data in conjunction with standard techniques such as magnetic resonance imaging. The cycloogygenase pathway has been identified as a key player in cancer progression, and several pathway products including thromboxane A_2 (TxA_2) are over expressed in a variety of human cancers, including gliomas.

The purpose of this pilot study was to determine levels of 11-dehydro-thromboxane B_2 ($11TxB_2$) (a stable hydrolysis product of TxA_2) in dogs with a variety of neurological and non-neurological diseases, using an enzyme immunoassay. Urine, serum, and CSF samples were stored immediately following collection at −20°C (or at 4°C for up to 7 days prior to −20°C storage).

$11TxB_2$ was reliably detected in urine, but not in serum or CSF. Storage at 4°C up to 7 days did not significantly affect urine TxB_2 levels, and concurrent corticosteroid or non-steroidal anti-inflammatory medication did not apparently affect levels within the study group as a whole. Overlap of $11TxB_2$ urine levels was seen between clinically normal and neurological animal groups. Although some of the highest $11TxB_2$ values were seen in animals with intracranial neoplasia, overlap with other neurological disease groups was present. Assay of $11TxB_2$ is feasible; however, additional analysis of a large study population will be necessary to determine whether a threshold urine $11TxB_2$ level may be useful as a diagnostic biomarker.

N07

LUMBAR FRACTALKINE AND M2 MICROGLIA INCREASE THROUGHOUT DISEASE PROGRESSSION IN CANINE DEGENERATIVE MYELOPATHY. Christine Sibigtroth[1], Maria Jones[2], Virginia Garcia[2], Gayle Johnson[3], Joan Coates[1], Eric Villalón[2], Michael Garcia[2]. [1]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA, [2]Division of Biological Sciences, College of Arts and Sciences, University of Missouri, Columbia, MO, USA, [3]Department of Veterinary Pathobiology, College of Veterinary Medicine, Univeristy of Missouri, Columbia, MO, USA

Canine degenerative myelopathy (DM) is an adult-onset neurodegenerative disorder. Clinical progression is homogeneous within and across breeds, thus four distinct disease stages have been identified (Kanazono et al., 2013; Coates and Wininger, 2010). DM shares similarities with some forms of amyotrophic lateral sclerosis (ALS), including underlying mutations in *superoxide dismutase-1* (*SOD1*). SOD1-mutant microglia play a central role in ALS progression in rodent models. Microglia behavior is complex, as expression patterns of classic M1 and M2 molecules are variable throughout disease and between species studied. The mechanism(s) underlying microglial phenotype determination within ALS is not known. Fractalkine, a neuronally produced chemokine, has been shown to suppress *SOD1* mutant microglial-mediated neurotoxicity in vitro. Thus, fractalkine is a possible contributor to microglial phenotype determination in ALS. Increased spinal cord microglia have been documented in end-stage DM-affected dogs, however, their response and phenotype throughout disease progression is unknown. The goals of this study were to (1) quantify and phenotype microglial cells in close proximity to lumbar spinal cord motor neurons of DM-affected dogs at each disease stage and compare these findings to age-matched, neurologically normal dogs and (2) correlate microglial phenotype with fractalkine protein levels in lumbar spinal cord throughout disease progression.

Spinal cord tissue was collected post-mortem from client-owned dogs donated to the University of Missouri for research purposes. Lumbar spinal cord microglia analyses were conducted with immunofluorescence and confocal microscopy. To identify microglia in close proximity to motor neurons (MNs), concentric circles

with increasing radii of 6 μM were applied to MNs of DM-affected dogs at each disease stage (stage 1 n = 120; stage 2 n = 118; late DM (3&4) n = 149) and age-matched control dogs (n = 145). Cells with positive immunoreactivity for Iba-1/iNOS (M1 microglia), or Iba-1/arginase-1 (M2 microglia), were quantified in a double-blind manner. Data were analyzed via Kruskal-Wallis ANOVA on ranks with *post-hoc* Dunn's method. Fractalkine total protein was quantified via western blot analysis of lumbar spinal cord from DM-affected dogs (stage 1 n = 2; stage 2 n = 4; late DM n = 3) and age-matched control dogs (n = 3). Relative optical densities were normalized to control dogs. Data were analyzed via ANOVA with *post-hoc* Holm-Sidak method.

A progressive increase in total microglia closely associated with lumbar motor neurons was observed in DM-affected dogs (stage1: 19 ± 0.7; stage 2: 25 ± 0.9; late DM: 28 ± 0.9; $P < 0.05$) compared to age-matched controls (13 ± 0.4; $P < 0.05$). Total M2 microglia were increased in stage 2 (7.5 ± 0.5) and late DM-affected dogs (6.2 ± 0.5) compared to age-matched controls (3.3 ± 0.7; $P < 0.05$). No differences were observed in total M1 microglia throughout disease progression. Lumbar spinal cord fractalkine protein was increased in stage 1 (2.2 ± 0.2) and late DM-affected dogs (2.2 ± 0.3) compared to age-matched controls (1 ± 0.1; $P < 0.05$).

These data suggest that M2, but not M1, microglia are progressively recruited to motor neurons during disease progression. Increased lumbar spinal cord fractalkine correlates with progressive M2 microglia in DM-affected dogs.

N08

INCREASED PHOSPHORYLATED NEUROFILAMENT HEAVY IN CEREBROSPINAL FLUID AS A DISEASE MARKER OF CANINE DEGENERATIVE MYELOPATHY. Christine Sibigtroth[1], Virginia Garcia[2], Gerry Shaw[3], Joan Coates[1], Patrick Pithua[1], Michael Garcia[2]. [1]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA, [2]Division of Biological Sciences, College of Arts and Sciences, University of Missouri, Columbia, MO, USA, [3]EnCor Biotechnology, Gainesville, FL, USA

Canine degenerative myelopathy (DM) is an adult-onset neurodegenerative disorder with four defined disease stages. DM initially manifests as spastic upper motor neuron paraparesis and general proprioceptive ataxia (stage 1). Progressive neurodegeneration results in non-ambulatory paraparesis/paraplegia (stage 2) and thoracic limb paresis (stage 3). End-stage disease culminates in flaccid tetraplegia, widespread muscle atrophy and signs of brainstem dysfunction (stage 4). The clinical spectrum of DM is homogeneous within and across breeds. Phosphorylated neurofilament heavy (pNF-H), a major structural protein of myelinated motor axons, has shown promise as a prognostic biomarker in other diseases of the nervous system such as amyotrophic lateral sclerosis (ALS). The goals of this study were to 1) quantify the concentration of pNF-H in the CSF of DM-affected dogs throughout disease progression and compare these finding to age-matched, neurologically normal dogs with and without *SOD1* mutations and 2) compare CSF concentrations of pNF-H between DM-affected and dogs with other chronic spinal cord disease that mimic DM.

Using an enzyme-linked immunosorbent assay (ELISA), we measured pNF-H concentration in CSF from aged control dogs (n = 10) and DM-affected dogs at all stages (stage 1 n = 9; stage 2 n = 6; stage 3 n = 6; stage 4 n = 3). Data were analyzed using Kruskal-Wallis ANOVA on ranks with *post-hoc* Dunn's method. Additionally, CSF pNF-H concentration was compared between DM-affected dogs (n = 24) and dogs with other chronic spinal cord disease (n = 7). Those data were analyzed by the student's t test. To evaluate diagnostic performance, a receiver operating characteristic curve was generated.

CSF concentrations of pNF-H were increased in DM-affected dogs at all disease stages compared to aged control dogs ($P = 0.002$); pNF-H concentrations did not differ between DM disease stages ($P = 0.704$). DM-affected dogs had increased CSF pNF-H compared to dogs with other chronic spinal disease ($P < 0.001$). Using a cut-off concentration of 1.5 ng/mL, sensitivity and specificity for diagnosis of DM were 0.83 and 0.95, respectively. Area under the receiver operator characteristic curve was 0.911.

These preliminary findings suggest that pNF-H in CSF is a sensitive and specific disease marker for DM. These data warrant further study of pNF-H in CSF and serum for diagnosis of disease and longitudinal monitoring of therapeutic efficacy in DM.

N09

ETIOLOGY OF FELINE JUVENILE ONSET SEIZURES. Muna Qahwash, Heidi Barnes Heller. University of Wisconsin, School of Veterinary Medicine, Madison, WI, USA

Seizure etiology are well documented in adult cats. Despite this awareness, little is published about the etiology and outcome of juvenile onset seizures in cats. Juvenile onset seizures are defined as seizures starting prior to 12 months of age for this study. The aims of this study were to 1) identify the common etiology and 2) describe the outcome (dead or alive) in cats with juvenile onset seizures. Inclusion criteria were 1) a confirmed or suspected seizure before 12 months of age and 2) complete medical records including a final diagnosis. The diagnosis was reclassified for each cat according to the 2010 ILAE guidelines. Fifteen cats met the inclusion criteria. Median age at onset was 24 weeks (range, 0.4 – 40 weeks). Six cats (40%) were diagnosed with structural epilepsy, 5 cats (33%) were diagnosed with unknown epilepsy, and 4 cats (26%) were diagnosed with reactive seizures. Generalized seizures were documented in 9 cats, 4 cats had focal seizures and 2 cats had both focal and generalized seizures. Six cats (40%) had cluster seizures, and status epilepticus was documented for 1 cat. Overall 8 cats (53%) were alive, 6 cats were dead and 1 cat was lost to follow up. (Follow up 16 – 173 months after diagnosis) Three cats died, or were euthanized, secondary to seizures or the underlying diagnosis and 3 cats died or were euthanized unrelated to their seizures or seizure diagnosis and 1 cat died due to unknown causes.

N10

COMPARISON OF SERUM TRACE NUTRIENT CONCENTRATION IN DOGS WITH PRIMARY GENETIC EPILEPSY VERSUS HEALTHY DOGS. Samantha Vitale, Devon Hague, Laura Selmic. University of Illinois Veterinary Clinical Medicine, Urbana, IL, USA

Primary genetic epilepsy is the most common cause of seizures in dogs, reportedly affecting 0.5–5.7% of the population. There have been several investigations regarding the serum concentrations of trace nutrients, including copper, selenium, zinc, manganese, and iron in human epileptics and animal models. However, no research of this nature is available in dogs with primary genetic epilepsy. This is a prospective pilot study, designed to compare the serum concentrations of several trace nutrients in non-epileptic dogs compared to dogs with epilepsy.

Blood samples were collected from 7 healthy control dogs and 8 dogs with primary genetic epilepsy. Serum was evaluated for concentrations of copper, selenium, zinc, cobalt, manganese, molybdenum, and iron using inductively coupled plasma mass spectroscopy. Mann U Whitney tests were used to compare normal and epileptic dog serum levels of the trace nutrient. A significantly higher level of copper ($P = 0.006$), selenium ($P = 0.018$), and manganese ($P = 0.017$) were found in epileptic dogs compared to normal dogs. No significant differences were found in levels of zinc ($P = 0.64$), cobalt ($P = 0.82$), molybdenum ($P = 0.60$), or iron ($P = 0.95$). These results suggest that there are differences in the concentrations of some trace nutrients in epileptic dogs compared to normal dogs, which warrants further investigation.

N11

SERUM LACTATE AS A PROGNOSTIC FACTOR IN DOGS AND CATS WITH HEAD TRAUMA: 93 CASES (2003–2014). Dana Sengewald, Devon Hague, Jason Tarricone, Maureen McMichael, Laura Selmic. University of Illinois Veterinary Clinical Medicine, Urbana, IL, USA

Previous studies identified associations of Modified Glasgow Coma Scale (MGCS) and hyperglycemia with survival in veterinary patients with head trauma. Lactate is the product of anaerobic glycolysis, and hyperlactatemia has classically been associated with decreased tissue perfusion. Some studies in dogs with various diseases, including trauma, have showed that higher initial lactate concentration in dogs is associated with increased risk of mortality. The main aim of this study is to determine if elevated serum lactate following head trauma in dogs and cats is associated with mortality and whether the degree of hyperlactatemia corresponds with clinical outcome.

Medical records for dogs and cats diagnosed with head trauma from the VetCOT Trauma Registry and searching the electronic medical record at the veterinary teaching hospital from 2003–2014 were examined. Cases were included if a diagnosis of head trauma was made and if blood lactate concentration was measured on the day of presentation. For each animal with head trauma included in the study, information about signalment, vital parameters, diagnostic testing, treatment and outcome was recorded. Based on information from the medical records, a MGCS was assigned to patients. Univariable logistic regression was used to evaluate association with survival between signalment, examination parameters, laboratory testing, and administration of treatment.

A total of 93 animals (78 dogs and 15 cats) met the inclusion criteria for this study. There was no evidence that hyperlactatemia ($P = 0.38$) or hyperglycemia ($P = 0.69$) were associated with mortality. The MGCS Score at initial evaluation was the only variable significantly associated with outcome ($P = 0.011$). Dogs and cats with head trauma may have elevated serum lactate levels, but the degree of hyperlactatemia is not associated with severity of head trauma or with outcome.

N12

COMPARISON OF FECAL MICROBIOMES BETWEEN DOGS WITH MENINGOENCEPHALOMYELITIS OF UNKNOWN ORIGIN AND CONTROLS. Nick Jeffery[1], Jan Suchodolski[2], Al Jergens[1], Jon Levine[2]. [1]Iowa State University, Iowa, IA, USA, [2]Texas A&M University, College Station, TX, USA

Recent experiments in laboratory rodents have suggested that the constituents of the fecal microbiome influence the activity of the immune system. Specifically, it has been shown that gnotobiotic rats are resistant to development of experimental autoimmune encephalitis (EAE), an immune-mediated disease of the CNS that mimics aspects of multiple sclerosis in humans and meningoencephalomyelitis of unknown origin (MUO) in dogs. Further experiments have shown that restoration of a normal microbiome abrogates this resistance to EAE.

In this study we investigated whether a similar susceptibility to MUO might exist in dogs that have specific microbiome constituents. Previous published work in other species suggests that *Faecalibacterium* spp, specifically *F. prausnitzii,* may modulate the immune system to reduce risk of inflammatory and immune-mediated diseases. Therefore, in this study we tested the hypothesis that a relative deficiency of *Faecalibacterium* spp, especially *F. prausnitzii,* would be associated with development of MUO.

We obtained fecal samples from dogs diagnosed with MUO and from controls, many of which were matched for breed, age and gender. Dogs that had been treated with antibiotics within the previous 4 weeks were excluded. The fecal microbiome was characterized using 16S rRNA sequencing by Illumina and qPCR assays targeting *Faecalibacterium* spp, and abundances were compared between affected dogs and controls.

In analysis of the entire sampled population there was a significant reduction (of log DNA/gram feces) in *Faecalibacterium* spp in affected dogs compared to controls (median 6.5 versus 3.8; Mann-Whitney test $P = 0.012$). However when affected dogs were compared only with matched controls, the median population of *Faecalibacterium* spp was smaller in the affected dogs (3.9 versus 6.4) but the Wilcoxon signed rank $P = 0.074$. Moreover, in 6/19 pairs the affected dogs had a smaller population of *Faecalibacterium* spp than the unaffected dogs. Similarly, although the population proportion of *F. prausnitzii* was smaller in affected dogs than controls (0.01% versus 0.1%), comparison using the Mann-Whitney test revealed $P = 0.089$. Examination of paired data again revealed lower median proportion in the affected dogs (0.03% versus 0.1%) but a Wilcoxon paired rank $P = 0.15$.

This study suggests that there is some, albeit limited, evidence to suggest that abundance of *Faecalibacterium* spp may influence the susceptibility of individual dogs to developing MUO. One possible explanation for our results is that the well-recognized breed susceptibility to MUO may be mediated, at least partially, through effects on gut microbiome. Here we examined just one highly specific hypothesis regarding gut microbiota in this disease; further analysis of the extensive fecal microbiome results available from this study may generate hypotheses that can be tested in future studies.

N13

CHARACTERIZATION OF IATROGENIC BLOOD CONTAMINATION ON LACTATE DEHYDROGENASE AND CREATINE KINASE IN CANINE CEREBROSPINAL FLUID. Jourdan Brune, Annie Chen-Allen. Washington State University, Pullman, WA, USA

Cerebrospinal fluid (CSF) analysis is regularly performed in patients with neurological disease but iatrogenic blood contamination can distort results. The effects of iatrogenic blood contamination on canine CSF lactate dehydrogenase (LD) and creatine kinase (CK) total and isoenzyme activity have not been previously evaluated. CK exists as 3 isoenzymes (CK-MM, CK-MB, CK-BB) and LD exists as 5 isoenzymes (LD1-5) in both serum and CSF. Investigating the effects of blood contamination on total LD and CK and their isoenzyme activity could allow clinicians to better interpret the diagnostic and prognostic value of LD and CK isoenzymes in canine neurologic patients.

CSF samples were contaminated with incremental amount of whole blood via serial dilution and LD and CK total and isoenzyme activity values were compared to an uncontaminated CSF sample and the established reference interval. Total LD and CK values were determined spectrophotometrically using a Sieman Dimension Xpand and isoenzyme activity was determined using gel electrophoresis and densitometric scanning. In contaminated samples, total LD and CK values were increased compared to the uncontaminated sample after contamination with > 2000 and > 4000 RBC/ul, respectively. Additionally, CSF isoenzyme activity began to emulate serum isoenzyme activity, where CK-BB and LD-5 banding became more prominent, when CSF was contaminated with > 4000 RBC/ul.

These findings suggest there is a threshold where blood contamination can artificially distort CSF LD and CK total and isoenzyme analysis. Therefore, these values will need to be interpreted with caution when there is iatrogenic blood contamination in CSF.

N14

OUTCOME COMPARISON IN DOGS WITH THORACOLUMBAR ACUTE NON-COMPRESSIVE NUCLEUS PULPOSUS EXTRUSION OR FIBROCARTILAGINOUS EMBOLIC MYELOPATHY. Lorenzo Mari[1], Anita Shea[1], Philippa Johnson[2], Luisa De Risio[1]. [1]Neurology and Neurosurgery Service, Centre for Small Animal Studies, Animal Health Trust, Newmarket, UK, [2]Section of Diagnostic Imaging, Cornell College of Veterinary Medicine, Ithaca, USA

The objective of the study was to compare the clinical outcome between dogs with acute non-compressive nucleus pulposus extrusion (ANNPE) or presumptive fibrocartilaginous embolic myelopathy (FCEM) affecting the thoracolumbar spinal cord segments.

The medical records of dogs presented to the Animal Health Trust between May 2006 and April 2015 and diagnosed with ANNPE or presumptive FCEM were retrospectively reviewed. A randomized list was generated of the MRI studies of 87 cases matching the inclusion criteria and submitted to two board-certified neurologists (LDR and AS) and one board-certified radiologist (PJ), blinded to any clinical data. Each observer was requested to make a diagnosis of ANNPE or FCEM based on previously published diagnostic criteria. Inter-observer agreement was evaluated with Kappa statistic. The clinical outcome of the consensus diagnoses was compared. Recovery of motor and autonomic function was evaluated via medical records and a telephone questionnaire with the owners. Clinical re-examinations were performed when possible.

Based on the review of the MRI studies, 72 cases were diagnosed with ANNPE and 15 with presumptive FCEM. The level of agreement between LDR-AS (95.3%; $\kappa = 0.853$) or AS-PJ (95.3%; $\kappa = 0.819$) was almost perfect while that between LDR-PJ (90.6%; $\kappa = 0.68$) was substantial.

Impaired urinary and/or fecal continence was reported only in the ANNPE group in 20/71 dogs (28%, $P = 0.033$); all these dogs had concurrent persistent motor deficits. Four dogs with impaired urinary and/or fecal continence were ambulatory at presentation. Inability to control the urge to defecate was the most frequently reported deficit in faecal continence.

All but one dog became or remained ambulatory without assistance. One dog diagnosed with presumptive FCEM presented paraplegic without nociception, did not recover an ambulatory status after 310 days and was euthanized. Only 20% dogs with ANNPE and 14% dogs with presumptive FCEM recovered a completely normal gait according to their owners, including 29% of the grade 2/5 dogs, 12.5% of the grade 3/5 dogs and none of the dogs that were plegic at presentation. The percentage of dogs with persistent motor deficits was not statistically different between the two groups. The time to recovery of some motor function in the most affected limb when presented with mono- or paraplegia was significantly longer in dogs with presumptive FCEM compared to dogs with ANNPE ($P = 0.025$); however only two cases with this variable were present in the FCEM group. Dogs diagnosed with presumptive FCEM were significantly younger ($P = 0.025$) and lighter ($P = 0.037$) than dogs with ANNPE and had a longer intramedullary hyperintense lesion in sagittal T2-weighted MRI images ($P = 0.001$). Dogs with ANNPE were more frequently reported to be performing physical activity ($P = 0.001$) and to vocalise ($P < 0.0001$) at the time of onset of the clinical signs and more frequently received anti-inflammatory drugs after diagnosis ($P = 0.012$) compared to dogs with presumptive FCEM.

In conclusion, dogs diagnosed with ANNPE affecting the thoracolumbar spinal cord segment developed impaired urinary/fecal continence significantly more frequently than dogs with presumptive FCEM. Impaired urinary/fecal continence can occur also in dogs that are ambulatory at presentation. The presence of persistent motor deficits is extremely common, particularly in dogs that presented non-ambulatory; however, no statistically significant difference was detected in the degree of recovery of motor function between the two groups.

N15

MORPHOLOGY OF THE CAUDAL FOSSA IN MESATICEPHALIC AND BRACHYCEPHALIC CATS AND ASSOCIATED CLINICAL SIGNS. Georgios (Yorgo) Varotsis[1], Tobias Schwarz[1], Rodolfo Cappello[2], Danielle Gunn-Moore[1], Katia Marioni-Henry[1]. [1]Hospital for Small Animals, R(D)SVS and the Roslin Institute, University of Edinburgh, Edinburgh, Midlothian, UK, [2]North Downs Specialist Referrals, Bletchingley, Surrey, UK

Morphological abnormalities of the caudal fossa are increasingly recognized as a cause of morbidity in many brachycephalic dogs but there is little information about these conditions in cats. The objective of this study was to investigate presence of similar morphological abnormalities of the caudal fossa of mesaticephalic and brachycephalic cats and possible association with clinical signs and final diagnosis.

Feline brain MRI studies at two referral centres were reviewed for cases without visible disease that could alter anatomic landmarks or raise intracranial pressure. T2 weighted median plane images of the brain and if available spinal cord were reviewed for the presence of caudal cerebellar indentation, coning, herniation, syringohydromyelia, and fluid accumulation in the middle ear. Area of the cerebral hemispheres and cerebellum was also measured Blind measurements were taken by two observers and reviewed.

There were 53 feline MRI studies meeting the inclusion criteria. Cerebellar herniation was found in 48% of mesaticephalic and 78% of brachycephalic cats. Syringohydromyelia was not identified in any of the MR studies including only the first two cervical spinal cord segments (36 cats) or more regions of the spinal cord (17 cats). High signal on T2-weighted transverse images of the middle ear was detected in 8% of mesaticephalic and 17% of brachycephalic cat. Information on presenting complaint, results of neurological examination and final diagnosis in cats with and without cerebellar herniation was reviewed.

Mesaticephalic breed of cats show indentation of the caudal aspect of the cerebellum and caudal cerebellar coning similar to brachycephalic breeds of cats. The dimensions of the foramen magnum planum and the area of the cerebral hemispheres and cerebellum measured on a T2W sagittal MR image of the brain were also similar between the two groups. However, brachycephalic cats had a higher percentage of herniation of the cerebellar vermis through the foramen magnum. Herniation of the cerebellar vermis does not appear to be associated with syringomyelia in cats.

N16

DIAGNOSTIC INVESTIGATION IN 13 CATS WITH SUSPECTED FELINE HYPERESTHESIA SYNDROME. Pablo Amengual[1], Clare Rusbridge[2], Tim Nuttall[1], Sarah Heath[3], Katia Marioni-Henry[1]. [1]Hospital for Small Animals, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Scotland, UK, [2]Faculty of Health & Medical Sciences, School of Veterinary Medicine, University of Surrey, Guildford, UK, [3]Behavioural Referrals Veterinary Practice, Chester, UK

Feline hyperesthesia syndrome (FHS) was first reported in 1979 and described as an accentuation of an otherwise normal behavior in cats consisting in episodes of tail chasing, biting or licking the lumbar area, flank, anal area or tail; skin rippling and muscle spasms; excessive and unusual vocalizations, wild and uncontrolled jumping and running and presumed hallucinations. FHS has been anecdotally reported in association with dermatological, behavioural, orthopaedic and neurological conditions. Treatment with phenobarbital, gabapentin, prednisolone, amitriptyline, fluoxetine, clomipramine and multivitamin supplements have been used with inconsistent responses. However, we are not aware of any clinical scientific investigation on Feline Hyperesthesia Syndrome.

We retrospectively collected data on the history, signalment, diagnostic workup and treatment from three referral veterinary centers. The inclusion criteria were a history of attacking or over-grooming the tail, flank or perineal area associated with either vocalization or lumbar hyperesthesia, manifested as rippling of the thoracolumbar skin occurring spontaneously or induced by gentle touch. Information on physical and neurological examination, and diagnostic work up were also necessary for inclusion in the study.

Thirteen cats were included in the study. The median age of presentation was 1 year (1–7 years). Eleven cats were attacking or over-grooming their tails and two cats their flank or perineal area. Tail mutilation was reported in 9/13 cats, rippling of the thoracolumbar skin in 10/13 cats and 6 cats presented with unusual vocalization during the episodes. The episodes occurred multiple times per day in 12/13 of the cases, and multiple times per week in 1/13. No consistent triggers were reported. Results of CBC and biochemistry were available for 12/13 cats, spinal radiographs for 7/13, magnetic resonance imaging for 6/13, cerebrospinal fluid analysis for 3/13, electromyography for 3/13, dermatological assessment for 7/13, and joint fluid analysis for one cat. The diagnostic work up led to the diagnosis of allergic dermatitis in 2 cases and immune-mediated polyarthritis in one case, but a definitive diagnosis was not reached in the remaining 10 cases. Treatment included corticosteroids in 7/13 of the cats, gabapentin in 8/13, clomipramine in 4/13, topiramate in 2/13, phenobarbital in 2/13

and cyclosporine in 6/13. The majority of the cases (10/13) received a combination of 2 or more drugs.

Our study confirmed previous anecdotal reports of FHS affecting young cats and the possibility of that FHS signs are secondary to inflammatory skin conditions. A definitive diagnosis was not reached in 10 cases. However, not all the cases had the same diagnostic work up and not all had a full behavioral and dermatological assessment. Based on the results of this retrospective study, specialists in neurology, behavior and dermatology have formulated a standardized questionnaire and proposed a rational diagnostic approach for investigation and treatment of this constellation of clinical signs in cats and as a platform for future prospective studies.

N17
CYSTOMETRIC CHARACTERIZATION OF URINARY BLADDER DYSFUNCTION IN CHRONICALLY PARALYZED DOGS.

Hilary Hu, Nick Jeffery, Chelsea Marko, Victoria Kichler. Iowa State University, Ames, USA

Increasing numbers of pet owners elect to keep dogs that have suffered permanent thoracolumbar spinal cord injury as pets. Although pelvic limb paralysis can be partially circumvented by using carts to allow greater mobility, care of the urinary bladder is often more problematic. In addition to urinary incontinence and recurrent urinary tract infections, it is possible that many such affected animals can suffer from detrusor hyper-reflexia. This is a risk factor for chronic elevation of bladder pressure and secondary renal damage in both human and canine spinal cord injury patients.

Here we describe cytometric quantification of bladder dysfunction in chronic paraplegic pet dogs. These dogs were <20 kg and had sustained severe T3-L3 spinal cord injury resulting in inability to walk or control urination persisting for at least three months post-injury. A dual-lumen sterile urinary catheter was used for cystometry: one lumen was used to infuse 0.9% sterile saline into the bladder while the other measured intravesicular pressure. Infusion was terminated when: (1) leaking was apparent at the external urethral orifice; or, (2) when the physiologic maximum holding capacity of 20 mL/kg was reached; or, (3) when the 50 mmHg intra-vesicular pressure threshold was reached.

Several cystometric metrics were measured in this study: (1) intravesicular pressure at leak; (2) bladder holding capacity expressed as a percentage of the physiologic maximum holding capacity of 20 mL/kg; and (3) bladder compliance, which was determined as change in bladder volume divided by change in intra-vesicular pressure. Two standard points were used for the calculation of bladder compliance: (1) the intra-vesicular pressure and bladder volume at the start of bladder filling (both were zero); and (2) the intravesicular pressure and bladder volume at cystometric capacity or immediately before the start of any detrusor contraction that resulted in urine leakage.

Cystometric data was recorded in 42 dogs with chronic spinal cord injury. Intravesicular pressure at leak ranged from 1 mmHg to 50 mmHg with a mean of 18.6 mmHg and median of 17 mmHg. In 2 (5%) patients, the intravesicular pressure reached the 50 mmHg threshold. The two additional parameters used to quantify detrusor hyper-reflexia were both highly variable within the population as a whole: (1) bladder compliance (mean = 27.3 mL/mmHg, median = 5.1 mL/mmHg, range = 0.4–180 mL/mmHg); and, (2) bladder holding capacity (mean = 68%, median = 68%, range = 9–100%).

We conclude that there is evidence that detrusor hyper-reflexia occurs in a small proportion of pet dogs paralyzed because of severe T3-L3 spinal cord injury. It is characterized by elevation in intravesicular pressure and reduction in bladder compliance and bladder holding capacity. This identification of a specific type of neurogenic bladder dysfunction permits timely interventions to mitigate renal damage. Potential therapies for treating detrusor hyper-reflexia can now be evaluated using these quantifiable bladder functional parameters derived from cystometry.

N18
EVALUATION OF KINEMATIC MAGNETIC RESONANCE IMAGING IN DOGS WITH OSSEOUS-ASSOCIATED CERVICAL SPONDYLOMYELOPATHY.

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Osseous-associated cervical spondylomyelopathy (OA-CSM) is a condition characterized by static and dynamic spinal cord compressions. The dynamic component of cervical spondylotic myelopathy in humans (hCSM) is evaluated with kinematic MRI (kMRI). The purpose of this study was to evaluate kMRI in dogs with OA-CSM using a positioning device that allowed controlled flexion and extension of the cervical vertebral column. Our hypothesis was that kMRI would reveal new compressive lesions not identified with standard positioning.

Ten Great Danes and 2 Doberman Pinschers with neurologic examination findings consistent with a cervical myelopathy were prospectively evaluated. All dogs underwent standard MRI in a neutral position (sagittal and transverse T1 and T2 weighted images) using a 3.0 T magnet. The patients were then placed in right lateral recumbency on a positioning device and the cervical vertebral column was first flexed and then extended. Sagittal and transverse T2 weighted images were acquired in both flexion and extension. Morphologic and morphometric assessments of the cervical vertebral column in neutral, flexion and extension were performed. Morphologic analysis included a modified spinal cord compression score (previously published), direction of spinal cord compression, signal intensity changes within the spinal cord, and worst site of spinal cord compression. Morphometric assessment included spinal cord height, intervertebral disc width, spinal cord width, spinal cord area, and vertebral canal height. A Fischer's exact test was used to evaluate the morphologic data and mixed-effects linear regression was used to analyze the morphometric data. Interobserver and intraobserver analyses were performed.

Given space constraints, only a portion of the results is reported. In neutral 4 patients had 1 compression, 4 patients had 2 compressions, 3 patients had 3 compressions, and 1 patient had 4 compressions (25 total compressions). In flexion 1 patient had 0 compressions, 4 patients had 1 compression, 4 patients had 2 compressions, 2 patients had 3 compressions, and 1 patient had 4 compressions (22 total compressions). In extension 2 patients had 1 compression, 1 patient had 2 compressions, 8 patients had 3 compressions, and 1 patient had 4 compressions (32 total compressions). Extension was associated with mild compression at C4–C5 ($P = 0.02$) that was not noted in neutral or flexion, and extension caused worsening of 28% (7/25 compressions) of the compressions that were present in neutral. There were 11/32 compressions (34%) in extension that were not present in neutral and 10/11 (91%) of these compressions had a dorsal component; the presence of dorsal compression with extension was significant at C4–C5 ($P = 0.01$). Flexion was associated with an increase in intervertebral disc width at C3–C4 ($P = 0.02$) and extension caused widening of the intervertebral disc at C6–C7; no other significant morphometric findings were noted. In 1 patient, a synovial cyst that was not identified in neutral was noted to cause mild spinal cord compression in extension.

Our results support the use of kMRI in patients with OA-CSM to reveal new compressive sites, dorsal compressions and to enhance visualization of extradural compressive lesions, such as synovial cysts.

N19
BARTONELLA SPP. PCR ASSAY RESULTS USING CEREBROSPINAL FLUID OF DOGS WITH CENTRAL NERVOUS SYSTEM DISEASE.

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A multitude of infectious agents are on the differential list for dogs with focal or multifocal neurological dysfunction. A number of *Bartonella* spp., including *B. henselae, B. vinsonii* subsp. *berkhoffii*, and *B. clarridgeaie,* are known to infect dogs. While multiple human cases of neurobartonellosis have recently been

described, the role these organisms play in clinical diseases of the central nervous system of dogs has not been widely explored. The purpose of this study was to use polymerase chain reaction (PCR) to amplify *Bartonella* spp. DNA from cerebrospinal fluid (CSF) of naturally exposed dogs in endemic areas meeting criteria for inflammatory central nervous system (CNS) disease.

CSF samples from 175 pure or mixed breed dogs were submitted to Colorado State University (www.dlab.colostate.edu) from either the Washington State University Neurology Department between January 2012 and September 2014 or the Colorado State University Veterinary Teaching Hospital between January 2012 and September 2015. The CSF samples were stored at −80°C until evaluated in this study. Dogs with neurologic examinations consistent with focal and multifocal neurologic dysfunction and CSF pleocytosis (total nucleated cell count >5 nucleated cells/μL and red blood cell <4,000 cells/μL) were included. Animals with normal neurologic examinations were also included if their CSF met our criteria. The CSF was thawed and centrifuged at 10,000 X g for 15 minutes. The supernatant was removed and the pellet assayed in a previously published PCR assay that targets the 16S-23S rRNA intergenic region. All positive amplicons were sequenced to determine the infective *Bartonella* spp.

A total of 67 dogs met the inclusion criteria, none of which were positive for *Bartonella* spp. DNA in CSF. Of the other 108 CSF samples, one was positive for *B. henselae* DNA. The CSF from this dog contained red blood cells (94 RBC/μL).

As *Bartonella* spp. have an intra-erythrocytic phase, we speculate that minimal peripheral RBC contamination in the CSF of dogs with systemic *Bartonella* spp. infection may lead to positive *Bartonella* PCR assay results in the absence of a CNS disease association. Thus, positive PCR for *Bartonella* spp. DNA in the CSF of dogs must be interpreted in light of number of RBCs within the sample as well as the presence of inflammation or systemic infection. Failure to amplify *Bartonella* spp. DNA from the CSF of the 67 dogs with inflammatory disease studied suggests the organism was not involved, the organism was in CNS tissues but not in the CSF, or the organism was present but in quantities undetectable by this PCR assay. The combination of PCR and culture has been found to be the most sensitive way to detect *Bartonella* spp. in samples from dogs and humans and the use of that technique should be considered in future studies.

N20

PREICTAL, POSTICTAL AND INTERICTAL BEHAVIORAL CHANGES IN DOGS WITH GENETIC EPILEPSY COMPARED TO CONTROL DOGS. Hilary Levitin[1], Devon Hague[1], Kelly Ballantyne[2], Laura Selmic[1]. [1]University of Illinois Veterinary Clinical Medicine, Urbana, IL, USA, [2]Medical District Veterinary Clinic at Illinois, Chicago, IL, USA

While anxiety related behaviors have been reported in humans diagnosed with epilepsy, only one veterinary study has shown an increased frequency of anxiety behaviors in epileptic dogs compared to other medical populations. The purpose of this study was to determine the presence and severity of anxiety related behaviors in dogs with genetic epilepsy when compared to control patients. The study's other aims were to investigate if an association exists between increased anxiety related behaviors in the preictal and postictal phases of a seizure episode compared to the interictal period and whether dogs with refractory epilepsy have more severe seizures, which could account for the increased anxiety related behavioral side effects as reported in humans and dogs.

This multi-institutional study used a questionnaire based on the Canine Behavioral Assessment and Research Questionnaire (CBARQ), that has been previously validated for its ability to analyze canine behavior. Owners of dogs that had been diagnosed with genetic epilepsy were asked to complete the questionnaire and a 10-point seizure severity scale. Sections of the CBARQ relating to separation, attachment and attention seeking, and obedience and training behaviors were modified to include additional parameters for detecting anxiety based behavioral changes associated with different phases of the seizure episode. The questionnaire was also distributed to primary care clients presenting dogs for wellness examinations and owners of dogs with intervertebral disc disease (IVDD). Veterinarians of the dogs in the study also completed a brief questionnaire as well to identify and verify the diagnoses of each dog that participated in this study. Fischer's exact tests were utilized, to assess for differences in behavioral traits exhibited between the epileptic, IVDD and primary care groups. If a significant difference between the groups was detected, then post hoc testing with Fischer's exact tests for pairwise comparisons was performed and a Bonferroni correction was applied to account for multiple comparisons.

Dogs in the epilepsy and IVDD groups were more likely to be fearful/anxious when approached by an unfamiliar dog than those dogs in the wellness group ($P = 0.0011$). Epileptic dogs with increasing seizure severity were significantly more likely to show fear and anxiety when approached by an unfamiliar dog ($P = 0.0129$) and showed significantly less excitement just before just before being taken on a car trip ($P = 0.0126$). Epileptic dogs receiving polytherapy had a significant decrease in excitement just before taking a walk ($P = 0.0007$) and just before being taken on a car trip when compared to dogs on monotherapy ($P = 0.027$). Dogs receiving polytherapy had significantly increased fear and anxiety when groomed or bathed by a household member ($P = 0.0197$), and had an increase in shaking, shivering, or trembling when left or are about to be left on their own ($P = 0.0004$).

Dogs receiving polytherapy had a significant increase in becoming agitated when their owner or others show affection for another person during preictal ($P = 0.005$), postictal ($P = 0.001$) and interictal ($P_r = 0.0083$) period and when their owner or others show affection for another dog or animal during preictal ($P = 0.0068$), postictal ($P = 0.0068$) and interictal ($P = 0.02$) period when compared to dogs on monotherapy. The finding of behavioral changes present throughout different seizure phases in this study raises further questions about how epilepsy affects canine patient's quality of life.

O01

THE ASSOCIATION OF ENDOTHELIN-1 SIGNALING WITH BONE ALKALINE PHOSPHATASE EXPRESSION AND PRO-TUMORIGENIC ACTIVITIES IN CANINE OSTEOSARCOMA (*VCS AWARD WINNER*). ZL Neumann[1], HC Pondenis[1], A Masyr[1], ML Byrum[1], KL Wycislo[2], TM Fan[1]. [1]Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL, USA, [2]Department of Pathobiology, University of Illinois, Urbana, IL, USA

Background: Canine osteosarcoma (OS) is an aggressive sarcoma characterized by pathologic skeletal resorption and pulmonary metastases. A limited number of negative prognostic factors, including bone alkaline phosphatase, have been identified in dogs diagnosed with OS; however, the biologic underpinnings for such observations have not been thoroughly investigated. Endothelin-1 mediated signaling is active during bone repair, and is responsible for osteoblast migration, proliferation, and bone alkaline phosphatase expression.

Hypothesis: The endothelin-1 signaling axis is active in canine OS cells, and this pathway is utilized by malignant osteoblasts for promoting cellular migration, proliferation, and bone alkaline phosphatase activities.

Animals: 45 dogs with appendicular OS.

Methods: The expressions of endothelin-1 and endothelin A receptor were studied in OS cell lines and spontaneous tumor samples. The biologic activities mediated by endothelin-1 signaling were investigated by characterizing responses in 3 OS cell lines. In 45 dogs with OS, bone alkaline phosphatase concentrations were correlated with primary tumor osteoproductivity.

Results: Canine OS cells express endothelin-1 and endothelin A receptor, and this signaling axis is functional and mediates OS migration, proliferation, and bone alkaline phosphatase activities. In OS-bearing dogs, circulating bone alkaline phosphatase activities were positively correlated with primary tumor relative bone mineral densities.

Conclusions and clinical importance: Canine OS cells express endothelin-1 and functional endothelin A receptors; with the potential for an autocrine or paracrine pro-tumorigenic signaling loop. Elevations of bone alkaline phosphatase are associated with osteoblastic OS lesions, and might be an epiphenomenon of active endothelin-1 signaling or excessive osteoproduction within the localized bone microenvironment.

O02

SAFETY EVALUATION OF COMBINATION DOXORUBICIN AND TOCERANIB PHOSPHATE (PALLADIA(R)) IN TUMOR BEARING DOGS: A PHASE I DOSE FINDING STUDY (*VCS AWARD WINNER*). MA Pellin[1], RM Wouda[2], K Robinson[1], K Tsimbas[1], ID Kurzman[1], DM Vail[1,3]. [1]Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA, [2]Kansas State University, College of Veterinary Medicine, Manhattan, KS, USA, [3]Carbone Cancer Center, University of Wisconsin-Madison, Madison, WI, USA

Introduction: Combination chemotherapy holds the promise of improved outcome in canine malignancy, but care must be taken to avoid overlapping toxicity.

Methods: Dogs with cytologically or histologically diagnosed tumors of any histology (excluding mast cell tumors) and any stage were allowed. A standard, open-label, phase I, 3 + 3 dose-cohort escalation design was employed. The toceranib dose remained constant at 2.75 mg/kg administered orally every other day. Doxorubicin was initiated at 20 mg/m2 administered IV every 21 days for four doses. The doxorubicin dose was escalated by 5 mg/m2 each cohort until MTD or 30 mg/m2 was reached. DLT was defined as any grade 3 adverse event with the exception of hematopoietic AEs for which a grade 4 AE was considered dose limiting. Any grade AE that was refractory to supportive care or persisted beyond seven days was also considered dose-limiting.

Results: Twenty-one patients were enrolled. Represented histologies included hemangiosarcoma, multicentric lymphoma, anal sac adenocarcinoma, epitheliotropic lymphoma, and prostatic carcinoma. The DLT was neutropenia occurring with doxorubicin 30 mg/m2. The MTD of the combination was determined to be doxorubicin 25 mg/m2 and toceranib 2.75 mg/kg. No grade 3 or 4 gastrointestinal or novel AEs were observed. Additionally, anti-tumor activity was observed in several cases.

Conclusion: The combination of doxorubicin 25 mg/m2 IV q21 days and toceranib 2.75 mg/kg PO EOD was well tolerated. Efficacy and longer term toxicity of this combination should be further investigated in phase II/III trials.

O03

COMPARISON OF SERUM CYTOKINE LEVELS BETWEEN DOGS WITH MULTICENTRIC LYMPHOMA AND HEALTHY DOGS. Jerome Calvalido, Paul Woods, Geoffrey Wood, Anthony Mutsaers, Darren Wood, William Sears. University of Guelph, Guelph, ON, Canada

In people, changes in the cytokine environment have been linked to the development of lymphoma, and multiple circulating cytokines have been shown to be relevant biomarkers for response to chemotherapy and prognosis. Therefore, the purpose of this study was to measure the serum levels of 13 cytokines in dogs with multicentric lymphoma and compare them to a population of healthy dogs. It was hypothesized there would be a significant difference in cytokine levels between dogs with lymphoma and healthy dogs, and between dogs with B cell and T cell lymphoma.

Thirty one dogs cytologically or histopathologically diagnosed with multicentric lymphoma were prospectively enrolled in the lymphoma group. CBC and biochemistry were performed in all dogs. Immunophenotype was determined by flow cytometry in all dogs, separating them into 2 groups: B cell (n = 21) and T cell (n = 10). Nineteen healthy dogs were enrolled in the control group. All dogs had similar exclusion criteria including previous administration of chemotherapy, systemic immunomodulatory medication (including corticosteroids and NSAID) within the last two weeks, and vaccination within the last four weeks.

T-test and Chi-square test were used to compare and match continuous variables (age, weight) and categorical variables (sex), respectively, between lymphoma and control groups. The cytokine concentrations were measured using a commercial canine multiplex magnetic bead-based (Luminex)® assay which measured IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, GM-CSF, TNF-α, IFN-γ, IP-10, KC-like, and MCP-1. The serum levels of each cytokine were compared amongst the three groups (B cell lymphoma, T cell lymphoma, healthy control) using ANOVA or Kruskal-Wallis tests, as appropriate. Statistical significance was set at $P < 0.05$.

There was no significant difference between the lymphoma and healthy control groups regarding sex, age and weight. IL-6, IL-10, KC-like, and MCP-1 were significantly higher in dogs with lymphoma compared to healthy dogs ($P = 0.01$, $P = 0.03$, $P = 0.01$, $P < 0.01$, respectively). IL-10, KC-like, and MCP-1 were significantly higher in the B cell lymphoma group than in the healthy group ($P = 0.01$, $P < 0.01$, $P = 0.01$, respectively). MCP-1, IL-6 and IL-15 levels were significantly higher in the T cell lymphoma group than in the healthy group ($P = 0.02$, $P < 0.01$, $P < 0.01$, respectively). Finally, IL-6 and IL-15 were significantly higher in the T cell lymphoma group than in the B cell lymphoma group ($P = 0.03$. $P = 0.02$, respectively). There were no significant differences in serum IL-2, IL-7, IL-8, IL-18, GM-CSF, TNF-α, IFN-γ, and IP-10 between the lymphoma and control groups.

In conclusion, this study found different serum cytokine profiles between dogs with lymphoma and healthy dogs, and also different serum cytokine profiles between dogs with B cell and T-cell lymphoma. Further study is necessary (and underway) to investigate the role of these cytokines in lymphoma pathogenesis, response to treatment, and prognosis.

O04

POST-CHEMOTHERAPY PERFORATION IN CATS WITH A DIAGNOSIS OF INTERMEDIATE OR HIGH GRADE GASTROINTESTINAL LYMPHOMA. Zachary Crouse[1], Brenda Phillips[2], Andi Flory[2], Jennifer Mahoney[1], Keith Richter[2], Linda Kidd[3]. [1]Angell Animal Medical Center, Boston, MA, USA, [2]Veterinary Specialty Hospital of San Diego, San Diego, CA, USA, [3]Western University of Health Sciences, Pomona, CA, USA

Whether solitary gastrointestinal (GIT) mass lesions caused by intermediate or large cell lymphoma in cats should be treated initially with surgical excision or chemotherapy is debated. There is a theoretical risk of perforation due to rapid cell death associated with chemotherapy. The prevalence of, and clinical findings associated with perforation have not been described.

The objective of this study was to document the prevalence and timing of post-chemotherapy perforation in cats with GIT masses caused by intermediate or large cell lymphoma. We hypothesized that tumor size, presence of hypoproteinemia, or suppurative inflammation within the mass at the time of diagnosis would be associated with perforation.

Cats with a diagnosis of intermediate to large cell lymphoma based on cytologic examination of a mass lesion of the GIT, and treated with variable chemotherapy were identified by searching the patient record database of two referral hospitals. Gastrointestinal perforation was confirmed with histopathology or using ultrasound and analysis of abdominal fluid.

Twenty-three cats with intermediate (n = 3) or large cell (n = 20) lymphoma were identified. Gastrointestinal perforation was confirmed in 4 of 23 cats (17.4%). Perforation occurred at 23, 56, 59, and 87 days after induction. There was no association between tumor size, the presence of hypoproteinemia, or suppurative inflammation within the mass at the time of diagnosis, and subsequent perforation.

Perforation is relatively common in cats with GIT mass lesions caused by intermediate or large cell lymphoma. However, the findings of this exploratory study suggest that acute perforation after induction of chemotherapy is not common.

O05

A RETROSPECTIVE STUDY ON THE INCIDENCE OF PROTEINURIA ASSOCIATED WITH THE USE OF TOCERANIB PHOSPHATE. Sindy Piscoya, Cheryl Balkman, Kelly Hume. Cornell University, Ithaca, NY, USA

Tyrosine kinase inhibitors (TKIs) are a commonly used therapy in both human and veterinary medicine. The incidence of proteinuria in humans receiving VEGFR-TKIs is reported to be as high as 18.7%. In veterinary medicine, the TKI masitinib mesylate has been shown to induce a protein-losing nephropathy in a subset of

patients. Documented reports of proteinuria with the use of toceranib phosphate are scarce. The purpose of this study was to describe the overall incidence of proteinuria associated with the use of toceranib phosphate. Medical records were retrospectively reviewed from September 2009 through October 2014 for canine patients with any type of malignancy receiving toceranib phosphate for a minimum of 3 weeks and a urinalysis and urine protein creatinine ratio (UPC) when indicated. Fifty-five cases met the inclusion criteria. The median dose of toceranib phosphate was 2.6 mg/kg (range, 2.1–2.8 mg/kg) given on Monday/Wednesday/Friday. Prednisone was used in conjunction with toceranib phosphate in 26 dogs with a median dose of 0.5 mg/kg (range 0.25–1.5 mg/kg) given on Tuesday/Thursday/Saturday/Sunday. In the toceranib phosphate plus prednisone group, proteinuria developed in 7 dogs at a median of 69 days (range, 15–122 days) with a median UPC of 0.8 (range, 0.6–1.1). In the toceranib phosphate alone group, 5 dogs developed proteinuria at a median of 47 days (range, 19–626 days) with a median UPC of 0.7 (range, 0.6–4.9). The use of toceranib phosphate was associated with development of proteinuria in a subset of patients and careful monitoring including frequent serial urine protein-creatinine ratios is recommended.

O06

GROWTH PATHWAYS IN FELINE ORAL SQUAMOUS CELL CARCINOMA. Krystal Harris, Howard Gelberg, Stuart Helfand. Oregon State University, Corvallis, OR, USA

The present study was designed to reach a better understanding of the biologic behavior of feline oral squamous cell carcinoma (FOSCC) by evaluating a variety of growth factor pathways known to contribute to aggressive behavior in many cancers. In addition, we evaluated a novel transcription factor, Bcl11b, a biomarker for head and neck squamous cell carcinoma (HNSCC).

FOSCC biopsies were graded and immunohistochemistry used to quantify relative expression of Bcl11b, EGFR, Ki67, and VEGF-D. Chi-square tests determined if Bcl11b expression correlated with expression of other receptors and growth factors. Western blots on FOSCC cell lines (SCCF1, SCCF2, SCCF3) were employed to verify expression of Bcl11b, EGFR, Src, ERK, and VEGF-D. Western blot on FOSCC cell lines determined phosphorylation of Bcl11b and the effect of a src inhibitor, dasatinib, on this activation. An MTT assay was applied on FOSCC cell lines following treatment with dasatinib. ELISA quantified VEGF production by FOSCC cells with and without dasatinib treatment.

Bcl11b staining intensity was moderate to strong in 79.4% (27/34) of FOSCC's. FOSCC biopsies with intense Bcl11b staining had the highest number of Bcl11b positive cells ($P < 0.001$). All biopsies expressed VEGF-D and 85.7% (18/21) expressed EGFR at varying intensities. A correlation between increasing Bcl11b and EGFR expression approached significance ($P = 0.05598$). All FOSCC cell lines expressed pBcl11b, pEGFR, pSrc, pERK, and VEGF-D. Dasatinib treatment lowered cell viability, inhibited activation of Bcl11b, and suppressed VEGF production. Ligand stimulation of EGFR resulted in increased activation of EGFR and ERK, increased VEGF/VEGF-D production, and partially rescued FOSCC cells from the effects of dasatinib.

This study represents the first description of Bcl11b expression in the cat and in FOSCC. We demonstrated activation of the Src pathway by FOSCC cells making Src a strategic therapeutic target that could be used in a clinical setting. Exposing FOSCC cells to dasatinib has inhibitory effects on cell proliferation, EGFR, and consistently decreased production of VEGF. Based on these results, dasatinib may have both anti-proliferative and anti-angiogenic effects in FOSCC. Since EGF is able to partially rescue cells from the effects of dasatinib, future studies should evaluate dual target inhibition of Src and EGFR, EGFR and VEGFR, as well as combining Src inhibition with standard chemotherapy agents.

O07

A RETROSPECTIVE ANALYSIS OF MULTIMODALITY TREATMENT FOR CANINE ORAL MELANOMA: 126 CASES. Michelle Turek[1], Tracy LaDue[2], Jayme Looper[3], Koichi Nagata[4], Keijiro Shiomitsu[5], Michele Keyerleber[6], Julia Buchholz[7], Tracy Gieger[5], David Vail[1]. [1]University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI, USA, [2]Southeast Veterinary Oncology and Internal Medicine, Orange Park, FL, USA, [3]Chicago Veterinary Specialty Group, Chicago, IL, USA, [4]University of Georgia, College of Veterinary Medicine, Athens, GA, USA, [5]Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA, USA, [6]Tufts University Cummings School of Veterinary Medicine, North Grafton, MA, USA, [7]Animal Oncology and Imaging Center, Hunenberg, Switzerland

Oral melanoma (OM) has a high propensity to metastasize to regional lymph nodes (LN) and lungs. Surgery and/or radiotherapy (RT) are effective local treatments, however most dogs succumb to distant metastasis. Immunotherapy represents an attractive strategy for this potentially immunogenic tumor. The objective of this multi-institutional retrospective study was to examine the clinical outcome of dogs with OM treated with ONCEPT™ melanoma vaccine, +/- surgery and/or RT.

Medical records of dogs with OM treated with ONCEPT™ were reviewed from seven veterinary radiotherapy facilities. Many dogs underwent concurrent surgery and/or RT (8 Gray X 4 weekly fractions). Dogs with distant metastasis and those receiving concurrent chemotherapy were excluded.

One hundred and twenty six dogs were included. All received ONCEPT™. Sixty had adequate local control (ALC; complete excision or irradiation of microscopic disease). Fifteen were treated in the microscopic setting. Fifty-one were treated in the gross disease setting, of which 39 underwent RT. Median time to progression, median progression-free survival and median overall survival were 304, 244 and 617 days, respectively. Dogs with ALC had improved clinical outcomes. The following also correlated with favorable clinical outcomes: rostral location, stage 1, absence of LN metastasis, low mitotic index, absence of bony lysis, absence of gross disease.

This is the largest reported series of dogs with OM treated with ONCEPT™. Clinical outcomes are similar to those reported recently for dogs treated with surgery and/or RT alone. Several prognostic indicators were confirmed. A prospective, randomized, controlled study is needed to determine the clinical benefit of ONCEPT™.

O08

ENHANCEMENT OF DOXORUBICIN EFFECTIVENESS WHEN COMBINED WITH SALINOMYCIN IN FISS CELL LINES. Lucia Borlle[1], Brittany Zumbo[2], Kelly R. Hume[1]. [1]Cornell University, Ithaca, NY, USA, [2]Michigan State University, MI, USA

Feline injection site sarcoma (FISS) is an aggressive neoplasia that remains a challenge for clinicians. Radical surgery is the first choice treatment but is not always feasible. Chemotherapeutic responses have been documented but are generally of short duration; tumors often develop resistance quickly. Salinomycin is an ionophore that has been shown to inhibit cancer stem cells and to increase the sensitivity of human soft tissue sarcoma cells to doxorubicin chemotherapy. The aim of our study was to determine if salinomycin could be used to increase the sensitivity of FISS cell lines to doxorubicin. Two primary cell lines cultured from feline patients with histologically confirmed FISS were used for this purpose. Since the threshold response to doxorubicin differs, we ranked the cells according to the sensitivity to doxorubicin and used one sensitive line (C10) and one resistant line (B4). Cell viability assays (MTT) were used to determine the response to single agent and combination therapy. Three independent assays were performed, with samples evaluated in triplicate in each assay. Student's t-tests were employed to compare the results of individual doxorubicin concentrations with and without salinomycin combination therapy. Addition of 5 uM salinomycin resulted in a statistically significant ($P < 0.05$) reduction in cell viability compared to single agent doxorubicin at multiple concentrations for both cell

lines. Our results highlight a potential chemotherapy protocol for the treatment of FISS. Further investigations need to be pursued to define the effect of this novel therapeutic combination and to validate our results *in vivo*.

O09
CLINICAL ADVANCEMENT OF A RNA-TRANSFECTED CD40-B CELL VACCINE FOR THE TREATMENT OF CANINE NON-HODGKIN'S LYMPHOMA. Nicola Mason, Martha MaloneyHuss, Josephine Gnanandarajah. University of Pennsylvania, Philadelphia, PA, USA

Canine Non-Hodgkin's lymphoma (NHL) occurs in approximately 30–100/100,000 dogs per year and accounts for up to 83% of canine hematopoietic cancers. Standard–of-care treatment for canine NHL comprises multi-drug chemotherapeutic protocols. 60–85% of dogs enter a complete clinical remission following induction chemotherapy, and median first-remission durations range from 140 to 385 days. Resistance to chemotherapy increases with each subsequent relapse and less than 10% of patients survive longer than 2 years from diagnosis. To prolong overall survival, more effective therapies are required to prevent relapse following successful induction chemotherapy.

Directing the immune system to specifically target malignant cells is a powerful strategy in the treatment of cancer. We have previously developed a vaccine strategy that utilizes autologous CD40-activated B cells as antigen-presenting cells electroporated with autologous tumor RNA as the antigenic payload to stimulate anti-tumor immunity in dogs with NHL. In our first clinical trial, we showed that dogs vaccinated three times at three week intervals following successful induction chemotherapy had a tendency towards prolonged remission times and overall survival when compared to historical controls. Furthermore, vaccinated dogs that did relapse showed a statistically significant increase in overall survival following rescue chemotherapy. These results coupled with immune analysis from vaccinated patients suggested that while RNA loaded CD40-activated B cells primed an anti-tumor immune response, it was insufficient to maintain remission. Furthermore, we hypothesized that rescue chemotherapy boosted vaccine primed tumor-specific immunity and lead to prolonged second remission times. We therefore hypothesized that repeat administrations of RNA-loaded CD40-activated B cells given every 2 months following an initial priming course would maintain anti-tumor immunity and prevent relapse.

To investigate this hypothesis we are currently performing a second phase I/II clinical trial using RNA-loaded CD40-activated B cells. Fifteen dogs will be recruited to this study. Eligibility criteria include a recent diagnosis of NHL, no prior treatment with chemotherapy or steroids, and no evidence of circulating blasts. At the time of diagnosis, peripheral blood mononuclear cells and malignant lymph node tissue are harvested to generate CD40-activated B cells and whole tumor RNA respectively. Dogs then undergo a 19-week CHOP-based chemotherapy protocol. Only dogs in complete clinical remission following induction chemotherapy are eligible to receive the autologous vaccine. Dogs are vaccinated every three weeks for three doses, and then every two months until clinical relapse. Induction and maintenance of anti-tumor immunity is being determined using an IFN-γ ELISpot assay. The primary endpoint of this trial is time to progression, and the secondary endpoints include duration of anti-tumor immunity, overall survival and lymphoma-specific survival.

To date, ten dogs have been recruited and CD40-activated B cells have been successfully generated for nine dogs. Briefly, PBMCs are co-cultured with feeder cells consisting of irradiated K562 cells transfected with human CD40L (KtCD40L) in the presence of cyclosporine and IL-4, to generate CD40-activated B cells. CD40-B cells are then electroporated with RNA extracted from autologous malignant lymph node tissue or with canine distemper virus Hemagglutinin antigen (CDV-HA) mRNA as a control vaccine. At the time of writing, 2/10 dogs have completed their induction chemotherapy and have received their initial priming vaccinations. No adverse events have occurred following vaccine administration, and neither dog has yet relapsed. The trial is ongoing and the preliminary data confirm the feasibility of autologous CD40-B cell vaccine generation and safety of administration. ELISpot analysis at baseline and at each vaccine time point are being performed to elucidate whether repeat vaccinations effectively maintain anti-tumor immunity.

O10
VITAMIN D STATUS AND ACUTE PHASE PROTEIN CONCENTRATIONS IN CANINE CANCER PATIENTS. Nicole Weidner[1], J. Paul Woods[2], Anthony J. Mutsaers[2], Julie Bayle[3], Adronie Verbrugghe[2]. [1]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [3]Royal Canin Research Center, Aimargues, France

Circulating concentrations of acute phase proteins (APP) have been used as biomarkers in canine oncology. Increased concentrations have been observed in cancer patients and decreased concentrations are linked to patient remission. In humans, increased APP concentrations have been linked with low vitamin D status in several disease states. Low vitamin D status has also been observed in human and canine cancer patients. Therefore, there is the possibility that increased APP concentrations will be linked with decreased vitamin D status in canine cancer patients. The objective of this study was to examine the relationship between blood vitamin D and APP concentrations in healthy dogs and dogs with cancer.

Dogs with lymphoma (n = 34), osteosarcoma (n = 21) and mast cell tumors (n = 26) diagnosed by cytology and/or histology presenting to one referral hospital for treatment, as well as client-owned healthy control dogs (n = 25), were enrolled. Blood samples were collected from all dogs prior to any treatment and analyzed for plasma 25-hydroxyvitamin D (25(OH)D) using radioimmunoassays, and haptoglobin (Hp), alpha-1-acid glycoprotein (AAG) and serum amyloid A (SAA) using ELISA kits. Statistical analyses were completed with SAS 9.3. Vitamin D and APP concentrations were compared between groups using ANOVAs followed by Tukey-Kramer post-hoc analyses. Pearson correlation coefficients were performed to determine relationships between vitamin D and APP concentrations within each group.

Mean±SD plasma 25(OH)D concentrations were significantly lower in dogs with lymphoma (102 ± 33 nmol/L) than in healthy dogs (129 ± 41 nmol/L, $P = 0.007$). Mean±SD plasma Hp and AAG concentrations were significantly higher in dogs with lymphoma (9.6 ± 7.3 ng/mL and 92.4 ± 71.0 ng/mL, respectively) than in healthy dogs (3.0 ± 1.7 ng/mL, $P < 0.001$ and 43.7 ± 30.8 ng/mL, $P < 0.001$, respectively). There were no significant differences in blood SAA levels among groups. Pearson correlation coefficients revealed a moderate inverse relationship between plasma AAG and 25(OH)D concentrations ($R = -0.39$, $P = 0.024$) in lymphoma patients only.

The lower plasma 25(OH)D and higher Hp and AAG concentrations in canine lymphoma patients compared to healthy dogs is consistent with previous reports. However, this study is the first to report these observations within the same population. Further research is needed to investigate whether decreased plasma 25(OH)D concentration is a factor in cancer development, or a consequence of cancer, and to determine the clinical relevance of these findings.

O11
RETROSPECTIVE EVALUATION OF METRONOMIC CYCLOPHOSPHAMIDE IN EPITHELIAL AND MESENCHYMAL MALIGNANT TUMOURS. J Ellis, RD Foale. Dick White Referrals, Six Mile Bottom, Cambridgeshire, UK

Continuous low dose chemotherapy administration has been reported to have anti-angiogenic effects with efficacy against multiple tumour types. This study aimed to retrospectively assess the use of metronomic cyclophosphamide for histopathologically confirmed solid malignant canine tumours.

Patient records from 2009 to 2014 were reviewed at a multidisciplinary referral hospital. Dogs without histological

confirmation of diagnosis were excluded. Thirty-seven cases of epithelial or mesenchymal malignancy in dogs treated with 10 mg cyclophosphamide daily/every other day were identified. This included 14 high grade soft tissue sarcomas, 5 soft tissue sarcomas of undefined grade, 4 intermediate grade soft tissue sarcomas, 3 splenic haemangiosarcomas, 3 incompletely excised low grade soft tissue sarcomas, 2 thyroid carcinomas, and 1 each of adenocarcinoma, bladder haemangiosarcoma, splenic fibrosarcoma, apocrine gland carcinoma, metastatic prostate carcinoma, and metastatic carcinoma of unknown origin. Two were lost to follow up. Twenty-two had at least one attempt at surgical excision and three underwent radiotherapy prior to initiation of chemotherapy.

Three of the dogs (8.6%) developed haematuria, but no other adverse effects were reported. The haematuria resolved in all three dogs after cessation of cyclophosphamide.

74% of dogs had stable disease or partial/complete remission >100 days with cyclophosphamide, including two dogs that had failed previous chemotherapy protocols and one that had received radiotherapy. Four dogs did not have resection of the primary tumour, and three of these remained clinically well for >100 days. 20 of 35 dogs (57%) had DFI >6 months with 13 (37%) still alive over one year later.

Of the dogs with intermediate/high grade sarcomas (n = 18) the median survival time (MST) was 362 days, with one dog lost to follow up and four still alive.

Of the dogs with carcinomas (n = 6) one was lost to follow up and two are still alive >1 year later with no clinical signs. One dog had a reduced rate of progression and stable disease for >100 days with no clinical signs of disease. Two dogs with thyroid carcinoma were still alive three years later. This is in contrast to the reported MST for invasive thyroid carcinoma treated with cisplatin of 98 days.

This study adds to the evidence that metronomic cyclophosphamide may have some use in the treatment of malignant mesenchymal and epithelial tumours. However, it is important to note that even at low doses side effects may occur.

O12
EVALUATION OF TOCERANIB PHOSPHATE (PALLADIA®) IN THE TREATMENT OF FELINE MAST CELL NEOPLASIA: 53 CASES.

Erika Berger[1], Chad Johannes[1], Gerald Post[2], Gillian Rothchild[2], Kai-Biu Shiu[3], Sarah Wetzel[3], Chelsea Tripp[4], Theresa Arteaga[5], Martin Crawford-Jukubiak[6], Anna Rita Serras[7], Sarah Gillings[8], Christine Swanson[9], Shawna Greene[10], Kelly O'Neill[11], Michelle Pressel[12]. [1]Iowa State University College of Veterinary Medicine, Ames, IA, USA, [2]The Veterinary Cancer Center, Norwalk, CT, USA, [3]Veterinary Specialty Center, Madison, WI, USA, [4]Veterinary Cancer Specialty Center, Seattle, WA, USA, [5]Animal Cancer Center, Monterey, CA, USA, [6]Sage Centers for Veterinary Specialty and Emergency Care, Concord, CA, USA, [7]Animal Health Trust, Newmarket, Suffolk, UK, [8]Summit Veterinary Referral Center, Tacoma, WA, USA, [9]Blue Pearl Specialty and Emergency Medicine for Pets, Grand Rapids, MI, USA, [10]Iowa Veterinary Specialties, Des Moines, IA, USA, [11]Saint Francis Veterinary Specialists, Decatur, GA, USA, [12]Pacific Veterinary Specialists, Capitola, CA, USA

The purpose of this study was to solicit and compile data from practicing veterinary specialists regarding their use of toceranib in cats with mast cell neoplasia and to provide initial assessment of possible clinical benefit and toxicity.

The American College of Veterinary Internal Medicine Oncology and Small Animal Internal Medicine listserves were used to solicit data pertaining to cases in which toceranib was used in the treatment of feline mast cell neoplasia. Cases were included if the following data were received: signalment (age, gender, breed), anatomic classification of disease (cutaneous or visceral), previous and concurrent treatment, toceranib dose (mg/kg) and schedule, duration of therapy, best response, and documentation of adverse events.

Case data from 53 cats with cutaneous (n = 25) or visceral (n = 28) mast cell neoplasia were received. Clinical benefit (CB) was seen in 81% (43/53), including 88% (22/25) with cutaneous (8CR, 12PR, 2SD) and 75% (21/28) with visceral (5CR, 13PR, 3SD) involvement. Most cats (n = 49) did not receive concurrent radiation or chemotherapy, though a majority (n = 38) received glucocorticoids during toceranib treatment. Mean duration of toceranib treatment in cats experiencing CB was 37 weeks (4–106) and 48 weeks (10–199) for cutaneous and visceral cases, respectively. Toceranib was administered at a median dose of 2.5 mg/kg in cats with CB; in 91% (39/43) the drug was given 3 times per week. Treatment was generally well tolerated with 57% (30/53) cats experiencing adverse effects at some point during toceranib treatment. The majority of these signs were low-grade (Grade 1 or 2) gastrointestinal or hematologic toxicities that resolved with treatment break and/or dose adjustment.

Toceranib appears to be well-tolerated in feline patients with mast cell neoplasia. Biologic activity of this drug is evident in the studied cats, however further prospective studies are needed to fully elucidate its role in treatment of this condition.

O13
EXPRESSION OF P GLYCOPROTEIN (ABCB1) IN CATS WITH T-CELL LYMPHOCYTIC GASTROINTESTINAL LYMPHOMA.

Valter de Medeiros Winkel, Bruno Cogliati, Archivaldo Reche Jr, Ana Luiza Nairismagi Alves, Sílvia Regina Ricci Lucas. University of Sao Paulo, Sao Paulo, SP, Brazil

Gastrointestinal is the most common anatomical form of lymphoma in cats and systemic chemotherapy is indicated for the treatment. Although most of cats can achieve a complete remission with chemotherapy, relapse can occurs and may be associated to the multidrug resistance (MDR). Cell mechanisms of MDR include activation of transmembrane proteins, that reduce intracellular concentrations of different chemical compounds, and alterations in drug target. The main protein related to MDR phenotype is P-glycoprotein. Its overexpression results in reduced concentration of certain drugs within the cell, which is linked to the resistance. The aim of this study was to investigate P glycoprotein (P-gp) expression in cats with T-cell lymphocytic gastrointestinal lymphoma. Immunohistochemistry was performed in 40 samples using the monoclonal antibodies mouse anti-PAX-5 (PAX5-Invitrogen), polyclonal rabbit anti-CD3 (DAKO) and mouse anti-P-glycoprotein (clone C494-Enzo Life Sciences). P-gp immunoreactivity was analysed by degree of staining intensity, between 0 (no expression) to 3 (strong expression). Lymphoma was considered to be positive for P-gp when more than 10% of the neoplastic population expressed the protein. Three cats had a strong and 7 had a moderate expression, comprising 25% of the cases. The median of overall survival time was 24 months for these 10 cats; 60% had complete and 40% partial response to the treatment, and it was the same median observed for all cats studied. In conclusion, the expression of P-gp was observed in 25% of the cats with T-cell lymphocytic gastrointestinal lymphoma and this expression was not related to the treatment response or overall survival time.

O14
EARLY EXPERIENCES WITH STEREOTACTIC RADIATION THERAPY FOR THE TREATMENT OF CANINE NON-LYMPHOMATOUS NASAL TUMORS.

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The purpose of this study was to describe initial experiences with a stereotactic radiation therapy (SRT) protocol (10 Gy x 3 daily fractions delivered with 6 MV photons via a linear accelerator) used as treatment for non-lymphomatous nasal tumors in dogs. A retrospective analysis of cases treated from August 2013 to October 2015 at NC State Veterinary Hospital was performed. Dogs were included if they had biopsy-confirmed non-lymphomatous nasal tumors. Dogs treated with chemotherapy were excluded. The gross tumor volume (GTV) was contoured using pre- and post-contrast 1–2 mm slice thickness CT image sets.

A clinical target volume (CTV) expansion was used to include the ipsilateral nasal cavity and sinuses as well as 0.5–1 cm of the contralateral nasal cavity. A PTV expansion was not used (CTV = PTV). Thirty Gray (Gy) was prescribed to 99% of the GTV and > 95% of the PTV with a planned simultaneous boost to the center of the GTV. Cone beam CT, indexed bite-block system, and a treatment couch with 6 degrees of freedom were used for set up and delivery. Cases were reviewed and information related to patient demographics, tumor characteristics, and radiation plan dosimetry was collected.

Twenty-five cases were included. Stages included T1 (n = 1), T2 (n = 10), T3 (n = 2), T4 (n = 12). None had evidence of distant metastasis. Nineteen had regional lymph node aspirates and metastasis was present in one. Tumor types included adenocarcinomas (n = 15), chondrosarcomas (n = 5), transitional nasal carcinomas (n = 3), intranasal squamous cell carcinoma (n = 1), and neuroesthesioblastoma (n = 1). Clinical signs improved in all cases. Twelve cases had recheck CT scans 3–4 months post-SRT and partial or complete tumor response was seen in all cases. Minimal acute toxicity was detected. The only late effect noted to date is oronasal fistula development in one dog 4 months post-SRT (this was anticipated due to tumor invasion into the palate at diagnosis). The median disease progression-free survival time has not been reached, and 52% of dogs are progression-free at 1 year. Nine dogs are deceased (median survival time 375 days) and 40% are alive 600 days post SRT.

Three fraction SRT has been used to treat non-lymphomatous nasal tumors in 25 dogs with limited adverse events. Continued accrual and follow-up will be necessary to confirm low toxicity, further elucidate effects of stage on outcome, and characterize clinical efficacy.

O15

TREATMENT OF CANINE APPENDICULAR OSTEOSARCOMA WITH AMPUTATION, CARBOPLATIN, AND TOCERANIB PHOSPHATE. Tracy Gieger[1], Dawn Clarke[2], Julie Nettifee-Osborne[1], Chad Johannes[3], Brianna Hallman[1], Michael Nolan[1], Laurel Williams[4]. [1]North Carolina State University, Raleigh, NC, USA, [2]University of Georgia College of Veterinary Medicine, Athens, GA, USA, [3]Iowa State University, Ames, IA, USA, [4]Veterinary Specialty Hospital of the Carolinas, Cary, NC, USA

Osteosarcoma (OSA) is locally invasive and highly metastatic. We hypothesize that standard-of-care carboplatin chemotherapy followed by continued administration of toceranib phosphate (TP) will result in improved survival in dogs with OSA as compared to historical controls that did not receive TP. In addition, we hypothesize that TP will decrease circulating levels of pro-angiogenic MMP-9, a downstream factor and surrogate marker for VEGF receptor inhibition.

Ten dogs with histologically confirmed OSA with no evidence of thoracic metastasis at diagnosis were treated with amputation followed by 4 doses of carboplatin chemotherapy (300 mg/m2 IV q 3 weeks). Fourteen days after chemotherapy was completed, TP was initiated at a dose of 2.75 mg/kg PO q 48 hours. Serum samples for VEGF and MMP-9, bloodwork to evaluate toxicity, screening for metastasis with thoracic radiographs, and quality-of-life (QOL) scores monitored via owner surveys were performed monthly. The Kaplan-Meier method was used to calculate OSA-free survival and overall survival (OS). A Friedman's test was used to compare VEGF and MMP values at baseline, pre-TP, and end of study. A Kruskal-Wallis test of repeated measures was used to compare platelet-corrected VEGF values at baseline, pre-TP, and end of study. P values of <0.1 were considered significant.

Three dogs required dose reductions of carboplatin secondary to neutropenia, and two required drug holidays during TP treatment. No dogs were withdrawn from the study due to toxicity. Seven dogs developed local recurrence or metastasis as the first event. One dog was withdrawn from the study due to owner wishes, one was euthanized due to osteoarthritis, and one developed lymphoma with no evidence of OSA metastasis. There was no significant difference in MMP or VEGF (absolute or platelet-corrected) at any time point, and there was no correlation of these values with OSA-free survival or OS. Median OSA-free survival was 183 days (range, 55–576) and OS was 250 days (range, 80–882).

Although the combination of carboplatin followed by TP was well-tolerated in dogs with appendicular OSA, survival times did not exceed previously published data from dogs treated with amputation followed by chemotherapy. Serum VEGF and MMP-9 levels remained unchanged from baseline despite the continued administration of TP. The results of this study do not support ongoing TP administration post amputation and standard-of care chemotherapy in dogs with appendicular OSA.

O16

SAFETY ASSESSMENT OF A NOVEL ONCOLYTIC MARABA VIRUS IN CATS. J. Paul Woods[1], Byram Bridle[1], Dorothee Bienzle[1], Josepha Delay[2], Annette Morrison[3], Michelle Cieplak[3], Jeff Hummel[4], Brian Lichty[4]. [1]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada, [3]Campus Animal Facilities, University of Guelph, Guelph, ON, Canada, [4]McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada

Oncolytic viruses are viruses whose replication is restricted to tumour cells leading to cytolysis. A growing number of viruses are being developed as oncolytic viruses and various strategies have been utilized to target tumour cells. Our group developed a biotherapeutic that combines two emerging cancer treatment modalities, tumour vaccination and oncolytic viruses. We recently determined that oncolytic viruses expressing tumour antigen transgenes are an excellent means to boost and enhance tumour vaccination. In this preliminary study, cats were first vaccinated with an adenovirus expressing a cancer gene, and then boosted with another virus (Maraba) also expressing this cancer gene. This vaccination protocol should elicit strong immunity to cancer cells, but should not cause any ill effects to the cats. The purpose of this study was to determine the safety (or toxicity) of a genetically altered oncolytic Maraba virus in cats.

Five young male (6–12 months old) purpose-bred cats were vaccinated intramuscularly with 9×10^9 PFU of a non-replicating human serotype 5 adenoviral vaccine vector (prime). Three weeks later the cats were administered 2.64×10^{11} PFU of MG1 Maraba virus intravenously (boost). Cats were monitored with physical exams observing for clinical signs, and by complete blood counts (CBC). Peripheral blood, saliva, urine, and feces were collected for virus isolation on days 1, 2, 3, 4 and 7 post- Maraba virus infusion.

There were no severe adverse events. Two cats had mild transient pyrexia (40 - 41.4 C) between 2 and 5 hours post Maraba virus infusion (typical of cytokine induction). The CBCs revealed a transient mild neutrophilia in 2 cats one day after Maraba virus infusion (14.92 and 8.73; reference interval $2.1–8.3 \times 10^9$ /L). Thrombocytopenia of 27×10^9/L (reference range $93–514 \times 10^9$/L) was noted in one cat 2 days post Maraba virus infusion, and in another cat thrombocytopenia of 9×10^9/L and 33×10^9/L on days 3 and 7, respectively, post Maraba virus infusion.

Viral genomes but not replication-competent viruses were detected in secondary lymphoid tissues (i.e. spleen) on day 39. Replication-competent virus was not detected in peripheral blood, saliva, urine, or feces.

Viral genomes in secondary lymphoid tissues without concurrent replication-competent virus is a finding similar to that in mice and primates, and reflects the propensity of Maraba virus to be taken up by antigen-presenting cells when delivered systemically, making it an ideal vector for boosting immune responses.

The lack of replication-competent virus in saliva, urine, feces or peripheral blood satisfied Canadian Food Inspection Agency (CFIA) concerns regarding potential shedding, and enabled initiation of a clinical trial utilizing Maraba virus to treat cats with mammary cancer. In this trial a heterologous prime:boost strategy targeting tumour antigens will be tested.

O17
ROLE OF MONOCYTE RECRUITMENT IN HEMANGIOSARCOMA METASTASIS IN DOGS (*VCS AWARD WINNER*). Daniel Regan[1], Andrea Escaffi[1], Jonathan Coy[1], Jade Kurihara[1], Steven Dow[1]. [1]Department of Clinical Sciences, Flint Animal Cancer Center, Colorado State University, Fort Collins, CO, USA

Introduction: Canine hemangiosarcoma is a highly malignant tumor, which is associated with poor long-term survival due to the development of early and widespread metastatic disease. Currently, little is known regarding the biology of canine hemangiosarcoma, and the mechanisms accounting for the highly metastatic nature of the tumor are poorly understood. In humans and rodents, monocytes have been shown to play key roles in metastasis through promotion of tumor cell extravasation, seeding, growth, and angiogenesis, as well as suppression of anti-tumor immunity. However, there has been little investigation into the role of monocytes in canine tumor metastasis. Therefore, we investigated the potential role of monocyte infiltration in the regulation of tumor metastasis in dogs.

Methods: To address this question, we initially performed immunohistochemistry for CD18 to determine the degree of monocyte infiltration in necropsy samples obtained from several common metastatic tumors of dogs, including hemangiosarcoma, osteosarcoma, and various carcinomas.

Results: We found that compared to other tumor types, hemangiosarcoma metastases had significantly greater infiltration of CD18+ monocytes. Next, migration assays were used to compare the ability of tumor cell lines to stimulate monocyte migration in vitro. Hemangiosarcoma cell lines were among the strongest at stimulating monocyte migration, and were also found to be the highest producers of the monocyte chemoattractant CCL2. In addition, hemangiosarcoma metastases in vivo were found to produce large amounts of CCL2, compared to other tumor metastases.

Conclusion: These results are consistent therefore with the hypothesis that overexpression of CCL2 and recruitment of large numbers of monocytes may explain in part the aggressive metastatic nature of canine hemangiosarcoma. Moreover, these findings suggest that immunotherapeutic interventions designed to block monocyte recruitment or mobilization may be an effective adjuvant strategy for suppressing tumor metastasis in dogs with hemangiosarcoma.

O18
PHASE I CLINICAL TRIAL OF THE TARGETED CHEMOTHERAPEUTIC DRUG, FOLATE-TUBULYSIN, IN DOGS WITH URINARY TRACT TRANSITIONAL CELL CARCINOMA (*VCS AWARD WINNER*). Nicholas Szigetvari[1], Deepika Dhawan[1], José A. Ramos-Vara[1], Christopher P. Leamon[2], Patrick J. Klein[2], Hock Gan Heng[1], Iontcho R. Vlahov[2], Philip S. Low[2], Lindsey M. Fourez[1], Deborah W. Knapp[1]. [1]Purdue University College of Veterinary Medicine, West Lafayette, IN, USA, [2]Purdue University College of Chemistry, West Lafayette, IN, USA

Introduction: Targeted chemotherapy can include repurposing drugs previously considered clinically irrelevant due to marked toxicity and low therapeutic index. Tubulysin is one such drug with compelling antiproliferative activity *in vitro*, but poor tolerability *in vivo*. High affinity folate receptor (FR) expression has previously been found in canine transitional cell carcinoma (TCC) and serve as a druggable target. A phase I clinical trial of Folate-Tubulysin was conducted in dogs with urinary tract TCC.

Methods: With PACUC approval, eligible dogs were enrolled in a 3 + 3 dose escalating cohort study of Folate-Tubulysin (Endocyte, West Lafayette, IN). Inclusion criteria were histologic diagnosis of TCC, presence of FRs on the TCC cells detected by immunohistochemistry and/or folate uptake in the TCC detected by nuclear scintigraphy, and informed pet owner consent. The initial dose was 0.2 mg/kg given intravenously every two weeks, with intra- and inter-cohort escalation by 0.02 mg/kg after at least two consecutive treatments. Escalation continued until dose limiting toxicity (DLT) was observed and the maximum tolerated dose (MTD) determined.

Results: Eleven dogs were enrolled to obtain the maximum tolerated dose (MTD). A total of twenty seven dogs were enrolled to further investigate toxicity and tumor response. The Folate-Tubulysin MTD was 0.26 mg/kg administered every two weeks. The most common DLTs were neutropenia, anorexia, and/or lethargy. Resolution of side effects occurred with minimal supportive care and in some dogs treatment delay. Early categorical tumor responses have included 3 partial remissions, 16 stable disease, 8 progressive disease.

Conclusion: Folate-Tubulysin was generally well tolerated when given at or below the MTD. Conjugation of Tubulysin to folate allows use of this compound that otherwise would not be possible due to toxicity.

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EN01
GLUCOSE HOMEOSTASIS DETERIORATES MORE RAPIDLY WITH AGE IN BURMESE CATS COMPARED TO NON-BURMESE. Margareth Rose Lederer[1], Jacquie Rand[2], Nubia Lopes[2], John Morton[3], Nick Jonsson[1]. [1]University of Glasgow, Glasgow, Scotland, UK, [2]University of Queensland, Gatton, Qld, Australia, [3]Jemora Pty Ltd., Geelong, Vic., Australia

Burmese cats are predisposed to diabetes mellitus in Australia, New Zealand and United Kingdom, and the disease is 3 to 4 times more prevalent in this breed compared to domestic cats. Mechanisms predisposing Burmese cats to diabetes remain unclear. This study compared hormonal and biochemical variables associated with glucose and lipid metabolism between mixed-age, lean to overweight, clinically healthy Burmese and non-Burmese cats. Burmese cats older than 3.5 years had higher fasting glucose compared to non-Burmese ($P = 0.02$), with 1.3 mmol/L higher at 3.5 years and 3.5 mmol/L higher at 10 years of age ($P < 0.001$), but not at 2 years of age. Mean and 2-hr glucose concentrations during a glucose tolerance test were higher in Burmese than non-Burmese cats ($P < 0.006$ to 0.031), indicating relative glucose intolerance. Lean Burmese cats had 4.8 μU/mL higher fasting insulin concentrations than non-Burmese, suggesting they are insulin resistant ($P = 0.031$). At time 10 minutes during the GTT, Burmese cats had lower insulin concentration compared to non-Burmese, but were higher at 120 minutes ($P = 0.066$ and $P = 0.046$). This is consistent with diminished first phase, and increased and prolonged second phase insulin release, a pattern of insulin secretion described in prediabetic, diabetic, obese humans and obese cats. Fasting triglyceride concentration was 0.2 mmol/L higher ($P = 0.015$) and fasting NEFA concentration was 0.18 mmol/L higher ($P = 0.016$) in Burmese compared to non-Burmese. These results demonstrate that Burmese cats have more rapid deterioration of β-cell function with age, and impaired glucose homeostasis and lipid metabolism occur concurrently in these cats.

EN02
PERTURBATIONS IN SERUM FRUCTOSAMINE LEVEL IN DIABETIC HYPERTHYROID CATS – A RETROSPECTIVE STUDY. Arnon Gal[1], Brie Trusiano[2], Adrienne French[3], Nicolas Lopez-Villalobos[4], Amy MacNeill[2]. [1]Institute of Veterinary, Animal and Biomedical Sciences, Palmerston North, New Zealand, [2]Colorado State University, Ft. Collins, USA, [3]New Zealand Veterinary Pathology, Palmerston North, New Zealand

Previous studies indicated that serum fructosamine level is decreased in hyperthyroid cats; however, its clinical utility in diabetic hyperthyroid cats was not reported. We hypothesized that hyperthyroidism in diabetic cats will result in a clinically significant decrease in serum fructosamine level compared to euthyroid diabetic cats. Data of serum total thyroxine (TT4) and fructosamine of hyperthyroid/euthyroid diabetic/nondiabetic cats from the New Zealand Veterinary Pathology and Colorado State University were retrieved and statistically analyzed. Serum fructosamine was significantly lower in hyperthyroid diabetic than euthyroid diabetic cats (mean 332 μmol/L 95% CI 291–379, n = 18 versus mean 527 μmol/L 95% CI 515–553, n = 186) while

not different between hyperthyroid diabetic and euthyroid nondiabetic cats (mean 332 μmol/L 95% CI 291–379, n = 18 versus mean 321 μmol/L 95% CI 296–345, n = 128). There was a significant negative correlation between serum TT4 concentration and serum fructosamine and glucose concentrations (n = 659, $P < 0.01$, and n = 297, $P < 0.01$, respectively). Hyperthyroid cats (diabetic or not) had significantly ($P < 0.05$) lower serum glucose than euthyroid cats (diabetic or not). Diabetes and random variability accounted for 49.7% and 45.4% of the change in serum fructosamine; whereas, age (3.1%) and population (1.8%) had minimal impact on serum fructosamine. In conclusion, in the current study, serum fructosamine concentration was significantly decreased in diabetic hyperthyroid cats and was similar to the fructosamine concentration in euthyroid nondiabetic cats. In addition, approximately 45% of the change in serum fructosamine was independent of diabetes. Future studies should be undertaken to explore specific factors that have an impact on fructosamine level.

EN03

PHARMACODYNAMIC AND PHARMACOKINETIC PROPERTIES OF INSULIN ASPART FOLLOWING SUBCUTANEOUS AND INTRAMUSCULAR INJECTION IN CATS. Hannah Pipe-Martin[1], Jon Fletcher[1], Chen Gilor[2], Michael Kearney[3]. [1]Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA, [2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, [3]Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

Insulin aspart (Aspart) is a rapid-acting insulin analog that has been shown to be an effective treatment for diabetic ketoacidosis (DKA) when administered by intermittent subcutaneous (SC) injection in humans. In cats, rapid-acting insulin analogs have not yet been evaluated, and the most commonly used protocols for DKA involve delivery of regular insulin via constant intravenous infusion, which can be technically challenging. The aim of this study was to determine the pharmacodynamics (PD) and pharmacokinetics (PK) of Aspart following SC and intramuscular (IM) injections in healthy cats.

Using the isoglycemic clamp method, time-action profiles were generated in eight healthy cats to determine pharmacodynamics of Aspart following SC and IM injection. Pharmacokinetics were determined by measuring the plasma Aspart concentration every 15 minutes during the clamp using the Mercodia® Iso-insulin ELISA kit.

Mean ± SEM time to onset of action and duration of action did not differ significantly between SC (11.12 ± 0.97 minutes and 154 ± 14.8 minutes) and IM injection (13.75 ± 3.1 minutes and 176 ± 19.8 minutes), and both methods of administration resulted in acceptable plasma concentrations and blood glucose lowering effect. Subcutaneous injection resulted in greater plasma Aspart concentration at 15 and 30 minutes and greater peak concentrations when compared to IM injection ($P < 0.0001$ and $P = 0.0005$ respectively).

Aspart is rapidly absorbed following SC and IM injection in healthy cats. The effective and predictable glucose lowering effect of insulin aspart may be advantageous in the treatment of DKA in cats.

EN04

THE EFFECT OF TETRA-HYDROXYLATED BILE ACID ON ADIPOCYTE SIZE AND INSULIN SENSITIVITY IN HEALTHY CATS. Isabelle Rast[1], Elena Salesov[1], Thomas Lutz[2], Eric Zini[1], Christian Wolfrum[3], Claudia Reusch[1]. [1]Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, [2]Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, [3]Laboratory of Translational Nutrition Biology, Swiss Federal Institute of Technology Zurich, Schwerzenbach, Switzerland

Obesity is a major risk factor for the development of diabetes in cats. Recently, retinoid-related orphan receptor gamma (RORγ) has been identified as an important transcription factor in the development of large insulin-resistant adipocytes. RORγ can be inhibited by its ligand tetra-hydroxylated bile acid (THBA). Oral supplementation of THBA reduces adipocyte size, improves insulin sensitivity and prevents hyperglycemia in obese mice. The purposes of this study were to investigate palatability and possible side effects of THBA, and the effects of THBA supplementation on adipocyte size and insulin sensitivity in healthy cats.

Six healthy purpose-bred cats were fed 5 mg/kg/day of THBA for 8 weeks. THBA was given in a small amount of food to each cat individually before the daily ration was offered. Complete blood count, biochemical profile and insulin tolerance test (ITT; 0.1 IU/kg insulin aspart IV) were performed at weeks 0 and 8. After 12–18 hours of fasting blood glucose concentration was recorded and the rate constant for the disappearance of glucose (K_{ITT}) was calculated. Subcutaneous fat tissue samples were taken in the umbilical area by a 4 mm biopsy punch, and adipocyte size was determined by histological and image based analysis of at least 10 sections per animal. Data were analyzed by parametric tests ($P < 0.05$).

Oral supplementation of THBA was well accepted in all cats, and no side effects were noted. None of the cats displayed abnormalities on complete blood count and serum biochemical profile. Fasting blood glucose concentration and average size of white subcutaneous adipocytes were significantly lower after 8 weeks of THBA administration, whereas K_{ITT} did not differ.

The reduction of adipocyte size in response to THBA treatment is in accordance with data from mice and underscores the possible role of RORγ signaling in the control of adipose tissue metabolism. While we observed a significant reduction in fasting glucose, overall insulin sensitivity did not improve. These findings might be explained by the fact that insulin sensitivity in normal weight subjects is mainly determined by liver and muscle cell function, while altered adipocyte size would not contribute significantly to the ITT. Future studies in obese cats and altered dosing regimes are required to assess whether THBA improves insulin resistance in cats, in addition to direct effects on adipocyte size.

EN05

THE EFFECT OF ADIPOSITY AND DIET ON SECRETION OF INCRETIN HORMONES IN CATS. Chen Gilor, Katie McCool, Adam Rudinsky, Valerie Parker, Prosper Boyaka. The Ohio State University, Columbus, OH, USA

Degree of adiposity and dietary macronutrient composition affect the secretion of incretin hormones but little is known about their effect in cats.

In this study, 8 overweight cats were fed a maintenance diet (MD) for 3 weeks followed by a weight control diet (WCD, lower fat, higher in carbohydrates and fiber). Cats were fed ad libitum initially and then food was restricted to achieve 1–2% loss of body weight weekly (11 weeks). When lean, cats were fed MD for 2 additional weeks. A standardized meal test (SMT) using a third diet was performed after at least 7 days on each diet, before and after weight loss (4 SMT's total). Glucose, insulin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) concentrations were measured immediately before and over 6 hours after feeding the SMT. Area under the concentrations curve (AUC) was compared using non-parametric tests.

Glucose, insulin and GLP-1 concentrations did not differ between SMT's at the 4 time points. Fasting GIP concentrations also did not differ but post-prandial GIP secretion was affected by degree of adiposity ($P = 0.025$) with a trend towards an effect of diet ($P = 0.085$). GIP secretion in lean cats fed WCD was higher than in obese fed MD ($P = 0.0062$).

In conclusion, compared to the overweight state, GIP secretion was increased in cats in response to SMT in the lean state. On-going exposure to WCD increased GIP responses to SMT, suggesting that dietary macronutrient content is important in determining GIP responses not only acutely but also on a long-term basis.

EN06

BREED, COAT COLOUR AND HAIR LENGTH AS RISK FACTORS FOR FELINE HYPERTHYROIDISM. Victoria J. Crossley, Yu-Mei Chang, Robert C. Fowkes, Jonathan Elliott, Harriet M. Syme. Royal Veterinary College, London, UK

Hyperthyroidism commonly affects geriatric cats, but its aetiology is poorly understood. Previous studies have shown Siamese, Himalayan and Burmese cats to have reduced risk of developing hyperthyroidism. These breeds have colourpoint coats as a result of temperature-sensitive mutations in the tyrosinase gene, which limit conversion of tyrosine to melanin pigment. Tyrosine is essential for synthesis of both melanin and thyroid hormones and, as such, coat pigmentation may impact relative tyrosine availability for thyroid hormone production. This study aimed to identify potential associations between coat phenotype and development of hyperthyroidism, by investigation of breed, coat colour and hair length as risk factors for feline hyperthyroidism.

This retrospective study of cats aged ≥10 years, referred to a single UK veterinary teaching hospital (2006–2014), used electronic patient records to obtain owner-reported data regarding breed, coat colour, age and sex, and clinical data to classify cats as hyperthyroid/euthyroid. Bayesian multivariable logistic regression was used to evaluate breed, age and sex as risk factors for hyperthyroid status in all the cats (purebred and non-purebred). To avoid interactions with coat length or colour in purebreds, the effect of coat colour/pattern, colour dilution, base pigment, white markings and hair length were assessed only in non-purebred cats in a separate analysis. Variables with $P < 0.2$ in univariate analyses were evaluated in multivariable models, with final variables significant at $P \leq 0.05$. Risk factors are quantified as odds ratio ((OR) P-value).

Of 3934 cats included in the final analysis, 885 were hyperthyroid and 3049 euthyroid. Multivariable results showed Burmese (OR 0.01, $P = 0.005$), Persians (OR 0.20, $P < 0.001$), Siamese (OR 0.32, $P = 0.006$), Abyssinians (OR 0.04, $P = 0.035$) and British shorthairs (OR 0.52, $P = 0.014$) had reduced risk of hyperthyroidism compared to domestic shorthairs. Domestic longhairs (OR 1.34, $P = 0.015$) showed increased risk of hyperthyroidism compared to domestic shorthairs, but coat colour, colour dilution, base pigment and white markings did not have a significant effect. Overall, females (OR 1.39, $P < 0.001$) had increased risk of hyperthyroidism compared to males, and, compared to 10 year olds, increased risk was found in cats aged 11–12 years (OR 1.45, $P = 0.007$), 13–14 years (1.83, $P < 0.001$) and 15–17 years (OR 1.50, $P = 0.004$).

To authors' knowledge this is the first study to report increased risk of hyperthyroidism in long haired, non-purebred, domestic cats. Consistent with a number of previous studies, increased risk of hyperthyroidism was found with increased age and female sex, and reduced risk in Burmese, Siamese and Persian breeds. However, this study also newly identified two further purebreds, Abyssinian and British shorthair, at reduced risk of hyperthyroidism, neither of which were exclusively colourpoint. Coat colouration was not found to be associated with risk of hyperthyroidism in the analysis of domestic cats, however, reliance on secondary data may have resulted in misclassification errors and coat phenotype may be a poor surrogate marker for melanin concentrations. Further studies are required to evaluate tyrosine availability as a potential factor in the aetiology of feline hyperthyroidism, and to investigate the apparent protective effect in certain purebreds and associations between hyperthyroidism and hair length.

EN07

DOES A LIMITED IODINE DIET AFFECT THE RESPONSE TO RADIOACTIVE IODINE THERAPY IN HYPERTHYROID CATS?. Allison Rowland, Karyn Harrell, Katharine Lunn. North Carolina State University, Raleigh, NC, USA

A retrospective study was performed to determine the outcome of radioactive iodine (131-I) therapy in cats previously treated with a limited iodine diet (Hill's Prescription Diet y/d®).

Medical records were searched for cats receiving 131-I therapy after documented feeding of the prescription diet for at least 14 days. Cats were excluded if the prescription diet had been supplemented with other foods, or if methimazole had been administered concurrently with, or subsequent to, the prescription diet. Owners were contacted directly to provide missing dietary information.

Nine cats fulfilled the study criteria. The prescription diet was fed for a median of 91 days (range: 19–371). The interval between discontinuing the diet and administering 131-I was 0 days in 3 cats, 4–5 days in 3 cats and 16–24 days in 3 cats. Highest serum total thyroxine (T4) without therapy was used in calculating 131-I dose; median highest T4 was 10.3 mcg/dl (range: 4.3–23.8 mcg/dl; reference range: 0.78–4.27 mcg/dl). The median dose of 131-I administered was 3.64 mCi (range: 3.06–5.26 mCi). The cats were initially evaluated 22–57 days after 131-I therapy and hyperthyroidism had resolved in all 9 cats, with a median post-therapy total T4 of 1.3 mcg/dl (range: 0.5–1.8 mcg/dl). Total T4 was below the reference range in 2 cats (0.5 and 0.55 mcg/dl) at the initial post-therapy evaluation, but was normal 22 and 34 days later (0.9 and 1.6 mcg/dl respectively).

In this small group of cats, pre-treatment with a limited iodine diet did not adversely affect the response to 131-I therapy.

EN08

EFFECTS OF LEVOTHYROXINE ADMINSTRATION AND WITHDRAWAL ON THE HYPOTHALAMIC-PITUITARY-THYROID AXIS IN EUTHYROID DOGS. Vincent Ziglioli, David Panciera, Edward Monroe, Gregory Troy, Katie Boes. Virginia Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Many dogs are misdiagnosed with hypothyroidism because of the vague presenting signs and limitations of thyroid function tests, leading to inappropriate treatment with levothyroxine. During chronic supplementation, the hypothalamic-pituitary-thyroid axis (HPTA) is suppressed and may make it difficult to accurately determine thyroid function following withdrawal of levothyroxine. We sought to determine if the HPTA is suppressed following levothyroxine administration in euthyroid dogs and the time required for resolution of any suppression. We hypothesized that levothyroxine administration would suppress the HPTA in euthyroid dogs and that the HPTA would recover within 8 weeks in all dogs, regardless of the duration of treatment.

Twenty-eight healthy euthyroid dogs were administered levothyroxine (mean dose 0.024 mg/kg every 24 hours) for either 8 weeks (Group 1) or 16 weeks (Group 2). The dose of levothyroxine was adjusted weekly until a target total thyroxine (T_4) serum concentration of 40–70 nmol/L was obtained 4–6 hours post pill. Serum concentrations of T_4, free thyroxine (fT_4) by equilibrium dialysis, thyrotropin (TSH), and 3,5,3′-triiodothyronine (T_3) were measured every 4 weeks during supplementation and for 4 months after levothyroxine was discontinued. A mixed model ANOVA followed by Tukey's procedure for multiple comparisons was used to compare thyroid function tests both within and between groups. The level of significance was set at <0.05.

Mean serum concentrations of T_4 and fT_4 were significantly higher and TSH was significantly lower during levothyroxine administration compared to baseline in both groups. Mean serum concentrations of T_4, fT_4 and TSH beginning 1 week after levothyroxine was discontinued were significantly different compared to values during levothyroxine administration but not compared to baseline values. When groups 1 and 2 were compared, there was no difference in mean T_4, fT_4, and T_3 concentrations between groups during or after levothyroxine supplementation. Suppression of the HPTA occurred during levothyroxine supplementation, with mean serum T_4, fT_4 and TSH concentrations returning to the reference interval by 1 week after discontinuation in both groups. It appears that assessing thyroid function tests 1 week after long-term levothyroxine supplementation may reliably establish dogs as being euthyroid.

EN09

VARIABILITY OF P450SCC AUTOANTIBODY PERSISTENCE IN DOGS AFFECTED WITH HYPOADRENOCORTICISM.

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Autoantibodies directed against the P450 side chain cleavage enzyme (P450scc) have been recently described in a proportion of dogs affected with hypoadrenocorticism, consistent with an immune-mediated disease process. In humans affected with Addison's disease, autoantibodies can have a predictive value, being detected prior to clinical signs developing, and have been shown to persist post-diagnosis. Furthermore, an autoantibody positive status post-diagnosis has been associated with successful remission of Addison's disease following B-cell depletion with rituximab, suggesting active pathology in these cases.

The current study explored changes in serum P450scc autoantibody status over time in dogs diagnosed with spontaneous hypoadrenocorticism. P450scc autoantibodies were measured by radioimmunoassay in an initial cohort of 213 dogs, indicating a prevalence of 24%. Thirty two of these dogs had repeat samples (n = 80 in total) available for analysis. Between two and six samples were available for each dog, obtained between two and 787 days apart.

Five dogs were consistently autoantibody positive in all samples, up to 425 days between sampling times, these comprised one male and four female dogs, with three crossbreeds, a Dogo Argentino and an English springer spaniel. Three dogs were initially autoantibody positive, then became negative at later time points, a 3 y 11 month old male entire bull terrier, a 4 y 5 month old female neutered beagle and a 2 y female entire crossbreed dog. One dog, a 1 y 8 month old female entire standard poodle, initially negative for P450scc autoantibodies, seroconverted 18 months after diagnosis. The remaining 23 dogs with multiple samples available were consistently autoantibody negative. Persistence was not associated with sex ($P = 0.673$).

This study demonstrates persistence of P450scc autoantibodies in a subset of dogs affected with hypoadrenocorticism and seroconversion over one year post-diagnosis. Whilst P450scc autoantibody positivity has been associated with sex, with females having a higher prevalence, there was no sex difference in persistence demonstrated. P450scc expression in the ovary may act as an additional source of antigenic stimulation in female dogs, explaining the later seroconversion observed in one individual. However, paralleling humans with Addison's disease, antibody persistence in dogs with hypoadrenocorticism might represent persistent pathology, due to residual antigenic stimulation and autoimmune inflammation in the adrenal gland.

EN10

JUVENILE HYPOADRENOCORTICISM IN THE NOVA SCOTIA DUCK TOLLING RETRIEVER: A RECESSIVE MONOGENIC AUTOIMMUNE DISEASE.

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While hypoadrenocorticism, or Addison's disease, can occur in a dog of any breed at any age, certain breeds, including Nova Scotia Duck Tolling Retrievers (NSDTRs), have an increased incidence of the disease. Specifically in the NSDTR, there appears to be at least two forms of Addison's disease in the breed: a juvenile onset form (JADD) occurring in dogs under 1 year of age, and an adult onset form occurring in dogs at 4.5 years of age on average. JADD differs from adult onset Addison's disease, as it can be a multisystemic illness and appears to involve an autoimmune component. This is supported by the identification of a CD3+ T cell infiltrate in histologic sections of a JADD affected adrenal gland sample and that many cases are often affected with other autoimmune diseases, including immune-mediated hemolytic anemia and thrombocytopenia, immune-mediated polyarthritis, and hypothyroidism. To identify a genetic basis of JADD, a genome-wide association study was performed using the Illumina Canine HD 173,000 SNP array with 14 NSDTRs diagnosed with Addison's disease less than 1 year of age and 33 healthy control NSDTRs over 6 years of age. All cases were definitively diagnosed through an adrenocorticotrophic hormone stimulation test. Genome-wide association analysis identified a 1.7 Mb associated haplotype on chromosome 27. Whole genome sequencing was performed on 2 NSDTRs with the associated haplotype, as well as 6 unaffected NSDTRs and 11 other dogs from 5 different breeds. Analysis of variants yielded 5 segregating SNPs in the associated region: 1 intergenic, 2 intronic, 1 coding, and 1 in the 3′ untranslated region of a gene. The only coding variant, a missense mutation causing an amino acid change from a proline to a leucine, occurs in a currently uncharacterized gene, but is predicted to be damaging and deleterious based on sequence conservation. In summary, genome-wide association and whole genome sequencing analysis has identified a novel gene and mutation implicated in multi-organ autoimmunity and juvenile onset Addison's disease in the NSDTR.

EN11

VARIABILITY IN POST ACTH STIMULATION SERUM CORTISOL FOLLOWING ADMINISTRATION OF CORTISONE ACETATE IN HEALTHY DOGS.

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The adrenocorticotropic hormone stimulation test (AST), often used for the diagnosis of canine hyperadrenocorticism, has a wide range of sensitivity and specificity. Thus, we hypothesized that standardized negative feedback on the hypothalamic-pituitary-adrenal-axis will result in large variability in the post AST serum cortisol concentrations. Therefore, this study aimed to evaluate the variability in the post AST serum cortisol in healthy dogs and to statistically examine parameters that may influence cortisol responses.

Fourteen healthy Harrier Hound dogs (mean age 7.1y±3.2; mean body weight 26.5 kg±2.7) were randomized by a Latin square design to receive 5 treatments [placebo, 50 mg, 37.5 mg, 25 mg, and 12.5 mg of cortisone acetate (CA)] in each of the five 7-day treatment periods, which were separated by 14-day washout periods. An AST was performed 24 hours after the last dose of CA for each of the 5 treatments and serum cortisol was analyzed by an electrochemiluminescence immunoassay.

A significant dose dependent decrease in the mean (±SEM) post AST serum cortisol was observed (placebo 365 ± 13 nmol/L, CA 12.5 mg 347 ± 13 nmol/L, CA 25 mg 335 ± 13 nmol/L, CA 37.5 mg 326 ± 13 nmol/L, CA 50 mg 308 ± 13 nmol/L). There was a significant ($P < 0.05$) between-week variability in post AST serum cortisol in the placebo and 25 mg and 37.5 mg CA groups. The overall variability in the post AST serum cortisol concentrations was attributed to the individual dog (31%), random unexplained variability (31%), dose of CA (16%), age (15%), body weight (4%), and timing of treatment (3%).

The wide range in the published specificity of the AST may be related to large variations in cortisol responses.

EN12
IONIZED HYPERCALCEMIA IN CATS: ETIOLOGIES AND ASSOCIATED CLINICAL SIGNS. Muzzammil Sayyid, Chen Gilor, Valerie Parker, Adam Rudinsky, Dennis Chew. The Ohio State University, Columbus, OH, USA

The etiologies, concurrent diseases, and clinical signs associated with hypercalcemia in cats have been reported in the past based only on measurement of total calcium and excluding cases of idiopathic hypercalcemia (IHC). Here we report on the relative frequency of various diagnoses and concurrent clinical signs associated with ionized hypercalcemia in cats. The medical records of an academic institution that treats primary and secondary cases was searched for cases treated between the years 2003–2013. Cases were included if serum or plasma ionized calcium concentrations were above the upper limit of the reference interval at least twice. Cats were excluded if ionized hypercalcemia was not present on follow-up examination or if a final diagnosis was not made.

Sixty-nine cases were identified. The most common diagnoses were IHC (48%) and chronic kidney disease (35%). Urolithiasis was diagnosed in 14% (43% calcium oxalate), neoplasia in 13% (46% lymphoma) and primary hyperparathyroidism in 3%. Other diagnoses included hyperthyroidism (8%), hypertrophic cardiomyopathy (4%), feline idiopathic cystitis (2%), and diabetes mellitus (2%). The average age at diagnosis of hypercalcemia was 10.0 ± 4.61 years. Most common clinical signs reported in the IHC group versus other diagnoses (in which clinical signs could be related to the primary disease, and not necessarily to hypercalcemia), were anorexia (21.7% versus 31.9%), vomiting (14.5% versus 26.1%), lethargy (10.1% versus 7.2%), and constipation (10.1% versus 1.4%).

In conclusion, ionized hypercalcemia in cats is frequently idiopathic. The association between feline ionized hypercalcemia and its etiologies and clinical signs should be further explored in case-controlled studies.

EN13
EVALUATION OF BASAL CORTISOL CONCENTRATIONS FOR THE DIAGNOSIS OF HYPOADRENOCORTICISM IN DOGS. Ashley Gold, Joe Hauptman, Daniel Langlois. College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA

Canine hypoadrenocorticism is a potentially life-threatening endocrine disorder characterized by glucocorticoid deficiency, with or without concurrent mineralocorticoid deficiency. The current standard for diagnosing hypoadrenocorticism is the ACTH stimulation test, but a simpler and less expensive test with similar accuracy would be desirable. Previous studies regarding the diagnostic utility of basal plasma or serum cortisol concentrations are limited by low numbers of affected dogs, and receiver operating characteristics (ROC) methods were not utilized to identify optimal cut-off points. The purpose of our study was to evaluate the diagnostic utility of basal cortisol concentrations for the diagnosis of hypoadrenocorticism in a large number of dogs by constructing an ROC curve to identify optimal cut-offs for sensitivity and specificity. Furthermore, the diagnostic utility was evaluated in two sub-populations of dogs: those with electrolyte abnormalities (Na:K ratio ≤28) and those without electrolyte abnormalities (Na:K ratio >28).

Medical records for dogs in which an ACTH stimulation test had been performed for a clinical suspicion of hypoadrenocorticism between 2005 and 2015 were retrospectively reviewed. Dogs that had received treatment for hyperadrenocorticism or recent glucocorticoid therapy were excluded. Five hundred and fourteen dogs met inclusion criteria, including 163 dogs with hypoadrenocorticism (post-ACTH stimulated cortisol concentration ≤55 nmol/L). An ROC curve was constructed for basal cortisol concentrations using commercially available software. The area under the ROC curve was 0.991. The ROC curves were nearly identical when evaluating dogs with and without electrolyte abnormalities. Accuracy was optimal at 22 nmol/L, which provided a sensitivity and specificity of 96.9% and 95.7%, respectively. At cut-offs of 55 nmol/L and 4 nmol/L, sensitivity and specificity were 99.4% and 99.7%, respectively. Using a previously estimated prevalence of 15%, cut points of 55 nmol/L and 4 nmol/L resulted in negative and positive predictive values of 99.8% and 97.9%, respectively. Even at lower prevalence rates, positive predictive values remained high at low cut points. Results of this study reaffirm the high sensitivity and negative predictive value of basal cortisol concentrations for the diagnosis of hypoadrenocorticism. However, the high specificity and positive predictive values at low cut points could be of major importance for veterinary practitioners. Prospective studies are needed to further substantiate these findings.

EN14
THYROID PROFILES IN HEALTHY KITTENS AGED TWO TO SIXTEEN WEEKS OF AGE. Christina Marino, Margret Casal. University of Pennsylvania, Matthew J. Ryan Veterinary Hospital, Philadelphia, PA, USA

Congenital hypothyroidism (CH) is associated with significant morbidity and mortality in pediatric felines. Thyroid hormones (TH) are essential for normal development of the nervous and skeletal systems. The paucity of information on TH levels and the inability to identify early histopathologic changes has led to CH underdiagnosis. The literature on TH levels in kittens is sparse and incongruent, comprised of mostly case reports. References state kitten total thyroxine (TT4) levels are 2 to 3 times higher than the adult cat TT4 level. They also make the assumption free thyroxine (fT4) levels are higher than the adult cat. An abstract was previously presented showing kitten TH levels did not exceed the adult cat normals. These values were measured with assays that are no longer used. The normal pediatric TH levels must be evaluated with the newer and currently available assays. The purpose of this study was to determine the TT4, fT4, total tri-idiothyronine (T3), free tri-idiothyronine (fT3) and thyroid stimulating hormone (TSH) levels in healthy kittens aged 2 through 16 weeks measuring levels at weekly intervals using the current available diagnostic methods.

Normal, healthy kittens from a research colony in an approved facility at the University of Pennsylvania, School of Veterinary Medicine were used. One half to one milliliter of whole blood based on body weight was drawn by jugular venipuncture each week. Serum was immediately obtained and frozen until analysis. The samples were submitted to the Michigan State University Diagnostic Laboratory for Animal and Population Health. All TH measurements were performed on serum. The TSH was measured using a canine chemiluminescent assay. The TT4, TT3 and fT3 were measured with radioimmunoassays and the fT4 was measured using equilibrium dialysis. All assays have been previously validated in cats.

Samples were collected at the same time in the morning at weekly intervals. All values are reported as the mean. The TT4 was initially above the adult cat reference range (ACRR; 10–47 nmol/L) at 2 weeks and peaked at 6 weeks (50.9 nmol/L and 64.6 nmol/L, respectively). The TT4 returned to within the ACRR at 8 weeks, was elevated again at 10 weeks (50.0 nmol/L), returned within the ACRR at 13 weeks and remained stable through 16 weeks. The fT4 remained within the ACRR (10–53 pmol/L) through all 16 weeks, but showed a similar peak at 6 and 10 weeks (50 pmol/L and 39.8 pmol/L, respectively). The TSH was within the ACRR (0–0.38 ng/mL) throughout the 16 weeks. The TSH remained stable at 0.16 ng/mL from 2 through 4 weeks, decreased within the ACRR at 8 weeks, peaked at 10 weeks (0.18 ng/mL) and then continuously decreased to 0.06 ng/mL at 16 weeks. The TT3 and fT3 remained within the ACRR through all 16 weeks. Both hormones peaked at 6 weeks and remained at that level through 16 weeks.

The TH levels in pediatric kittens were measured with the newer assays and with the exception of TT4, all TH levels were within the ACRR. The TT4 did not elevate more than twice the upper limit of the ACRR at any time point and was consistently within the ACRR after 12 weeks of age. A peak in TT4, fT4, TT3 and fT3 was seen at 6 weeks and a peak in TT4 and fT4 was also seen at 10 weeks. TSH levels using the canine TSH assay have been validated for use in adult cats, but these values have not yet been reported in kittens. The TSH levels remained within the ACRR and it showed a similar peak at 10 weeks. These patterns likely occur due to an increased growth and development at 6 and 10 weeks.

EN15

SPECTROPHOTOMETRY AND ULTRACENTRIFUGATION FOR MEASUREMENT OF PLASMA LIPIDS IN DOGS WITH DIABETES MELLITUS. Eileen Seage, Kenneth Drobatz, Rebecka Hess. Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA, USA

Conflicting results have been reported regarding lipid concentrations in dogs with diabetes mellitus (DM). Some studies have reported that low-density lipoprotein-cholesterol (LDL-C) is not significantly different in dogs with and without DM. The goal of this study was to report total cholesterol (TC), total triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), very low-density lipoprotein-cholesterol (VLDL-C), and LDL-C in fasted dogs with and without DM.

Twenty-two dogs with DM and nine healthy control dogs with normocholesterolemia were prospectively enrolled following a minimum eight-hour fast. Plasma lipid fractions were measured by ultracentrifugation (TC, HDL-C, VLDL-C, and LDL-C) or direct sample analysis (TG) coupled with enzymatic reactions and spectrophotometric end points (Roche COBAS c311). Median lipid concentrations in dogs with and without DM were compared using a two-sample Wilcoxon rank-sum test. A P-value <0.05 was considered significant.

In dogs with DM, median TC (343 mg/dl), TG (98 mg/dl), HDL-C (196 mg/dl), VLDL-C (39.5 mg/dl), and LDL-C (67.5 mg/dl) concentrations were significantly higher than concentrations in healthy dogs (197 mg/dl, 57 mg/dl, 168 mg/dl, 12 mg/dl, 16 mg/dl, respectively, $P < .05$ for all comparisons). The greatest difference was measured in LDL-C concentration, which was 4.2 times higher in dogs with DM compared to controls. VLDL-C, TC, and TG concentrations were 3.3, 1.7, and 1.7 times higher, respectively, in dogs with DM compared to healthy dogs. The smallest difference was noted in HDL-C concentrations.

It is concluded that the increase in LDL-C is an important component of dyslipidemia in dogs with DM. Future studies of therapeutics focused at decreasing LDL-C in dogs with DM may be warranted.

EN16

EFFECT OF HYDROCORTISONE ADMINISTRATION ON LEPTIN AND ADIPONECTIN SYNTHESIS IN HEALTHY DOGS. Hye-Ryung Choo, Changhwan Ahn, Woon-Bum Baek, Bohye Shin, Hakhyun Kim, Ji-Houn Kang, Eui-Bae Jeung, Mhan-Pyo Yang. Chungbuk National University College of Veterinary Medicine, Cheongju, Chungbuk, Republic of Korea

The objective of the present study was to examine whether circulating concentrations of leptin and adiponectin, distribution of abdominal fat, and mRNA expressions of leptin and adiponectin in abdominal adipose tissue were affected by hydrocortisone administration. Six laboratory dogs each received hydrocortisone (8.5 mg/kg) orally every 12 hours for 90 days. We measured the serum concentrations of leptin and adiponectin using canine-specific ELISA kits. We quantified visceral fat using CT scanning, and we analyzed the mRNA expressions of leptin and adiponectin in abdominal fat using real-time PCR.

Hydrocortisone administration resulted in increased abdominal visceral fat mass on days 30, 60, and 90 compared with fat mass before administration (day 0). Additionally, visceral fat area at the L3 level also was increased during hydrocortisone administration. Serum leptin concentrations began to increase on day 1 of hydrocortisone administration and were significantly increased on days 1, 3, 7, 30, 60, and 90 compared with concentrations on day 0. Serum adiponectin concentrations on days 1, 3, 7, 30, 60, and 90 were decreased compared with concentrations on day 0. The mRNA expressions of leptin and adiponectin in the abdominal fat were increased on day 30 compared with expressions on day 0, but the expressions were decreased on days 60 and 90 compared with those on day 30. We identified a correlation between serum leptin and adiponectin concentrations and visceral fat distribution. This study showed that hydrocortisone administration affected visceral fat distribution and serum concentrations of leptin and adiponectin by dysregulating leptin and adiponectin expression.

EN17

USING PURIFIED FELINE INSULIN TO EVALUATE CROSS-REACTIVITY WITH A HUMAN INSULIN ANALOG ELISA. Jon Fletcher, Hannah Pipe-Martin, Chin-chi Liu. Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

Recombinant DNA technology has been used to modify the structure of human insulin to yield analogs that are more rapidly-acting than regular insulin or have a longer duration of action than NPH insulin. The long-acting insulin analogs have been studied in cats and are routinely used to manage feline diabetics. More recently, the isoglycemic clamp method has been used to study the metabolic effect of a rapid-acting insulin analog in healthy cats. Generating time-action profiles to study the pharmacodynamic properties of insulin formulations does not require measurement of plasma insulin concentrations, but if measured, they provide useful pharmacokinetic data that can be used to validate the time-action profiles. The Iso-Insulin ELISA (Mercodia AB, Uppsala, Sweden) is used to measure the concentration of insulin analogs in plasma. A previous study using actual feline serum samples with a wide range of insulin concentrations measured by a commercial ELISA failed to detect cross-reactivity of feline insulin with the Iso-Insulin ELISA.

The purpose of this study was to evaluate the cross-reactivity of feline insulin with the Iso-Insulin ELISA using purified feline insulin standards. Five purified feline insulin standards with known concentrations ranging from 9.13 ng/L to 702 ng/L that are used as calibrators for the commercial feline insulin ELISA (Mercodia AB, Uppsala, Sweden) were used. The ELISAs were performed in duplicate according to the manufacturer's recommendations. The mean absorbance values of the feline insulin ELISA calibrators were below the mean absorbance value of the lowest Iso-Insulin calibrator (0.087) except for the first calibrator, which had a slightly higher mean absorbance of 0.099. The slightly higher absorbance for the first calibrator was likely the result of a minor technical issue with this very sensitive ELISA since all calibrators with higher insulin concentrations consistently had absorbances at or below the lowest Iso-insulin calibrator.

These results demonstrate that purified feline insulin concentrations up to 702 ng/L are not detected by the Iso-Insulin ELISA, and therefore will not interfere with insulin analog measurements in feline plasma.

EN18

STEROID HORMONE PANEL TEST AND TRILOSTANE AND MELATONIN THERAPY IN POMERANIAN DOGS WITH ALOPECIA X. Melissa Sanches[1], Marcia Jericó[2], Eric Januário[1]. [1]Pet Care Centro Veterinário, São Paulo, SP, Brazil, [2]Universidade Anhembi Morumbi and Clinica Veterinária Alto da Lapa Sao Paulo, SP, Brazil

Alopecia X is a skin disease of unknown etiology characterized by non-inflammatory, bilateral and symmetric alopecia, with hyperpigmentation and absence of pruritus. This condition is most frequently observed in young dogs of Nordic breeds, and is thought to be related to abnormal adrenal gland function, with trilostane and melatonin having been proposed as possible treatment alternatives. This study aims to evaluate the comprehensive steroid panel and therapeutic response of Pomeranian dogs diagnosed with Alopecia X. Eighteen Pomeranian dogs admitted at veterinary clinics (São Paulo, Brazil) with a chief complaint of symmetric and bilateral alopecia were studied. These included 12 males and 6 females, 16 of which were neutered, aging from 2 to 5 years. All animals were subjected to thorough laboratory screening: complete blood count with differential leukocyte count, liver function enzymes, urea, creatinine, triglycerides, cholesterol, blood glucose, and thyroid panel, as well as urinalysis and abdominal ultrasonography; all exam results were within the reference values. Subsequently, blood samples were collected to quantify by radioimmunoassay at Provet Laboratories (Brazil) and Tennessee University the following hormones, both before and after synthetic ACTH stimulation (single dose 0.05 mg/kg/EV): 17-hydroxiprogesterone, aldosterone, androstenedione, cortisol, estradiol, progesterone and testosterone. Eight dogs were then

subjected treatment A (trilostane 1 mg/kg PO BID), seven dogs were subjected to treatment B (trilostane 1 mg/kg PO BID and melatonin 3 mg/dog PO BID) and two dogs received treatment C (melatonin 3 mg/dog PO BID); three animals were lost to follow-up. In comparison to reference values established by the laboratories for both neutered and non-neutered dogs, the animals with Alopecia X presented higher levels in three of the seven hormones both before and after the ACTH stimulation test. Abnormally elevated hormone levels were observed prior to and following ACTH stimulation in respectively: 3 (17%) and 16 dogs (89%) for 17-hydroxiprogesterone, 9 (50%) and 10 dogs (55%) for androstenedione, and 2 (11%) and 16 dogs (89%) for progesterone. Also, the abnormally elevated testosterone levels were observed in 6 dogs (33%) prior to ACTH stimulation and 8 dogs (44%) following ACTH stimulation. Two dogs presented abnormally decreased estradiol levels, both prior to and following ACTH stimulation. On the other hand, abnormally elevated cortisol levels were observed in 3 dogs (17%) prior to ACTH stimulation, and these elevated levels were maintained by only one dog (6%) following ACTH administration. In contrast, all dogs had aldosterone levels within reference ranges, with the exception of one dog with slightly elevated aldosterone levels prior to ACTH stimulation. Of the dogs subjected to treatment A, four (50%) had complete hair regrowth and three (37.5%) had partial regrowth after three months of treatment. Of the dogs subjected to treatment B, one (14%) had complete hair regrowth, four (57%) had partial regrowth, and one (14%) did not show signs of regrowth. Treatment for one of the two dogs subjected to treatment C had to be withdrawn due to emesis. Pomeranian dogs affected by Alopecia X presented increased levels of 17-hydroxiprogesterone, androstenedione, testosterone and progesterone, both prior to and following stimulation with synthetic ACTH, suggesting an adrenal gland increased function in the sex hormones production. Due this, trilostane, as a steroidogenesis blocker, was found to be a positive therapeutic option for Alopecia X, with or without the association of melatonin.

EN19

METABOLITE DIFFERENCES BETWEEN HEALTHY SENIOR BURMESE AND NON-BURMESE CATS, AND ASSOCIATIONS BETWEEN METABOLITES AND MEASURES OF GLUCOSE METABOLISM. Mia Reeve-Johnson[1], Jacquie Rand[1], Stephen Anderson[2], Diane Vankan[4], Daniel Dias[3], Berin Boughton[3], Alysha De Livera[3], Katsumi Ishioka[4], Ute Roessner[4]. [1]School of Veterinary Science, University of Queensland, Brisbane, Australia, [2]School of Biomedical Sciences, University of Queensland, Brisbane, Australia, [3]Centre for Molecular, Environmental, Genetic and Analytic (MEGA) Epidemiology, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia, [4]School of Veterinary Nursing & Technology, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, Tokyo, Japan

Phenotypic identification of cats at risk of developing diabetes has yet to be defined. Burmese cats are reported to have a dyslipidemia and are 3 to 4 times more likely to develop diabetes than other breeds.

To identify plasma metabolite differences between healthy senior Burmese and matched non-Burmese cats ranging from lean to obese, and to determine if significant correlations exist between these metabolites and measures of glucose metabolism, hormonal and biochemical related to diabetes.

Of the 69 cats tested, 49 non-Burmese; 20 lean (BCS 4-5/9), 12 overweight (BCS 6-7/9) and 17 obese (BCS 8-9/9) and 20 were Burmese (6 lean, 12 overweight and 2 obese).

Food was withheld 18 - 24 hours, and a cephalic vein catheter implanted. A venous blood sample was collected and the serum and plasma separated and frozen at −80°C. A glucose tolerance test (glucose 0.5 g/kg) was performed. Biochemical (screening, fasting and 2-hr blood glucose in the glucose tolerance test, triglyceride), hormonal (leptin, adiponectin, leptin:adiponectin ratio, insulin, glucose:insulin ratio) and enzymatic (fPLI, MCP-1) analyses were performed on plasma and serum samples. After extraction, analyses using GC-MS was performed. Linear modeling was used to identify statistically significant (adjusted P-value<0.05) metabolites associated with obesity and whether these responses were affected by sex and age. Pearson correlations were used to identify relationships between metabolites and other parameters.

GC-MS analyses identified 25 metabolites significantly ($P < 0.05$) different between Burmese and non-Burmese cats. In Burmese cats, metabolites that are involved in amino acid metabolism were either increased (phenylalanine, tyrosine, valine, serine, threonine, cysteine, proline and arginine) or decreased (cysteine, alanine and b-alanine) compared to non-Burmese cats. Some metabolites in carbohydrate metabolism were decreased (glucose, fructose, lactic acid), as were some involved in lipid metabolism (glucose 3 phosphate, phosphoric acid, stearic acid, cholesterol). When these metabolites were correlated with known measures of glucose metabolism, numerous amino acids were positively correlated with insulin and triglyceride (tyrosine, phenylalanine cysteine, proline, glutamine and arginine were all positively correlated with insulin, and tyrosine and phenylalanine were also correlated with triglyceride). These results agreed with human studies where future risk of developing diabetes was found to be associated with these changes in metabolites. The amino acid metabolites alanine, threonine and tyrosine were negatively correlated with adiponectin and glucose and fructose were positively associated with 2-hr blood glucose following a glucose tolerance test. However, some metabolites considered discriminators of impaired fasting glucose and increased in humans at risk of diabetes (phosphoric acid, alanine and stearic acid) were decreased in Burmese cats, as were metabolites of glycerolipid metabolism (glycerate, glycerol 3 phosphate, glucose and fructose).

This study is the first to report metabolite differences between healthy non-Burmese and Burmese cats and identifies some biomarkers involved in amino acid, carbohydrate and lipid metabolism that are altered in Burmese cats in a similar pattern to those identified in humans at risk of diabetes. Further research is required to determine if these are useful as markers of metabolic dysfunction in cats at risk of developing diabetes.

EN20

A GENOME-WIDE ASSOCIATION STUDY IDENTIFIES NOVEL CANDIDATE GENES FOR SUSCEPTIBILITY TO DIABETES MELLITUS IN DSH CATS (*ESVE AWARD WINNER*). Y Forcada[1], M Boursnell[2], B Catchpole[1], DB Church[1]. [1]The Royal Veterinary College, North Mymms, UK, [2]Animal Health Trust, Newmarket, UK

Diabetes mellitus (DM) is a common feline endocrinopathy and pathophysiologically similar to human type 2 diabetes (T2DM). T2DM occurs due to a combination of insulin resistance and β-cell dysfunction. Several studies have identified environmental and genetic susceptibility factors for T2DM. In cats, environmental factors such as obesity and physical inactivity have been linked with DM; however, identification of genetic factors has been challenging. To date, MC4R is the only gene shown to be associated with increased susceptibility to DM in overweight domestic short hair (DSH) cats. The aim of the present study was to perform a genome-wide association study (GWAS) to identify loci associated with DM in lean DSH cats.

Illumina Infinium 63k iSelect DNA arrays were used to interrogate genomic DNA samples from 200 lean diabetic DSH cats from the Royal Veterinary College Feline DM Archive and 400 control DSH cats. The data was analysed using PLINK whole genome data analysis toolset. Significance was established at $P < 1 \times 10^{-5}$. SNPs with a minor allele frequency below 0.05 and a call rate below 95% and individuals with a genotyping rate < 90% were excluded from analysis.

A total of 49,930 SNPs were available for analysis. After excluding cats with low genotypic rate, 389 control DSH and 192 lean diabetic DSH cats were evaluated. Diabetic cats had a mean (SD) age of 11.62 (3.44) years; 123 (63%) were male, 71 (37%) female. Nondiabetic cats had a mean (SD) age of 14.83 (2.06) years; 216 (54%) were female, 183 (46%) male. Control cats were significantly older than diabetic cats ($P < 0.0001$; t-test). Five significant SNPs were identified: chrA2.4150731 ($P = 1.4 \times 10^{-7}$);

chrUn17.115508 ($P = 7 \times 10^{-7}$); chrUn17.394136 ($P = 3 \times 10^{-7}$); chrUn17.314128 ($P = 3 \times 10^{-7}$) and chrUn17.7283 ($P = 9 \times 10^{-6}$). The first SNP is located within chromosome A2; the others are located within a 0.8 Mb region towards the end of chromosome A3. The SNP in chromosome A2 is located 3 kb upstream of dipeptidyl-peptidase-9 (DPP9), a peptidase similar to DPP-4, involved in incretin inactivation. Within the identified region of chromosome A3, genes of interest include TMEM18 and ACP1; both have been associated with T2DM in humans, most likely causing insulin resistance. This is the first GWAS of DM in cats. A number of significant SNPs have been identified, some of which are located in proximity to genes that have been associated with T2DM in humans; others could be involved in pathophysiology related to DM. Further investigation of these candidate genes is warranted.

Disclosures: Disclosures to report.

SNP chips for the GWAS were provided by the Morris Animal Foundation.

GI01
SERUM PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATIONS IN DOGS WITH GASTROINTESTINAL FOREIGN BODIES.

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Elevated serum canine pancreatic lipase immunoreactivity (cPLI) concentrations are highly specific for pancreatitis. Additionally, some studies in dogs with primary gastrointestinal (GI) disease have suggested a negative outcome in dogs with increased serum cPLI concentrations. GI disease, including foreign bodies (FBs) can mimic clinical signs of pancreatitis. GI FBs have also been proposed as a risk factor for the development of pancreatitis in dogs. To date the prevalence of pancreatitis in dogs with GI FBs is unknown. The primary aim of this study was to determine the prevalence of elevated cPLI concentrations (as measured by Spec cPL®) in dogs with GI FBs, and to correlate Spec cPL concentrations with patient survival. A secondary aim was to compare Spec cPL concentrations among dogs based on age, FB type, FB location, and type of removal procedure.

Serum Spec cPL was measured in 49 dogs prospectively enrolled with endoscopically or surgically confirmed GI FBs from May 2014 to May 2015. Samples were obtained the day of the procedure prior to anesthetic induction. Dogs were retrospectively grouped based on age (≤4 years, n = 22; 5–8 years, n = 11; ≥9 years, n = 16), FB type (linear, n = 14; discrete, n = 35), FB location (stomach, n = 21; duodenum, n = 3; jejunum, n = 12; involving multiple segments, n = 13) and type of removal procedure (endoscopy, n = 19; surgery, n = 30). The prevalence of a serum Spec cPL above the upper limit of the reference interval (≥ 200 μg/L) or the suggested cut-off value for pancreatitis (≥ 400 μg/L) was determined in all dogs. Serum Spec cPL concentrations were compared amongst groups using Kruskal-Wallis or Mann-Whitney U tests, as appropriate. Statistical significance was set at $P < 0.05$.

A Spec cPL≥ 200 μg/L was identified in 12/49 dogs (24.5%), 9 of which (18.4%) had a Spec cPL≥ 400 μg/L. Spec cPL (median, range) was higher in dogs 5–8 years (125 ug/L, <30–2885 μg/L) and ≥9 years (233 μg/L, 43–2816 μg/L) of age than in dogs ≤4 years (59 μg/L, <30–246 μg/L; $P < 0.0001$) of age. Spec cPL was higher in dogs with linear FBs (376 μg/L, <30–2885 μg/L) than in dogs with discrete FBs (68 μg/L, <30–837 μg/L; $P = 0.0038$). Spec cPL was higher in dogs with FBs affecting multiple GI segments (569 μg/L, <30–2885 μg/L) than in dogs with gastric FBs (60 μg/L, <30–185 μg/L; $P = 0.0159$). Spec cPL was higher in dogs undergoing surgery (108.5 μg/L, <30–2885 μg/L) than in dogs undergoing endoscopy (63 μg/L, <30–185 μg/L; $P = 0.0322$). Two of 49 dogs (4%) were euthanized during the course of the study. Both dogs had evidence of septic peritonitis at the time of euthanasia. In both dogs, Spec cPL concentrations were elevated (1292 μg/L and 2816 μg/L, respectively).

The overall prevalence of Spec cPL ≥ 200 μg/L and ≥ 400 μg/L in dogs with GI FBs was relatively low (24.5% and 18.4%, respectively). Spec cPL was higher in middle aged and older dogs, dogs with linear FBs, and dogs that needed to undergo surgery for FB removal. A Spec cPL≥ 400 μg/L was noted in both dogs that were euthanized. Due to this small number, additional studies would be needed to confirm if an elevated Spec cPL is associated with a negative outcome in dogs with GI FBs.

GI02
SERUM PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATIONS AFTER CHRONIC ADMINISTRATION OF SUPRAPHYSIOLOGIC DOSES OF GLUCOCORTICOIDS TO DOGS.

Sarah Cocker[1], Keith Richter[1], Joerg Steiner[2], Jonathan Lidbury[2], Jan Suchodolski[2]. [1]Veterinary Specialty Hospital, San Diego, CA, USA, [2]Gastrointestinal Laboratory, Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

Prednisone and other glucocorticoids (GCs) are commonly used as long-term medications to treat a multitude of immune mediated, inflammatory, and neoplastic diseases in dogs. Patients with many of these diseases may have clinical signs that overlap with those of patients with pancreatitis. The effect of long-term GC administration on canine serum Spec cPL concentrations has not been adequately assessed. Therefore, the aim of this study was to determine if long-term administration of GCs at supraphysiologic doses affects serum concentrations of Spec cPL in dogs with a variety of different diseases.

Dogs were prospectively enrolled at the Veterinary Specialty Hospital of San Diego between February 2015 and October 2015. Inclusion criteria for enrollment included prescribed long-term (≥ 3 weeks) administration of at least 0.5 mg/kg/day prednisone-equivalent of GCs. Serum Spec cPL concentration was measured prior to initiation of GC therapy so each dog served as its own control. A second measurement of serum Spec cPL concentration was obtained in all dogs after at least 3 weeks of treatment with GCs. Only dogs that had no dosage change within one week of sampling were evaluated. The dose of GCs recorded was the dose that was being administered at the time that the second serum sample was collected. Cases were excluded if there was poor owner compliance with GC administration (defined as missing more than two consecutive doses of GCs) or if there was administration of other medications known or suspected to increase serum Spec cPL concentrations (e.g. potassium bromide or phenobarbital) or GC administration within the 3 months prior to enrollment. Comparison of serum Spec cPL between pre GC administration and ≥ 3 weeks post GC administration was performed using Wilcoxon matched-pairs signed rank test. Statistical significance was set as $P < 0.05$.

A total of 23 dogs were enrolled. Eight cases were excluded due to euthanasia (n = 3), lack of follow up for second sample (n = 3), GC dose <0.5 mg/kg/day at time of second sample collection (n = 1), and change in GC dose < 1 week before second sample collection (n = 1). Fifteen cases met the inclusion criteria. Of the 15 cases enrolled reasons for chronic GC administration included: immune mediated thrombocytopenia (n = 4), immune mediated hemolytic anemia (n = 4), protein losing enteropathy (n = 3), histiocytic sarcoma (n = 1), suspected immune mediated pancytopenia (n = 1), myelofibrosis (n = 1), and mast cell tumor (n = 1). The median Spec cPL concentrations for pre and post GC administration were 68 μg/L (30 – 1,997 μg/L) and 165 μg/L (30 – 1,628 μg/L), respectively. There was no statistically significant difference of serum Spec cPL concentrations between baseline and post GC therapy of ≥ 3 weeks in this group of patients ($P = 0.19$; Figure 1).

The results of this study did not reveal a statistically significant difference in SPEC cPL concentrations in dogs prior to administration of GC and ≥ 3 weeks after administration of supraphysiologic doses of GCs. Thus, the administration of GCs should not compromise the clinician's ability to interpret results of SPEC cPL concentrations.

GI03

SERUM IL-2, IL-6, IL-8, AND TNF-A CONCENTRATIONS IN DOGS WITH INCREASED SERUM SPEC CPL® CONCENTRATIONS. Agostino Buono[1], Andrea Petrelli[2], Jonathan Lidbury[1], Jan Suchodolski[1], Joerg Steiner[1]. [1]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX, USA, [2]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Italy

The role of cytokines in pancreatitis is well characterized in human medicine. However, limited information is available about their role in dogs with pancreatitis. The aim of this study was to describe serum concentrations of interleukin (IL) -2, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) in dogs with increased serum concentrations of pancreatic lipase immunoreactivity (cPLI; as measured as Spec cPL®).

Forty-nine surplus serum samples from dogs with a serum cPLI concentration >1000 μg/L were used for this study. Ten healthy dogs with serum cPLI concentration <200 μg/L were used as controls. Serum cytokine concentrations were measured using previously analytically validated electrochemiluminescence immunoassays. Data were analyzed using nonparametric statistics. Significance was set at $P < 0.05$.

The cytokine concentrations were significantly increased in dogs with serum cPLI concentrations >1000 μg/L when compared to healthy controls, (increased cPLI group median [minimum-maximum] versus healthy group median [minimum-maximum]): IL-2 (18.9 pg/mL [0–559.2] versus 7.4 [1.7–56.8], $P = 0.0167$); IL-6 (17.0 pg/mL [0–1085] versus 4.5 [0.6–88] $P = 0.0003$); IL-8 (6054.4 pg/mL [667.9–19664.9] versus 2849.8 [1538.5–4247.5], $P = 0.0029$); TNF-α (1.2 pg/mL [0–115.1 pg/mL] versus 0.3 [0.1–0.6], $P = 0.0014$). No correlation was found between age, sex, or serum cPLI concentration and any of the serum cytokine concentrations.

In conclusion, serum IL-2, IL-6, IL-8, and TNF-α concentrations were significantly increased in dogs with serum cPLI concentrations >1,000 μg/L. Prospective studies are needed to assess the utility of these cytokines as early diagnostic markers or prognostic markers in dogs with pancreatitis.

GI04

FISHHOOK FOREIGN BODIES IN DOGS AND CATS: 107 CASES (2004–2015). Austin Hardegree[1], James Barr[1], Micah Bishop[2], Medora Pashmakova[1]. [1]Texas A&M University, College Station, TX, USA, [2]Animal Specialty Hospital, Naples, FL, USA

Fishhook foreign bodies are a common presentation in certain geographic areas and often require emergent removal from the gastrointestinal tract or skin. In the authors' institution, endoscopic retrieval is considered highly successful in contrast to previous literature. The objective of this study was to describe location, retrieval, complications, and outcomes associated with fishhooks.

Electronic medical records of cats and dogs admitted between 2004 and 2015 for fishhook foreign bodies were searched for radiographic location of fishhook, retrieval method, and documentation of complications and outcome. Data was tested for normality. A Mann-Whitney test or odds ratios were constructed to compare association between different variables.

One hundred and seven cases (5 cats and 102 dogs) with a total of 111 fishhooks were included. Single shanked hooks comprised 52/111 (46.85%) and multiple shanked hooks comprised 59/111 (53.15%) of cases. Most common locations were the pharyngeal/oral region 38/111 (34.26%)), cervical esophagus (25/111 (22.52%)), and stomach (27/111 (24.32 %)). Three hooks were cutaneously embedded. Hooks with multiple shanks were 2.314 times more likely to be associated with esophageal damage than single shanked hooks (95% CI: 1.038–5.159; P = 0.0478). Endoscopic retrieval was successful in 60/69 (87.0%) cases in which it was attempted. Esophageal perforation occurred in 2 endoscopic cases and a total of 9 cases necessitated surgery. Survival was 100%.

Our study supports that endoscopic retrieval is highly successful and survival is 100%, even in cases requiring surgery. While multiple shanked fishhooks are more likely to cause esophageal damage, neither type of hook nor location affected outcome.

GI05

PERTURBATIONS OF THE INTESTINAL MICROBIOTA AND BILE ACID METABOLISM IN DOGS WITH DIABETES MELLITUS. Alana Redfern-Allen[1], Blake Guard[2], Jan Suchodolski[2], Joerg Steiner[2], Jonathan Lidbury[2], Albert Jergens[1]. [1]Iowa State University, Ames, IA, USA, [2]Texas A&M University, College Station, TX, USA

The intestinal microbiota has been recently linked to the development of human metabolic diseases including diabetes mellitus (DM), due to its role in energy balance, gut permeability, and host inflammatory state. Microbial imbalances may also affect bile acid (BA) metabolism through altered profiles of primary and secondary BA, which modulate signaling pathways involved in insulin resistance via GLP-1 induction. Characterization of the intestinal microbiota and BA in dogs with DM has not been systematically evaluated. Therefore, the aim of this study was to investigate the fecal microbiota and BA composition of healthy dogs and dogs with DM.

Fecal samples were collected from 27 heathy control dogs and 23 diabetic dogs. The fecal microbiota was evaluated prior to intervention using quantitative polymerase chain reaction (qPCR) for *E. coli*, *Streptococcus*, *Turicibacter*, *Faecalibacterium*, *Fusobacterium*, and *Blautia*. The fecal concentrations of primary (i.e., cholic and chenodeoxycholic acid) and secondary (i.e., litho-, deoxy-, and ursodeoxycholic acid) BA were measured using gas chromatography with mass spectrometry. Non-parametric Mann-Whitney *U* tests were used to compare these parameters between healthy dogs and dogs with DM. Statistical significance was set at $P < 0.05$.

Results indicate that DM dogs exhibit imbalances in their fecal microbiota characterized by decreased numbers of *Turicibacter* ($P = 0.026$) and *Faecalibacterium* (P = 0.002) as compared to healthy dogs. Perturbations in BA metabolism were observed in the DM cohort and involved an increase in primary BAs and a decrease in secondary BAs ($P < 0.001$ for both).

We conclude that dogs with DM have significant imbalances in intestinal microbiota and also altered bile acid metabolism, which may contribute to disease pathogenesis and morbidity. Further studies are warranted to elucidate mechanisms behind these alterations, and whether they are primarily due to disease or secondary to environmental factors including diet and antibiotics.

GI06

VARIATION OF THE MICROBIOME AND METABOLOME ALONG THE CANINE GASTROINTESTINAL TRACT. JB Honneffer, JM Steiner, JA Lidbury, JS Suchodolski. Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA

The gastrointestinal tract (GIT) of the dog is a complex ecosystem and its biological and biochemical compositions vary along the length of the GIT. The analysis of fecal samples is frequently used as a surrogate indicator for gastrointestinal disease occurring in more proximal intestinal sections. While the canine gastrointestinal biogeography of the microbiome has previously been described, corresponding metabolite profiles at various points along the GIT of healthy dogs have not been characterized to our knowledge. Therefore, the aim of this study was to perform 16s rRNA gene sequencing and untargeted metabolomics on samples from multiple sites to characterize the metabolome and microbiome along the GIT of healthy dogs.

Samples of the duodenal, ileal, colonic, and rectal contents were collected from six adult dogs immediately after humane euthanasia for another study. The microbiota was characterized using Illumina sequencing of the 16S rRNA gene. The metabolome was characterized by untargeted mass spectrometry-based methods. Inter-animal variation was statistically addressed by blocking in the software JMP, with subsequent Wilcoxon tests and Benjamini-Hochberg correction for multiple comparisons. Statistical significance was set at $P < 0.05$.

Predominant phyla throughout the samples were Proteobacteria, Firmicutes, Fusobacteria, and Bacteroidetes. Percentages of reads identified as Proteobacteria decreased aborally ($P = 0.017$;

duodenum, 62%; ileum, 48%; colon, 9%; rectum, 6%), while those of Firmicutes increased ($P = 0.032$; 20%, 43%, 68%, 69%). On the gas chromatography platform, 530 unique compounds were detected, and 199 of these were assigned to named metabolites. 134 named metabolites had at least one site-pair comparison reaching statistical significance. Key fermentation compounds such as sugars and amino acids had varied site distributions: glucose increased progressively along the GIT along with 6-deoxyglucose ($P = 0.047$ and 0.034, respectively). Nearly all amino acids exhibited a decrease between the ileum and colon (*e.g.*, alanine, citrulline, glutamine, glycine, lysine, methionine, tryptophan, and valine, all $P < 0.03$).

In conclusion, the metabolome and microbiome vary along the canine GIT and further studies are needed to explore the effect of local disease at specific sites, to determine the host versus microbial origin of these metabolites, and possibly to enhance extrapolation from fecal assay results to improve our understanding of GI diseases.

GI07

EFFECTS OF A HYDROLYZED PROTEIN DIET AND METRONIDAZOLE ON THE FECAL MICROBIOME AND METABOLOME IN HEALTHY DOGS. Jan Suchodolski[1], Erin Olson[2], Julia Honneffer[1], Blake Guard[1], Amanda Blake[1], AlShawaqfeh Mustafa[1], Jörg Steiner[1], James Barr[1], Frederic Gaschen[2]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA, [2]Louisiana State University, Baton Rouge, LA, USA

The abnormal interaction between the GI microbiome and the immune system is a major contributing factor to IBD. Treatment often includes the use of antimicrobials like metronidazole or the use of an elimination diet (i.e., hydrolyzed protein diet), but their effects on the intestinal microbiota are not well studied. Recent epidemiological studies in humans have linked gut dysbiosis due to antibiotics as risk factor for various chronic diseases, such as obesity, diabetes, and asthma. A better understanding of the functional effects of antibiotics and diets on the microbiome is needed. The aim of this study was to prospectively evaluate the impact of metronidazole administration versus hydrolyzed protein diet on the metabolome of healthy dogs.

Twenty-four healthy pet dogs were assigned to one of 3 groups (8 dogs each): control dogs with no intervention (group 1, G1), dogs treated with a soy-based hydrolyzed diet (Purina HA) for 12 weeks with administration of metronidazole 15 mg/kg PO q12 h during weeks 6 to 8 (G2), and dogs maintained on their usual diet and administered the same dose of metronidazole for 2 weeks (G3). Feces and blood were collected at day (D) 0, 21 and 42 in G1; and D0, 14, 28 and 42 days in G2 and G3. Microbial communities were analyzed by Illumina sequencing of 16S rRNA genes, and the software PICRUSt was used to predict functional gene families. The serum and fecal metabolome was assessed by an untargeted approach combining various mass spectrometry platforms. All assessed parameters were adjusted for multiple comparisons using the Benjamin Hochberg adjustment, and an adjusted $P < 0.05$ was considered significant.

No significant differences in any of the evaluated parameters were observed in G1 (control group). Also, in G2, the dietary switch to the hydrolyzed diet (between baseline and week 6) did not lead to any significant changes in any of the evaluated parameters. However, when dogs in G2 and the dogs in G3 received metronidazole, significant changes were observed. Because the responses in these 2 groups were similar, they were combined for statistical analysis. Microbiome structure and diversity were significantly altered between baseline and time of completion of metronidazole treatment, and also 4 weeks after end of metronidazole treatment (ANOSIM: $P = 0.002$). The most significant changes were increases in *E. coli* and decreases in Firmicutes. Functional gene families found to be significantly more abundant during antibiotic administration included: lipopolysaccharide biosynthesis, glutathione metabolism, recombination and repair proteins, tryptophan metabolism, and fatty acid metabolism ($P < 0.0001$ for all). At cessation of antibiotic administration (D14), functional gene families found to be significantly more abundant included: bile secretion, xylene degradation, dioxin degradation, and signal transduction mechanisms ($P < 0.0001$ for all). Metronidazole lead to alteration in 98 out of 469 measured fecal metabolites. Major changes observed were reduction in secondary bile acids ($P < 0.001$), increases in oxidative stress pathways ($P < 0.0001$), and changes in tryptophan-indole pathways ($P < 0.001$). While most of the changes were reversed 14 days after the end of antibiotic administration, some of the evaluated bacterial taxa and metabolites remained significantly altered up to 4 weeks after end of administration (end of study).

In conclusion, metronidazole has a profound effect on the fecal microbiome and metabolome, which only partially resolves after 4 weeks. Further studies are warranted to assess whether the dysbiosis and these disrupted metabolic pathways due to metronidazole may pose a risk factor for development of some chronic diseases. In contrast, the hydrolyzed diet had no noticeable effect on the microbiome and metabolome of healthy dogs.

GI08

ALTERED FECAL BILE ACID METABOLISM IN DOGS WITH CHRONIC ENTEROPATHY. Blake Guard[1], Linda Toresson[2,3], Julia Honneffer[1], Amanda Blake[1], Yuri Lawrence[1], Jonathan Lidbury[1], Joerg Steiner[1], Jan Suchodolski[1]. [1]Texas A&M University, College Station, TX, USA, [2]Evidensia Specialist Animal Hospital, Helsingborg, Sweden, [3]Helsinki University, Helsinki, Finland

Chronic enteropathy in dogs has been characterized as a multifactorial disease that is thought to involve inappropriate and ongoing activation of the mucosal immune system in addition to a microbial dysbiosis. Recently, studies in humans have suggested that in some patients with inflammatory bowel disease, microbial dysbiosis drives bile acid (BA) dysmetabolism. Primary BAs are synthesized by the liver and secreted into the intestinal lumen, where enzymatic reactions catalyzed by bacteria lead to BA biotransformation. Additionally, BAs participate in gastrointestinal mucosal defense. For example, secondary BAs have been shown to be anti-inflammatory as they have the potential to decrease the synthesis of proinflammatory cytokines such as TNF-α. Therefore, the aim of this study was to investigate the fecal BA profiles of dogs with chronic enteropathy.

Fecal samples were collected from healthy dogs (n = 13) and dogs with chronic enteropathy with histologically confirmed inflammation (n = 13). Fecal bile acids were analyzed by gas chromatography coupled with mass spectrometry. A Mann-Whitney *U* test was used to compare concentrations of BAs between groups. Cholic acid and chenodeoxycholic acid were among the primary bile acids measured, while deoxycholic acid, lithocholic acid, and ursodeoxycholic acid were among the secondary bile acids measured. Statistical significance was set at $P < 0.05$.

No significant differences in fecal concentrations of primary bile acids were found between healthy dogs and dogs with chronic enteropathy. Deoxycholic acid was significantly decreased in dogs with chronic enteropathy (median [min-max]: 0.26 μg/mg [0.11–13.18 μg/mg]) compared to healthy dogs (median [min-max]: 4.64 μg/mg [0.11–18.94 μg/mg]; $P = 0.0240$). Lithocholic acid was also significantly decreased in the dogs with chronic enteropathy (median [min-max]: 0.07 μg/mg [0.01–2.94 μg/mg]) compared to healthy dogs (median [min-max]: 1.05 μg/mg [0.03–3.21 μg/mg]; $P = 0.0159$).

In conclusion, this study revealed a decrease in fecal secondary bile acids in dogs with chronic enteropathy. Further studies are needed to investigate the interplay between specific bacterial groups and altered bile acid metabolism as well as their relationship with intestinal inflammation in dogs with chronic enteropathy.

GI09

ALTERED FECAL STEROL PROFILES IN DOGS WITH CHRONIC INFLAMMATORY ENTEROPATHY. JB Honneffer[1], L Toresson[2,3], BC Guard[1], R Lopes[1], AB Blake[1], JA Lidbury[1], JM Steiner[1], JS Suchodolski[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA, [2]Evidensia Specialist Animal Hospital, Helsingborg, Sweden, [3]University of Helsinki, Finland

Diseases of chronic inflammation of the gastrointestinal tract, including idiopathic inflammatory bowel disease (IBD), are thought to be perpetuated by bacterial dysbiosis and dysregulation of the mucosal immune system in both humans and animals. Gastrointestinal (GI) absorption of metabolites is altered by inflammation, with concurrent changes in microbial metabolism within the GI tract. Cholesterol is the primary sterol in mammals; it is incorporated into cell membranes throughout the body and used as a building block for endogenous hormones, steroids, and bile acids. Some phytosterols have shown anti-inflammatory properties and have been shown to decrease disease severity in a murine colitis model. Therefore, the aim of this study was to compare fecal sterol profiles of dogs with chronic enteropathy to those of healthy dogs.

Fecal samples were collected from healthy dogs (n = 13) and dogs with chronic enteropathy (n = 13) with biopsy-confirmed inflammation. Sterols in lyophilized feces were subjected to trimethylsilyl ether derivatization and analyzed by gas chromatography-mass spectrometry (GC/MS) operating in Single Ion Monitoring (SIM) mode. Target analytes included cholesterol, cholestanol, β-sitosterol, sitostanol, fucosterol, stigmaterol, and campesterol. Fecal concentrations of sterols were expressed as μg/mg of lyophilized feces. A Mann-Whitney *U* test was used for comparison between animal groups with statistical significance set at $P < 0.05$.

Fecal cholesterol and cholestanol were not significantly altered between healthy dogs and dogs with chronic enteropathy. However, phytosterols were significantly decreased in dogs with chronic enteropathy (healthy group median [min-max] versus chronic enteropathy group median [min-max]): β-sitosterol ($P = 0.0066$, 2.53 μg/mg [1.41–4.62] versus 1.34 [0.084–3.70]); sitostanol ($P = 0.0001$, 0.54 μg/mg [0.17–1.14] versus 0.08 [0.01–0.45]); fucosterol ($P = 0.0138$, 0.23 μg/mg [0.10–0.33] versus 0.09 [0.01–0.80]); stigmasterol ($P = 0.0138$, 0.46 μg/mg [0.23–0.66] versus 0.30 [0.01–0.52]); and campesterol ($P = 0.0313$, 0.84 μg/mg [0.45–1.76] versus 0.53 [0.14–1.79]).

In conclusion, this study suggests that the fecal sterol profile in dogs with chronic enteropathy is characterized by decreased phytosterols. Additional studies are required to explore the effect of dietary supplementation of phytosterols and to investigate how these sterols are related to specific bacterial groups that digest dietary plant material in these patients.

GI10

THE FECAL MICROBIOME OF DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY. Anitha Isaiah, Joseph Cyrus Parambeth, Jonathan A Lidbury, Joerg M Steiner, Jan S Suchodolski. Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA

Exocrine pancreatic insufficiency (EPI) in dogs is a syndrome of inadequate synthesis and secretion of pancreatic enzymes, which leads to clinical signs of maldigestion. Previous reports show that small intestinal bacterial dysbiosis occurs in dogs with EPI and is reversed with pancreatic enzyme therapy. However, there are no studies evaluating the fecal microbiome of dogs with EPI. The objective of this study was to compare the fecal microbiome of healthy dogs (n = 18), untreated (n = 7) dogs with EPI, and dogs with EPI treated with enzyme replacement therapy (n = 19).

To be included into the study, the dogs had to be at least 1 year of age, have clinical signs of EPI, a serum cTLI concentration ≤2.5 μg/L, and be free from any other concurrent disease. Three naturally voided fecal samples collected over three consecutive days were frozen immediately after collection and pooled. Fecal samples were collected in a similar manner from healthy dogs without any clinical signs of gastrointestinal disease. Extracted DNA from fecal samples was used for Illumina sequencing of the bacterial 16S rRNA gene and analyzed using Quantitative Insights Into Microbial Ecology (QIIME). The analysis of similarities (ANOSIM) function in the statistical software package PRIMER 6 (PRIMER-E Ltd., Luton, UK) was used on the unweighted UniFrac distance matrix to determine if any groups of samples contained significantly different bacterial communities. There was a significant difference in fecal microbial communities when healthy dogs were compared to treated ($P = 0.001$) and untreated ($P = 0.001$) dogs with EPI. Quantitative Insights Into Microbial Ecology (QIIME). The analysis of similarities (ANOSIM) function in the statistical software package PRIMER 6 (PRIMER-E Ltd., Luton, UK) was used on the unweighted UniFrac distance matrix to determine if any groups of samples contained significantly different bacterial communities. There was a significant difference in fecal microbial communities when healthy dogs were compared to treated ($P = 0.001$) and untreated ($P = 0.001$) dogs with EPI. Alpha diversity was significantly decreased in untreated and treated EPI dogs when compared to the healthy dogs ($P < 0.01$). The families Bifidobacteriaceae ($P = 0.006$), Enterococcaceae ($P = 0.035$), and Lactobacillaceae ($P = 0.001$) were significantly increased in the untreated and treated dogs with EPI when compared to healthy dogs. In contrast, Lachnospiraceae ($P < 0.001$), and *Blautia* ($P < 0.001$) were significantly decreased in dogs with EPI.

In conclusion, this study suggests that the fecal microbiome of dogs with EPI (both treated and untreated) is different from that of healthy dogs.

GI11

EFFECT OF WEIGHT LOSS AND DIET ON FECAL MICROBIOTA AND FECAL METABOLOMICS IN CATS. Adam Rudinsky[1], Katie McCool[1], Valerie Parker[1], Prosper Boyaka[1], Josh Daniels[1], Rosana Lopes[2], Joerg Steiner[2], Jan Suchodolski[2], Chen Gilor[1]. [1]The Ohio State University College of Veterinary Medicine, Columbus, OH, USA, [2]Texas A&M University College of Veterinary Medicine, College Station, TX, USA

Intestinal microbiota and short chain fatty acids (SCFA) are important in a variety of metabolic disorders. We hypothesized that obesity and diet affect intestinal microbiota and SCFA in cats.

Eight cats were used in a repeated-measures study. Cats were fed a maintenance diet (M-diet) from study days -21-0 then switched to a weight-loss diet (WL-diet) on day 0 without calorie restriction until day 7. On days 8–84, daily intake was restricted to achieve 1–2% weight-loss per week. On days 84–98, feeding the M-diet resumed while maintaining stable body condition score (BCS). Fecal samples were collected and stored at −80°C until analysis. Samples were collected on day −7 and 0 (Obese/M-diet), 5 and 7 (Obese/WL-diet), 77 and 84 (Lean/WL-diet) and 86, 89, 91 and 98 (Lean/M-diet) for microbiota and SCFA profiles. Body weight, BCS, muscle condition score, and body fat percentage were assessed throughout the study.

Microbiota and SCFA profiles revealed changes associated with diet and BCS. Microbiota diversity and species richness alterations were noted. Acetic and butyric acid production was significantly different between obese and lean cats. Diet and BCS had significant interaction in the statistical model for remaining SCFA. On post-hoc analysis, isobutyric, valeric and isovaleric acids were significantly different between obese and lean cats on the maintenance diet. Valeric and isovaleric were different between the M-diet and WL-diet in obese cats.

Microbiota and SCFA profile alterations were noted based on body condition. Further study is warranted to determine the impact of these changes on inflammation and metabolic disorders.

GI12

PROBIOTIC MIXTURE VSL#3 INCREASES BENEFICIAL FECAL AND MUCOSAL MICROBIOTA IN CANINE INFLAMMATORY BOWEL DISEASE. Jan Suchodolski[1], Robin White[2], Blake Guard[1], Todd Atherly[3], Peter Sciabarra[1], Steve Hill[4], Craig Webb[5], Curtis Mosher[1], Giacomo Rossi[6], Albert Jergens[1]. [1]Texas A&M University, College Station, TX, USA, [2]Iowa State University, Ames, IA, USA, [3]USDA-ARS, Ames, IA, USA, [4]Veterinary Specialty Hospital, San Diego, CA, USA, [5]Colorado State University, Fort Collins, CO, USA, [6]University of Camerino, Camerino, Italy

Canine inflammatory bowel disease (IBD) is an immune-mediated enteropathy likely triggered by environmental and immunoregulatory factors in genetically susceptible dogs. Previous studies suggest a pivotal role for intestinal bacteria in disease pathogenesis since luminal microbial composition is markedly altered at diagnosis. It has been suggested that probiotic bacteria are effective and promising agents for the treatment of IBD in humans and animals. However, the effects of probiotic bacteria that directly interact with the host's resident intestinal microbiota are poorly understood. The aim of the present study was to investigate the impact of multi-strain probiotic VSL#3 supplementation (Visbiome; Exegi Pharma) at a dose of 50 billion bacteria/kg/day on the fecal and intestinal mucosal microbiota in dogs with IBD. Nineteen dogs diagnosed with moderate-to-severe IBD (CIBDAI ≥ 6) were randomized to receive standard IBD therapy (ie, elimination diet and prednisone) with or without probiotic VSL#3 for 8 weeks. Dogs were evaluated clinically at 0, 3, and 8 weeks and endoscopically and histopathologically at 0 and 8 weeks. The fecal and mucosal microbiota were investigated before and during treatment using 16S rRNA based quantitative polymerase chain reaction (qPCR) and fluorescence in situ hybridization (FISH), respectively. Both cohorts of IBD dogs showed a reduction in GI signs after 8 weeks of therapy compared to baseline CIBDAI scores ($P < 0.05$). Using qPCR, the placebo group failed to show significant changes in any of the bacterial groups analyzed. In contrast, VSL#3 treated dogs showed a significant ($P = 0.001$) increase in total lactic acid bacteria (*Bifidobacteria*, *Streptococci*, *Lactobacilli*) at treatment weeks 3 and 8 versus baseline values. *Bifidobacteria* were also significantly ($P = 0.018$) increased at 3 and 8 weeks post-VSL#3 treatment. FISH analysis showed that VSL#3 altered the number and spatial distribution of most colonic mucosal bacterial groups including total bacteria and the number of *Bifidobacteria*, *Fecalibacteria*, *Lactobacilli*, *Streptococci*, and *Enterobacteriaceae* ($P < 0.05$ for each). The number of mucus-laden and attaching *Bifidobacteria* and *Streptococci* were also significantly ($P < 0.05$) increased in dogs receiving VSL#3. In conclusion, probiotic therapy of IBD dogs with VSL#3 increases the number of fecal and mucosal probiotic genera, especially the lactic acid bacteria. IBD dogs receiving standard therapy (placebo) showed little change in the composition of microbial communities despite exhibiting clinical improvement.

GI13

CHANGES IN INTESTINAL MACROPHAGE POPULATIONS FOLLOWING CLINICAL RESOLUTION IN DOGS WITH CHRONIC ENTEROPATHY. Julien R.S. Dandrieux[1], Wayne Kimpton[1], Barbara Bacci[2], Karin Allenspach[3], Albert E. Jergens[4], Caroline Mansfield[1]. [1]University of Melbourne, Werribee, Vic., Australia, [2]University of Surrey, Guildford, Surrey, UK, [3]Royal Veterinary College, London, UK, [4]Iowa State University, Ames, IA, USA

Chronic enteropathy (CE) is a multi-factorial disease, which involves aberrant immune responses to commensal bacteria or dietary antigens. Although macrophages have been shown to play an important role in human disease very little information is available in dogs. CD163 is a scavenger receptor specific for macrophages, and has been detected in canine duodenum. The aim of this study was to determine if intestinal CD163 positive cell ($CD163^+$) populations alter after successful treatment of CE. Calprotectin was used as an additional marker for macrophages.

Dogs were prospectively enrolled and underwent standard diagnosis and treatment trial for CE. Duodenal biopsies were taken before and after long-term resolution of clinical signs (≥2 months). Serial histologic sections were stained for CD163 (using AM-3K) and calprotectin. Positively stained cells in five villous and crypt regions were manually counted. The percentages of positively stained cells from the total nucleated cells per 10,000 μm^2 before and after treatment were compared with repeated-measure ANOVA.

Five dogs had dietary-responsive and three steroid-responsive CE. $CD163^+$ significantly increased following treatment in the villi (from 4.8% [0–12.6] to 6.2% [0.0–12.4], P = 0.043), whereas $calprotectin^+$ significantly decreased (from 3.7 [0.0–11.7] to 2.0 [0.0–9.7]). Increased $CD163^+$ (5.3 [1.0–9.7] to 6.2 [0.8–12.5]) and decreased $calprotectin^+$ (1.3 [0.0–4.2] to 0.9 [0.0–4.5]) was also observed following treatment in the crypts, although this was not significant. Similar changes were seen in food and steroid responsive dogs.

These results suggest that macrophages may play a role in canine CE. Further characterization of macrophages is warranted to understand their role in CE.

GI14

HISTOPATHOLOGIC CHARACTERISTICS OF INTESTINAL BIOPSIES FROM DOGS WITH CHRONIC ENTEROPATHY WITH AND WITHOUT HYPOALBUMINEMIA. Sara Wennogle[1], Craig Webb[1], Simon Priestnall[2]. [1]Colorado State University, Fort Collins, CO, USA, [2]Royal Veterinary College, Hertfordshire, UK

The term protein-losing enteropathy (PLE) is often applied to cases of chronic enteropathy (CE) when the disease process results in hypoalbuminemia. Multiple previous studies have identified hypoalbuminemia as a risk factor for a negative outcome in dogs with chronic enteropathy, yet it has not been determined whether the histopathology differs between CE dogs with and without hypoalbuminemia. The goal of this retrospective study was to compare histopathologic findings (as determined by the WSAVA scoring system) in dogs with CE with and without hypoalbuminemia.

Electronic medical records at Colorado State University were reviewed for dogs having intestinal biopsy performed between January 2010–July 2015. Included dogs had clinical signs compatible with CE of at least 3 weeks duration. Dogs with significant concurrent disease, potential non-GI causes of hypoalbuminemia, intestinal lymphoma, or for which a complete medical record could not be obtained were excluded. 83 cases were suitable for inclusion. Dogs were placed in either the CE (albumin ≥ 3.0 gm/dL), or CE w/ PLE (albumin <3.0 gm/dL) group. Recorded data included signalment, presenting complaint, clinical signs, additional clinicopathologic abnormalities, tissue types available, and method of biopsy procurement. A blinded pathologist (SLP) was asked to review all biopsies and apply the WSAVA scoring system to each intestinal biopsy. In cases where more than one tissue type was available (13/83), the tissue type with the highest total WSAVA score was used in the analysis.

Results are summarized in the table below. The most notable discrepancies between groups occurred in the categories of crypt distension (CD), lacteal dilation (LD), villous stunting (VS), and lamina propria neutrophils (LP N), with a greater proportion of dogs in the CE w/ PLE exhibiting those changes on histopathology. Villous epithelial injury (VEI) and intraepithelial lymphocytes (IEL) were also appreciably more common in dogs in the CE w/ PLE group. Lamina propria lymphocytes and plasma cells (LP L/PC) and lamina propria eosinophils (LP E) were observed in both groups. Mucosal fibrosis (MF) was uncommon in both groups.

Dogs with PLE secondary to CE had a greater percentage of lacteal dilation, injury to the villi, crypt distension, and lamina propria neutrophils than dogs with CE that had normal albumin.

Furthermore, although lacteal dilation was noted in 76% of PLE cases, it was listed as a primary diagnosis in only 5 cases. Further investigation is required to determine if these differences represent a difference in underlying etiology, or should alter treatment strategy in specific cases.

	LP L/PC	LP E	LP N	IEL	LD	VS	VEI	CD	MF
CE	22/46 (48%)	12/46 (26%)	7/46 (15%)	1/46 (2%)	16/46 (35%)	23/46 (50%)	4/46 (9%)	5/46 (11%)	0/37 (0%)
CE w/ PLE	23/37 (62%)	11/37 (30%)	17/37 (46%)	11/37 (30%)	28/37 (76%)	35/37 (95%)	14/37 (38%)	24/37 (65%)	2/37 (5%)
Total	45/83 (54%)	23/83 (28%)	24/83 (29%)	12/83 (14%)	44/83 (53%)	58/83 (70%)	18/83 (22%)	29/83 (35%)	2/83 (2%)

GI15

HUMAN GRANULOCYTE IMMUNOFLUORESCENCE ASSAY FOR ANTI-NEUTROPHIL ANTIBODIES SHOWS STRONG ASSOCIATION WITH CANINE FOOD RESPONSIVE DIARRHEA. Karin Allenspach[1], Shayna Streu[2], Valerie DiMuro[3], Anna Riddle[1], Joseph Kirk[1], Lucrezia Perazzotti[4], Regina Wagner[5], Elisbabeth Mueller[5], Jessica Florey[1]. [1]Royal Veterinary College, London, UK, [2]Michigan State University, East Lansing, MI, USA, [3]Arcadia University, Philadelphia, PA, USA, [4]Euroimmun UK Ltd, Wimbledon, UK, [5]Laboklin AG, Bad Kissingen, Germany

Perinuclear anti-neutrophilic antibodies (pANCA) have previously been shown to be serum markers in canine IBD, and more likely to be associated with food responsive diarrhea (FRD) cases than steroid responsive disease (SRD). The indirect immunofluorescence assay used in previous publications is based on isolation of canine granulocytes from donor dogs, which are subsequently fixed in ethanol on microscope slides, and then incubated with the patient serum and secondary antibody. This assay is time consuming and has been shown to have low inter-observer agreement. In this pilot study we therefore sought to investigate whether a commercially available human granulocyte indirect immunofluorescence assay could be used to detect pANCA in dogs and to evaluate preliminary sensitivity and specificity of the assay for groups of dogs with FRD *versus* SRD.

Forty-four dogs with FRD and 20 dogs with SRD were included in the study. Granulocyte Mosaic Biochip slides (EUROIMMUN AG) containing human granulocytes fixed in ethanol, human granulocytes fixed in formalin, purified myeloperoxidase protein (MPO), purified proteinase-3 protein (PR-3), and HEp-2 cells were used for the assay. These biochips allow detection of pANCA, cytoplasmic antineutrophil antibodies (cANCA), as well as anti-nuclear antibodies (ANA) on the same slide. The assay was performed according to manufacturer's instructions. Briefly, biochips were incubated with 30ul of 1:10 and 1:100 diluted serum samples for 30 Min, then washed and incubated with secondary anti-dog IgG FITC-labeled antibody for 30 Min. After washing, the slides were embedded in medium, covered and evaluated under fluorescence light at 20–40× magnification by two independent observers.

The number of pANCA or cANCA positive results in the group of FRD dogs was 27/44 (61%). To the contrary, none of the dogs in the SRD group had evidence of pANCA or cANCA seroreactivity (0/20). Consequently, sensitivity of the new assay to predict FRD amongst a group of dogs with chronic enteropathies was 0.61 (95% CI 0.45–0.75); and specificity was 1.0 (95% CI 0.89–1.0). This is comparable to previous studies using the canine granulocyte method (sensitivity 0.51; specificity 0.95). Fishers exact test revealed a statistically significant association of a positive pANCA or cANCA test with FRD ($P < 0.0001$). Inter-observer agreement for the assay was good (kappa 0.75). Furthermore, in 8 of the ANCA positive cases, it was possible to identify either MPO or PR-3 as antigens on the biochips.

In conclusion, the human granulocyte immunofluorescence assay used in this study was able to identify sero-positivity for ANCA in a high percentage of dogs with FRD. The data suggests that this assay could be used as an alternative to the canine assay for detection of ANCA in dogs.

GI16

SERUM CITRULLINE CONCENTRATIONS IN DOGS WITH CHRONIC ENTEROPATHY. Rosana Lopes, Yuri Lawrence, Robert K. Phillips, Jonathan A. Lidbury, Jan S. Suchodolski, Jörg M. Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA

Citrulline, a non-proteinogenic amino acid, has recently been described as a biomarker of enterocyte function in humans. Decreased concentrations of plasma citrulline have been reported in patients with short bowel syndrome, Crohn's disease, viral enteritis, or in critically ill patients. In dogs, the relationship between serum citrulline concentration and small intestinal dysfunction has not been well characterized. Citrulline is mainly synthesized from glutamine in enterocytes and is metabolized in the duodenum and jejunum. Therefore, intestinal injury can lead to decreased serum citrulline concentrations. The aims of this study were to analytically validate a GC/MS method for the measurement of citrulline in canine serum and to assess the concentration of this amino acid in dogs with chronic enteropathy.

Surplus serum samples from 40 dogs were used for analytical validation of the citrulline assay. Thirty-one surplus serum samples from healthy dogs and dogs with chronic enteropathy were used to evaluate possible alterations in serum citrulline concentrations in dogs with chronic enteropathy. Validation variables included the lower limit of detection, linearity, spiking recovery, and precision. Serum citrulline concentrations from 19 dogs with chronic enteropathy were compared to those from 12 healthy dogs. A Mann-Whitney *U* test was performed to compare serum citrulline concentrations between healthy and diseased dogs. Statistical significance was set as $P < 0.05$.

The lower limit of detection for citrulline in serum was 4.9 μmol/L. Observed-to-expected ratios for citrulline dilutional parallelism (n = 6) were 83.2% to 116.7%. Recoveries for spiking concentrations of citrulline in serum samples (n = 5) were between 84.9% to 119.4%. Mean intra- and inter-assay coefficients of variation (%CVs) for the citrulline assay were 5.4% and 10.9%, respectively. Dogs with chronic enteropathy had significantly lower serum citrulline concentrations (median [minimum - maximum]: 10.5 μmol/L [2.8 - 46.0]) than healthy dogs (median [minimum - maximum] 32.5 μmol/L [20.0 - 44.0]; $P < 0.0001$).

The assay evaluated here was shown to be linear, precise, accurate, and reproducible. Dogs with chronic enteropathy had significantly lower serum citrulline concentrations than healthy dogs. Further studies are warranted to assess the clinical utility of the serum citrulline as a biomarker for enterocyte function in dogs.

GI17

ORAL VERSUS PARENTERAL COBALAMIN SUPPLEMENTATION IN DOGS WITH CHRONIC ENTEROPATHIES AND HYPOCOBALAMINEMIA. Linda Toresson[1], Joerg Steiner[3], Jan Suchodolski[3], Thomas Spillmann[2]. [1]Evidensia Specialist Animal Hospital, Helsingborg, Sweden, [2]Helsinki University, Helsinki, Finland, [3]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA

Hypocobalaminemia is a common sequel to chronic enteropathies (CE) in dogs and has been reported to be associated with a negative outcome. Current supplementation protocols for

cobalamin (cbl) in dogs call for parenteral (PE) supplementation, although no protocol has been validated. In humans, several studies have reported equal efficacy of per oral (PO) and PE administration of cbl. Further, this group has previously reported about successful PO cbl supplementation in dogs with CE and hypocobalaminemia in a retrospective study. Thus, the purpose of this prospective randomized study was to validate the current PE and a new PO cbl supplementation protocol and compare the efficacy of PO versus PE cbl supplementation in dogs with hypocobalaminemia.

Dogs with symptoms of CE and a serum cbl below 285 ng/L (reference interval: 244–959 ng/L) were included from Evidensia Specialist Animal Hospital, Helsingborg, Sweden, Helsinki University Hospital, Finland, and from two other Swedish Veterinary Clinics. Dogs were randomized to treatment with either daily PO cbl tablets during the whole study period (cyanocobalamin (Behepan) 1 mg/tablet; dogs up to 10 kg received ¼ tablet, dogs 10 to 20 kg ½ tablet and dogs ≥ 20 kg 1 tablet/day) or PE cbl according to a protocol currently suggested by a diagnostic laboratory (http://vetmed.tamu.edu/gilab/research/cobalamin-information#-dosing). A block-randomized schedule was performed by an external statistician prior to the start of the study in March 2014. The study was approved by the Swedish Board of Agriculture and the Viikki Campus Research Ethics Committee, Finland. Serum cbl for follow-up was analyzed 28 ± 5 days and 90 ± 15 days after initiation of supplementation using an automated chemiluminescence immunoassay (Immulite 2000, Siemens Healthcare Diagnostics).

41 dogs of 22 breeds were included with a median age of 6.2 (range 1.7–13.1) All of the dogs had symptoms of CE. Concurrent medical treatment and diet was given based on clinical judgement. Intestinal biopsies from the small and large intestine confirming chronic inflammation were available from 25/41 dogs. There was no statistically significant difference in serum cbl concentrations at baseline between the two groups ($P = 0.25$, Mann Whitney test). Serum cbl increased in all dogs after supplementation. In the group receiving PO supplementation, median serum cbl concentrations was 245 ng/l (150–285) at inclusion (n = 22), 970 ng/L (564–2026) after 28 days (n = 22), and 1334 ng/L (768–3921) after 3 months (n = 20). The difference between baseline and 28 days was statistically significant ($P < 0.0001$; Wilcoxon matched-pairs signed rank test) as well as the difference between 28 days and 3 months (<0.006; Wilcoxon matched-pairs signed rank test). In the group receiving PE cbl, mean cbl concentration was 221 ± 43 ng/L at inclusion (n = 19), 1686 ± 757 ng/L after 28 days (n = 19), and 803 ± 275 ng/L (n = 13) after 3 months. The difference between baseline and 28 days was statistically significant ($P < 0.0001$; paired t-test) as well as the difference between 28 days and 3 months ($P = 0.0003$, paired t-test). Comparing the increase in serum cbl concentrations at 28 days to baseline, the increase was significantly higher in the PE group than the PO group (mean increase 1465 ± 745 ng/L and 888 ± 449 ng/L, respectively, $P = 0.004$, unpaired t-test). However, after 3 months, the median (range) increase in serum cbl concentrations compared to baseline group were significantly higher in the PO than the PE group (970 ng/L (538–3663) and 608 ng/L (38–997), respectively, p = 0.0001, Mann Whitney test).

We conclude that both PO and PE cbl supplementation appears to effectively increase serum cbl concentrations in dogs with CE and hypocobalaminemia. Further studies comparing cellular cobalamin status after PO or PE supplementation are warranted.

GI18
PLASMA ESSENTIAL TRACE ELEMENT CONCENTRATIONS IN DOGS WITH CHRONIC ENTEROPATHY. Nozomu Yokoyama, Hiroshi Ohta, Hazuki Mizukawa, Yumiko Kagawa, Shouta Nakayama, Noboru Sasaki, Keitaro Morishita, Kensuke Nakamura, Yoshinori Ikenaka, Mayumi Ishizuka, Mistuyoshi Takiguchi. Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

Chronic enteropathy (CE) is a common cause of malnutrition in dogs. Essential trace elements (ETE) are defined as micronutrients that are required for proper biochemical processes in the body. To date, little is known about plasma ETE status in dogs with CE. The aim of this study was to determine whether ETE deficiency occurred in dogs with CE.

Dogs with CE that were presented to Hokkaido University Veterinary Teaching Hospital between July 2014 and June 2015 were recruited. Fourteen laboratory beagles were used as controls. Plasma ETE concentrations (Chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum) were measured by inductively coupled plasma-mass spectrometry. Plasma ETE concentrations in CE dogs were compared with those of controls. In CE dogs, correlation between plasma ETE concentrations and canine chronic enteropathy activity index (CCECAI), plasma albumin concentration and histopathologic score calculated according to the World Small Animal Veterinary Association (WSAVA) criteria.

Twenty-two dogs with CE were enrolled in this study. There were no significant differences between plasma ETE concentrations of CE dogs and those of controls, except for molybdenum. Plasma molybdenum concentration in CE dogs (median 6.9 ng/mL, range 0.38–60 ng/mL) was higher than that of controls (median 3.2 ng/mL, range 0.72–4.7 ng/mL) ($P = .015$). However, plasma molybdenum concentration didn't correlate with CCECAI, albumin concentration and histopathologic score.

These results indicate that there might be no obvious plasma ETE deficiency in dogs with CE. Further study is necessary to clarify the reason for increased plasma molybdenum concentration in dogs with CE.

GI19
VALIDATION OF ULTRASONOGRAPHIC MEASUREMENT OF GASTRIC EMPTYING TIME IN HEALTHY CATS USING RADIONUCLIDE SCINTIGRAPHY. Roman Husnik, Jon Fletcher, Lorrie Gaschen, Frederic Gaschen. Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

The prevalence of gastric emptying (GE) disorders in cats is unknown due to lack of clinically applicable diagnostic test. This study assesses agreement for GE times between ultrasonography and the gold standard in 7 healthy cats.

For scintigraphy, 60-second static acquisitions were performed in right and left lateral recumbency at 0, 5, 15, 30, 45, and 60 minutes following a 4 mCi Tc99M-mebrofenin solid test meal (20% of daily energy requirements), and at 30-minute intervals thereafter. Mean total counts from a gastric region of interest were measured from both images. Geometric means of the decay-corrected counts were calculated, and the amount of gastric radioactivity was expressed as a percentage of the initial count. Using nonlinear regression analysis, 25%, 50%, 75% and 90% GE times were determined. Sonographic assessment of GE was repeated three times after the same meal (non-labeled). Using the same time points, mean cross-sectional area of transverse images of the relaxed antrum was obtained and expressed as a percentage of the maximal area. The area under the curve was calculated and 25%, 50%, 75% and 90% GE times determined.

Good correlation was found between scintigraphy and sonography for the 75% (287 ± 67 [mean ± SD] and 306 ± 28 minutes respectively, r = 0.85, $P < 0.02$) and 90% GE times (340 ± 77 and 381 ± 29 minutes respectively, r = 0.85; $P < 0.02$). Ultrasound evaluation of antral cross-sectional area is a valid alternative to scintigraphy in the late phase of GE in healthy cats.

GI20
EFFECT OF MOSAPRIDE ON POSTPRANDIAL GALLBLADDER MOTILITY AND PLASMA LEVELS OF MOTILIN IN DOGS. Toshiaki Kakimoto, Hideyuki Kanemoto, Kenjiro Fukushima, Koichi Ohno, Hajime Tsujimoto. The University of Tokyo, Tokyo, Japan

Impairment of gallbladder motility might be responsible for the pathogenesis of gallbladder mucocele and biliary sludge in dogs

and the improvement of the gallbladder motility has a possibility to exert the preventive effects on the progression of the gallbladder diseases. However, little has been studied on the treatments to enhance the gallbladder motility in dogs. It was reported that 5-HT_4 receptor agonist improves gallbladder emptying in human gallstone patients, and that mosapride, which is a selective 5-HT_4 receptor agonist practically used for prokinetics of canine gastrointestinal disease, induces the increase of motilin, which is a physiological inducer of gallbladder motor activity. In this study, we investigated the effects of mosapride on the postprandial gallbladder motility and the plasma levels of motilin in dogs with or without biliary sludge. Furthermore, we examined the expression of 5-HT_4 receptor using gallbladder tissue of a normal dog.

Nine apparently healthy beagles, owned by the University of Tokyo, were enrolled in this study. Of these, 5 did not have any gallbladder abnormality (group N), and 4 had biliary sludge (group S) based on the assessment by ultrasonography. Gallbladder ejection fractions (EF) were compared between dogs that were given diet with and without simultaneous single oral administration of mosapride 1 mg/kg. EF was estimated by ultrasonography, as described in the previous study. Briefly, dogs were fasted at least 12 hours before examination. Once the fasting gallbladder volume was ultrasonographically measured, they were given a test diet with and without mosapride. Postprandial gallbladder volume was recorded at an hour after the feeding. Before each ultrasonography, blood was collected for the measurement of plasma motilin levels. Experiments and animal care complied with the policies outlined in the Guide to Animal Use and Care of the University of Tokyo. The expression of 5-HT_4 receptor was evaluated by Western blotting using an archived canine gallbladder.

In the group N, median EF of the dogs treated with mosapride was significantly higher than those without the treatment [46.1% (range: 44.1 to 60.6) versus 38.1% (24.2 to 43.5%), respectively, $P < 0.05$]. Such tendency was also observed in the group S, although the difference was not significant [treatment: 43.3% (33.9 to 63.2%) versus without treatment: 34.8% (23.6 to 37.7%), $P = 0.06$]. However, the median degree of the increase of the plasma motilin concentrations after feeding was not significantly different between the dogs with and without mosapride in either group [Group N: mosaporide: −1.6 pg/mL (range: - 123.3 to 168.3 pg/mL) versus feeding only: 65.0 pg/mL (- 25.0 to 415.0 pg/mL), Group S: mosaporide: 37.0 pg/mL (range: - 273.0 to 95.0 pg/mL) versus feeding only: 173.3 pg/mL (5.0 to 363.3 pg/mL)]. In Western blotting, an immunoreactive band was observed at the position of 45 kDa using the protein purified from the archived gallbladder, suggesting that 5-HT_4 is expressed in the canine gallbladder.

These results suggested that mosapride enhances postprandial gallbladder motility. Although the mechanisms of this effect are still to be clarified, our results suggested that mosapride enhances gallbladder motility possibly via gallbladder 5-HT_4 receptor and motilin does not play a role in this effect. Further studies are needed to support our results.

GI21
EVALUATION OF THE PRESENCE AND ROLE OF CYSTEINE PROTEASE 30 IN FELINE *T. FOETUS*.

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Tritrichomonas foetus (Tf) is a flagellated protozoan parasite that is recognized as a significant cause of diarrhea in domestic cats with a prevalence rate as high as 30%. No drugs have been shown to consistently eliminate Tf infection in all cats. Cysteine proteases (CPs) have been identified as mediators of Tf-induced adhesion-dependent cytotoxicity to the intestinal epithelium. Thus, CPs represent novel targets for the treatment of feline trichomonosis. However, cats also produce CPs that are part of life-critical systems. Thus, parasitic CPs need to be selectively targeted to reduce the potential for host toxicity. Previous studies have demonstrated the importance of a specific CP, CP30, in mediating bovine and human trichomonad cytopathogenicity. Thus, the aims of this study were to characterize the presence of CP30 in feline Tf isolates and to evaluate the effect of targeted inhibition of CP30 on feline Tf-induced adhesion dependent cytotoxicity.

The presence of CP30 in 4 different feline Tf isolates was evaluated by indirect immunofluorescence and flow cytometry using a rabbit polyclonal antibody that targets bovine Tf CP30 (α-CP30). Feline Tf treated with rabbit IgG and/or secondary Ab in the absence of α-CP30 Ab and bovine Tf were used as negative and positive controls, respectively. The effect of inhibition of CP30 activity on Tf adhesion-dependent cytopathogenicity was determined using CFSE-labeled feline Tf that were allowed to adhere to monolayers of porcine intestinal epithelial cells (IPEC) in co-culture. The effect of CP30 inhibition on feline Tf adhesion and cytotoxicity was evaluated by immunofluorescence quantification assays and crystal violet spectrophotometric analysis. A minimum of 6 replicates were performed for each co-culture experiment. Data were analyzed using Systat software ($P < 0.05$).

CP30 expression was identified in all feline Tf isolates tested by indirect immunofluorescence and flow cytometry. Treatment of feline isolates with α-CP30 Ab resulted in significantly decreased Tf adhesion to and cytotoxicity towards IPEC-J2 monolayers compared to rabbit IgG-treated Tf isolates.

These studies establish that CP30 is expressed by feline Tf isolates and may be an important virulence factor in the cytopathogenicity of feline Tf. The results of these studies provide strong evidence-based justification for investigation of CP30 as a novel target for the prevention and/or treatment of feline trichomonosis.

GI22
EVALUATION OF THE EFFECT OF OMEPRAZOLE ON SERUM CALCIUM, MAGNESIUM, GASTRIN AND BONE IN CATS.

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Chronic proton pump inhibitor administration has been associated with electrolyte imbalances and cobalamin deficiency, disrupted bone homeostasis, serum hypergastrinemia and rebound acid hypersecretion in humans. These side effects could have important clinical implications in cats. However, no studies have explored if cats receiving prolonged oral omeprazole (e.g., > 4 weeks in duration) experience similar side effects. We hypothesized that prolonged oral omeprazole administration would result in altered bone homeostasis and/or serum calcium, magnesium, cobalamin and/or gastrin concentrations in healthy cats.

In a within subjects, pre to post design, 6 adult healthy colony cats received either omeprazole (0.83–1.6 mg/kg PO q12 h) or placebo (250 mg lactulose PO q12 h) for 60 days. Analyses of serum calcium, magnesium, cobalamin and gastrin concentrations were performed on days 0, 30, and 60, with dual energy x-ray absorptiometry performed at days 0 and 60 for each treatment. On day 60 of omeprazole treatment, intragastric pH capsules were placed in two of the cats to measure the effects of rapid omeprazole cessation. Continuous data were analysed using a two-way ANOVA and tested to account for the effects of phase of treatment, time of measurement and the interaction of phase and time. Log transformation of serum gastrin, bone mineral density (BMD) and bone mineral content (BMC) were required for the data to meet ANOVA normality assumptions. To reduce the possibility of a Type I error due to analysis of multiple study variables, the Bonferroni correction was applied ($\alpha = 0.006$). SAS 9.4 was used to perform all data analyses and generate all descriptive statistics.

No significant changes were observed between treatment and placebo for any parameters, except serum gastrin, which was significantly higher during omeprazole treatment in comparison to placebo ($P < 0.002$). Although the change was not significant, all cats experienced a decrease in BMC by the end of the omeprazole treatment arm. Evidence of gastric hyperacidity was seen in both cats following cessation of omeprazole.

In conclusion, the results of this study provide evidence that omeprazole may be relatively safe when administered to cats for 60 days or less, but slow withdrawal of the medication is recommended based on the hypergastrinemia and rebound hyperacidity

demonstrated in this pilot trial. However, further studies involving more cats are needed to confirm these results.

GI23
GRAVITY-ASSISTED ESOPHAGEAL TRANSIT CHARACTERISTICS IN DOGS WITH MEGAESOPHAGUS. Jillian Haines[1], Alison Khoo[2], John Thomason[3], Erin Brinkman[3], Andrew Mackin[3]. [1]Washington State University, Pullman, WA, USA, [2]Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada, [3]Mississippi State University, Mississippi State, MS, USA

Treatment of megaesophagus (ME) often relies on feedings in a vertical position. Little is known about the behavior of food material in the esophagus during transit to the stomach in dogs with ME. The aims of our project were to standardize a contrast video fluoroscopy technique to evaluate esophageal transit time in dogs with ME while sitting in a vertical position, and to use this information to determine an appropriate duration an individual patient with ME should remain vertical after drinking and feeding.

Twelve dogs with ME were included in the study. Six dogs had congenital ME, 4 dogs had idiopathic acquired ME, and 2 dogs had ME secondary to myasthenia gravis. All dogs were placed in a vertical position using adjustable size-appropriate Bailey chairs. Bailey chairs were constructed from thin wood which allowed them to be radiolucent. In three different feeding sessions, dogs were given standardized amounts of liquid barium, canned food meatballs mixed with barium, or their normal diet also mixed with barium (if the diet was a different consistency than meatballs, generally a slurry). Fluoroscopy was performed in 5 minute increments to visualize transit of food or barium from time to ingestion until full clearance of material from the esophagus, or for a maximum of 20 minutes for liquid barium and 30 minutes for food material.

In dogs that were accustomed to the Bailey chair, performance of contrast video fluoroscopy was technically straightforward. Significant individual variation was seen between dogs for transit time of all three ingesta types. Ingesta consistency affected transit time, but the consistency that allowed the most rapid transit time varied by individual. Mean transit time for liquid was 15.4 mins with 58% (7/12) of dogs having no clearance of liquid by 20 mins. Mean transit time for meatballs was 24.6 mins with 67% (8/12) having incomplete clearance of meatballs at 30 minutes. Mean transit time for the slurry consistency was 26.3 mins with 75% (6/8) of tested dogs having incomplete clearance of food at 30 mins. Three dogs were identified as having gastroesophageal reflux and one dog was found to have a concurrent swallowing disorder. Based on individual imaging results, recommendations were made to alter management strategies for 8/12 dogs; change food consistency in 50%, change time kept upright in 25% of dogs, and alter pharmacologic therapy in 17%.

Fluoroscopy performed utilizing vertical positioning in modified Bailey chairs in dogs with ME provided clear information that allowed for individual evaluation of the most ideal food consistency, anticipated behavior of ingested water, and overall esophageal transit time for ingested food material. This information permitted informed decision-making with regards to what food type appeared to be best for individual dogs, whether providing drinking water at times separate to feeding was likely to be well tolerated, and how long the dog would need to remain in a vertical position after eating or drinking. The imaging also allowed for identification of concurrent disorders affecting food tolerance and allowed for more specific management strategies in the majority of dogs.

GI24
FEASIBILITY OF MEASURING GASTROINTESTINAL TRANSIT TIME IN HEALTHY DOGS USING ALICAM. Jill S. Pomrantz[1], Jonathan A. Lidbury[2], Brian T. Hardy[3], Jörg M. Steiner[2], Jan S. Suchodolski[2], Brigitte B. Mcatee[2], Jeffrey A. Solomon[1]. [1]Infiniti Medical, LLC, Menlo Park, CA, USA, [2]Texas A&M University, College Station, TX, USA, [3]University of California, Davis, Davis, CA, USA

The lack of a simple and widely available test for assessing gastrointestinal motility in dogs can make diagnosing disorders difficult. Ambulatory light-based imaging is an easy to use, novel gastrointestinal imaging technique performed by oral administration of a fully automated capsule-sized camera device (ALICAM) that is propelled by natural peristalsis. The time required for passage of the capsule through the stomach and small intestine can be determined based on correlation of images with an internal clock. The aim of this study was to evaluate the feasibility of using ALICAM to measure gastric transit time (GTT) and small intestinal transit time (STT) in healthy dogs.

The study subjects were 10 clinically healthy dogs between 3 and 8 years of age that weighed 17–32 kg. Dogs were fasted for 16–24 hours before and 8 hours after capsule administration. Images were downloaded and independently reviewed by 3 board-certified internists. Each reviewer determined GTT and STT. The reproducibility of the results was compared between reviewers.

The median (min-max) GTT was 86 (13–218) minutes. The median (min-max) STT was 124 (34–168) minutes. There was agreement among the reviewers with an intra-class correlation coefficient of >0.999 and 0.969 for GTT and STT respectively. The upper limits of the 95% confidence intervals for GTT and STT in healthy dogs were 243 and 179 minutes, respectively.

Ambulatory light-based imaging can be used to assess gastrointestinal transit time in healthy dogs. Further studies are needed to determine its role in dogs with gastrointestinal disease.

GI25
COMPARISON OF GASTRIC TRANSIT TIME IN HEALTHY DOGS AND DOGS WITH SIGNS OF GASTRIC HYPOMOTILITY. Jill S. Pomrantz[1], Jonathan A. Lidbury[2], Brian T. Hardy[3], Jörg M. Steiner[2], Jan S. Suchodolski[2], Brigitte B. Mcatee[2], Jeffrey A. Solomon[1]. [1]Infiniti Medical, LLC, Menlo Park, CA, USA, [2]Texas A&M University, College Station, TX, USA, [3]University of California, Davis, Davis, CA, USA

Ambulatory light-based imaging is a gastrointestinal imaging technique performed by oral administration of a fully automated camera device contained in a capsule (ALICAM) that is propelled by natural peristalsis. Gastric transit time (GTT) can be determined by visualization of the capsule's passage from the stomach into the duodenum and correlating images with the clock function of the device. The aim of this study was to use the ALICAM system to compare the GTT in healthy dogs with the GTT in sick dogs with clinical signs that could be consistent with gastric hypomotility.

The study population consisted of a group of 10 clinically healthy dogs and a group of 26 dogs with retching, regurgitation, and/or vomiting. All dogs were fasted for 16–24 hours before and 8 hours after capsule administration. Studies were reviewed by a board-certified internist. For each case, GTT was determined. A Wilcoxin rank sum test was used to compare the two groups.

The median GTT (min-max) in healthy dogs was 86 (13–218) minutes. The median (min-max) GTT in sick dogs was 187 (2–1405) minutes. In 7 sick dogs, the capsule remained in the stomach for the duration of the study (435–1405 minutes) and the recorded time represented the minimum GTT. The GTT for sick dogs was significantly longer than for healthy dogs ($P = 0.046$).

The results of this study suggest that gastric hypomotility occurs in a subset of dogs presenting with retching, regurgitation, and/or vomiting. Further studies are needed to determine if these dogs would benefit from prokinetic therapy.

GI26

FEASIBILITY OF A NOVEL GASTROINTESTINAL IMAGING DEVICE FOR USE IN DOGS. Jill S. Pomrantz[1], Brian T. Hardy[2], Ajay Sharma[3], Jeffrey A. Solomon[1]. [1]infiniti Medical, Llc, Menlo Park, CA, USA, [2]University of California, Davis, Davis, CA, USA, [3]University of Georgia, Athens, GA, USA

The purpose of this study was to test the safety and feasibility of a disposable and fully automated ingestible camera system that images the gastrointestinal tract in ambulatory dogs.

Five ambulatory light-based imaging (ALI) devices were constructed and contained within a translucent 11x31 mm capsule. The key components were a battery, a light source, 4 auto-focusing cameras, an internal memory system, and a microprocessor with an accelerometer that synchronized camera activity with device motion.

Five client-owned dogs were food restricted for 24 hours before and 8 hours after capsule administration. Capsules were administered using a direct pilling technique. Normal activity and access to water were permitted throughout the study. After recovery, capsules were inspected for damage. Images were then downloaded and independently reviewed by three board-certified internists.

Capsules were successfully administered to 5/5 dogs and were recovered within 24 to 36 hours. There were no adverse events. Median (min-max) study duration was 16 (8–18) hours. Capsules recorded a median (min-max) of 19,713 (8572–51,683) images. Median (min-max) gastric and small intestinal transit times were 89 (10–110) minutes and 134 (68–177) minutes respectively. Visualization of the mucosa and image quality were described as excellent for the stomach and small bowel. The colon was poorly imaged due to retained feces.

ALI is safe and feasible in dogs. It produced high quality images throughout the majority of the gastrointestinal tract. Visualization of the colon might be feasible with additional preparation. This non-invasive technique may expand the role of imaging in dogs with gastrointestinal signs.

GI27

CORRELATION BETWEEN NOVEL IMMUNOHISTOCHEMICAL MARKERS, HISTOPATHOLOGY AND CLINICAL SCORES IN CATS WITH CHRONIC ENTEROPATHY. Giacomo Rossi[1], Alison Manchester[2], Jan Suchodolski[3], Craig Webb[2]. [1]University of Camerino, Matelica, Italy, [2]Colorado State University, Fort Collins, CO, USA, [3]Texas A&M University, College Station, TX, USA

A number of studies have demonstrated a poor correlation between histopathology and the clinical severity of chronic enteropathy in dogs, as well as the potential contribution of various immunohistochemical (IHC) markers to enhance interpretation of intestinal biopsies. Although a standardized scoring system has been developed for feline enteropathy using clinical parameters and histopathologic severity, a similar investigation, including the utility of IHC markers, has not yet been performed in cats. The purpose of this study was to identify correlations between histopathologic severity, immuno-histochemical markers, and clinical scoring of cats with chronic enteropathy.

A modified feline chronic enteropathy activity index score (FCEAI), a standard panel of gastrointestinal serum parameters, a standardized quantification of histopathology, a quantitative fecal score, and a group of immunohistochemical (IHC) markers were determined in 29 cats presented for signs consistent with chronic enteropathy. Study participants were from a client-owned population of cats experiencing diarrhea and/or vomiting of at least 3-weeks duration; had no evidence of a non-gastrointestinal disease based on history, physical examination, biochemical profile, complete blood count, urinalysis, total T4, and fPLI and fTLI; and had endoscopy with biopsies and histopathology of the small intestine as part of their diagnostic work-up. Serum cobalamin and folate concentrations were also determined. Histopathologic grading and IHC quantification was performed by a single, board certified pathologist blinded to any additional information regarding the case. Immunohistochemistry was performed to identify the prevalence of cellular staining for TGF-β, CD3, Foxp3, and MAC387. The degree of cellular staining was measured at 3 random and separate areas and those 3 values were averaged to give the final immunohistochemical score for each particular marker. Biochemical parameters, FCEAI scores, and histopathologic grading scores were tested for normality using the Kolmogorov-Smirnov test, and since the vast majority of data was not normally distributed, non-parametric statistics were used for analysis.

Nonlinear regression analysis demonstrated a significant positive correlation between the FCEAI score and all 4 IHC markers: TGF-beta (r^2 0.24, $P = 0.008$), CD3 (r^2 0.24, $P = 0.011$), Foxp3 (r^2 0.17, $P = 0.026$), and MAC 387 (r^2 0.25, $P = .006$). The histopathologic severity score was found to have a significant negative correlation with the IHC for TGF-beta (r^2 0.29, $P = 0.004$) and Fox p3 (r^2 0.25, $P = 0.007$) and a significant positive correlation with CD3 (r^2 0.40, $P = 0.0007$), but not MAC 387. This study failed to demonstrate a significant correlation between the histopathologic severity score and either the fecal score or the modified FCEAI scoring index.

As with dogs, there appears to be an absence of a significant correlation between histologic severity and clinical scorings such as a clinical severity index or fecal score in cats with chronic enteropathy. A significant correlation was, however, identified between a novel set of IHC markers and both the histopathology and clinical severity scores. The pathophysiologic relevance and clinical utility of these markers remains to be determined.

GI28

FECAL D-/L-LACTATE CONCENTRATIONS AND ABUNDANCE OF LACTIC ACID BACTERIA IN DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY. Amanda Blake, Joseph Cyrus Parambeth, Anitha Isaiah, Blake Guard, Jonathan Lidbury, Jorg Steiner, Jan Suchodolski. Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA

Exocrine pancreatic insufficiency (EPI) in dogs is characterized by maldigestion as a result of inadequate synthesis and secretion of pancreatic enzymes. Previous studies have shown that dogs with EPI have small intestinal dysbiosis (SID) and may require concurrent antibiotic therapy in addition to pancreatic enzyme replacement therapy. In humans, patients with short bowel syndrome, which is also characterized by maldigestion and SID, have been reported to have increased fecal lactate concentrations. Therefore, the aim of this study was to compare the abundance of lactic acid-producing bacteria and D- and L-lactate concentrations in feces from healthy dogs (n = 18), dogs undergoing enzyme replacement therapy for EPI (n = 17), and untreated dogs with EPI (n = 5).

Inclusion criteria for dogs with EPI were: a serum cTLI concentration $\leq$ 2.5 μg/L, age $\geq$ 1 year, clinical signs of EPI, and no other concurrent disease. Healthy dogs had no clinical signs of gastrointestinal disease. Fecal samples were obtained for three consecutive days, pooled, and DNA was extracted using the PowerSoil®DNA Isolation Kit (MoBio Laboratories) for analysis of lactic acid producing bacteria by quantitative PCR. Fecal samples were deproteinized and fecal D- and L-lactate were measured by enzymatic methods (R-Biopharm, D-/L-lactic acid kit). A Kruskal-Wallis test followed by Dunn's post-test was used to compare lactate concentrations and bacterial abundances between groups. Statistical significance was set at $P < 0.05$.

D-lactate was significantly increased in both the treated (median [min-max]: 4.34 mM [0.16–20.77 mM]; $P = 0.0081$) and untreated (11.45 mM [5.65–26.49 mM]; $P = 0.0013$) dogs with EPI when compared to the healthy controls (0.35 mM [0.13–0.74 mM]). L-lactate was significantly increased in both the treated (median [min-max]: 18.25 mM [0.29–32.61 mM]; $P = 0.0023$) and untreated (23.31 mM [13.28–24.76 mM]; $P = 0.0019$) dogs with EPI when compared to the healthy controls (0.47 mM [0.18–2.79 mM]). *Lactobacillus* and *Bifidobacterium* were significantly increased in both treated ($P = 0.0007$and $P = 0.0019$, respectively) and untreated dogs ($P = 0.0120$ and $P = 0.0076$, respectively) with EPI when compared to healthy controls.

In conclusion, this study suggests that fecal D- and L-lactate are increased in dogs with exocrine pancreatic insufficiency, regardless of enzyme replacement treatment status. Also, lactate-producing bacterial groups were altered in dogs with EPI when compared to healthy control dogs.

GI29

EVALUATION OF SERUM BETA-HYDROXYBUTYRATE CONCENTRATIONS IN DOGS WITH CHRONIC ENTEROPATHIES. G Esposito[1], K Allenspach[2], JM Steiner[1], JA Lidbury[1], JS Suchodolski[1]. [1]Gastrointestinal Laboratory, College of Veterinary Medicine Texas A&M University, College Station, TX, USA, [2]Royal Veterinary College, London, UK

Beta-hydroxybutyrate (BHB), a ketone body, is synthesized in the liver from acetyl CoA and is utilized as an energy source when glucose supplies are limited. BHB has been demonstrated to play a role in the oxidative stress response by the binding of histone deacetylase and in the suppression of inflammatory disease by antagonizing the cryopyrin inflammasome (NALP3). Previous untargeted metabolomics studies of both human and canine patients with idiopathic inflammatory bowel disease have shown significantly higher serum concentrations of BHB in diseased patients than in healthy controls, suggesting that BHB may be useful as a biomarker for inflammatory bowel disease. In this study, we investigated serum BHB concentrations in healthy dogs and dogs with two types of chronic enteropathy: food responsive diarrhea and steroid-responsive diarrhea.

Serum samples from dogs with food responsive diarrhea (FRD, n = 30), steroid responsive diarrhea (SRD, n = 14), and a group of healthy control dogs (n = 30) were used. All samples were surplus material from a previous study. Dogs were diagnosed as having food responsive or steroid responsive diarrhea based on response to treatment. Serum concentrations of BHB were measured in serum samples using a colorimetric assay kit (beta-hydroxybutyrate colorimetric assay kit, Cayman Chemicals). A Kruskal-Wallis was performed to compare serum concentrations between groups.

Median serum BHB concentrations were (minimum – maximum): 0.45 mmol/L (0.02 – 0.11) for healthy dogs, 0.45 mmol/L (0.00 – 0.27) for dogs with SRD, and 0.03 mmol/L (0.01 – 0.13) for dogs with FRD. There was no significant different between the three groups ($P = 0.328$).

These results suggest that serum concentrations of BHB are not significantly increased in dogs with chronic enteropathies compared to healthy dogs.

GI30

EFFECT OF SERUM CREATININE ON FELINE SERUM DGGR-LIPASE AND SERUM PANCREATIC LIPASE IMMUNOREACTIVITY. Ellen Everson, Anthony Abrams-Ogg, Helen Kocmarek, Kristiina Ruotsalo. University of Guelph, Guelph, ON, Canada

The objective of this study was to examine the influence of kidney function (indicated by serum creatinine level) on serum 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6′methylresorufin) ester (DGGR) lipase and pancreatic lipase immunoreactivity (PLI) in cats.

Using electronic record searches, all cats were identified that had had a serum biochemistry profile ordered at the Ontario Veterinary College between January 1, 2010 and April 14, 2015. Serum creatinine had been measured at the Animal Health Laboratory (AHL), University of Guelph, by enzymatic UV method (Randox) on a Cobas 6000 c501 analyzer (Roche Diagnostics). Serum lipase had been measured at AHL by the DGGR colorimetric assay (Cobas) on the same analyzer. In some cases, an aliquot of the same serum sample had been submitted for Spec fPL® at IDEXX laboratories, Markham, ON. Laboratory results were retrieved and corresponding medical records reviewed. Data obtained from the records included, when available, hydration status and body condition score as assessed by the attending clinician.

Data were first assessed as independent events by analyzing only the results of the first serum biochemistry profile obtained for each cat (n = 1314). The median lipase value in the group of cats with creatinine values above reference interval (>190 μmol/L, n = 162) was 18.5 U/L (range 7–594 U/L), which was higher ($P < 0.001$) than the median lipase value of 14 U/L (range 5–747 U/L) in the group of cats with creatinine values ≤190 (n = 1152), although ranges overlapped substantially. The same trend ($P = 0.06$) was observed for PLI in these respective groups: median was 16.6 μg/L (range 1.5–50 μg/L) for cats with creatinine >190 μmol/L (n = 9); median was 3.85 μg/L (range 0.5–50 μg/L) for cats with creatinine ≤190 μm/L (n = 55). There was a significant, but weak, correlation between creatinine and lipase values across all cats (rho=0.2, $P < 0.001$, n = 1314), but no correlation was present for the group of cats with creatinine values >190 μmol/L (n = 162). There was no correlation between creatinine and PLI. Multivariate linear regression was used to assess the influence of creatinine, hydration, and body condition on lipase over all cats for which this information was available (n = 313); none of these variables demonstrated a significant effect ($P = 0.09$, 0.22, and 0.97, for creatinine, hydration, and body condition, respectively). The data were next assessed by MANOVA for variation over time within individual cats for which repeat serum biochemistries and medical record data were available (n = 279). Hydration demonstrated a significant effect on creatinine ($P < 0.001$), and a trend towards effect on lipase ($P = 0.07$). Creatinine did not demonstrate any further relationship with lipase ($P = 0.39$). PLI was repeated in 15 cats; values moved in the same direction as creatinine in only 4 cases.

The data indicate that kidney function has a mild effect on DGGR-lipase level in cats, which may in part be due to hydration status. The study may be underpowered to show this effect in all analyses and on PLI. Creatinine may underestimate decreased kidney function in underweight cats, affecting results, but the overall weak associations between creatinine and lipase, and creatinine and PLI, suggest that elevated lipase or PLI should not be attributed solely to decreased kidney function in azotemic cats. This may be because the magnitude of increases in lipase and PLI in pancreatic disorders overwhelms the magnitude of effect of kidney function.

GI31

PREVALENCE OF *HELICOBACTER* SPECIES AND THEIR ASSOCIATION WITH GASTRIC PATHOLOGY IN DOGS WITH GASTROINTESTINAL DISEASE. Roman Husnik[1], Jiri Klimes[2], Simona Kralova-Kovarikova[3], Petr Fictum[4], Michal Kolorz[5], Frederic Gaschen[1]. [1]Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA, [2]Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic, [3]Department of Small Animal Internal Medicine, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic, [4]Department of Pathological Morphology And Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic, [5]Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

The prevalence and pathogenic role of different species of *Helicobacter* in the dog have not been definitely documented to date. This prospective study included 84 privately owned dogs for which the prevalence of gastric *Helicobacter* species and associated gastric lesions was determined, and the accuracy of different diagnostic techniques was compared.

Inclusion criteria consisted of chronic GI clinical signs (vomiting, diarrhea, anorexia, weight loss), exclusion of non-GI diseases and discontinuation of all medications for at least 2 weeks. Biopsy specimens were obtained by gastroduodenoscopy. The presence of *Helicobacter* spp. was detected using genus- and species-specific PCR (investigated species were *H. heilmannii* sensu stricto [HH s.s.], *H. bizzozeronii* [HB], *H. felis* [HF], *H. salomonis* [HS] and *H. pylori* [HP]), cytology, rapid urease test, and histologic examination with hematoxylin-eosin and silver staining of gastric biopsy samples.

PCR detected *Helicobacter* DNA in 60 dogs (71.4%). Comparing the diagnostic value of cytology, rapid urease test and histology to PCR, cytology yielded a sensitivity of 88.3% and a specificity of 91.7%. The rapid urease test had a sensitivity of 85% and a specificity of 91.7% and histology had a sensitivity of 81.7% and a specificity of 83.3%. Using a combination of diagnostic tests for *Helicobacter* spp. including rapid urease test, cytology, histology and PCR, 65 dogs (77.4%) had one or more positive result. For 61 dogs (72.6%) diagnostic tests were concordant. Single infections with HH s.s. was detected by PCR in 23.3%, HB in 15%, HS in 6.7% and HF in 1.7% of 60 PCR

positive dogs. Mixed infection of HH s.s. and HB was found in 38.3% and combination of HH s.s. and HF was detected in 1.7% of the dogs. *Helicobacter* species remained undetermined in 13.3% of the patients. No HP was found in animals examined. In most dogs, the endoscopic appearance of the gastric mucosa was normal. Overall, the most common endoscopic lesions were erosions or ulcers and increased granularity of gastric mucosa. Gastritis was diagnosed histologically in 36.7% of dogs positive for *Helicobacter* spp. and in 41.7% of dogs without evidence of *Helicobacter* colonization. Severe lymphocytic-plasmacytic gastritis (11.1%) and acute purulent-necrotic gastritis (1.5%) were found in single HB infections and moderate lymphocytic-plasmacytic gastritis (14.3%) in single HH s.s. infections. An association between the presence of mixed infection (including HH s.s. and HB) and the lower severity of gastric inflammation was detected when compared to single *Helicobacter* species infection.

This is a large study evaluating association of the individual *Helicobacter* species with histologically confirmed gastritis in dogs. Dogs are frequently colonized by *Helicobacter* spp. other than HP, and may represent a reservoir for human infection with non-HP *Helicobacter* spp. The combination of cytology and rapid urease test performed on gastric biopsy samples represents a reliable diagnostic method suitable for clinical practice. Competitive inhibition may occur when 2 *Helicobacter* species are simultaneously detected in gastric biopsies, and the presence of one *Helicobacter* species may interfere with the virulence of other *Helicobacter* species.

GI32

NORMAL AND ABNORMAL FINDINGS IN THE CANINE GASTROINTESTINAL TRACT USING AMBULATORY LIGHT-BASED IMAGING. Jill S. Pomrantz[1], Brian T. Hardy[2], Jeffrey A. Solomon[1]. [1]Infiniti Medical, LLC, Menlo Park, CA, USA, [2]University of California, Davis, Davis, CA, USA

Ambulatory light-based imaging (ALI) is a new imaging modality that allows for non-invasive endoluminal visualization of the gastrointestinal mucosa. ALI is performed by oral administration of a capsule containing a fully automated camera (ALICAM) that is propelled by natural peristalsis. The capsule is retrieved from the dogs' feces and images are downloaded. Familiarity with normal and abnormal findings is essential to accurate interpretation of these studies, but these have yet to be documented in veterinary patients. The aim of this study was to present a gallery of normal and abnormal images of the canine gastrointestinal tract using ALICAM.

ALICAM studies from 65 dogs were evaluated retrospectively by a board-certified internist. The average study consisted of 21,003 images obtained over 16 hours. Patients evaluated included 55 dogs with clinical signs of gastrointestinal disease and 10 asymptomatic dogs. The patients ranged in age from 4.5 months to 13.7 years old and in weight from 7.8 to 72 kg. Images representative of normal mucosa and common lesions were saved and logged.

The most common reasons for ALICAM administration were vomiting, diarrhea, signs of gastrointestinal bleeding, or a combination of several gastrointestinal signs. Gastric lesions identified in dogs with gastrointestinal signs included irregular mucosa, polyps, ulcers, erosions, masses and foreign material. Small intestinal lesions identified included irregular mucosa, dilated lacteals, ulcers, erosions, masses, and parasites (tapeworms and hookworms). Colonic lesions included masses and erosions.

Establishing characteristics of normal and abnormal findings is an important step in the evolution of ALI as a diagnostic test.

GI33

FECAL α_1-PROTEINASE INHIBITOR CONCENTRATIONS IN DOGS WITH CARDIAC DISEASE. Joseph Cyrus Parambeth, Jordon P. Vitt, Jan S. Suchodolski, Jonathan A. Lidbury, Ashley B. Saunders, Joerg M. Steiner. Department of Small Animal Clinical Sciences, College Station, TX, USA

Protein-losing enteropathy (PLE) has been documented in various cardiac diseases (i.e., congestive heart failure, constrictive pericarditis, and congenital heart diseases) typically related to increased interstitial pressure and intestinal lymphangiectasia in humans. Fecal α_1-proteinase inhibitor ($f\alpha_1$-PI) is a stable protein lost into the gastrointestinal tract at a rate comparable to that of albumin and therefore serves as an endogenous marker of gastrointestinal protein loss. While PLE has been suspected in dogs with cardiac disease, no published studies are available. The aim of this study was to evaluate $f\alpha_1$-PI concentrations in dogs with variety of cardiac diseases.

Single, naturally voided fecal samples from 27 adult dogs (≥1 year of age) and 15 puppies (<1 year of age) were collected from dogs with congenital and acquired cardiac diseases. Two dogs, one puppy and one adult, had serial fecal evaluations (i.e., before and after occlusion of their patent ductus arteriosus (PDA) with an Amplatz® canine ductal occluder (ACDO) device). Each dog in the study had an echocardiogram performed by a board certified cardiologist or a resident under the supervision of a board certified cardiologist. Fecal samples were frozen upon collection and $f\alpha_1$-PI concentration was measured using an in-house radioimmunoassay and was compared with an established reference interval for healthy adult dogs. A $f\alpha_1$-PI concentration of ≥21.0 μg/g feces was considered abnormal.

Three of 27 adult dogs (11%) all with congenital cardiac disease had an increased $f\alpha_1$-PI concentration (median [minimum - maximum]: 10.1 μg/g feces [1.8 - 45.6]). Also, nine of 15 (60%) of the puppies, all with congenital heart diease, had an increased $f\alpha_1$-PI concentration (median [minimum - maximum]: 24.8 μg/g feces [6.6 - 79.1]). Some of the dogs had multiple congenital heart defects on echocardiographic evaluation. Also, the severity of cardiac disease varied in each patient. For the two PDA dogs for which samples from two time points were available, there was a reduction in $f\alpha_1$-PI concentration post coil embolization; from 79.1 to 21.5 μg/g feces in the puppy, and from 36.5 to 14.1 μg/g feces in the adult. The increased $f\alpha_1$-PI concentrations in puppies have to be interpreted with caution as higher $f\alpha_1$-PI concentrations have been reported previously in puppies and a specific reference interval for puppies has not been established.

Limitations of this study include the small number of dogs, the lack of intestinal biopsies to rule out any other causes of increased gastrointestinal protein loss, the lack of multiple fecal samples to account for day to day variability in $f\alpha_1$-PI concentrations reported earlier, the variety of cardiac diseases, and lack of complete serum chemistry profiles for all dogs. However, this study shows that $f\alpha_1$-PI concentrations may be increased in a subset of dogs with cardiac disease. Prospective studies are needed to establish if increased $f\alpha_1$-PI concentrations in dogs occur consistently with congenital heart diseases, are increased in dogs with acquired heart diseases that increase interstitial pressure, and if elevations have any influence on treatment or prognosis.

GI34

EVALUATION OF THE EFFECTS OF PRE-CONDITIONING ON FEMALE CANINE ADIPOSE-DERIVED MESENCHYMAL STEM CELL CYTOKINE PRODUCTION. Rebecca Timmons, Stephanie Smith, Craig Webb, Tracy Webb. Colorado State University, Fort Collins, CO, USA

Clinical use of mesenchymal stem cell (MSC) therapy may be optimized through pre-conditioning methods that would enhance their immunomodulatory functions. We investigated the effects of pre-conditioning strategies on cytokine production of female canine adipose-derived MSC that may optimize clinical effects of MSC application in this species. Adipose-derived MSC were generated from healthy adult female dogs and subjected to 6 conditions. Supernatants were harvested after 1, 6, 12, 18, and 24 hours, and levels of cytokines present in the supernatants were determined by ELISA. IL-10 and TGFβ were not released in measurable levels within a 24 hour period under any of the tested culture conditions. Large amounts of MCP-1 (mean=10.2 ng/mL) and VEGF (mean=2.9 ng/mL) and very small amounts of IL-8 (mean=40.9 pg/mL) were constitutively produced by the cells at 24 hours. All of the pre-conditioning strategies except TGFβ altered measured cytokine production in the MSC from control levels at 24 hours: poly I:C increased IL-8 and MCP-1 secretion; IFNγ increased MCP-1 and decreased VEGF and IL-8 secretion;

serum-free decreased IL-8, MCP-1, and VEGF secretion; and the mixed hypoxic environment inhibited MCP-1, VEGF, and IL-8 secretion. All changes in cytokine secretion were evident at the 6 hour time point with most at maximal levels by 12 hours suggesting that 12 hour culture periods are sufficient to induce relevant changes in MSC cytokine secretion. This initial study shows that specific pre-conditioning methods hold promise for tailoring and augmenting canine MSC immunomodulatory properties to treat specific diseases, although further study is necessary and currently on going.

HM01
REPRODUCIBILITY, STABILITY AND BIOLOGICAL VARIABILITY OF THROMBIN GENERATION USING CALIBRATED AUTOMATED THROMBOGRAPHY IN HEALTHY DOGS. Benoit Cuq[1], Shauna Blois[1], Darren Wood[1], Anthony Abrams-Ogg[1], Christian Bédard[2]. [1]Ontatio Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Faculté de Médecine Vétérinaire, Université de Montréal, St Hyacinthe, QC, Canada

Thrombin plays a central role in hemostasis and thrombosis, and is involved in all *in vivo* hemostatic pathways. Calibrated automated thrombography (CAT), a thrombin generation assay, may therefore be a valid physiological test for hemostatic disorders in dogs. Our objectives were to establish reference intervals for CAT thrombin generation, and assess the effects of pre-analytical and biological variability on this assay in healthy dogs.

Lag time (lag), time to peak (ttpeak), peak thrombin generation (peak), and endogenous thrombin potential (ETP) were measured in 41 clinically healthy dogs. Both direct jugular venipuncture and winged-needle catheter-assisted saphenous venipuncture were used to collect samples from each dog. Storage stability at −80°C was assessed over 2 months in a subset of samples. Biological variability of CAT was assessed via nested ANOVA using samples obtained weekly from 9 dogs for 4 consecutive weeks.

Samples for CAT analysis were stable at −80°C for up to 2 months. Samples collected via winged-needle catheter venipuncture showed poor repeatability compared to direct venipuncture samples; there was also poor agreement between the two sampling methods. Intra-individual variability of CAT variables was low (≤7.8%); inter-individual variability ranged from 4.8–76.8%. Analytic precision goals were met for ttpeak, peak and ETP. Indices of individuality ranged from 0 to 0.12.

In conclusions, thrombin generation seems to be stable and repeatable in healthy dogs. Direct venipuncture sampling is recommended for CAT. Indices of individuality were low for all CAT variables, suggesting limited utility of population-based reference intervals for this assay.

HM02
DEVELOPMENT AND VALIDATION OF A NOVEL CANINE IMMUNE THROMBOCYTOPENIA BLEEDING SCORE. Kelly Makielski[1], Marjory Brooks[2], Chong Wang[1], Jonah Cullen[1], Annette O'Connor[1], Dana LeVine[1]. [1]Iowa State University, College of Veterinary Medicine, Ames, IA, USA, [2]Cornell University, College of Veterinary Medicine, Ithaca, NY, USA

A method of objective quantification of clinical bleeding in canine immune-mediated thrombocytopenia (ITP) is needed because ITP patients have variable bleeding tendencies that do not directly relate to severity of thrombocytopenia. A consistent description of their bleeding would allow more direct comparisons of this bleeding heterogeneity in multi-institution studies. Application of a standardized bleeding assessment tool would help stratify ITP patients in clinical trials based on their bleeding severity to provide an objective comparison of patient outcomes among treatment protocols. In this study we aimed to develop and validate a daily canine ITP bleeding assessment tool (DOGiBAT) and to evaluate the utility of a training course for improving accurate implementation of the DOGiBAT.

A novel bleeding assessment tool, DOGiBAT, was developed for canine ITP comprising nine different anatomic site-specific bleeding grades ranging from 0 (none) to 2 (severe). An online training course was developed for application of the DOGiBAT to score clinical cases. A case-based quiz set of still images was used to assess the trainees' ability to apply the DOGiBAT tool. Additionally, to assess the efficacy of the training course, 70 veterinary student volunteers were randomized to take the quiz with (n = 35) or without (n = 35) the training. All students scored all sites from all cases and site scores were considered correct if they agreed with the investigators' pre-specified classification. The frequency of correct scores was compared between trained and untrained students. A logistic regression model was used to assess the association between training and score, while adjusting for correlated responses from sites within cases.

Clinicians (n = 13) and technicians (n = 3) taking the quiz following training were able to correctly apply the DOGiBAT, scoring 100% of responses correctly. 86.1% of responses from students that received training were correctly scored, compared to only 78.2% of responses from students that did not receive training. The odds of trained students giving correct answers were higher than untrained students ($P < 0.0001$). The anatomic site for which training was most important for correct scoring was oral bleeding (82.9% with training, 47.6% without; $P < 0.0001$). Similarly, scoring of ocular (75.2% with training, 58.1% without; $P = 0.0097$) and cutaneous bleeding (84.8% with training, 66.7% without; $P = 0.003$) were improved by the training course. Students with more clinical experience were better able to apply the DOGiBAT correctly (fourth years, 84.2%; third years, 80.1%; $P < 0.0149$).

Our study suggests that the DOGiBAT is simple scoring tool for classification of bleeding severity in dogs with ITP. A short online training course was shown to improve the accuracy and consistency of DOGiBAT scoring. Adoption of the DOGiBAT scoring system for future clinical trials of canine ITP patients will facilitate more rigorous assessment of treatment-effect based on standardized bleeding outcomes.

HM03
EFFECTS OF ASPIRIN DOSE ESCALATION ON CANINE PLATELET FUNCTION AND URINARY THROMBOXANE AND PROSTACYCLIN LEVELS. Natalie McLewee, Todd Archer, Robert Wills, Andrew Mackin, John Thomason. Mississippi State University, MS, USA

Aspirin is commonly used in an effort to prevent thrombus formation in dogs. High-dose aspirin (10 mg/kg, PO, q 12 h) reliably inhibits platelet function and thromboxane A_2 (TXA_2) synthesis, but can be associated with unacceptable side effects, including inhibition of prostacyclin synthesis, which counteracts the effects of inhibition of TXA_2. Lower doses of aspirin, in contrast, inhibit platelet function and TXA_2 synthesis, have few side effects, and allow ongoing prostacyclin synthesis. However, low-dose aspirin therapy at standard dosage (0.5–1 mg/kg, PO, q 24 h), does not reliably inhibit platelet function, a phenomenon known as "aspirin resistance". Aspirin-associated platelet dysfunction has been shown to be highly dose-dependent, suggesting that traditional "low-doses" of aspirin may be under-dosing canine patients. Our study used incremental increases in dosages to determine the dose of aspirin that consistently inhibited platelet function and TXA_2 synthesis without inhibiting prostacyclin synthesis.

Eight healthy adult research dogs were separated into one of 5 groups, with each group given different oral doses of aspirin: Dose A, 0.5 mg/kg, q24 h; Dose B, 1 mg/kg, q24 h; Dose C, 2 mg/kg, q24 h; Dose D, 4 mg/kg, q24 h or Dose E, 10 mg/kg, q12 h. Prior to aspirin therapy (Day 0) and again after one week (Day 7) of drug administration, blood and urine were collected to assess platelet function (turbidimetric aggregometry and PFA-100® (closure time)) and urine 11-dehydro-thromboxane B_2 (11-dTXB$_2$, a stable metabolite of TXA_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF1$_\alpha$, a stable metabolite of prostacyclin) synthesis. A dog was considered to be an aspirin responder if there was >25% reduction in maximal amplitude on aggregometry, or the Day 7 PFA-100® closure time was >300 seconds.

On aggregometry, for Doses A-E, there was a mean decrease in maximum amplitude of 3.8%, 41.3%, 81.3%, 75.9%, and 87.8%,

respectively. Compared to Day 0, there was a significant decrease on Day 7 for Doses B-E. The maximum amplitude was decreased >25% (indicating aspirin responsiveness) in all dogs receiving Dose C or greater. Similar to aggregometry, when the PFA-100® closure times on Day 0 were compared to Day 7, there was a significant increase for Doses B-E. On Day 7, for Doses A-E, mean PFA-100® closure time was >300 seconds (indicating aspirin responsiveness) in 0%, 25%, 62.5%, 87.5%, and 87.5%, respectively. Compared to Day 0, there was a significant decrease on Day 7 for the concentrations of 11-dTXB$_2$ ($P = 0.0003$) and 6-keto-PGF1$_\alpha$ ($P = 0.0075$). However, the changes in 11-dTXB$_2$ and 6-keto-PGF1$_\alpha$ were not dependent on aspirin dose.

The results of our study suggest that an aspirin dose of 2 mg/kg, PO, q24 h reliably inhibits platelet function in normal dogs based on the established "gold standard" assay, platelet aggregometry. Additionally, even though all aspirin doses inhibited prostacyclin synthesis to some degree, there was no difference in prostacyclin concentration between a dose of 2 mg/kg, PO, q24 h and the traditionally used lower doses. Based on our results, an increased standard aspirin "low-dose" of 2 mg/kg, PO, q24 h may minimize the risk of aspirin resistance in dogs.

HM04

B CELL ACTIVATING FACTOR AS A BIOMARKER IN DOGS WITH PRIMARY IMMUNE MEDIATED THROMBOCYTOPENIA. Jessica Pritchard[1], Michael Wood[2], Adam Birkenheuer[1], Henry Marr[1]. [1]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA, [2]Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin – Madison, Madison, WI, USA

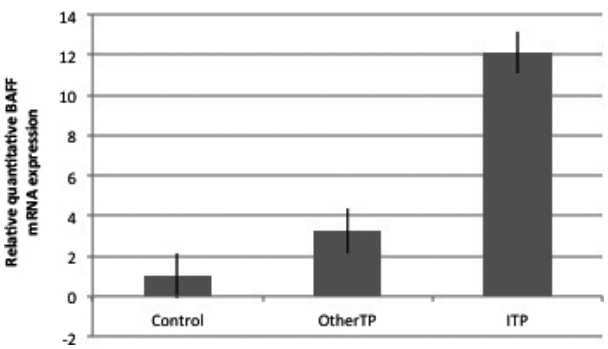
There is a critical need for a biomarker that differentiates active primary immune mediated thrombocytopenia (pITP) from secondary causes of thrombocytopenia, and the concentration of which correlates with disease status in dogs. Such a biomarker would provide a valuable diagnostic tool to modulate therapy, evaluate risk of relapse, and reduce patient exposure to potentially harmful immunosuppressive medications. B cell activating factor (BAFF) is a cytokine within the tumor necrosis factor family that plays a crucial role in B cell maturation, survival, and class switching. Increased concentrations of BAFF in mice and people have been linked to immune-mediated diseases such as systemic lupus erythematosus, rheumatoid arthritis, and ITP. Our central hypothesis is that BAFF production will be significantly and specifically increased in dogs with untreated pITP.

The purpose of this pilot study was to quantify and compare the transcription of BAFF in the blood of dogs with untreated pITP, dogs with secondary thrombocytopenia, and healthy control dogs.

Quantitative real-time PCR (qRT-PCR) was used to assess BAFF mRNA expression. Total RNA was isolated from 6 dogs with untreated pITP, 5 healthy dogs, and 15 dogs with secondary thrombocytopenia. pITP was defined as dogs that had platelet counts <20,000 cells/mL, were PCR and serology negative for *Babesia*, *Ehrlichia,* and *Anaplasma*, had no evidence of neoplasia on abdominal and thoracic imaging, and no history of drug administration (antibiotics, corticosteroids, vaccines, or chemotherapeutics). Secondary thrombocytopenic control dogs had platelet counts below the reference interval due to diagnoses other than pITP, and healthy dogs presented for wellness exams and had normal platelet counts. qRT-PCR was performed using intron-spanning primers to amplify a 129 base pair region of the mRNA. β-actin was used as a reference gene.

The relative mRNA expression of BAFF in pITP dogs was increased 12.13-fold and 3.25-fold compared to healthy control dogs and dogs with secondary thrombocytopenia, respectively ($P < 0.01$) (Figure 1).

These results indicate that relative expression of BAFF mRNA is increased significantly and specifically in dogs with pITP compared to both healthy dogs and dogs with other causes of thrombocytopenia, similar to previous findings in people. Thus, BAFF may be a useful biomarker to distinguish dogs with pITP from those with other causes of thrombocytopenia. Using these primers our group has also identified a canine macrophage cell line (030-D) that expresses BAFF and is utilizing them for protein identification and quantitation as well as further characterization of BAFF mRNA splice variants.



Levels of plasma BAFF mRNA in healthy dogs (control), dogs with secondary thrombocytopenia (other TP), and dogs with ITP. The ratios of BAFF mRNA in dogs with ITP compared to those with secondary thrombocytopenia and healthy dogs are 3.25 and 12.13, respectively.

HM05

COMPARISON OF MULTIPLATE, PLATELET FUNCTION ANALYZER-200, AND PLATELETWORKS IN DOGS TREATED WITH ASPIRIN AND CLOPIDOGREL. Sophie Saati, Anthony Abrams-Ogg, Shauna Blois, Darren Wood. University of Guelph, Guelph, ON, Canada

In dogs, the antiplatelet drugs aspirin (ASA) and clopidogrel may be used in an effort to prevent thromboembolism. Response to these drugs might be variable as in humans, therefore platelet function testing may be warranted to monitor treatment effect. This study aimed to evaluate the effects of ASA, clopidogrel, or combination therapy, in healthy laboratory Beagles using three platelet function tests: Multiplate Analyzer (MP), Platelet Function Analyzer-200 (PFA), and Plateletworks (PW). Secondary goals were comparison of PW using two hematology analyzers: Vetscan HM5 (using impedance) and ADVIA 2120 (using flow cytometry); and evaluation of the novel INNOVANCE P2Y cartridge for PFA to evaluate clopidogrel-induced platelet inhibition.

Six dogs were given ASA 1 mg/kg/day and placebo, clopidogrel 2 mg/kg/day and placebo, or a combination of both drugs, for 1 week each. Drugs were given in a randomized crossover design (Latin square). Each dog was randomized to one of the three treatments in three different phases such that each dog received each treatment. Investigators were blinded to the treatments. A washout period of 2 weeks occurred between phases. Blood samples were collected on days 0 and 7 of each phase, and analyzed using MP (adenosine diphosphate [ADP], arachidonic acid [AA], collagen [COL] agonists), PFA (P2Y, COL-ADP, COL-Epinephrine [EPI] cartridges), and PW (ADP, AA, COL agonist tubes) using the two hematology analyzers.

Significant ($P < 0.05$) changes in mean values pre and post treatments were: For MP - Area under the curve (AUC) units (U) was decreased with COL only with combination therapy (pre 19 U, post 6 U). AUC was decreased with AA with either clopidogrel (pre 41 U, post 19 U) or combination therapy (pre 44 U, post 9 U). AUC was decreased with ADP with all treatments (ASA pre 62 U, post 52 U; clopidogrel pre 59 U, post 13 U; combination pre 58 U, post 12 U). For PFA – closure time (CT) was increased with the P2Y cartridge with clopidogrel (pre 95 seconds, post >300 seconds) and combination therapy (pre 119 seconds, post >300 seconds). CT was increased with the COL-ADP cartridge with clopidogrel (pre 65 seconds, post 185 seconds) and combination therapy (pre 68 seconds, post 215 seconds). CT was increased with the COL-EPI cartridge only with ASA (pre 167 seconds, post 235 seconds). For PW using the HM5 analyzer - % aggregation (% Agg) with AA was decreased for all treatments (ASA pre 80%, post 7%; clopidogrel pre 90%, post 4%; combination pre 78%, post 12%). With ADP, % Agg was decreased for

clopidogrel (pre 45%, post 9%) and combination therapy (pre 47%, post 9%). PW results for HM5 and ADVIA 2120 showed almost perfect agreement (kappa 0.83–0.98). Although the results above revealed changes in mean values with treatments, results were variable for individual dogs. The only tests for which every dog's individual values followed the changes in mean values were: MP with COL with combination therapy; PFA with the P2Y cartridge with clopidogrel or combination therapy; PW with ADP for clopidogrel or combination therapy, and PW with AA for all treatments.

All three platelet function tests detected ASA and clopidogrel effects in some dogs and may have utility for monitoring antiplatelet therapy. The P2Y cartridge was superior to the COL-ADP cartridge for detection of clopidogrel effect with PFA. PW can be performed with either Vetscan HM5 or ADVIA 2120, and PW using AA was the only test that detected all drug effects.

HM06

EVALUATION OF THE RISK OF RELAPSE OF CANINE IMMUNE-MEDIATED THROMBOCYTOPENIA AFTER ROUTINE VACCINATION. Jenny Ellis, Patricia M. Ward, Robert D. Foale. Dick White Referrals, Six Mile Bottom, Cambridgeshire, UK

Vaccination has been suggested as a cause of initial development and relapse of immune-mediated thrombocytopenia (ITP), although this relationship has not been definitively established in dogs. This study aimed to identify cases of canine primary ITP that had been vaccinated after cessation of treatment and to assess if any of these vaccinated cases showed signs of relapse.

Records of animals diagnosed with ITP between 2004 and 2014 were retrospectively reviewed at a multi-disciplinary hospital. Cases were included if a diagnosis of presumptive primary ITP had been made with a platelet count of below $50x10^9$/l (ref 200 – 500). Dogs diagnosed with secondary ITP were excluded, as were cases that did not have assessment for underlying disease, with minimum investigation required comprising full haematological and biochemical assessment including blood film evaluation, abdominal ultrasonography and thoracic radiography. Infectious disease PCR tests were also performed in some cases at the discretion of the clinician involved. Referring veterinary practices were contacted for follow-up information regarding length of treatment, ongoing vaccination protocols including dates and vaccination administered, and survival data as required.

One hundred and forty-six thrombocytopenic dogs were identified, with 32 dogs not surviving to discharge. Sixty-eight dogs were excluded due to detection of an underlying infectious or neoplastic disease. Of the dogs with presumptive ITP, 11 were lost to follow-up and 14 dogs were still receiving prednisolone or other immunosuppressive agents. Four of these dogs remained on long-term prednisolone due to relapse when the dose was tapered.

Of the 21 dogs that were in remission with all immunosuppressive treatment withdrawn, 9 did not receive any vaccinations after diagnosis. None of these dogs showed signs of relapse during the follow-up period. The remaining 12 dogs received at least one vaccination a minimum of one month after discontinuing all immunosuppressive treatments. One of these dogs had initially relapsed early in the treatment course, prompting a longer treatment protocol before withdrawal of treatment. None of the dogs receiving vaccinations once immunosuppressive treatment was withdrawn showed signs of relapse, including those receiving multiple vaccinations over several years. Vaccinations administered included a combination of core boosters, intranasal vaccination for *Bordetella bronchiseptica*, and one dog received a Rabies booster for travel.

These results failed to show an association between relapse of presumptive primary ITP in dogs and vaccination, although the possibility cannot be excluded. Future studies assessing higher numbers of dogs would be required to further assess a potential association.

HM07

PREDICTING IN VIVO RESPONSE TO LOW-DOSE ASPIRIN IN HEALTHY DOGS USING IN VITRO PLATELET AGGREGOMETRY. Rachel Hegedus, Pamela Lee, Michael Court, Jillian Haines. Washington State University, Pullman, WA, USA

Aspirin, or acetylsalicylic acid (ASA), is a cyclooxygenase inhibitor that prevents the synthesis of hemostatically active prostaglandins. At low doses aspirin is used to inhibit platelet function and ultimately thrombus formation. Some human patients appear non-responsive to these antiplatelet effects, termed "aspirin resistance." This same phenomenon has been identified in dogs and could be a contributing factor to the formation of thromboemboli in some at risk patients. Accurately predicting aspirin response prior to treatment could prevent inappropriate drug therapy and devastating thrombus formation in otherwise manageable diseases.

The objective of our study was to determine whether the ASA concentration needed to cause 50% inhibition of platelet aggregation (EC50) measured using dog blood collected before ASA treatment (i.e. the *in vitro* EC50*)* could be used to predict the EC50 value measured in the same dogs after administration of low-dose oral aspirin (i.e. the *in vivo* EC50*)*.

Twenty client-owned healthy dogs were utilized for this study. *In vitro* testing was performed on two separate occasions at least 1 week apart to determine individual repeatability. Blood was incubated with six concentrations of aspirin and aggregation was measured using a multiple electrode impedance aggregometer (Multiplate instrument). *In vivo* testing was performed at least 1 week after *in* vitro testing. The same dogs received a single dose of 1 mg/kg aspirin orally and aggregation was assessed at baseline prior to administration, then 20 minutes, 40 minutes, and 180 minutes after administration. Additionally, plasma concentrations of ASA and salicylic acid (SA) were measured at each time point using liquid chromatography–mass spectrometry (LC-MS). SA is the major ASA metabolite and may be a better indicator of ASA exposure (bioavailability) than ASA.

Calculation of an *in vitro* EC50 allowed dogs to be separated into four different response groups. Sixteen of the 20 dogs (80%) had *in vitro* EC50 values below 250 umol/L consistent with an appropriate aspirin response with mean ± SD (range) values of 84.5 ± 36.0 (34.8–151.4) umol/L. When these values were evaluated for repeatability a mean difference of 44.9% was found with the highest difference between independent measurements for each dog of 95.1%. One dog showed a consistently poor response with *in vitro* EC50 values between 250–500 umol/L at both times measured. Two dogs had substantial variation in their *in vitro* EC50 values between the two time points and were classified as variable responders. One additional dog showed no significant inhibition of platelet aggregation at any time up to an ASA concentration of 3300 umol/L and was classified as a non-responder. *In vivo* aspirin dosing at 1 mg/kg did not cause measureable inhibition of platelet aggregation in any of the dogs after a single dose at any time point. An *in vivo* EC50 could not be established based on these findings as 50% inhibition was never achieved at this dose. Plasma ASA and SA concentrations were available for 18 of 20 dogs. The mean ± SD (range) maximum plasma concentrations (Cmax) for ASA were 0.23 ± 0.21 (0 - 0.77) umol/L and for SA were 21 ± 5 (14 - 34) umol/L. Two dogs did not have ASA concentrations above the detection limit (0.03 umol/L).

This study suggests individual variation in aspirin response does exist in dogs as assessed by multiple electrode aggregometry. A single low dose (1 mg/kg) of aspirin was not sufficient to cause measurable inhibition of platelet aggregation in healthy dogs and resulted in ASA and SA concentrations that were substantially less than the *in vitro* EC50. Low-dose (1 mg/kg) aspirin therapy may not be a reliable antithrombotic therapy after only a single dose thus necessitating the potential need for additional therapies during the first 24 hours. Further research looking at higher oral aspirin doses or multiple day dosing is warranted to better establish an *in vivo* EC50 and determine the predictive value of *in vitro* EC50 determination prior to commencement of therapy.

HM08
RETROSPECTIVE EVALUATION OF ANEMIA AND ERYTHROCYTE MORPHOLOGY IN DOGS WITH LYMPHOMA AND INFLAMMATORY BOWEL DISEASE. Cyril Parachini-Winter, Lisa Carioto, Carolyn Gara-Boivin. School of Veterinary Medicine of the University of Montreal, Saint-Hyacinthe, Canada

The differentiation between intestinal lymphoma and inflammatory bowel disease (IBD) can be diagnostically challenging. Anemia is a frequent paraneoplastic syndrome in dogs with lymphoma, and eccentrocytes have also been reported in canine lymphoma. The purpose of this study was to assess the prevalence of anemia and various RBC morphologic anomalies in the peripheral blood of dogs with a diagnosis of lymphoma or IBD, to determine if there is an association with the specific disease and with the severity of lymphoma.

Medical records of 32 dogs diagnosed with all forms of lymphoma, 23 dogs diagnosed with IBD and 28 control dogs, presented to the Centre Hospitalier Universitaire Vétérinaire between 2006 and 2014 were retrospectively reviewed. Each blood smear was blindly re-assessed by a board-certified pathologist. A comparison between the following 3 groups: healthy dogs, dogs with IBD and dogs with lymphoma, as well as within the lymphoma group (stages 1,2,3 versus 4/5, sub-stage a versus b, and small versus intermediate/large cells) was performed for the following hematologic parameters: hematocrit, hemoglobin, erythrocyte count, mean corpuscular volume, and mean corpuscular hemoglobin concentration. The total numbers and each specific RBC anomaly were compared between these 3 groups. RBC morphologic anomalies were also categorized by the underlying pathologic process (regenerative anemia, oxidative stress, or other morphologic changes) and compared between the 3 groups.

Prevalence of anemia was significantly higher in dogs with lymphoma than dogs with IBD ($P = 0.02$) and healthy dogs ($P = 0.0001$), and significantly higher in stages 4 and 5 lymphoma as compared to stage 1, 2 or 3 ($P = 0.045$). There was a significantly higher total number of RBC morphological anomalies in the peripheral blood of dogs with lymphoma as compared to dogs with IBD ($P = 0.02$) and healthy dogs ($P < 0.0001$). Dogs with sub-stage b lymphoma also had a higher prevalence of total RBC morphologic anomalies as compared to sub-stage a ($P = 0.04$). When categorized by pathologic etiology, dogs with lymphoma had a significantly higher number of RBC anomalies associated with regenerative anemia ($P < 0.0001$), oxidative stress ($P = 0.002$), and other RBC anomalies ($P < 0.0001$) when compared to healthy dogs, but no significant difference when compared to dogs with IBD. The prevalence of eccentrocytes was significantly higher in dogs with lymphoma compared to IBD ($P = 0.03$), as well as healthy dogs ($P = 0.002$). No differences were noted between small, intermediate and large cell lymphoma for all parameters studied.

In conclusion, observation of anemia, of an increased total number of RBC morphological anomalies on blood smear, and specifically the presence of eccentrocytes could increase the clinical suspicion of lymphoma when compared to IBD in dogs with a suggestive clinical presentation.

HM09
AN IN-VITRO ASSESSMENT OF CANINE TO FELINE RED BLOOD CELL XENOTRANSFUSION. Michele Wilkinson, Ian McClure, Patricia Kaufman. Animal Blood Resources International, Stockbridge, MI, USA

Canine to feline xenotransfusions are increasingly reported in the veterinary literature as a temporary but questionably effective means of addressing life threatening anemia in recipient cats. This study was conducted to determine the in-vitro compatibility of feline red blood cells (RBCs) with canine sera and conversely canine RBCs with feline sera. Each cat and dog had a saline assessment initially conducted to determine if pre-existing auto-agglutination existed. Both major and minor cross matches were performed, with major defined as feline sera against canine RBCs and minor defined as feline RBCs against canine sera. All cross matches were performed via manual technique at 4, 25 and 37 degrees Centigrade. The end assessment of incompatibility was based on scored assessments of macroscopic or microscopic agglutination and/or hemolysis of RBCs. The first group of cross matches consisted of 19 blood Type A cats against five dogs. The dogs were selected to comprise a broad spectrum of the Dog Erythrocyte Antigen (DEA) possibilities. Each of the 19 cats had a major and minor cross match with each of the five dogs at all three temperatures for a total of 570 data points. None of the cross matches were compatible (100% incompatibility). To expand the database of canine bloodtypes, a second novel group of seven blood Type A cats were major and minor cross matched against ten dogs, five dogs of which were cohorts from the first set of cross match data. This yielded 420 data points for assessment of compatibility. Similar to the first data set, none of the cross matches were compatible (100% incompatibility). This data suggests there is an inherent incompatibility between dog and cat blood regardless of components tested. Clinicians contemplating canine to feline xenotransfusions should be cautious of utilizing this technique even in the presence of life threatening anemia.

HM10
CHARACTERIZATION OF THE IMMUNOPHENOTYPE OF DOGS WITH IMMUNE-MEDIATED HEMOLYTIC ANEMIA. James Swann, Julia Wu, Barbara Glanemann, Oliver Garden. Royal Veterinary College, London, UK

Whereas the clinical features of immune-mediated hemolytic anemia (IMHA) have been described extensively in dogs, the immunologic phenotype of this disease has yet to be fully characterized. We hypothesized that this phenotype would differ between dogs with primary IMHA and dogs with inflammatory diseases that did not have an immune-mediated etiology.

Serum and EDTA-anticoagulated blood samples were collected from healthy control dogs (HC), dogs with primary IMHA (IMHA), and dogs with inflammatory diseases (INF). Dogs were considered to have primary IMHA if they were anemic (with hematocrit less than 35%) and fulfilled one of the following criteria: significant spherocytosis on fresh blood smear, persistent agglutination of erythrocytes after dilution in saline, or a titer of greater than 1:16 in a direct antiglobulin test. Dogs were excluded if any underlying cause of disease was detected by serum biochemical testing, imaging of the thorax and abdomen and testing for infectious agents. Dogs in the INF group had diseases that were not considered to have an immune-mediated etiology and had no previous history of autoimmune disease. Dogs in the HC group had no previous history of autoimmune disease and had unremarkable physical examinations. Owners of dogs consented for collection and use of samples, and the study was approved by an institutional ethical review committee.

After lysis of red blood cells, peripheral blood mononuclear cells (PBMCs) were stained with fluorophore-conjugated antibodies specific for CD4, CD5, CD8, CD79b and FoxP3 and analyzed by flow cytometry to identify major lymphocyte subsets. Total RNA was extracted from PBMCs for quantitative reverse transcriptase polymerase chain reactions using primers for three reference genes and for interferon gamma (IFNγ) and interleukin (IL)-10. The serum concentrations of five cytokines (IL-2, IL-6, IL-8, IL-10 and TNFα) were measured using enhanced chemiluminescent assays. Results of complete blood cell counts and serum biochemical profiles were recorded for all INF and IMHA cases.

Samples were obtained from 25 IMHA dogs, in addition to 20 HC and 11 INF dogs; these groups had similar age and sex distributions. There was no difference in the proportion of regulatory T cells, helper T cells or cytotoxic T cells between groups ($P = 0.226$), but the serum concentrations of IL-2 ($P < 0.001$) and TNFα ($P < 0.001$) were significantly greater in IMHA dogs compared to either of the other groups. The serum concentrations of IL-6 ($P = 0.001$) and IL-8 ($P = 0.001$) were significantly greater in IMHA dogs compared to HC, but there was no difference compared to the INF group. There was no difference between groups in the serum concentration of IL-10 ($P = 0.731$), nor in the relative expression of IL-10 ($P = 0.805$) or IFNγ ($P = 0.670$). Principal component analysis revealed three major factors underlying the variability in the dataset, of which one, positively correlated with IL-2, IL-8, TNFα, blood monocyte count, serum total bilirubin concentration and negatively

correlated with hematocrit, differed between IMHA and INF dogs ($P = 0.001$).

Immunophenotyping of dogs with IMHA revealed increased concentrations of some pro-inflammatory cytokines compared to HC and INF dogs. Characterization of factors explaining variability in all parameters suggested development of different underlying inflammatory processes in IMHA and INF groups. Further work is required to determine whether these changes are associated with survival and whether interventions that ameliorate the effects of IL-2 and TNFα might improve outcome in dogs with IMHA.

HM11

A RETROSPECTIVE STUDY ON USE OF LEFLUNOMIDE IN DOGS WITH IMMUNE MEDIATED DISEASES. Masahiko Sato, Julia Veir, Marie Legare, Michael Lappin. Colorado State University, Fort Collins, CO, USA

Leflunomide is an immunomodulating agent labeled for the treatment of rheumatoid arthritis and psoriatic arthritis in humans. The drug inhibits the synthesis of ribonucleotide uridine monophosphate pyrimidine which ultimately lessens lymphocyte replication. This effect could be of benefit in the treatment of a variety of immune mediated diseases in dogs. The pharmacokinetics of leflunomide are known in dogs and the drug can suppress lymphocyte proliferation in this species. However, little clinical information concerning use of the drug in dogs is available. The purpose of this retrospective study was to report safety and efficacy of leflunomide for the treatment of naturally occurring immune mediated diseases in dogs.

Medical records from 1995–2014 at a USA Veterinary Teaching Hospital were retrospectively searched for dogs with immune mediated diseases administered leflunomide. Data that were extracted from the medical records included signalment, bodyweight, underlying indication for leflunomide, dose of leflunomide, treatment duration, concurrent medications, treatment response, and adverse events. Dogs with immune mediated polyarthritis (IMPA) were assessed for response to treatment on the basis of clinical signs of IMPA and physical examination findings. Dogs with immune mediated thrombocytopenia (IMTP) were assessed for response to treatment based on platelet count. A dog with cutaneous histiocytosis (CH) was assessed for treatment response on the basis of gross appearance of the skin lesion.

A total of 92 cases were included. The mean dose of leflunomide was 1.79 ± 0.8 mg/kg/day. The median duration of the use of leflunomide was 23.5 weeks. Adverse events which could be related to leflunomide administration included diarrhea (3 of 92, 3.3%), lethargy (2 of 92, 2.2%), suspect blood dyscrasia (3 of 92, 3.3%), thrombocytopenia (2 of 31, 6.5%) and increased liver enzyme activities (1 of 16, 6.3%). Leflunomide was discontinued due to the possible adverse events in 6 dogs (6.5%). Significant dose differences between dogs with adverse events (n = 11, 2.6 ± 0.8 mg/kg/day) and dogs without adverse events (n = 81, 1.7 ± 0.8 mg/kg/day) were found ($P < 0.001$). The treatment response could be evaluated in 9 dogs with suspected IMPA, 7 dogs with IMTP, and 1 dog with CH. All of the 9 dogs with IMPA, 1 dog with IMTP and 1 dog with CH received leflunomide without administration of concurrent immunosuppressant drugs. Six of the dogs with IMTP received leflunomide with concurrent immunosuppressant drugs, the doses of which were unchanged when leflunomide was added to the treatment. Of these 17 dogs, 12 dogs (70.5%; 7 dogs with IMPA, 4 dogs with IMTP, 1 dog with CH) had an apparent positive response to the use of leflunomide. There was no significant difference ($P = 0.18$) in doses between dogs that responded to leflunomide (2.0 ± 0.8 mg/kg/day) and those that did not respond (1.5 ± 0.4 mg/kg/day).

A lower starting dose of leflunomide of 2 mg/kg/day than the current suggested dose of leflunomide of 3–4 mg/kg/day is recommended based on the findings in this study.

HM12

EFFECTS OF LEUKOREDUCTION AND STORAGE ON EICOSANOID CONCENTRATIONS IN UNITS OF CANINE PACKED RED CELLS. Samantha Muro, Jung Hwa Lee, Matthew Ross, Robert Wills, Todd Archer, Andrew Mackin, John Thomason. Mississippi State University College of Veterinary Medicine, Starkville, MS, USA

Storage of blood products creates an unnatural environment that can accelerate product degradation and pose risk to the recipient. Eicosanoids are arachidonic acid-derived bioactive molecules that are involved in inflammation and can accumulate inside units of packed red blood cells (pRBCs). Since leukocytes and platelets are the primary sources of eicosanoid synthesis within units of pRBCs, removal of these cells via leukoreduction may decrease eicosanoid synthesis and potentially reduce the risk of transfusion-related complications.

In this crossover study, units of blood were collected from 8 healthy dogs. In half of the units, leukocytes and platelets were removed via leukoreduction. Units of pRBCs were then created via centrifugation and stored for 10 or 21 days. Initial baseline plasma samples were collected after processing and before storage (Day 0). Additional samples were collected following storage for 10 or 21 days, both after removal from refrigeration and again after 5 hours at room temperature (to simulate transfusion conditions). Concentrations (ng/mL) of arachidonic acid (AA), prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), thromboxane B_2 (TXB_2, stable metabolite of TXA_2), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$, stable metabolite of prostacyclin) and leukotriene B_4 (LTB_4) were quantified by liquid chromatography-mass spectrometry.

There was no difference in AA, PGE_2, PGD_2, and LTB_4 concentrations at any time point, and there was no difference in the concentrations between leukoreduced and non-leukoreduced units.

Prior to storage (Day 0), there were significantly greater concentrations of TXB_2 in units of leukoreduced pRBCs compared to non-leukoreduced units. In all leukoreduced samples, except for Day 10 post-transfusion, the TXB_2 concentrations decreased compared to Day 0. Except for the pre-storage samples, there was no difference in TXB_2 concentrations between leukoreduced and non-leukoreduced units. On both Days 10 and 21, there was a significant increase in 6-keto-$PGF_{1\alpha}$ concentrations for both leukoreduced and non-leukoreduced units. There was no difference in the 6-keto-$PGF_{1\alpha}$ concentrations between leukoreduced and non-leukoreduced units, except for the Day 10 post transfusion samples, in which the leukoreduced units were increased compared to the non-leukoreduced units.

Prior to storage, the $PGF_{2\alpha}$ concentrations were significantly greater in units of leukoreduced pRBCs compared to non-leukoreduced units. However, for the remaining time points, the $PGF_{2\alpha}$ concentrations decreased compared to Day 0, and there was no difference between leukoreduced and non-leukoreduced units.

In units of pRBCs, leukoreduction markedly influenced some eicosanoid concentrations. In general, removal of platelets and white cells tended to decrease production of most eicosanoids during storage. For some eicosanoids, however, this effect was counteracted by the passage of blood through the leukoreduction filter, which lead to marked increases in eicosanoid levels in units of pRBCs prior to storage. Further studies will be needed to determine what impact the complex effects of leukoreduction on eicosanoids in stored pRBCs will have on the incidence of transfusion reactions.

HM13

INCIDENCE OF DEA 5 IN CANINE POPULATION USING NOVEL CANINE ANTISERA. Ian McClure, Michele Wilkinson, Patricia Kaufman. Animal Blood Resources International, Dixon, CA, USA

Dog Erythrocyte Antigen (DEA) 5 is a red cell membrane receptor, and is one of the six blood groups that have been described having clinical implications[1,2]. Up until recently, blood typing was unavailable for this antigen. Previous supplies of the DEA 5 antisera had been exhausted, and the ability to create more was seriously hindered. A novel antisera was recently discovered, bringing back the ability to accurately blood type for the DEA 5 antigen.

A proprietary testing methodology was developed for this antisera using known facts about the DEA 5 antigen. The antisera was tested against canines previously known to be positive for the DEA 5 antigen. The antisera proved to identify the DEA 5 antigen with 100 percent positivity. Blinded in-house trials were performed as well, and technicians could correctly identify canines with the DEA 5 antigen.

Once the testing methodology was codified, and a Standard Operating Procedure (SOP) written, testing for the DEA 5 antigen moved into phase two, blind clinical testing. Two closed canine colonies were tested for the DEA 5 antigen, one comprised entirely of unrelated greyhounds, the other of mixed canines. Previously, a portion of these mixed canines had been typed for the DEA 5 antigen, so the sample set had known positives mixed with unknowns. Samples were blinded via identification number. All canines were infectious disease screened, up to date on vaccinations and physical examinations. Testing on the two canine colonies revealed an interesting find. Previously, it was thought that greyhounds had up to a 30% incidence rate of the DEA 5 antigen[3]. However, in testing of the closed greyhound colony, the incidence rate proved to be zero. In a mixed breed closed canine colony, the incidence of the DEA 5 antigen proved to be 21.6%. All previously known DEA 5 antigen positive mixed canines were correctly identified by laboratory technicians.

In phase three of the testing methodology, clinical samples sent to the laboratory were typed for the DEA 5 antigen to establish an incidence rate. Over a period of two months, the laboratory used convenience sampling to test for the DEA 5 antigen. At the closing of phase three, a total of 777 random canine samples were tested, with a total of 63 canines testing positive for the DEA 5 antigen, resulting in an incidence rate of 8.1 %, consistent with previously known incidence rates. Therefore, at the conclusion of phase three, it was determined the novel antisera was valid for the detection of the DEA 5 antigen.

With the re-introduction of a novel anti-DEA 5 reagent, a more complete profile of canine blood types can be achieved. This can result in better clinical decision making regarding transfusion in the canine patient, and therefore better outcomes, including the avoidance of delayed transfusion reactions when a DEA 5 incompatibility exists.

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HM14

PROSPECTIVE STUDY IN THE TREATMENT OF NONREGENERATIVE IMMUNE-MEDIATED ANEMIA IN 8 DOGS.

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Nonregenerative immune-mediated anemia (NRIMA) is a common hematologic disorder in dogs. Because the bone marrow of the affected dogs shows erythroid hyperplasia and/or erythroid maturation arrest, it has been recognized as immune-mediated destruction of erythroid precursor cells. A variety of immunosuppressive agents have been used in the treatment of this disease, but information regarding the therapeutic efficacy is limited due to the lack of prospective research. The aim of this pilot study was to assess the treatment outcome to the pre designed immunosuppressive regimen including corticosteroids, cyclosporine and mycophenolate mofetil.

8 dogs with NRIMA referred to Hokkaido university teaching hospital between 2012 and 2015 were included. Inclusion criteria in this research were a minimum 5-day history of anemia, a severe nonregenerative anemia (Hct < 20%) with absolute reticulocyte count < $60 \times 10^3/\mu l$, and absence of any identifiable underlying disease which could induce severe anemia. The following data were collected for each dog: age, breed, sex, results of hematological analyses performed on admission, results of bone marrow examination. First treatment regimen included prednisolone (2 mg/kg/day) and cyclosporine (10 mg/kg/day) for 8 weeks. Good response were defined as an absolute reticulocyte count > $60 \times 10^3/\mu l$ or increasing Hct. Dogs that responded within 8 weeks continued to be treated with gradual tapering of dose, and re-evaluated after 6 month. Dogs that did not respond to the first regimen proceeded to second regimen including prednisolone and mycophenolate mofetil (15 mg/kg, twice a day). Similarly, good response for second regimen was determined as explained above and dogs that did not respond to both regimens were defined as poor response.

Dogs ranged 5 to 9 years old (median age, 9.5 years), and included 5 castrated males, 2 intact females and 1 spayed female. There were 6 miniature dachshunds, 1 Shih-tzu and 1 Papillon. Median Hct and absolute reticulocyte count were 16.3% and 59,640/μl, respectively. Bone marrow erythroid series were evaluated as hyperplasia in 5 dogs, euplasia in 2 dogs and hypoplasia in 1 dog. Erythroid maturation arrest was observed in all dogs. 6 of 8 dogs underwent core biopsy and 2 dogs showed myelofibrosis. 5 of 8 dogs responded to the first regimen and time to response was between 28 and 52 days. 3 dogs stopped receiving the first regimen due to anorexia that might be induced by Cyclosporine. 6 month prognoses of the other 2 dogs were complete remission (CR: Hct increased to reference range) and partial remission (PR: Hct did not reach the reference range), respectively. 3 dogs that did not respond to first regimen were proceeded to the second regimen. 1 of 3 dogs showed response after 29 days and 6 month prognosis was PR. The other 3 dogs that discontinued receiving Cyclosporine were also proceeded to the second regimen, and 6 month prognoses were CR in 2 dogs and PR in 1 dog. 2 dogs that did not show any response to both treatments had myelofibrosis.

In this research, response to both regimens could be evaluated in 6 dogs and median response time was 35 days (range 28–52). These results may be valuable for practitioners in consideration of changing immunosuppressive agent.

HM15

VALIDATION OF RAPID THROMBOELASTOGRAPHIC ANALYSIS ON CITRATED AND NATIVE WHOLE BLOOD FROM HEALTHY DOGS.

Hye-Bin Wang[1], Hyeri Shin[1], Jin-Young Chung[2], Kun-Ho Song[1], Kyoung-Won Seo[1]. [1]Chungnam National University, Daejeon, Korea, [2]Kangwon National University, Chuncheon, Korea

Thromboelastography (TEG) is rapid and non-invasive diagnostic instrument that has been used in veterinary medicine for evaluation of haemostatic function in whole blood (WB). Recently, rapid thromboelastography (r-TEG) has been recognized as an overall evaluation of coagulation abnormalities in human medicine and become available faster than conventional coagulation tests (CCTs). Although r-TEG is becoming more generally used in human medicine, there are no studies describing the use of r-TEG in veterinary medicine. The objectives of this study were to 1) validation of r-TEG values with native and citrated WB samples from healthy dogs, and to compare stability and repeatability between two types of samples, and 2) examine whether the use of r-TEG could accelerate the coagulation pathway and decrease in taken time in comparison to kaolin activated TEG. Blood was collected from 16 clinically healthy Beagle dogs. TEG analysis was performed on kaolin-activated, citrated r-TEG, and native r-TEG blood samples. TEG activated clotting time (ACT), kappa value (k), alpha angle (α), and maximum amplitude (MA) were recorded. Coefficients of variation (CV) of native r-TEG and citrate r-TEG for TEG ACT, K, α angle, and MA were 13.4/18.8%, 11.1/16.6%, 4.2/5.1% and 10.0/10/0%, respectively. The mean time for the citrated and native r-TEG were 1313.9 ± 250.9/1351.3 ± 264.6 seconds (mean ± SD) respectively, in comparison with 1779.9 ± 197.0 seconds for kaolin activated TEG.

The native and citrated r-TEG tests were taken significantly less time than the kaolin activated TEG test ($P < 0.05$). Based on the results of this study, the r-TEG can evaluate haemostatic status rapidly than other coagulation tests and it is expected to be usefully used in critical setting in veterinary medicine.

HM16

SERIAL EVALUATION OF THROMBOELASTOGRAPHY AND PLATELET AGGREGOMETRY IN HEALTHY DOGS. Sarah Shropshire, Christine Olver, Michael Lappin. Colorado State University, Fort Collins, CO, USA

The coagulation system is comprised of many complex interactions where a balance between the pro-thrombotic and fibrinolytic arms is necessary to maintain normal hemostatic function. Thromboelastography (TEG) and platelet aggregometry are specialized assays that help to evaluate the viscoelastic properties of blood clotting and platelet function, respectively. If interindividual variability is high, serial evaluation within an individual rather than single measurements may provide greater sensitivity to detect clinically significant changes. If the intraindividual variability is greater than the interindividual variability however, then serial evaluation would not offer increased sensitivity. The purpose of this study was to compare interindividual and intraindividual variability over time for TEG and platelet aggregometry variables in healthy dogs.

Tissue factor activated TEG was performed in six specific pathogen free (SPF) sex and age-matched healthy Beagles at three different time points where the variables reaction time (R), clotting time (K), rate of clot formation (α), and maximum amplitude (MA) were recorded. Adenosine diphosphate (ADP)-induced and arachidonic acid (AA)-induced platelet aggregometry in addition to platelet count, hematocrit, and fibrinogen were also performed concurrently. APTT, OSPT, antithrombin activity, and D-dimer concentrations were measured on the first day of the study. A one-way random effects model was used to analyze the following variables; R, K, α, MA, area under the curve for ADP (AUC_{ADP}), and area under the curve for AA (AUC_{AA}). Variance components were created from the random effects model and used to calculate coefficients of variation for interindividual variability (CV_G) and intraindividual variability (CV_I). A repeated measures ANOVA was performed on the platelet count, hematocrit, and fibrinogen over the three time points with $P < 0.05$ considered significant.

The intraindividual variability was lower than the interindividual variability over time for MA, AUC_{ADP}, and AUC_{AA} however the intraindividual variability was higher than the interindividual variability for the TEG variables R, K, and α. There were no statistical differences in the platelet count, hematocrit, and fibrinogen measurements over time.

Based on the results of this study, serial measurements for ADP and AA-induced platelet aggregometry and the TEG variable MA may provide a more sensitive method to detect relevant changes when monitoring patients. However, due to the high intraindividual variability for the TEG variables R, K, and α, serial measurements may not be more beneficial than a single measurement and comparing it to population based reference intervals.

HP01

FLUORESCENCE IN SITU HYBRIDIZATION IDENTIFIES OCCULT BACTERIAL INFECTION IN GALLBLADDER MUCOCELES. Sara Wennogle[1], David Twedt[1], Kenneth Simpson[2]. [1]Colorado State University, Fort Collins CO, USA, [2]Cornell University, Ithaca NY, USA

Gallbladder mucocele is a commonly recognized form of gallbladder disease in the dog that is characterized by cystic mucinous hyperplasia and insidious accumulation of viscous bile and mucus within the gallbladder. Although several predisposing factors have been proposed, the underlying etiology of gallbladder mucocele formation is unknown. Previous studies have found limited associations with cholecystitis or bacterial infection. The aim of this study was to utilize culture-independent, fluorescence in situ hybridization (FISH) to determine the presence of bacteria in gallbladder mucoceles with and without concurrent cholecystitis.

Electronic medical records at Colorado State University were reviewed for cases of histopathologically confirmed gallbladder mucocele between December 2010 and January 2015. 29 cases were available and suitable for evaluation. Case data included patient signalment, clinical signs, clinicopathological abnormalities, ultrasonographic findings, histopathology, and bacterial culture of bile and liver. Formalin-fixed, paraffin-embedded gallbladder sections were mounted on charged glass slides and evaluated by FISH using a eubacterial probe (5Cy3-EUB-338) concurrently with a control probe (56 FAM-Non-EUB-338). Sections were examined on a BX51 epifluorescence microscope, and images were captured with an Olympus DP-7 camera.

Dogs undergoing cholecystectomy for treatment of gallbladder mucocele ranged in age from 6–14 years old. The most common breeds were mixed (n = 10), Shetland Sheepdog (n = 3), Miniature Schnauzer (n = 2), Pomeranian (n = 2), and Miniature Dachshund (n = 2). Ultrasound was performed in all 29 cases, with 22/29 (76%) exams concluding the appearance of the gallbladder was consistent with a mucocele. Histopathology revealed cystic mucinous hyperplasia in all cases, with associated cholecystitis noted in 13/29(45%) cases. Bacteria were detected by eubacterial FISH in 8/29 (28%) cases. Bacteria were visualized as multifocal clusters of short rods within the mucus (n = 1), adherent to the wall (n = 1), or within the parenchyma (n = 4) in 6/29 (21%) gallbladders. In 2 cases, a single bacterium was observed within the gallbladder parenchyma. Of the 6 cases with multifocal bacteria, 4 had concurrent cholecystitis. Bacterial culture of mucocele contents was positive in only 1/24 cases, yielding moderate growth of *Escherichia coli*. FISH analysis of this case showed diffuse colonization of mucocele contents and mucosa by *E. coli*.

FISH detected the presence of bacteria in 28% of mucoceles evaluated. FISH was more sensitive than bacterial culture, which was positive in only 1/8 cases with visible bacteria. Our results reveal that bacterial infection is more common than previously thought, and support the need for further studies to examine the relationship between gallbladder mucoceles, cholecystitis, and bacterial infection.

HP02

ASSOCIATION OF GALLBLADDER MUCOCELE FORMATION WITH OCCULT HYPOTHYROIDISM IN DOGS: A MATCHED CASE-CONTROLLED STUDY. Kathleen Aicher, John Cullen, Gabriela Seiler, Katharine Lunn, Kyle Mathews, Maria Correa, Jody Gookin. North Carolina State University, Raleigh, NC, USA

Gallbladder mucocele (GBM) formation results from accumulation of viscous bile that obstructs or ruptures the gallbladder. After cholecystectomy a median of 27% of dogs die before hospital discharge. The cause of GBM formation is unknown. Retrospective studies identify 14–17% of dogs with GBM have a diagnosis of hypothyroidism compared to 5–9% of normal dogs. Whether hypothyroidism is an independent risk factor for GBM formation or the consequence of an underlying metabolic syndrome is unknown. This distinction has important implications regarding the cause of GBM formation and may establish a rationale for diagnosis or treatment of hypothyroidism in these dogs. We undertook a prospective case controlled study of dogs newly diagnosed with GBM formation and lacking clinical signs of hypothyroidism to test a hypothesis that occult hypothyroidism exists at a high prevalence in these dogs and that the cause may differ from classic lymphocytic thyroiditis. Dogs diagnosed with GBM by ultrasound and healthy, age, breed and sex-matched dogs with normal gallbladder ultrasound were enrolled. Exclusion criteria included clinical evidence, prior diagnosis or treatment for hypothyroidism; drugs interfering with thyroid function, intact reproductive status, or emergent disease. Testing included a CBC, chem panel, UA, thyroid function testing including serum total T4, total T3, free thyroxine by equilibrium dialysis (FT_4), free triiodothyronine (FT_3), thyrotropin (TSH) and autoantibodies to T4AA, T3AA and TGAA. Dogs were stratified by disease severity into 1) no clinical signs, 2) mild clinical disease, 3) moderate to require hospitalization, and 4) severe requiring intensive care (incl. emergent surgery). Thyroid histology of GBM dogs and controls was examined by a pathologist blinded to the diagnosis. Parametric data were analyzed by t-test and non-parametric data by Mann-Whitney U test. $P < 0.05$ was considered significant. Of 106 screened dogs, 38 with GBM met study inclusion criteria and ranged in age from 6–16 years (median 10.5 yrs). Breeds included 11 Shetland sheepdogs, 4 Cocker Spaniels, 3 Chihuahuas, 3 Beagles, and 16 other pure breeds. Illness severity scores were: 37% absent; 21% mild; 21% moderate; and 21% severe. Of 18 healthy dogs screened, 12 were enrolled. Dogs with GBM had significantly

lower serum T_4 (median 11, range 0–44 nM), T_3 (median 0.7, range 0–1.7 nM), FT_4 (median 15, range 1–27 pM), and FT_3 (median 3, range 0–10.5 pM) compared to control dogs. Relative to control dogs, dogs with GBM frequently had severely low individual thyroid hormone concentrations. Serum T4 was below reference range (RR) in 50% of GBM dogs compared to 8% controls. Four (10.5%) GBM dogs had a T4 = 0. Serum T3 was below RR in 47% of GBM dogs compared to 25% controls. Three (8%) GBM dogs had a T3 = 0. Serum FT4 was below RR in 12% of GBM dogs compared to 0% controls. Serum FT3 was below RR in 17% of GBM dogs compared to 0% controls. Significant differences in T4AA, T3AA or mean TSH were not identified. However 23.5% of GBM dogs and only 8% control dogs had an elevated TSH. Serum TGAA was elevated in 17% of control dogs and 0% of GBM dogs. No inflammation was detected in the thyroids of any of 12 dogs with GBM undergoing autopsy. These results identify a high prevalence of occult, often severe, decreases in individual serum thyroid hormone concentrations in GBM dogs. Influence of non-thyroidal illness must be considered for some of these abnormalities. Lack of serum TGAA and absent histologic inflammation in thyroids of GBM dogs is noteworthy. These findings suggest that an alternative mechanism for abnormal thyroid function in dogs with GBM should be considered.

HP03

INTEROBSERVER AGREEMENT FOR HISTOLOGICAL SCORING OF CANINE HEPATIC FIBROSIS. Jonathan Lidbury[1], Aline Rodrigues Hoffmann[1], Fabiano Olivera[2], Brian Porter[1], Thomas Van Winkle[3], Jan Suchodolski[1], Guy Grinwis[4], Renata Ivanek[1], John Cullen[5], Joerg Steiner[1]. [1]Texas A&M University, College Station, TX, USA, [2]Antech Diagnostics, College Station, TX, USA, [3]University of Pennsylvania, Philadelphia, PA, USA, [4]Utrecht University, Utrecht, The Netherlands, [5]North Carolina State University, Raleigh, NC, USA

Hepatic fibrosis is an important complication of canine chronic hepatitis. To the authors' knowledge, the interobserver agreement associated with the histological scoring of fibrosis from canine hepatic biopsy specimens has not previously been reported. Therefore, the aim of this study was to evaluate the interobserver agreement for the histological scoring of canine hepatic fibrosis.

Paraffin-embedded liver specimens from 50 dogs were selected from the tissue archives at Texas A&M University and North Carolina State University. Two contiguous sections were cut from each specimen, one was stained with hematoxylin and eosin (H&E) and one with picrosirius red (PR). Six board-certified veterinary pathologists assigned a fibrosis score (0: absent, 1: mild, 2: moderate, 3: marked, or 4: very marked) to each section using a scoring system adapted from the Ishak scoring system that is used in humans. Interobserver agreement for the H&E and PR stained sections was evaluated using the kappa and weighted-kappa statistics. The median fibrosis score assigned to H&E and PR stained sections from each dog were compared using the Wilcoxon signed-rank test.

Multiuser kappa (95% CI) for H&E stained sections was 0.35 (0.26 – 0.44) and multiuser weighted-kappa was 0.59 (0.50 – 0.70). Pathologists were in complete agreement 49% of the time, differed by one score level 41% of the time, and differed by more than 1 score level 11% of the time. Multiuser kappa (95% CI) for PR stained sections was 0.39 (0.30 – 0.49) and multiuser weighted-kappa was 0.64 (0.55 – 0.73). Pathologists were in complete agreement 53% of the time, differed by one score level 42% of the time, and differed by more than one score level 5% of the time. There was no significant difference in the median fibrosis scores assigned by the 6 pathologists between H&E and PR stained sections (both medians were 2; $P = 0.248$).

There was only fair interobserver agreement when veterinary pathologists assessed canine hepatic fibrosis from H&E or PR stained sections. However, because there was frequent partial agreement between pathologists, this scoring system may be acceptable for clinical use. A clear benefit of the routine staining with PR for the assessment of hepatic fibrosis was not demonstrated in this study.

HP04

EMBOLIZATION OF INTRATRAHEPATIC PORTOSYSTEMIC SHUNTS IN DOGS WITH A PROTOTYPE COIL. Matthias Schneider, Andreas Stosic, Stephan Bayer. Small Animal Clinic, Giessen, Germany

Surgical treatment of intrahepatic portosystemic shunts (iPSS) is challenging. Frequently, multiple interventions are needed to avoid portal hypertension and to achieve a complete closure. Complications seen in catheter-assisted embolization with commercial coils include coil migration, portal hypertension (rapid thrombotic occlusion), and incomplete occlusion. Attenuation of hepatic vein by means of stent-supported coil embolization may lead to the development of intrahepatic venous collaterals. In the present study we postulated that implantation of a single prototype coil combined with immediate unfractionated heparin therapy (UHT) is an effective treatment for iPSS in dogs and has fewer complications than reported with open surgery.

Dogs with single iPSS with or without intrahepatic venous collaterals were included. A percutaneous access to the right jugular vein was used for retrograde portal vein catheterization. Portal vein pressure and iPSS diameter were measured unblocked and blocked (balloon catheter: 8F wedge catheter or 20/27 mm occlusion catheter). The prototype coil with polyester fibers was selected to be either at least 1 mm larger in diameter than the blocked iPSS or twice the diameter of the unblocked iPSS, and was implanted using an 8F guiding catheter. Coagulation times were assessed by activated coagulation time (ACT) at baseline and during UHT. Heparin was started before coil implantation with a bolus of 100 IU/kg (injected into the portal vein), followed by continuous rate infusion (25 IU/kg/h). Postoperatively, UHT was administered 150 IU/kg q6 h subcutaneously and intermittent 50 IU/kg intravenously to achieve an ACT of 150 to 200 seconds, and then adjusted based on sonographic proven development/resolution of ascites. Functional closure was judged according to the result of the oral ammonia tolerance test. Descriptive statistics and Kaplan-Meier analysis was used.

The prototype coil was implanted in a total of 25 dogs. Most common breeds were Hovawart (n = 8) and Golden Retriever (n = 5). Median age was 10 months (range 6 – 35) and median body weight 26 kg (range 12 – 48). iPSS originated from the right lateral (n = 1), right medial (n = 7) or left portal vein branch (n = 17). 19/25 dogs showed portal hypertension during shunt blocking. Unblocked and blocked shunt diameter were 5.2 - 13.0 mm (median 8.3; n = 25) and 9.0 - 21.0 (median 13.3; n = 24), respectively. Intervention was successful at the first (n = 24) or second (n = 1) intervention. Major complications developed in 5 dogs leading to death in 2 dogs. Minor complications were common and were mostly associated with bleeding problems. One dog was lost to follow-up after three months. Of the remaining 22 dogs, 5 experienced complications associated with change to a non-protein restricted diet: severe neurological abnormalities leading to euthanasia (n = 1), moderate symptoms leading to re-intervention (n = 2), mild symptoms which could be treated successfully by changing to a moderately protein restricted diet (n = 2). All dogs without portal hypertension showed functional closure at 3 months follow-up. In dogs with portal hypertension the cumulative proportion of functional closure reached 81% at 24 months follow-up.

In conclusion, stiffness and size of the prototype coil were responsible for the high procedural success and functional closure rates. Unfractionated heparin treatment needs optimization to reduce complications, but the general treatment regimen seems promising.

HP05

INVESTIGATION OF HEPATIC COPPER ACCUMULATION IN DOGS FROM TWO TIME PERIODS (1982–1988 AND 2009–2015). Ryan Schultz, Rebecca Smedley, John Buchweitz, Katie Barnes, Sarah Abood, Daniel Langlois. Michigan State University College of Veterinary Medicine, East Lansing, MI, USA

Canine copper-associated hepatitis (CCAH) has become an increasingly recognized cause of progressive and potentially fatal

liver disease in dogs; however, the exact etiology remains unclear. To date, no broad epidemiologic investigations quantifying hepatic copper concentrations over time in both predisposed (to CCAH) and non-predisposed breeds have been conducted. The purpose of our study was to determine and compare quantitative hepatic copper concentrations in breeds that are, or are not, predisposed to CCAH from two study periods. We hypothesized that hepatic copper concentrations have increased for all dogs over time, but the relative increase will be greater in predisposed breeds. A retrospective search of the histopathology database at the Michigan State University Diagnostic Center for Population and Animal Health was conducted for two study periods: 1982 through 1988, and 2009 through 2015. Doberman Pinschers, Labrador Retrievers, and West Highland White Terriers were considered predisposed to CCAH. Dalmatians, Skye Terriers, and Bedlington Terriers were excluded from analysis due to a rarity of cases, limited information on predisposition, or a known genetic etiology, respectively. Cases with archived liver tissue available were randomly selected, reviewed, and then classified by breed and presence or absence of inflammatory liver disease. Hepatic copper concentrations (parts per million, dry weight basis) were measured in all specimens using inductively coupled plasma mass spectrometry. Comparisons were made within and across groupings using commercially available statistical software.

A total of 373 cases were evaluated, including 207 dogs predisposed to CCAH and 166 non-predisposed dogs. Independent of breeds groupings and inflammatory activity, mean hepatic copper concentrations in the recent time period were 225 ppm greater than those in the historical time period ($P < 0.05$). This effect was predominantly due an increase of 386 ppm over time in predisposed breeds ($P < 0.001$). The mean hepatic copper concentration of 338 ppm in non-predisposed breeds in recent years was greater than the mean hepatic copper concentration of 271 ppm in non-predisposed breeds in the historical period, but this trend did not reach significance ($P = 0.08$). In the recent period, the mean hepatic copper concentration of 519 ppm in predisposed breeds with non-inflammatory liver histology was greater than the mean hepatic copper concentration of 297 ppm in non-predisposed breeds with non-inflammatory liver histology ($P < 0.001$). This difference was not observed in the historical time period. In both periods, dogs with hepatitis had higher hepatic copper concentrations than those without hepatitis, but this difference was magnified in recent years. In the aggregate, these findings confirm that hepatic copper concentrations have changed over time. The etiology of these changes is unknown, but suggestive of changes in environmental copper exposure. Detailed investigations of potential sources of copper exposure, namely drinking water and diet, are needed to further elucidate a potential environmental etiology. Additional investigations also are needed to determine if these changes are important for all breeds of dog.

ID01

EVIDENCE FOR GENETIC PREDISPOSITON TO *BORRELIA BURGDORFERI* INFECTION IN PURPOSE BRED BEAGLES. Elena Contreras[1], Scott Moroff[2], Michael Lappin[1]. [1]Department of Clinical Sciences, Colorado State University Veterinary Teaching Hospital, Fort Collins, CO, USA, [2]Antech Diagnostics, Lake Success, NY, USA

Borrelia burgdorferi (BB) and *Anaplasma phagocytophilum* (AP) are transmitted by *Ixodes* spp., and seroprevalence rates in endemic areas can be greater than 15% in dogs. A genetic predisposition to infection (Bernese mountain dogs) or clinical illness associated with BB has been suggested, particularly in people and dogs with Lyme nephritis (Retriever breeds). In vaccine studies, wild caught *I. scapularis* adults placed in feeding chambers and allowed to feed for seven days usually results in at least a 75% BB infection rate. Recently, an *I. scapularis* infestation study using a previously reported model and ticks with a similar BB carriage rate from the same collection area previously used, resulted in only a 29.2% infection rate. The purpose of this report is to describe the genetic relatedness of the dogs used in that study.

A total of 10 female and 14 male purpose-bred beagles, 9–12 months of age, and negative for antibodies against *A. phagocytophilum*, *B. burgdorferi*, and *E. canis* (Accuplex 4 BioCD system; Antech Diagnostics), were infested for 7 days with 13 female and 12 male wild-caught *I. scapularis* with an estimated 48% BB infection rate and a 15% AP infection rate. Blood was collected from all dogs prior to tick attachment and then on Weeks 1–12 and Week 18 after tick attachment and screened for BB and AP antibodies as described. Dogs that seroconverted were assumed to have been infected. Statistical analyses were performed with commercial software; $P \leq 0.05$ was considered statistically significant.

Antibodies against BB were detected in 7 of 24 dogs (29.2%) between 4–8 weeks post-infestation; all positive dogs were females ($P = 0.0003$). When positive and negative dogs were compared, there were no significant differences in the ages, number of ticks recovered, or number of fed ticks. Antibodies against AP were detected in the serum of 10 of 24 dogs (41.7%). Results using the same source of dogs, ticks, and model in previous publications were 77.8% and 55.5%% for BB and AP, respectively. The differences in infection rates between the current and historical studies were similar for AP but significantly lower in the current study for BB ($P = 0.004$). The most recent common ancestor (MRCA) for 6 of the 7 (86%) BB-positive dogs was either a sibling or parent. Dogs A and B were siblings and had the same father as Dog C. The mother of Dog D was the maternal grandmother of Dogs A and B. The father of Dog E was the paternal great grandfather of Dogs A, B, C. The mother of Dog F was the maternal grandmother of Dog E. The MRCA for 8 of the 17 (47.1%) BB-negative dogs was either a sibling or parent. None of the BB positive dogs had a MRCA sibling or parent in common with any of the BB negative dogs.

These findings provide further evidence that a genetic predisposition to *B. burgdorferi* infection exists. As studies have investigated common genotypes and alleles among humans with Lyme borreliosis and arthritis, this should also be considered in future studies of canine Lyme borreliosis.

ID02

RAPID DIAGNOSIS OF *BABESIA GIBSONI* USING POINT-OF-CARE INSULATED ISOTHERMIC POLYMERASE CHAIN REACTION ASSAY. Kirsten Cooke[1], Patrick Frenzer[1], Julie K. Levy[1], Sylvia Tucker[1], P. Cynda Crawford[1], Sarah Kirk[2]. [1]University of Florida, Gainesville, FL, USA, [2]The American Society for the Prevention of Cruelty to Animals, New York, NY, USA

Babesia gibsoni is commonly identified in pit bull-type dogs, particularly those involved in dogfights. Dogs seized from fighting operations undergo health and behavior evaluations to assess suitability for adoption. Adoption carries the risk for sending dogs harboring infectious diseases to unsuspecting owners or to regions where the infections are not endemic. Screening large numbers of dogs for infectious diseases is expensive and it may be days before results are available. A more rapid and cost-effective diagnostic test would make identification and treatment of *B. gibsoni* infected dogs more feasible for rescue organizations. Recently, the development of insulated isothermic PCR (iiPCR) has made mobile PCR a reality. The purpose of this study was to determine performance of the POCKIT® iiPCR system for diagnosis of *B. gibsoni* in dogs rescued from fighting organizations.

Whole blood was collected from 260 pit bull-type dogs rescued during a federal dogfighting investigation. Real-time PCR was performed for *Babesia* species, and positive samples underwent a second round of species-specific PCR in a commercial diagnostic laboratory (IDEXX Laboratories, Inc.). Residual whole blood samples were frozen and stored at -80 C pending subsequent analysis. POCKIT® iiPCR was performed twice on each sample, once with a kit specific for *B. gibsoni* and once with a kit that detects both *B. gibsoni* and *Babesia canis* (Babesiosis). The sensitivity and specificity of each test were calculated using the commercial test as the gold standard. Positive (PPV) and negative (NPV) predictive values were calculated for theoretical 5%, 15%, and 30% prevalences. The study was approved by the UF IACUC.

A total of 80 dogs (31%) were positive for *B. gibsoni* by the gold standard commercial PCR test. Of these, 72 were identified as positive by the POCKIT® iiPCR *B. gibsoni* kit, and 70 were identified as positive by the POCKIT® iiPCR *Babesiosis* kit. Sensitivity and specificity of the *B. gibsoni* kit were 90% and 99%, respectively. Sensitivity and specificity of the *Babesiosis* kit were 88% and 98%,

respectively. The PPVs of the B. gibsoni kit at 5%, 15%, and 30% prevalence were 82%, 94%, and 97% respectively. The NPVs of the *B. gibsoni* kit were 99%, 98%, and 96% respectively. The PPVs of the *Babesiosis* kit were 70%, 89%, and 96% respectively. The NPVs of the *Babesiosis* kit were 99%, 98%, and 95% respectively.

The POCKIT® iiPCR system was quick to learn, portable, and had a short run time of approximately 1 hour. There were few false-positive results, indicating that positive results are likely to represent true infections when tested in high-risk animals. Approximately 10–15% of infected dogs would be missed by the POCKIT® iiPCR system. However, the portability and speed with which results can be obtained may result in more infected dogs being diagnosed than in the current situation in which testing is rarely performed in dog-fighting cases due to cost and logistics of sending samples to outside laboratories. This system may also be used for *B. gibsoni* treatment monitoring using a hybrid approach. For example, following initial diagnosis with either the POCKIT® system or a commercial laboratory, the POCKIT® system could be used for testing during treatment. Once a negative result was obtained, a sample could be submitted to a commercial laboratory to confirm treatment efficacy. Although this study focused on mass screening for *B. gibsoni*, the portable iiPCR platform has potential to aid in rapid detection of a variety of infections under field conditions.

ID03

PREVENTION OF *BORRELIA BURGDORFERI* AND *ANAPLASMA PHAGOCYTOPHILUM* TRANSMISSION FROM *IXODES SCAPULARIS* TO DOGS BY SAROLANER. Nicole Honsberger, Robert Six, Thomas Heinz, Angela Weber, Sean Mahabir, Thomas Berg. Zoetis, Kalamazoo, MI, USA

The efficacy of sarolaner (Simparica™, Zoetis) to prevent transmission of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* from infected wild-caught *Ixodes scapularis* to dogs was evaluated in a well-controlled laboratory study. Twenty-four purpose-bred laboratory Beagle dogs seronegative for *B. burgdorferi* and *A. phagocytophilum* antibody were allocated randomly to one of three oral treatment groups: placebo administered on Days 0 and 7, sarolaner administered on Day 0 (28 days prior to tick infestation), or sarolaner administered on Day 7 (21 days prior to tick infestation). Sarolaner tablets were shaved and/or sanded based on each dog's individual bodyweight to provide a dosage of 2 mg/kg. On Day 28 each dog was infested with approximately 25 female and 25 male wild caught adult *I. scapularis* that were determined by random sampling to have infection rates of 57% for *B. burgdorferi* and 6.7% for *A. phagocytophilum* by PCR. *In situ* tick counts were conducted on Days 29 and 30. On Day 33, all ticks were counted and removed. Blood samples collected from each dog on Days 27, 49, 63, 77, 91 and 104 were tested for the presence of *B. burgdorferi* and *A. phagocytophilum* antibodies using the SNAP® 4Dx® Plus Test, and quantitatively assayed for *B. burgdorferi* antibodies using an ELISA test. Skin biopsies collected on Day 104 were tested for the presence of *B. burgdorferi* by bacterial culture and PCR. Acaricidal efficacy was calculated based on the reduction of geometric mean live tick counts in the sarolaner-treated groups compared to the placebo-treated group for each tick count.

Geometric mean live tick counts for placebo-treated dogs were 14.8, 12.8 and 19.1 on Days 29, 30 and 33, respectively. For the group treated with sarolaner 21 days prior to infestation, the percent reductions in mean live tick counts on Days 29, 30, and 33 were 86.3%, 100%, and 100%, and for the group treated with sarolaner 28 days prior to infestation were 90.9%, 97.1%, and 100%. Geometric mean live tick counts for both sarolaner-treated groups were significantly lower than those for the placebo group on all count days ($P < 0.0001$).

Successful transmission of *B. burgdorferi* to all eight placebo-treated dogs was confirmed by positive antibody (6 of 8 dogs), PCR (7 of 8 dogs), and/or culture result (7 of 8 dogs). Similarly, transmission of *A. phagocytophilum* was confirmed by the presence of antibodies in four placebo-treated dogs. In contrast, a single oral dose of sarolaner prevented transmission of *B. burgdorferi* from infected ticks to dogs infested 21 or 28 days after treatment as demonstrated by negative antibody, PCR, and culture results. Prevention of *A. phagocytophilum* transmission was also demonstrated by negative antibody in all sarolaner-treated dogs. There were no adverse reactions to treatment with sarolaner.

ID04

MOLECULAR ANALYSIS ON CANINE BABESIA PREVALENCES AND EXPOSURE TO ANAPLASMA, EHRLICHIA, BORRELIA AND DIROFILARIA IN MOST POPULATED SERBIAN REGION – BELGRADE AREA. Milica Kovacevic Filipovic[1], Anja Ilic[1], Zorana Milanovic[1], Dragan Bogdanovic[2], Vladimir Radonjic[3], Jelena Francuski Andric[1], Nenad Andric[1], Brendon Thatcher[4], Christian Leutenegger[4], Phyllis Tyrrell[4], Antony Mestek[4], Chandrashekar Ramaswamy[4]. [1]Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia, [2]State University of Novi Pazar, Novi Pazar, Serbia, [3]Private Veterinary Practice Petrovac", Belgrade, Serbia, [4]IDEXX Laboratories, Westbrook, Maine, USA

Babesiosis is endemic in many parts of Serbia. Other tick borne diseases are less commonly diagnosed because their clinical signs are nonspecific and there is no published data indicating their prevalence. The aim of this study was to determine molecular prevalence of *Babesia canis canis* and *B. gibsoni*, molecular and seroprevalence of *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, *E. ewingii*, *E. chaffeensis* and *Borrelia burgdorferi* and *Dirofilaria sp.* microfilariae in different outdoor canine populations.

Blood samples from apparently healthy stray (n = 38), shelter (n = 39) and hunting dogs (n = 35) and outdoor pet dogs suspected to have tick borne diseases (n = 50) were tested using real time PCR and serological assays. Real-Time PCR assays were used to amplify *Babesia*, *Anaplasma* and *Ehrlichia* DNA from blood samples. An experimental SNAP® Multi-Analyte Assay (SNAP® M-A) (IDEXX Laboratories, Inc. Westbrook, Maine, USA) was used to screen all sera for antibodies to *Anaplasma* and *Ehrlichia* genus peptides and *A. phagocytophilum*, *A. platys*, *B. burgdorferi*, *E. canis*, *E. ewingii*, and *E. chaffeensis* species-specific peptides. Modified Knott test was used to identify dogs with *Dirofilaria sp.* microfilaria presence.

In total, prevalence of *Babesia sp.* infections among apparently healthy dogs was 16% and among sick pet dogs, was 65%. Prevalence of *B. canis* was the highest in shelter (25%) and the lowest in stray dogs (8%). *B. gibsoni* was rare among shelter, stray and pet dogs, while it was not detected among hunting dogs. Molecular analysis did not reveal the presence of dogs with active anaplasmosis, ehrlichiosis or borreliosis. However, 32% of stray and shelter dogs, 12% of hunting dogs and 4% of sick pet dogs had antibodies to *A. phagocytophilum*. Only one dog (hunting group) was seropositive to *A. platys*, one dog to *E. ewingii* (stray group) and two dogs to *B. burgdorferi* (stray group). None of the dogs was seropositive to *E. canis* and *E. chaffeensis*. Also, 33% of pet, 18% of hunting, 11% of stray and 3% of shelter dogs had microfilariae in the blood. Number of dogs exposed to two and three pathogens was the highest among shelter dogs (31% and 10%, respectively), and number of dogs exposed to four pathogens was the highest among stray dogs (5%).

In Belgrade area, *B. canis* and *A. phagocytophilum* are the most prevalent vector-borne pathogens in apparently healthy outdoor dog population, while *B. canis* and *Dirofilaria sp.* are the most important pathogens among sick pet dogs.

ID05

ENHANCED SEROLOGIC SURVEILLANCE TO DETECT PREVALENCE OF CANINE VECTOR-BORNE INFECTIONS ON THE ISLAND OF ST. KITTS, WEST INDIES. Diana Scorpio[1], Jennifer Ketzis[1], Linda Shell[1], Brendon Thatcher[2], Ramaswamy Chandrashekar[2]. [1]Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis, [2]Idexx Laboratories, Westbrook, Maine, USA

The island of St. Kitts (West Indies) has a large feral and semi-feral dog population. Semi-feral dogs present a unique opportunity for study since they are mostly outdoors but are comparatively well

taken care of compared to feral dogs. Care tends to range from minimal to adequate, though many owners use the Ministry of Agriculture Veterinary Services for preventative medicine rather than a veterinary hospital setting. Historically, dogs on the island are endemically infected and infested with internal and ectoparasites, which are also associated with a number of vector-borne diseases. Due to the high prevalence of the vector Rhipicephalus sanguineus, it is not unusual to see high prevalence of Ehrlichia canis and *Anaplasma platys*. Samples collected from an island-wide field study from last year revealed an abundance of serologic data. These samples have been further tested utilizing a research-based serology platform (IDEXX Laboratories) and additional canine vector-borne infections data has been collected from these analyses.

Over a period of 3 months, veterinarians and students from RUSVM visited all 9 parishes of St. Kitts, covering a diverse geographic area with a wide diversity in ecosystem landscape. One hundred and eleven dogs were identified and visited, and were given a complete physical exam, then blood and feces were collected. Demographic data was collected, including breed, age, housing, other animals in household, evidence of ticks or fleas, deworming and vaccine administration. Blood was analyzed using a research-based broader canine vector-borne infection serologic platform (SNAP® M-A, IDEXX Laboratories), which tests for specific antibodies against *E. ewingii, E. canis, E. chaffeensis, Anaplasma platys, A. phagocytophilum,* and *Borrelia burgdorferi*. This testing platform is also based on a rapid ELISA SNAP® technology.

Analyses using the new SNAP® M-A serologic platform revealed no evidence of exposure to *E. ewingii, E. chaffeensis, or Borrelia burgdorferi*. However, as expected due to the endemic nature of the tick vector, the platform identified *A. platys* exposures of approximately 20.7%. *E. canis* exposures were detected at a prevalence of approximately 32%. Of interest is that the SNAP®

M-A test also identified 19 dogs (17%) with antibodies to *A. phagocytophilum*, not previously described on St. Kitts due to the absence of *Ixodes* tick vectors.

Using the SNAP® M-A serologic platform, there was a robust capacity to detect up to 2 *Anaplasma* species, in addition to having a sensitivity which corroborates previously published prevalence rates of *E. canis*. Of note is the presence of *A. phagocytophilum* exposures, and additional studies are warranted to understand and explain these findings. The data collected in this study supports the importance of including an exhaustive list of differentials when suspecting canine vector-borne infections, in addition to aggressive and consistent use of environmental and topical parasiticides for tick control.

ID06
PERFORMANCE OF POINT-OF-CARE ASSAYS FOR FELV AND FIV.

Julie Levy. Maddie's Shelter Medicine Program, University of Florida, Gainesville, FL, USA

At least 5% of cats in the US are infected with FeLV or FIV, contagious retroviruses that predispose to bone marrow suppression, cancer, chronic inflammatory conditions, immune dysfunction, and wasting syndromes. This equates to more than 5 million cats affected by these preventable viruses and at risk for disease and premature death. Although vaccination plays a role in prevention, the cornerstone of control is identification and segregation of infected cats. Recently, new point-of-care screening tests have become available, but independent comparison of test performance is needed. The purpose of this study was to determine the performance of currently available FeLV antigen/FIV antibody combination test kits.

For assessment of FeLV tests, serum or EDTA whole blood samples were collected from animal shelters or reference laboratory and tested with two microtiter plate ELISAs for the detection of FeLV p27 antigen (ViraCHEK®/FeLV and PetChek® FeLV Antigen Test) to establish a gold standard. Only samples with concordant results were included in the study. For assessment of FIV tests, plasma samples previously collected from naturally or experimentally infected cats and verified by virus culture and from uninfected SPF cats were included. None of the cats had been vaccinated against FIV. The final study set included 146 FeLV+, 154 FeLV-, 94 FIV+, and 97 FIV- samples. Point-of-care tests evaluated in this study included SNAP® Combo FeLV Ag/ FIV Ab Test, WITNESS® FeLV-FIV Test Kit, Anigen® Rapid FIV Ab/FeLV Ag Test Kit, VetScan® Feline FeLV/FIV Rapid Test. All test results were visually read by two blinded observers.

	SNAP®	WITNESS®	ANIGEN®	VETSCAN®
FeLV Sensitivity (95% CI)	100% (96.9-100)	89.0% (82.8-93.2)	91.8% (86.1-95.4)	85.6% (78.9-90.5)
FeLV Specificity (95% CI)	100% (97.1-100)	95.5% (90.8-98.0)	95.5% (90.8-98.0)	85.7% (79.3-90.48)
FIV Sensitivity (95% CI)	97.9% (92.1-99.9)	94.7% (87.9-98.0)	96.8% (90.6-99.3)	91.5% (83.9-95.8)
FIV Specificity (95% CI)	99% (93.8-100)	100% (95.4-100)	99% (93.8-100)	99% (93.8-100)

The SNAP® test had the overall best performance. In high-stakes testing, test kits should be selected for both high sensitivity and specificity. When tests lack sensitivity, infected cats may escape detection and remain at risk for infecting other cats. When specificity falls, uninfected cats may be unnecessarily segregated or even euthanized. In low infection prevalence, such as observed in FeLV and FIV, decreased specificity has the largest impact on erroneous test results, leading to decreased positive predictive value (increased false-positives).

ID07
DOES A DIVA TEST EXIST FOR DIFFERENTIATING FIV INFECTION FROM FIV VACCINATION?.

Cynda Crawford. Maddie's Shelter Medicine Program, University of Florida, Gainesville, FL, USA

Diagnosis of FIV infection is based on detection of circulating FIV antibodies. Introduction of FIV vaccines created a diagnostic dilemma because vaccine antibodies were indistinguishable from those induced by infection using existing serological assays. New point-of-care tests for FIV antibody are now available to practitioners. The purpose of this study was to determine the accuracy of FIV diagnostic tests in FIV-vaccinated cats and whether any of the tests could serve as a DIVA test to differentiate infected from vaccinated animals.

Plasma samples were collected from 104 uninfected SPF cats vaccinated 3 times with a killed dual-subtype FIV vaccine (Fel-O-Vax, Boehringer Ingelheim) according to manufacturer instructions. Age at vaccination ranged from 2.5 to 13 months. The interval between vaccination and sample collection ranged from 1.75 to 14 months. The FIV-free infection status of all cats was confirmed by virus culture. Three FIV-infected cats were similarly vaccinated and tested. The plasma samples were tested in 4 commercial point-of-care assays: SNAP® Feline Combo FeLV Ag/FIV Ab Test, WITNESS® FeLV-FIV Test, Anigen® Rapid FIV Ab/FeLV Ag Test Kit, and VetScan® Feline FeLV/FIV Rapid Test. All testing was performed by personnel blinded to sample status. Each test result was independently interpreted by 2 personnel.

The SNAP® and VetScan® tests detected FIV antibodies in 102/104 and 88/104 uninfected vaccinated cats, respectively. This would lead to misclassification of most of the vaccinated cats as infected. The WITNESS® and Anigen® tests had very high specificity, indicating that nearly all vaccinated cats were correctly identified as uninfected. All 4 tests correctly identified the 3 FIV-infected cats that were vaccinated against FIV.

Specificity and 95% Confidence Intervals for 4 Point-of-Care Tests for FIV Infection Performed in 104 Uninfected Cats that were Vaccinated against FIV

WITNESS®	ANIGEN®	VETSCAN®	SNAP®
98.1% (92.8-99.9)	98.1% (92.8-99.9)	21.2% (14.3-30.0)	1.9% (0.1-7.2)

Accurate detection of FIV-infected cats is essential to providing appropriate care and segregation from uninfected cats. Since euthanasia is a tool employed in the control of FIV, especially in animal shelters where the previous vaccination history of cats is usually unknown, misidentification of vaccinated cats as being infected may have unwarranted fatal consequences. Based on this study, the WITNESS® FeLV-FIV Test and the Anigen® FIV Ab/FeLV Ag Test are the most accurate for differentiating FIV-infected from FIV-vaccinated cats and meet the criteria for DIVA tests.

ID08

AN EPIDEMIOLOGICAL STUDY OF GAMMAHERPESVIRUSES IN DOMESTIC CATS IN JAPAN. Morihiro Tateno, Yasuyuki Endo, Masashi Takahashi. Kagoshima University, Kagoshima, Japan

Gammaherpesviruses (GHVs) are members of an emerging subfamily of the Herpesvividae. The two major human GHVs, Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are clinically important pathogens because those viruses possess tumorigenesis potential especially in immune-compromised patients. In feline practice, we often experience tumor cases, especially lymphomas. Feline leukemia virus (FeLV) was a major cause for the development of lymphoma in the past days, however, the majority of lymphoma cases are shifting to FeLV-free cats. But the mechanism of tumorigenesis in these "spontaneously" developed lymphomas has not been well understood. Under this situation, GHV became a candidate which explains the lymphomagenesis in domestic cats. Recent study identified a novel GHV in domestic cats (Felis catus GHV, FcaGHV), and epidemiological surveys found that FcaGHVs are distributing in several countries including the United States, Australia, Singapore, Germany and Austria. In the present study, a molecular epidemiological study was conducted to investigate the prevalence of GHVs in Japan using large numbers of samples which were previously used to a nation-wide epidemiological survey for two feline retroviruses in Japan (Nakamura Y et al., J Vet Med Sci, 72:1051–1056, 2010).

GHV-derived DNA in blood samples was detected by degenerate nested pan-GHV PCR targeting gB gene as previously reported. Nucleotide sequences of obtained DNA fragments were determined and BLAST analysis was carried out. Phylogenic analysis was also conducted. Risk factors relating to GHV infection on clinical parameters, including age, sex, status of retrovirus infections, bite wound history and clinical signs, were statistically analyzed using univariable (Fisher's exact test) and multivariable (Logistic regression model) analyses.

GHV-derived DNA was detected in 2.6% of Japanese domestic cats. BLAST analysis revealed that all of them showed high similarity with FcaGHVs which were isolated from domestic cats in the United States. Phylogenic analysis also revealed that all Japanese isolates formed one cluster with FcaGHV. Age (> 2 years old) (Odds ratio [OR], 6.02; 95% confidential interval [CI], 0.79–46.0; $P = 0.05$) and feline immunodeficiency virus (FIV) infection (OR, 5.41; 95% CI, 1.93–15.1; $P < 0.01$) were found to be a risk factor in univariable analysis. In multivariable analysis, only FIV infection (OR, 3.74; 95% CI, 1.28–10.94; $P = 0.016$) was a significant risk factor.

In this study, the prevalence of GHVs in Japanese domestic cats was surveyed and risk factor for GHV infection was determined. FcaGHV prevalence in Japan (2.6%) was lower than previously reported prevalence in other countries and areas (US, 16%; Europe, 16.2%). It was unable to determine the cause of this difference. However, Japanese isolates were identified to have close genetic relationship to domestic cat derived FcaGHV. This finding suggested that the virus is highly conserved in each animal species and GHV is harboring in host specific manner as well as other herpesviruses. The risk factor for GHV infection in Japanese cats was FIV infection and it was same as previously reported. But we have to clarify how FIV enhances the GHV infection and/or viremia. The clinical importance of GHVs infection in feline practice is still unknown. Further investigation will be necessary to identify the pathogenicity of GHVs in domestic cats.

ID09

CANINE INFLUENZA H3N2 INFECTION IN FOUR DOGS. Anne Cohen[1], Jill Richardson[2], Amy Glaser[3], Edward Dubovi[3], Nyssa Reine-Salz[2]. [1]Chicago Veterinary Emergency and Specialty, Chicago, USA, [2]Merck Animal Health, Madison, USA, [3]Cornell University, Ithaca, USA

The H3N2 influenza virus is of avian origin and was first isolated from clinically ill dogs in China in 2006 and South Korea in 2007.[1] Canine H3N2 influenza virus has been associated with severe respiratory signs and other clinical signs such as fever, reduced body weight, and interstitial pneumonia.[1] This case report presents four confirmed, naturally infected clinical cases of Canine Influenza H3N2 in the United States. The cases presented were all pet dogs that had histories of recent exposure to other dogs in a social setting including dog parks and doggie day cares. None of the dogs originated from, or had traveled to, typical regions endemic for this viral disease. All dogs were presented at different stages of illness attributed to infectious respiratory disease.

One dog , a 1.5 year old Corgi, was evaluated for lethargy, anorexia, and loose stool for 2 days. He had also developed a cough on the day of presentation and had mild increases in respiratory rate. On physical examination, he had a fever with mildly increased respiratory sounds. The patient was re-presented the next day for worsened signs of coughing, lethargy and hyporexia. Thoracic radiographs were performed which showed severe bilateral bronchopneumonia. Based on his poor response to supportive care, the owners elected humane euthanasia. Necropsy was performed at the New York State Veterinary Diagnostic Laboratory at Cornell University. Necropsy revealed Severe, acute, locally extensive necrohemorrhagic interstitial pneumonia with epithelial necrosis.

Another dog, a 8 year 10 month old Miniature Pincher, was evaluated after a 9 day history of coughing, wheezing, weakness, ataxia, hyporexia and one day of clear nasal discharge. On physical examination, the patient was weak, ~7% dehydrated, had serous nasal discharge, muffled heart sounds, poor peripheral femoral pulses, and harsh bilateral thoracic sounds with crackles and wheezes. Thoracic radiographs were performed which showed right caudodorsal and left cranial bronchoalveolar bronchopneumonia. The patient experienced cardiopulmonary arrest and upon return of spontaneous circulation the owners elected humane euthanasia. Necropsy results were severe, acute, multifocal to coalescing necrohemorrhagic pneumonia with hyaline membrane formation and epithelial necrosis. Enteric lesions were also noted with moderate, diffuse, chronic lymphoplasmacytic enteritis with multifocal crypt necrosis.

Another dog, a 4 year old Greater Swiss Mountain dog, was referred for hospitalization due to right cranial and middle lung lobe consolidation, fever, dyspnea, gagging with phlegm production, exercise intolerance and hyporexia. On physical examination, he was quiet, febrile at 105.1 degrees Fahrenheit, had an increased respiratory rate of 60 breaths per minute with harsh bronchovesicular lung sounds. He became oxygen dependent with a pulse oximetry of 89–91%. Recheck thoracic radiographs were performed on the fourth day of hospitalization which showed a worsened alveolar pattern in the right and left cranial thorax. He was hospitalized for 7 days, making gradual improvements until discharge. H3N2 was confirmed via PCR at Cornell University.

The fourth case, a 10 year old terrier mix breed, was presented for evaluation of a productive cough for two days and one day of lethargy, anorexia and weakness. On physical examination, harsh lung sounds with mild increased respiratory rate was appreciated. The patient vomited a large volume of bile material after examination. A severe fever of 107.1 degrees Fahrenheit was appreciated with a respiratory rate of 48 breaths per minute and ~5% dehydration. Thoracic radiographs were performed which showed a mild to moderate bronchointerstitial pattern most concentrated in the caudodorsal lung fields. The patient was hospitalized for 4 days and was eventually tapered off of oxygen and sent home on oral antibiotic therapy. A respiratory panel was sent to IDEXX Reference Laboratory. The patient was positive for an Influenza A Assay, negative for an H3N8 PCR and positive for H3N2 PCR.

All four of the dogs were diagnosed with Canine Influenza H3N2 through PCR, three through the New York State Veterinary Diagnostic Laboratory and one through Idexx Diagnostic Laboratory. In patients with characteristic clinical features, Canine Influenza H3N2 virus infection should still be considered as differential diagnosis.

ID10

DEMONSTRATION OF PROTECTION AGAINST CANINE INFLUENZA VIRUS H3N2 INFECTION FOLLOWING VACCINATION WITH INACTIVATED CIV H3N2. Rhonda LaFleur[1], Tamara Davis[1], Patrick Tuma[1], Huchappa Jayappa[1], Mike Francis[2], Ian Tarpey[2]. [1]Merck Animal Health, Elkhorn, NE, USA, [2]MSD Animal Health, Milton Keynes, UK

A study was conducted in dogs to evaluate the efficacy of an inactivated Canine Influenza Virus (CIV) H3N2 vaccine following experimental challenge with a virulent heterologous strain of CIV H3N2, isolated from the recent CIV H3N2 outbreak in the United States. Eleven dogs, 7–8 weeks of age, were vaccinated with 2 doses of an inactivated CIV H3N2 vaccine, 3 weeks apart, and 19 dogs were vaccinated with a placebo. Two weeks after the second vaccination, all dogs were challenged intranasally with virulent CIV H3N2 and then monitored daily for 10 days for clinical signs including fever, nasal discharge, sneezing, coughing, depression, and dyspnea. Nasal swabs were collected to evaluate viral shedding, and serum samples were collected at various time points to determine antibody titers. At necropsy, lungs were scored for consolidation. Following the booster vaccination, the placebo-vaccinated control dogs remained seronegative (<10) to CIV H3N2, while 10 of the 11 vaccinated dogs developed an antibody titer to CIV H3N2 (GMT = <80; Range = <10 - 320). Antibody titers in dogs from both treatment groups increased following challenge, but the increase was greater in the vaccinated dogs

(GMT = >1452; Range = 40 - >10,240). Following challenge, 8 (42%) of the 19 placebo-vaccinated control dogs were euthanized prior to the 10-day post-challenge observation period due to severe clinical signs, including difficulty breathing, depression, fever, and severe coughing with retching; whereas, none (0%) of the vaccinates had to be euthanized ($P = 0.014$). Clinical signs were evaluated based on a weighted scoring system. The mean clinical score for the placebo-vaccinated control group was 24.9, compared to only 8.7 for the vaccinated group ($P = 0.036$). The placebo-vaccinated control group shed CIV H3N2 virus for a mean of 1.9 days, compared to 1.4 days for the vaccinated group ($P = 0.507$). The median lung consolidation score for the placebo-vaccinated control group was 7.4, compared to 0.0 for the vaccinated group ($P = 0.026$). Results of this study demonstrate that this inactivated CIV H3N2 vaccine significantly protects dogs against severe clinical disease and lung consolidation associated with a virulent CIV H3N2 infection.

ID11

PREVALENCE OF CANINE INFECTIOUS RESPIRATORY DISEASE COMPLEX PATHOGENS IN DOGS IN GEORGIA AND NORTH CAROLINA. Jill Richardson[1], Amy Glaser[2], Edward Dubovi[2], Nyssa Reine-Salz[1]. [1]Merck Animal Health, Madison, NJ, USA, [2]Cornell University, Ithaca, NY, USA

Canine infectious respiratory disease complex (CIRDC) is caused by many different viruses and bacteria. In June 2015, veterinarians around Atlanta, Georgia and also western areas of North Carolina began to notice an unusual increase in dogs presenting to their clinics with signs of infectious respiratory disease. Nasal and pharyngeal swabs from dogs exhibiting clinical signs were submitted to the Animal Health Diagnostic Center (AHDC) at Cornell University. A canine respiratory polymerase chain reaction (PCR) screening panel was utilized which allows identification of the following CIRDC pathogens: *Bordetella bronchiseptica*, *Mycoplasma cynos,* canine adenovirus 1 and 2, canine distemper, canine influenza A, H3N8 and H3N2, parainfluenza virus 5, pneumovirus and respiratory coronavirus.

Between June 1 and August 7, 2015, Merck Animal Health's Diagnostic Support Program tested over 175 samples from dogs with clinical signs of respiratory disease from Georgia and North Carolina. Of those dogs, 51 tested positive for Canine Influenza virus H3N2 - 29 of these cases were identified from Georgia and 22 cases were from North Carolina. None of the tested dogs were confirmed to have the H3N8 canine influenza strain.

Results of the testing in these regions also identified 38 cases of parainfluenza virus, 10 cases of *B. bronchiseptica*, four cases of adenovirus type 2, 29 cases of pneumovirus, 33 cases of respiratory coronavirus, and one case of canine distemper. Further evaluation of the positive cases of parainfluenza showed that 34 of the 38 had received parainfluenza vaccination with the injectable distemper, adenovirus type 2, parvovirus and parainfluenza virus combination vaccine. Also, of the 38 parainfluenza positive dogs, 21 had been vaccinated with a monovalent *B. bronchiseptica* vaccine (oral, injectable or intranasal). Seven of these dogs had been vaccinated with a *B. bronchiseptica* and parainfluenza combination intranasal product, of which two had been recently vaccinated and the parainfluenza sampled may have been of vaccine origin.

The information gathered from this testing program documents the spread of Canine Influenza virus H3N2 to two new locations in the US. In addition, data collected supports the role of parainfluenza virus as a major preventable pathogen in CIRDC and route of vaccination should be considered in vaccination protocols.

ID12

FREQUENCY, BENEFITS AND HEALTH RISKS OF ANIMALS IN NURSING HOMES: CROSS-SECTIONAL STUDY OF OHIO FACILITIES. Jason Stull, Timothy Landers. The Ohio State University, Columbus, OH, USA

Animals are argued to play an important role in nursing homes, providing health benefits, but may also be a source of infections and injuries for animals and patients. To-date little is known of the involvement of animals in nursing homes, practices in place to protect pet and resident health, or specific health benefits and risks. The objective of this study was to determine the frequency and avenues in which animals are utilized in the nursing home environment, practices to safeguard health, and benefits and risks of resident-animal interactions. An anonymous 15-minute online questionnaire was developed and delivered to a convenience sample of nursing home administrators in Ohio, USA from November 2014 through March 2015.

Responses from 95 facilities (approximately 5% of all registered facilities in OH) of varying sizes and geographic locations were received and adequately completed to allow for analysis. Nearly all respondents (94; 99%) allowed animals in their facilities (live-in, visiting, or both), with dogs being the most frequent animal involved with facility activities (99%), followed by cats (89%), birds (74%), fish (57%), and rodents (28%). High-risk species such as reptiles/amphibians and "petting zoo farm animals (e.g., pigs, goats, chickens)" were often permitted in facilities (27% and 12%, respectively). Few facilities had restrictions targeting particular species (n = 9) or age (n = 1) of animals. Animals were reported to visit most (71%) facilities weekly or more often. One respondent (1%) reported an animal (cat) was mistreated by a resident or staff in the preceding 12 months. No known or suspected animal-associated infections were reported for the preceding 12 months. Perceived benefits of animals in facilities were high (e.g., all participants agreed/strongly agreed that "residents appeared to be happier after interacting with an animal" and "animals were useful for calming agitated residents").

These results highlight the frequent use and perceived benefits of involving animals in nursing homes, but also common gaps in established policies and best practices important for reducing resident and animal health risks. Findings can assist in the development of directed guidance documents for nursing homes to better facilitate safe interactions between residents and animals.

ID13

EFFECT OF QUORUM QUENCHING WITH AZITHROMYCIN ON *PSEUDOMONAS AERUGINOSA* ASSOCIATED OTITIS EXTERNA/MEDIA IN DOGS. Modest Vengust[1], Javid Hosseini[1], Darja Kusar[1], Gabrijela Tavcar Kalcher[1], Irena Zdovc[1], Rok Blagus[2], Tina Kotnik[1]. [1]Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia, [2]Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

Many bacteria can express their virulence via quorum sensing (QS); in *Pseudomonas aeruginosa* (PA) the QS signal molecules are

N-acyl-homoserine lactones (PA-AHLs). The use of QS blockers or quorum quenching (QQ) to attenuate bacterial growth and virulence is a new strategy for treatment of resistant bacterial pathogens. Azithromycin (AZ) expresses in-vitro and in-vivo QQ effect, and is used clinically to reduce PA virulence. Hypotheses were tested that topical treatment with AZ will: 1) Attenuate the formation of most common PA-AHLs: N-butyryl-L-homoserine lactone (BHL), N-decanoyl-L- homoserine lactone (HHL) and N-(3-oxo-dodecanoyl)-L-homoserine-lactone (OdDHL); 2) Enhance PA clearance from the ear canal; 3) Have a positive effect on ear canal sample cytology; and 4) Reduce the severity of clinical signs related to PA associated otitis externa/media (PAOEM) in dogs.

This was a randomized double blind placebo controlled study. Samples were collected from 21 dogs with PAOEM, which were treated with topical AZ (1 mL AZ solution at the concentration of 50 μg/mL, SID) or placebo (1 mL sterile 0.9% saline) for 56 days. All dogs were prescribed standard PAOEM treatment and regularly evaluated by their veterinarians. For the purpose of this study dogs were clinically evaluated and ear canal samples taken on days 0, 7, 14, 21, 28, 42 and 56. Samples were obtained by rinsing ear canals with 30 mL of sterile saline. The concentration of BHL, HHL and OdDHL in ng/mL was determined by liquid chromatography coupled with tandem mass spectrometry. Bacteriological and cytological analyses were performed. Clinical signs (ear canal edema, pain, stenosis and erythema) were graded from 0–4; grades were then summarized to form a single clinical sign grade. Data were analyzed using linear mixed effects model for continuous outcomes and general linear mixed effects model for the binary outcomes; a P-value of <0.05 was considered significant.

The concentration of BHL, HHL and OdDHL were not affected by time ($P \geq 0.1$) or AZ ($P \geq 0.2$). The presence of PA in ear canal samples was affected by time ($P = 0.002$) but not AZ ($P = 0.06$). The presence of rod shaped bacteria, cocci and neutrophils in the sample were affected by time ($P \leq 0.02$) but not AZ ($P \geq 0.08$). Macrophages were not affected by time or AZ. Clinical signs and, therefore, the duration of the disease was influenced by time ($P < 0.0001$). Azithromycin had no effect on clinical signs and the duration of the disease ($P = 0.5$).

Detection of PA-AHLs in this study showed that QS acts as a promoter of PA virulence in otitis externa/media. PAOEM in dogs is, therefore, an appropriate disease model to study the effects of QQ on PA. Neither of our hypotheses was verified. Azithromycin had no effect on PA-AHLs and the elimination of PA from the ear canal, nor had any effect on cytology of the sample or the outcome of the disease. Only a weak tendency towards quicker elimination of PA from the ear canal was noted in this study.

ID14
COMPARATIVE EVALUATION OF FIVE IN-CLINIC RAPID TESTS FOR FELINE LEUKEMIA VIRUS INFECTION.

Jiayou Liu[1], Melissa Beall[1], Tom O'Connor[1], Ramaswamy Chandrashekar[1], Mike Lappin[2]. [1]IDEXX Laboratories, Westbrook, ME, USA, [2]Colorado State University, Fort Collins, CO, USA

The feline leukemia virus is a potentially life-threatening oncogenic retrovirus that is transmitted horizontally by close contact or vertically by in-utero transmission. The p27 viral core protein is produced by the virus in infected cells and is found in the cytoplasm in several blood cells as well as free in the serum and plasma of infected cats. Reference laboratories and veterinary clinics commonly use some form of enzyme-linked immunoassay (ELISA) or particle-based immunoassay to detect the presence of the p27 protein antigen in samples obtained from blood. The purpose of this study was to compare the performance of several in-clinic tests: the SNAP® Feline Triple® Test (IDEXX), the VetScan® FeLV/FIV Rapid Test (Abaxis), the Witness® FeLV-FIV Test (Zoetis), Anigen® Rapid FIV/FeLV Test (BioNote), Speed Duo® FeLV/FIV Test (Virbac).

The sample population (84 positive and 101 negative) consisted of serum and plasma samples submitted to IDEXX's worldwide Reference Laboratory for feline retrovirus testing. Virus isolation and reverse transcriptase polymerase chain reaction results were not available and so samples were judged to be positive or negative based on the results of the ViraCHEK® FeLV (Zoetis) microtiter plate assay. ViraCHEK® was used as the reference test because it was reported in a published study to have high sensitivity and specificity (Hartmann et al. 2007) and it detects the same FeLV antigen as does each of the in-clinic tests evaluated in this study.

When compared to the ViraCHEK® microtiter plate assay results, the SNAP® Feline Triple® Test had the highest sensitivity among all in-clinic tests evaluated (Table below).

In-clinic Test	Comparative Sensitivity (95% CI)	Comparative Specificity (95% CI)
SNAP® Feline Triple FeLV Test	97.6% (91.1-99.8)	100% (95.5-100)
WITNESS® FeLV Test	76.2% (66.0-84.0)	97.0% (91.2-99.3)
VetScan® FeLV Test	69.0% (58.5-77.9)	97.0% (91.2-99.3)
Anigen® FeLV Test	66.7% (56.0-75.8)	97.0% (91.2-99.3)
Speed Duo® FeLV Test	51.2% (40.7-61.6)	99.0% (94.0-100)

These findings are important because in a clinical setting positive test results may be the only mechanism to identify infected cats – false negative results could delay supportive care and result in transmission to causal-contact naïve cats. Additional studies are needed to determine the clinical sensitivity and specificity of these tests using samples from cats characterized by PCR and/or viral culture.

ID15
DETECTION OF GIARDIASIS IN DOGS:COMPARISON OF THREE RAPID DIAGNOSTIC TESTS.

Hannah Bewsey, Jan Drexel, Jiayou Liu, Brendon Thatcher, Tom O'Connor, Ramaswamy Chandrashekar. IDEXX Laboratories, Westbrook, ME, USA

Giardiasis is one of the most common intestinal parasitic infections affecting both dogs and cats. Infections may be subclinical or cause severe diarrhea. Dogs become infected by ingestion of *Giardia* cysts in contaminated water or feces. A single fecal flotation has a diagnostic sensitivity of approximately 70% because cysts can be few in number, are shed intermittently, and can be difficult to distinguish from other fecal matter by inexperienced observers. Fecal flotation combined with fecal antigen tests that detect soluble cyst wall antigen results in a diagnostic sensitivity of approximately 97%. While new *Giardia* antigen assays are now available for veterinary use, comparison studies are for the most part lacking.

The present study compared the performance of three in-clinic tests, the SNAP® *Giardia* Test, the VetScan® Canine *Giardia* Rapid Test (Abaxis), and the Anigen® Rapid CPV/CCV/*Giardia* Antigen Test (BioNote). Canine fecal samples used were submitted to IDEXX Reference Laboratories for ova and parasite testing by centrifugal flotation. After testing, the samples were stored at −20°C. The samples were then evaluated for soluble cyst wall antigen using the Thermo Scientific™ ProSpecT™ *Giardia* Microplate Assay. Positive samples were defined as those testing positive by both reference methods while negative samples were defined as those testing negative on both reference methods. A total of 95 positive samples and 81 negative samples were identified and tested on all three in-clinic tests. Six samples were excluded from sensitivity/specificity analysis because they failed to produce positive control lines on the VetScan® Test, despite producing valid results on the other two in-clinic tests. Results were compared to the consensus reference method results for calculation of sensitivity and specificity.

In-clinic Test	Comparative Sensitivity (95% CI)	Comparative Specificity (95% CI)
SNAP® Giardia Test	89.2% (81.1-94.2)	100.0% (94.2-100.0)
VetScan® Giardia Test	71.0% (61.0-79.2)	83.1% (73.0-89.9)
Anigen® Giardia Test	78.5% (69.0-85.7)	70.1% (59.1-79.2)

Using these criteria, the SNAP *Giardia* Test had numerically greater sensitivity and specificity than the other 2 tests. This study used another antigen test in the definition of true positive and true negative results since the samples were not fresh fecals; and not tested using a different test method like immunofluorescent antibody assay.

ID16
SEROPREVALENCE OF ANTIBODIES TO ANAPLASMA PHAGOCYTOPHILUM AND BORRELIA BURGDORFERI IN DOMESTIC CATS.

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Anaplasma phagocytophilum (*Aph*) and *Borrelia burgdorferi* (*Bb*) are pathogenic bacteria vectored by *Ixodes* spp. and are known to infect cats. While associations between *Bb* infections and feline clinical syndromes are unclear, studies have demonstrated *Aph* infection to be associated with fever, severe lethargy, and thrombocytopenia in cats. The present study aimed to evaluate the seroprevalence of antibodies to *Aph* and *Bb* in a large population of domestic cats from across the United States to better understand their risk of exposure to these agents.

A total of 5,416 samples submitted between September 2014 and February 2015 for Complete Blood Count (CBC)-inclusive test profiles were obtained from IDEXX Reference Laboratories located in *Ixodes* spp.-endemic areas. Samples originated from 26 states with California (24%), Illinois (19%), and Massachusetts (13%) contributing most to the study population. The population had a median age of 12.0 years (range: 1 month – 25 years) with similar representation between males (51%) and females (49%). All samples were screened by *Aph*-specific and *Bb*-specific peptide immunoassays and the results were evaluated based on various test and demographic parameters.

Overall, 9.7% of the feline sera tested in this study population contained antibodies to *Aph* while 2.8% had antibodies to *Bb*. Additionally, 1.2% of cats tested had antibodies to both *Aph* and *Bb*. As observed in dogs and humans, antibody prevalence rates among domestic cats varied by geography. The highest positive rates for *Aph* and *Bb* were observed in Connecticut (20.2%, n = 337) and Maine (14.0%, n = 50), respectively, while the lowest seroprevalance for both *Aph* and *Bb* was observed in Nevada (*Aph* = 2.1%, *Bb* = 0.0%, n = 47).

These results corroborate findings in previous studies which indicate that cats can be infected with *Aph* and *Bb* and suggest that tick prevention is an important consideration in cats as well as dogs.

ID17
VALIDATION OF A HIGH-THROUGHPUT SEROLOGICAL ELISA METHOD FOR FELV P27 ANTIGEN DETECTION.

Genevieve Clark, Roberta Cahill, Brendon Thatcher, Peter F. Smith, Thomas P. O'Connor, Ramaswamy Chandrashekar, Melissa J. Beall, Jesse S. Buch. IDEXX Laboratories, Inc., Westbrook, Maine, USA

Feline leukemia virus (FeLV) is a globally-distributed oncogenic retrovirus that can cause fatal disease in cats. Immunoassays that detect FeLV p27 antigen and are readily used to aid in the diagnosis of FeLV have been commercially-available for more than two decades. While single-sample rapid test formats are preferred for in-clinic use, the microtiter plate format enzyme-linked immunosorbent assay (ELISA) is the methodology most commonly employed in reference laboratory settings to facilitate batch testing. However, current commercially-available microtiter plate format ELISAs for FeLV are not well suited for this purpose because they rely on manual wash steps, drop-wise addition of reagents from reagent bottles, and visual interpretation of results. The purpose of this study was to validate a microtiter plate format ELISA designed specifically to maintain performance while enhancing throughput in a reference laboratory setting.

The FeLV microtiter plate format ELISA method validated in this study facilitates high throughput operation by combining parallel fluid transfer processes with automated data acquisition and results interpretation. The assay method is based on sequential orthogonal screening and confirmatory protocols. The screening protocol utilizes two distinct anti-FeLV p27 mouse monoclonal antibodies that produce a colorimetric response in samples containing FeLV p27 antigen. The confirmatory protocol requires neutralization of positive samples in a separate set of controlled assays using an anti-FeLV polyclonal antibody that blocks binding of the mouse monoclonal antibodies to FeLV p27 antigen and thereby inhibits color generation. The confirmatory step offers increased assurance of specificity by enabling discrimination between infected true positives and false positives associated with several factors including anti-mouse heterophilic antibodies in patient samples.

Precision of the screening assay was determined using 3 samples (negative, low positive, and high positive). The intra-assay coefficient of variation (CV) ranged from 3.9% to 7.9% while the inter-assay CV ranged from 6.0% to 8.6%. For the confirmatory assay, the same low positive and high positives samples were used demonstrating an intra-assay CV which ranged from 3.0% to 4.7% and an inter-assay CV between 7.4% and 9.7%. No interference was observed for either protocol at bilirubin, hemoglobin, and lipid concentrations up to 14.4 mg/dl, 550 mg/dl, and 6.4 $OD_{660\ nm}$, respectively. The analytical sensitivity for FeLV p27 antigen was established at 1.7 ng/mL for inactivated whole FeLV and at 1.0 ng/mL for purified recombinant FeLV p27. Discriminating analytical specificity was demonstrated based on the absence of cross-reactivity to related retrovirus antigen from murine leukemia virus and Feline RD-114. Overall, the assay exhibited 100% diagnostic accuracy in 83 patient samples (n = 63 negative; n = 20 positive) with FeLV status defined on the combined basis of PCR (FeLV RealPCR; IDEXX Laboratories, Inc.) and independent ELISA (ViraCHEK FeLV; Zoetis, Inc.).

Results of this study validate that the new microtiter plate format ELISA is a highly specific, precise, and accurate method for detection of FeLV p27 antigen in feline patient samples and is appropriate for high-throughput applications in a reference laboratory setting.

ID18
INVESTIGATION OF WHETHER LEPTOSPIRA VACCINAL ANTIBODIES REACT WITH BORRELIA PEPTIDES USED IN A COMMERCIAL ASSAY.

Amber Caress[1], Scott Moroff[2], Michael Lappin[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]Antech Diagnostics, Lake Success, NY, USA

In small animal veterinary medicine, two of the most common pathogenic spirochete genera are *Borrelia* spp. and *Leptospira* spp.. While antibodies that develop against these organisms over the course of infection in dogs are thought to be genus specific, studies evaluating for cross-reactive antibodies against individual *Borrelia* spp. or *Leptospira* spp. peptides are for the most part lacking. The purpose of this study was to determine if *Leptospira* spp. antibodies induced by administration of one of four commercial vaccines would cross-react with the *B. burgdorferi* antigens used in a commercially available assay.

Staff and student owned dogs were recruited at a Veterinary Teaching Hospital in a *B. burgdorferi* non-endemic area. Inclusion criteria stipulated that dogs were apparently healthy, weighed at least 10 kg, and had not been administered a *Leptospira* spp. vaccine within the past year. The dogs were randomized to be administered one of four commercially available *Leptospira* spp. vaccines that all contained serovars *Canicola, Gryppotyphosa, Icterohemorrhagiae,* and *Pomona.* Blood was collected prior to vaccine administration on Week 0 and Week 3 and then again on Weeks 4, 8, and 12. *Leptospira* spp. microagglutination titers (MAT) were determined using a commercially available service. After confirming the maximal *Leptospira* spp. titers occurred on Week 4, an aliquot of this sera was shipped to Antech Diagnostics for analysis of *B. burgdorferi* antibodies against OspA, OspC, and OspF with the Accuplex 4 BioCD system.

The Week 4 sera from all 31 dogs had an MAT titer of 1:100 for at least 1 *Leptospira* spp. serovar. MAT titers of 1:800 or greater were detected against serovar *Gryppotyphosa* and serovar *Pomona* in 27 dogs. None of these 31 samples contained antibodies against the *B. burgdorferi* OspA, OspC, and OspF peptides used in the commercially available assay.

In conclusion, the *B. burgdorferi* peptides used in the Accuplex 4 BioCD system do not recognize antibodies induced by the commercially available *Leptospira* spp. vaccines administered in this study. However, lateral flow device manufacturers and laboratories providing *B. burgdorferi* serological assays have specific sources of peptides that may vary antigenically from those used here and so the results of this study may not be the same for all laboratories.

ID19

CLINICAL AND LABORATORY FINDINGS IN DOGS WITH *IXODES*-INDUCED CHRONIC *ANAPLASMA PHAGOCYTOPHILUM* INFECTION AFTER PREDNISOLONE ADMINISTRATION. Elena Contreras[1], Kristy Dowers[1], Scott Moroff[2], Michael Lappin[1]. [1]Department of Clinical Sciences, Colorado State University Veterinary Teaching Hospital, Ft Collins, CO, USA, [2]Antech Diagnostics, Lake Success, NY, USA

Anaplasma phagocytophilum (AP) is transmitted by *Ixodes* spp. and is the cause of granulocytic anaplasmosis in many species including dogs, cats, horses, and people. In endemic areas, antibodies against AP are detected in the serum of up to 40% of dogs and some healthy dogs are AP PCR positive. The effect of prednisolone administration on dogs infected with AP after *I. scapularis* infestation has not been determined.

A total of six young laboratory-reared beagles that were infected by AP, but no other vector borne agents, as evidenced by detection of AP DNA in blood by PCR assay (FastPanel, Antech Diagnostics) and AP antibodies in serum (Accuplex 4 BioCD system; Antech Diagnostics) after infestation with wild-caught *I. scapularis* were selected for study. On Week 20 after *I. scapularis* infestation, the dogs were placed into two Groups, each containing one AP PCR positive, AP antibody positive dog and two AP PCR negative, AP antibody positive dogs. Group 1 was administered doxycycline at 5 mg/kg, PO, twice daily for 4 weeks. After sample collection the morning of Week 24, all dogs were administered prednisolone at 2 mg/kg, PO, daily for 2 weeks. The dogs were evaluated daily for clinical abnormalities and by CBC, AP PCR and AP antibody assay Weeks 24–28. Sera from 5 dogs was assayed for AP antibodies on Week 48.

Clinical abnormalities were not detected over the course of the study. The only CBC abnormalities noted were anemia, thrombocytosis and neutropenia in one Group 1 dog and one Group 2 dog on Week 24, prior to prednisolone administration. After Week 20, only one dog was positive for AP DNA in blood (Group 2; Week 24 and Week 25). One Group 1 dog was negative for AP antibodies in all samples collected after Week 20, and the other two Group 1 dogs were negative for AP antibodies Week 48. In contrast, all dogs in Group 2 were AP antibody positive on Week 48.

The 2 dogs with anemia, thrombocytosis and neutropenia on the day prednisolone was initiated were clinically normal, AP PCR negative, and were normal in all other CBC evaluations, suggesting these results were spurious. The one dog in Group 2 that was positive for AP DNA on Week 25, was also positive on Week 24 prior to prednisolone administration. None of the other samples assessed by CBC and AP PCR assay after initiation of prednisolone administration showed evidence for activation of AP infection, suggesting that this protocol is unlikely to adversely affect dogs with chronic AP infection in the field. Whether other immune suppressive protocols would activate chronic AP infection remains to be proven. The only dogs that became AP seronegative by Week 48 were administered doxycycline, suggesting treatment may aid in the elimination of chronic AP infection, even after prednisolone administration.

ID20

SERUM NEUTRALIZATION OF US FELINE CALICIVIRUS ISOLATES FOLLOWING VACCINATION WITH A NON-ADJUVANTED, KILLED, FCV-BIVALENT VACCINE. LeMac Morris[2], Jennifer Hess[2], Michael Lappin[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]Boehringer Ingelheim, St. Joseph, KS, USA

The objective of this study was to determine the proportion of feline calicivirus (FCV) field isolates that would be neutralized in vitro by sera from cats that were routinely vaccinated with a product containing two caliciviruses. Serum from cats hyperinoculated against 2 feline caliciviruses (FCV) has been shown to cross neutralize a greater proportion (90.2%) of 61 FCV field isolates from the United States when compared to serum from cats hyperinoculated with 1 FCV isolate (23.0%)

At 8 and 11 weeks of age, each of the 44 kittens was administered an experimental vaccine containing modified-live panleukopenia, modified-live feline herpesvirus 1, and non-adjuvanted inactivated calicivirus strains FCV-255 and FCV-DD1. Serum was collected from each kitten 7 and 14 days after the second dose of vaccine and pooled by day.

The ability of the pooled sera to neutralize a total of 33 FCV strains collected from cats in the USA was determined. The specific FCV serum neutralization titer for each FCV was defined as the serum dilution causing a 50% inhibition of virus replication. Using the Day 7 post-vaccine pooled sera, 24 of 33 (72.7%) FCV isolates were neutralized. Using the Day 14 post-vaccine pooled sera, 20 of 33 (60.6%) FCV isolates were neutralized. The placebo serum pool had undetectable antibody titers against all 33 FCV field strains that were screened.

Cross-neutralization of USA field strains of FCV was > 60% using pooled sera from kittens that were only administered 2 doses of an inactivated non-adjuvant vaccine containing FCV-DD1 and FCV-255 strains. These results suggest that cats vaccinated with products containing more than one FCV isolate will likely have broad heterologous protection if exposed to field strains of FCV.

ID21

RISK FACTORS ASSOCIATED WITH *GIARDIA* AND *CRYPTOSPORIDIUM* IN PET DOGS AND CATS IN THE USA. Hanaa Thigeel[1], Francisco Olea-Popelka[1], Valeria Scorza[1], David Aucoin[2], Michael Lappin[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]Antech Diagnostics, Irvine, CA, USA

Giardia spp. and *Cryptosporidium* spp. are protozoans that colonize and reproduce in the intestines of many domesticated animals, including dogs and cats. Outcomes range from subclinical infection to severe diarrhea. *Cryptosporidium* spp. infection rates in dogs and cats are largely unknown as sensitive diagnostic procedures were not previously available. Polymerase chain reaction (PCR) assays are now available to amplify *Giardia* spp. and *Cryptosporidium* spp. DNA from feces. The purpose of this study was to report fecal PCR assay results for *Giardia* or *Cryptosporidium* in a sample set of dogs and cats in the USA.

All fecal samples that were submitted to a commercial laboratory (ANTECH® Diagnostics) between 2010 and 2015 for evaluation with a PCR panel of assays that amply DNA of a select group of infectious agents including *Giardia* spp. (dogs and cats), *Cryptosporidium* spp. (dogs and cats), and *Cryptosporidium felis* (cats only) were analyzed. Descriptive, univariable, and multivariable logistic regression analyses were conducted to assess associations amongst age, sex, region, and season and the probability of testing positive to either *Giardia* spp. or *Cryptosporidium* spp. in pet dogs and cats.

Giardia spp. and *Cryptosporidium* spp. fecal PCR assay results were available for 23,042 dogs and 16,326 cats.

Cryptosporidium spp. DNA was amplified from feces of 336 of 1,762 *Giardia* spp. DNA positive dogs (19.1%) and 132 of 843 *Giardia* spp. DNA positive cats (15.7%). Of the 843 *Giardia* spp. DNA positive cats, 97 (11.8%) were positive for *Cryptosporidium felis* DNA. Logistic regression models results showed that age (young) and region were associated with presence of *Giardia* spp. DNA and *Cryptosporidium* spp. DNA in feces of dogs and cats. In addition, presence of *Giardia* spp. DNA in feces of dogs varied by season.

Species	Pathogen	Percent	95% CI
Dog	*Giardia* spp.	7.7	7.3-8.0
	Cryptosporidium spp.	5.4	5.1-5.7
Cat	*Giardia* spp.	5.2	4.9-5.5
	Cryptosporidium spp.	7.4	7.0-7.9
	Cryptosporidium felis	5.1	4.7-5.4

The results indicate that *Giardia* spp. DNA, *Cryptosporidium* spp. DNA, and *Cryptosporidium felis* DNA are commonly amplified from feces of dogs and cats, coinfections are common, and positive rates can vary by age, season, and region. Either of the agents should be suspected more highly in young animals. Additional studies will be required to evaluate for associations of positive test results with clinical findings and to determine the likelihood dogs or cats are carrying zoonotic *Giardia* spp. or *Cryptosporidium* spp.

ID22

CLINICALLY SIGNIFICANT DIFFERENCES IN SENSITIVITY LEVELS OF IN-CLINIC RAPID TESTS FOR ANTIBODIES TO *ANAPLASMA* SPP. IN DOGS. Jiayou Liu[1], Ramaswamy Chandrashekar[1], Mathew Eberts[2], Mary Menard[3], Katie Erswell[4]. [1]IDEXX Laboratories, Westbrook, ME, USA, [2]Lakeland Veterinary Clinic, Baxter, MN, USA, [3]Borador Animal Hospital, Salem, NY, USA, [4]Pine Point Animal Hospital, Scarborough, ME, USA

Tick-borne diseases, including Lyme disease, ehrlichiosis, and anaplasmosis, are becoming increasingly prevalent as tick distributions expand through climate change, wildlife migration, and increased relocation of companion animals. Granulocytic anaplasmosis is caused by the obligate intracellular bacterium *Anaplasma phagocytophilum*, which is transmitted by *Ixodes* spp. of ticks. Both dogs and humans are susceptible to this infection. In dogs common signs of the infection, such as lethargy, anorexia, lameness and fever, are non-specific. Differential diagnosis is supported by evidence of morulae or DNA, especially in acute cases, and by detection of specific antibodies using IFA or ELISA-based tests.[1] A related but distinct bacterium, *A. platys*, also infects dogs and causes infectious cyclic thrombocytopenia.[2]

The present study compared the performance of two in-clinic rapid tests, the SNAP® 4Dx Plus® Test (IDEXX), and the VetScan® Canine *Anaplasma* Test (Abaxis). Random canine samples sourced from endemic regions for *A. phagocytophilum* were characterized by *A. phagocytophilum* Immunoflourescence assay (IFA) which was performed by a commercial reference laboratory. *A. platys*-specific samples were sourced from dogs living in the southwest, an *A. platys*-endemic region. These samples were characterized using A. *platys*-specific peptide ELISA.[3] The characterized samples were then blinded and randomized before testing with the two in-clinic rapid tests. Results are shown in the table below.

		Relative Sensitivity		Relative Specificity	
Organism	Reference Test (sample size - pos/neg)	SNAP 4Dx Plus	Abaxis VetScan Test	SNAP 4Dx Plus	Abaxis VetScan Test
A. phagocytophilum	IFA (143/90)	86.0%	32.2%	96.7%	88.9%
A. platys	ELISA (68/147)	85.3%	63.2%	98.0%	89.1%

Notably, the VetScan® Test failed to detect more than 60% of the medium to high titer (1:400 and above) *A. phagocytophilum* samples.

Independent evaluation of SNAP 4Dx Plus and VetScan *Anaplasma* Tests was performed in three different veterinary practices (Maine, New York and Minnesota) using samples obtained from pet dogs. Similar results were obtained - the VetScan Test failed to detect over half of the IFA positive dogs detected by the SNAP 4Dx Plus Test in each of the three clinics.

These results demonstrate the SNAP 4Dx Plus Test has better sensitivity for the detection of antibodies to both *A. phagocytophilum* and *A. platys* when compared to the Abaxis VetScan *Anaplasma* Test.

1. Carrade et al. J Vet Intern Med. 2009, 23:1129.
2. Bowman DD, Topics in Companion Anim Med. 2011, 26:173.
3. Qurollo BA et al. Infect Ecol Epidemiol. 2014, 4: 24699.

ID23

PREVALENCE OF SELECT INFECTIOUS DISEASE AGENTS IN CLIENT OWNED CATS IN MOSCOW, RUSSIA. Katie MacMillan[1], Natalia Volgina[2], Michael Lappin[1]. [1]Colorado State University, Fort Collins, Colorado, USA, [2]Private Practice, Moscow, Russia

Client-owned cats in Moscow, Russia can be exposed to fleas and ticks and some develop fever, hemolytic anemia, thrombocytopenia, gingivitis, and other clinical problems noted in cats of other countries. However, limited data are available concerning prevalence rates for infectious agents common to eastern and western Europe. The overall objective of this study was to determine the estimated prevalence of select feline infectious disease agents in a convenience sample population of cats from Moscow, Russia.

Remnant blood in EDTA and serum from client owned cats in Moscow that were evaluated for reasons independent to the study were collected with permission and stored a 4C until evaluated. Using previously validated assays, sera were assayed for *Toxoplasma gondii* IgG (Toxo), *Bartonella* spp. IgG (Bart), FeLV antigen (Ag), and FIV antibody (Ab). Total DNA was extracted from blood samples and DNA of *Anaplasma* spp., *Bartonella* spp., *Ehrlichia* spp., and haemoplasmas were amplified by previously validated PCR assays with *Mycoplasma hemofelis* (Mhf) amplicons confirmed by sequencing.

None of the cats were positive for DNA of *Anaplasma* spp. or *Ehrlichia* spp. but many of the cats showed evidence of exposure or current infection with one of more of the other agents. *Bartonella* spp. antibodies were most common in serum and *Candidatus* M. hemominutum (Mhm) DNA was most common in blood.

Serology (positive/tested [%])				PCR (positive/tested [%])			
Toxo IgG	Bart igG	FeLV Ag	FIV Ab	Mhm alone	Mhf alone	Mhm/Mhf	Bart PCR
24/96 (25%)	30/96 (30%)	4/93 (4.3%)	1/93 (1.1%)	14/100 (14%)	2/100 (2%)	1/100 (1%)	2/100 (2%)

Toxoplasma gondii IgG and *Bartonella* spp. IgG titers ranged from 1:64 to 1:2048. Of the 81 cat samples evaluated in all assays, single, dual, triple, and quadruplicate infections were documented in 31, 11, 2, and 2 samples, respectively. Using this subset of samples, 57.8% of the cats had evidence of infection or exposure to one or more of the select agents.

The results from this sample population suggest cats in Moscow are exposed to intermediate hosts or undercooked meat (*T. gondii*), fleas (*Bartonella* spp. and hemoplasmas), and come in direct contact with infected cats (hemoplasmas, FeLV and FIV). These infectious agents should be on appropriate clinical differential lists, processed foods should be fed, ectoparasite control should be maintained, and feline-feline contact should be avoided when possible.

ID24

COXIELLA BURNETII DNA NOT IDENTIFIED IN FLEAS FROM DOMESTIC CATS IN AUSTRALIA AND THE USA. Alison Manchester[1], Jennifer Hawley[1], Julia Beatty[2], Vanessa Barrs[2], Michael Lappin[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]Valentine Charlton Cat Centre, University of Sydney, Sydney, Australia

Coxiella burnetii is a rickettsial pathogen with serious zoonotic implications (Q fever) transmitted by direct contact and vectored by numerous tick species. Cats infected *C. burnetii* have been implicated in human infections in Australia and the United States but minimal information exists concerning routes of feline infection. Recently, *C. burnetii* DNA was isolated from *Ctenocephalides felis* collected from wildlife in Cyprus; this flea commonly infests cats. The purpose of this study was to evaluate select groups of fleas from cats in the Australia and United States for the presence of *C. burnetii* DNA using a previously optimized PCR assay.

The DNA samples utilized in the study had been extracted from fleas infesting 96 cats in previously published *Bartonella* spp., hemoplasmas, and *Rickettsia felis* studies. They were maintained at −80°C until the present study. The fleas were collected from cats in eastern Australia (86) and the southern United States (4 Alabama, 6 Florida) and pooled in groups comprised of a maximum of 5 fleas before DNA extraction. A previously reported conventional PCR assay utilizing primers pairs targeting the IS-1111 (IS-5, IS-9, IS-14 and IS-20) insertion sequences transposase elements in the *C. burnetii* genome was used. If positive amplicon was detected, genetic sequencing would be performed to confirm the presence of the pathogen.

For this study, fleas were not speciated before DNA extraction. However, previous studies in both countries suggest *C. felis* was most likely. The detection sensitivity of the assay was shown to be 3.14 ng total *C. burnetii* genomic DNA per PCR reaction utilized in all 4 primer sets. While all positive and negative controls performed as expected, none of the 96 pooled flea extracts were positive for *C. burnetii* DNA.

Coxiella burnetii DNA was not recovered from fleas collected from cats in eastern Australia or the southern United States. The results suggest that fleas infesting domestic cats in these regions are not important vectors for *C. burnetii* and are unlikely to play a role in the transmission of the organism to humans with Q fever.

ID25

RISK OF HEARTWORM INFECTION IN DOMESTIC CANINES OF NORTHWESTERN OREGON. Kirk Miller[1], William Rausch[2]. [1]Oregon State University College of Veterinary Medicine, Corvallis, OR, USA, [2]Portland Veterinary Cardiology, Portland, OR, USA

Canine infection with *Dirofilaria immitis*, the parasitic agent responsible for causing heartworm disease, is easily and safely prevented via the periodic administration of one of a number of preventative agents. Many veterinarians in Oregon do not routinely recommend the use of heartworm prophylactics; therefore, dogs are commonly not on a preventative. Confusion regarding the use of heartworm preventatives in Oregon is understandable, given the paucity of incidence data in our region. The decision between a veterinarian and a client on whether or not to start a preventative is often made based on a cost-benefit analysis, where only the cost is known. The purpose of this study was to evaluate the risk of developing heartworm infection amongst unprotected dogs in Northwestern Oregon. This information will enable veterinarians and their clients to make more informed decisions regarding heartworm prevention.

Between March 2010 and August 2015 owners of all dogs surrendered to the Oregon Humane Society filled out a short questionnaire relating to their dogs' histories. This questionnaire was used to determine the eligibility of each dog for this study. Criteria for inclusion in the study included that the dogs: (1) had been in the possession of the surrendering party since they were 6 months of age or younger; (2) were at least 12 months of age; (3) had no history of travel outside of the state of Oregon; and (4) were never administered heartworm preventative. Dogs that did not meet all four of these criteria were excluded from the study. Additional information gathered from the records included breed, age, sex and spay/neuter status, location (by zip code), and the average number of hours the dog spent outdoors. If a dog did meet the study criteria then blood was drawn and tested for the presence of *D. immitis* using a commercially available ELISA test (IDEXX Snap 4Dx Plus).

A total of 5,103 dogs were surrendered to the Oregon Humane Society during this period. Of these, 298 met the inclusion criteria and were included in the study. None of these 298 dogs tested positive for *Dirofilaria immitis*. The tested dogs had spent a median of 2 years with their previous owners (range 6 months to 16 years) for a combined total of 1300.45 years of exposure. Using a likelihood-based method, the maximum likelihood estimate (MLE) for the risk is 0 (95% CI: [0, 0.0023]) per year.

The findings of this study suggest that the likelihood of unprotected Northwestern Oregon dogs developing heartworm infection is currently very low. Heartworm transmission prediction models based on climate data (Knight/Lok) have shown that the climate conditions in the area can support heartworm transmission. Data compiled by the Companion Animal Parasite Council (Capcvet.org) confirm that *Dirofilaria immitis* has been diagnosed within the state of Oregon, although only 0.18% of all positive cases of heartworm in the U.S. are in the state of Oregon. It is important to consider that these data reflect a point in time. As the population of Oregon continues to grow, more heartworm infected dogs will invariably arrive. Changes in climate patterns will also influence the transmission of this disease. Continued monitoring and vigilance are recommended.

ID26

ECTOPARASITES AND VECTOR-BORNE PATHOGENS OF DOGS IN BAJA CALIFORNIA SUR. Cody Minor, Dana Hill, Danielle Straatmann, Michael Lappin. Colorado State University, Fort Collins, CO, USA

In the last several years, clinical illness in a number of people in Baja California Sur, México, has been suspected to be associated with rickettsial disease agents such as *Rickettsia rickettsii*, *Rickettsia felis*, and/or *Ehrlichia* species. The objective of this study is to determine the prevalence of select vectors and vector-borne disease agents carried by companion dogs of the region.

During planned community outreach sterilization and vaccination clinics for local dogs with consenting owners, samples of ectoparasites were collected from infested dogs and preserved in alcohol for identification, and blood samples were collected in EDTA and non-additive tubes retained for testing. Sera were assayed for antigen of *Dirofilaria immitis* and antibodies against *Ehrlichia canis*/*E. ewingii*, *Anaplasma phagocytophilum*/*A. platys*, and *Borrelia burgdorferi* using a commercial kit (SNAP® 4DXPlus®, IDEXX Laboratories). Sera were also assayed for antibodies against *Rickettsia* species by indirect fluorescent antibody (IFA) testing (*R. rickettsia* antigen source). Total DNA was extracted from blood and evaluated with conventional polymerase chain reaction (PCR) assays to amplify DNA of *Anaplasma* species, *Babesia* species, *Bartonella* species, *Ehrlichia* species, *Rickettsia* species, and hemotropic *Mycoplasma* species (Hemoplasmas). Positive amplicons were sequenced to ascertain the species.

Samples from 67 dogs were available. From these, ectoparasites *Rhipicephalus sanguineus* (33 dogs; 49.3%), *Ctenocephalides felis* (11 dogs; 16.4%), and *Pulex irritans*/*simulans* (four dogs; 6.0%) were identified. *Dirofilaria immitis* antigen (one dog; 1.5%) and antibodies against *A. phagocytophilum*/*A. platys* (10 dogs; 14.9%) and *E. canis*/*E. ewingii* (31 dogs; 46.3%) were detected in the serum of some dogs. Two samples had probable antibodies against *R. rickettsii* through IFA testing and are being confirmed. Evaluation of DNA from 41 dogs has been completed to date; *E. canis* DNA was amplified from eight dogs (19.5%), *A. platys* DNA from six dogs (14.6%), *Babesia canis vogeli* DNA from three dogs (7.3%), and *Mycoplasma haemocanis* DNA from three dogs (7.3%). Coinfections were confirmed in four dogs (9.8%) and consisted of *D. immitis*/*E. canis* (one dog), *E. canis*/*A. platys* (one

dog), *E.* canis/M. *haemocanis* (one dog), and *E. canis*/*A. platys*/*M. haemocanis* (one dog).

Vector-borne agents detected to date likely reflect common exposure to *R. sanguineus*, as this tick vectors each of the PCR-confirmed agents. Further information will be gained by completion of the PCR assay analysis of the blood, fleas, and ticks.

ID27

IDENTIFYING AGREEMENT AND BARRIERS TO PROPOSED CANINE INFECTIOUS DISEASE GUIDELINES FOR DOG GROUP SETTINGS. Jason Stull[1], Michelle Evason[2], Jennifer Kasten[1]. [1]Ohio State University, Columbus, OH, USA, [2]Rayne Clinical Nutrition Canada, Burnaby, BC, Canada

Canine group settings, locations or events where dogs temporarily come together in a shared environment (e.g., shows, sporting events, dog parks) pose an increased risk for infectious disease transmission. Despite this increased risk, few guidelines exist to provide recommendations for reducing disease risk in these settings. During 2014–2015 a panel of canine infectious disease experts reviewed the current literature and drafted a set of 44 evidence-based recommendations for prevention of infectious diseases for dogs in group settings. In August 2015 a survey of attendees at the AKC Canine Health Foundation Parent Organization conference was completed to determine agreement with and perceived barriers to these recommendations. The 15-minute self-administered survey was provided to 238 Conference attendees and consisted of a series of Likert-type and open-ended questions (online and paper format) seeking feedback on 22 of the recommendations. The survey was completed by 185 individuals (78%), and all responses were reviewed, summarized, and open-ended comments categorized by theme. Respondents self-identified as: participants, judges, and breeders in a variety of local, national, and international canine group events. Most respondents (> 40%) agreed with all but three of the panel's recommendations, yet a majority of respondents stated the recommendations would be difficult or very difficult to implement in their setting (primarily dog shows). Common survey result themes related to difficulty of implementation included: administrative concerns (cost, human resources/manpower), enforcement issues, ethical concerns, privacy concerns, and strong need for official outreach to promote awareness and education related to canine infectious diseases. Survey responses identified needs for: further refinement of recommendations to aid comprehension and clarity (especially around ecto- and endoparasite control), and education to promote culture changes related to disease risk prevention. In order to raise awareness of canine infectious disease in group settings amoungst event participants, attendees, and organizers; an online freely available canine infectious disease risk calculator tool is being developed.

ID28

CORRELATION OF *MYCOPLASMA* QUANTITATIVE PCR TO SEVERITY OF CONJUNCTIVITIS IN CATS. Alexis Dubin, Jennifer Hawley, Cynthia Powell, Michael Lappin, Julia Veir. Colorado State University, Center for Companion Animal Studies, Fort Collins, CO, USA

Mycoplasma species are one of the most common infectious causes of conjunctivitis in cats. *Mycoplasma felis* is commonly implicated as a primary pathogen, but other *Mycoplasma* species have also been detected in clinically ill cats. Findings from previous studies using conventional PCR (cPCR) to investigate the role of *Mycoplasma* species in causation of feline conjunctivitis have been mixed as *Mycoplasma* can be carried by apparently normal cats. Therefore, the purpose of this study was to determine if increasing severity of conjunctivitis in cats correlates with higher *Mycoplasma* species copy numbers using qPCR.

A total of 77 conjunctival swabs collected from 29 shelter cats with conjunctivitis and confirmed to contain *Mycoplasma* species DNA using cPCR were selected for study. The severity of conjunctivitis at the time the samples were acquired was determined using a grading scheme from 0 - 9. The samples were evaluated using a previously validated qPCR to determine the *Mycoplasma* copy number. Statistical methods consisted of using the Spearman's rho test to determine if severity of conjunctivitis was correlated to qPCR *Mycoplasma* species copy number.

The results revealed the severity of conjunctivitis significantly correlated to qPCR *Mycoplasma* copy number (Spearman's correlation coefficient −0.32, $P = 0.0042$), however, the strength of this correlation was only mild to moderate.

Based on the results of this study, future investigation of the impact of *Mycoplasma* species other than *M. felis* on the correlation of qPCR and severity of conjunctivitis in cats should be performed.

NM01

EFFECT OF THE HYPER-IMMUNE EGG YOLK SUPPLEMENTATION ON WEIGHT GAIN IN NEONATE PUPPIES. Hanna Mila[1], Alexandre Feugier[2], Claire Mariani[2], Aurelien Grellet[2], Sylvie Chastant-Maillard[1]. [1]École nationale vétérinaire de Toulouse, UMR 1225 Interactions Hôte-Pathogènes, INP, Toulouse, France, [2]Royal Canin, Aimargues, France

Colostrum provides puppies with most of their passive immune transfer, as in dogs only 5% of immunoglobulin G (IgG) is acquired via transplacental transfer. Inadequate colostrum intake during the first day of life will deprive puppies not only of immunoglobulins, but also from many hormones, growth factors and nutrients. Hence, it increases the risk for neonatal morbidity and mortality. Supplementation during the first hours of life with canine serum or plasma increased blood IgG concentration[1] in colostrum deprived puppies as well as improved their growth during the entire neonatal period (0–3 weeks)[2]. This study aimed to evaluate the effect of exogenous specific antibodies administrated via egg yolk before the intestinal barrier closure on growth in preweaning puppies.

Specific antibodies against canine parvovirus type 2 (CPV2) and *E. coli* were obtained in eggs from hens vaccinated separately against one of the mentioned agents[3]. Egg yolk was flash-dried and tested for the presence of CPV2 and *E. coli*-specific antibodies. Hyper-immune solution was then prepared by mixing egg powder with a commercial milk replacer (Babydog Milk, Royal Canin, Aimargues, France; 1 g of egg powder with CPV2 antibodies and 1 g of egg powder with *E. coli* antibodies with 12 mL of reconstituted milk). A total of 334 puppies from 16 different breeds, enrolled in one breeding kennel, were included in the study. Depending on the expected adult body weight, puppies were classified into small breed dogs (S; adult weight < 25 kg), and large breed dogs (L; > 25 kg). Within each litter and taking into account the birth weight, puppies were assigned into supplemented or control group. Each puppy from the supplemented group received orally 1.5 mL/100 g bw of hyper-immune solution at once within the first 8 hours after birth. Puppies from the control group received at the same dose (1.5 mL/100 g) and time after birth (< 8 h) the milk replacer only. All puppies were weighed at birth and at 7, 14, and 21 days of life. Linear mixed models (MIXED procedure; SAS Institute Inc., Cary, NC, USA) with litter modeled as a random effect were performed to determine the variables affecting birth weight and weight gain during the neonatal period: breed size (small; large), age (0–7; 7–14; 14–21 days), supplementation (supplemented or control group). All the interactions between mentioned fixed effects were also tested.

L represent 38.3% (128/334) of the included puppies. Among L and S, 65 (50.8%) and 104 (50.5%) were supplemented, respectively. The weight at birth was significantly higher in L compared with S (median: 370 g [interquartile range: 325; 408 g] versus 200 g [156; 248 g]; $P < 0.001$). Birth weight was found not different between supplemented and control puppies whatever the breed size ($P = 0.14$): supplemented L 366 g [330; 412 g] versus 375 g [322; 405 g] in controls; supplemented S 199 g [155; 247 g] versus 201 g [159; 248 g] in controls. Weight gain during the neonatal period was influenced by time ($P < 0.001$), supplementation ($P = 0.031$) and the interactions between the breed size and supplementation ($P = 0.027$) and time and breed size ($P = 0.001$). L gained 176 g [67; 294 g] during the 1st week, 223 g [158; 324 g] during the 2nd week and 260 g [160; 382 g] during the 3rd week

of life, with significantly greater weight gain compared with S only during the 1st week (S = 116 g [66; 172 g]; $P = 0.001$). Whatever the period concerned, supplemented L gained more weight during the entire neonatal period than the controls (841 g [485; 1087 g] versus 623 g [436; 858 g]; $P = 0.048$). No difference was evidenced between supplemented and control S ($P = 1$).

In our study large breed puppies supplemented at birth with the hyper-immune egg yolk had greater weight gain during the entire neonatal period. Retarded growth at the early stage of life increases the risk of morbidity and mortality in puppies[4]. Thus it could be hypothesized that better growth in supplemented puppies reflects a better health. Nevertheless, further studies are needed in order to confirm our findings in other breeding kennels and on large number of individuals.

[1]Poffenbarger et al., 1991; [2]"Canine health product containing antibodies against canine parvovirus type 2" WO2015004181 A1.; [3]Van Nguyen et al., 2006; [4]Mila et al., 2012.

NM02

EFFECTS OF DIETARY MEDIUM CHAIN TRIGLYCERIDES ON VOLUNTARY ACTIVITY IN DOGS AND CATS. Yuanlong Pan, Hui Xu, Sandeep Bhatnagar, Janet Jackson. Nestle Purina Research, St Louis, MO, USA

Decline in cerebral glucose metabolism is a common feature of aging in people and animals including rats, dogs, and monkeys, and it is closely associated with age-dependent cognitive decline. We had confirmed in a previous study that dietary medium chain triglycerides (MCTs) can enhance cognitive functions in dogs by providing the brain with an alternative energy source. In this study, we investigated the effects of dietary MCTs on voluntary activity in dogs and cats.

In the cat study, sixteen middle-aged and senior cats were fed 100% of their maintenance energy requirements (MERs) with a control diet for one week, and then were switched to the MCT-containing diet for 28 days. Their daily activity was monitored during the feeding trial with activity monitors. In the dog study, twenty senior dogs were fed 100% of their MERs with a control diet for 6 days, and then were assigned to one of 2 MCT-containing diets with 10 dogs per diet for 27 days. Then the dogs were switched to the alternative MCT diet for 32 days. Daily activity was recorded with activity monitors during the feeding trial. In cats, the MCT-containing diet significantly increased both daytime (17381.23 ± 2551.18 versus 22628.55 ± 2790.61, $P < 0.0001$) and night time activity (4415.56 ± 654.71 versus 5926.66 ± 786.88, $P = 0.002$). Interestingly, in dogs, both MCT diets significantly increased only daytime activity (80349.46 ± 9703.34 versus 99254.33 ± 12163.83; 80349.46 ± 9703.34 versus 105120.38 ± 11550.91, $P < 0.05$). The results show that both dogs and cats became more active when fed a diet containing MCTs

NM03

EFFECTS OF DIETARY MACRONUTRIENT CONTENT AND FEEDING PATTERN ON LEPTIN CONCENTRATIONS IN LEAN HEALTHY CATS. Dagmar Tarkosova[1], Jacquie Rand[2], Heidi Farrow[2], Marcia Coradini[2], John Morton[3]. [1]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, [2]The University of Queensland, Brisbane, Australia, [3]Jemora Pty Ltd, Geelong, Australia

Excessive weight gain is associated with metabolic and hormonal changes that predispose cats to insulin resistance and other disorders. The adipokine leptin is an important regulator of energy metabolism, and circulating leptin concentrations correlate with fat mass in cats and other species. In cats, leptin is associated with insulin resistance, independent of bodyweight. Although dietary factors such as energy density, fat content and feeding pattern increase the risk of obesity, there is limited information on how dietary factors such as feeding, fasting, and macronutrient content influence leptin concentrations in cats. The aims of this study were to compare leptin concentrations in lean, healthy cats fed diets high in protein, fat and carbohydrate, to assess associations between leptin concentrations and insulin, glucose, and NEFA concentrations, and to determine the effects of feeding pattern on baseline and postprandial leptin concentrations.

A controlled trial was conducted with clinically healthy cats (n = 24). Mean body weight was 4.9 kg and all cats had ideal body condition score of 3 on a 5-point scale. All cats were fed a "washout" diet (commercial feline diet) for five weeks, and were then fed one of three test diets high in one of protein, fat or carbohydrate for five weeks. Diets were dry extruded formulations, and each test diet provided approximately 50% of energy from the test macronutrient, and 25% of energy from each of the other two test macronutrients. Leptin concentrations were measured during two feeding patterns: a meal-feeding test (once daily feeding) and an *ad libitum*-feeding test, conducted 4 weeks after commencement of the test diets. During the meal-feeding test, cats ate 90 - 100% of the 12 hour *ad libitum* intake as a single meal in 0.5 hour. Blood samples (4 mL), were collected over 24 hours in the meal feeding test and 12 hours in the *ad libitum* test.

Distributions of leptin variables in the *ad libitum* and meal-feeding tests were similar across dietary groups after consumption of the washout diet for 5 weeks. Mean baseline concentrations (average of −30 and −5 minutes values), mean concentrations over 24 hours (mean 24-h), and peak leptin concentrations in the meal feeding test varied significantly by diet (overall $P < 0.001$ to 0.027). Baseline, mean 24-h and peak leptin concentrations for the high fat diet were significantly higher than for the high protein diet, and for baseline and mean 24-h, for the high carbohydrate diet. A similar pattern was observed in the *ad libitum* feeding test with highest leptin concentrations in cats consuming the high fat diet. In general, leptin concentrations after consuming the high carbohydrate diet were not significantly different from the high protein diet, but if they differed, leptin concentration for the high carbohydrate diet was higher compared to the high protein. There was no significant effect of diet on the time to peak leptin concentration in the meal-feeding test (overall $P = 0.855$), and median times to peak leptin concentration were 15 hours for all diets. In the meal-feeding test, only 3/8 cats significantly exceeded their baseline leptin concentration for each of the high protein and high fat diets, but 7/8 did so for the high carbohydrate diet. In general, leptin concentrations decreased significantly approximately 2 hr after eating in the meal feeding test, but remained relatively constant during *ad libitum* feeding. When data for all diets were combined, leptin was significantly positively correlated with insulin concentrations during *ad libitum* feeding ($P = 0.008$ to 0.036), but only at baseline and at 24 hours in the meal feeding test ($P = 0.025$ and <0.001, respectively). There were no correlations between leptin and either glucose or NEFA across all diets. Glucose, insulin and NEFA did not account for the decline in leptin soon after feeding in the meal feeding test.

In conclusion, independent of feeding pattern, leptin concentrations tend to be highest in lean cats consuming a diet with 50% of energy from fat, compared to diets high in protein or carbohydrate. Leptin concentrations vary minimally over 12 hours of *ad libitum* feeding, whereas leptin decreases approximately 2 hours after feeding following a fast, and this decrease is not accounted for by changes in insulin, glucose or NEFA concentrations. Further investigation is needed to understand the interactions between hormones associated with satiety, dietary factors and weight gain in cats.

NM04

EFFECT OF HIGH SODIUM DIET ON BLOOD PRESSURE AND CARDIAC FUNCTION IN HEALTHY ADULT DOGS. Hui Xu, Dottie Laflamme, Sandeep Bhatnagar, Xuemei Si, Grace Long. Nestle Purina PetCare Research, St. Louis, MO, USA

Promoting water intake is recommended for managing dogs with lower urinary tract disease. Increased dietary salt has been used for this purpose in veterinary therapeutic diets. The objective of this study was to evaluate potential adverse effects of dietary salt (sodium chloride) on blood pressure and cardiac function in dogs.

Following a two week baseline period where dogs were fed a Control Diet containing 0.13 g sodium/100 kcal ME, twenty healthy dogs (6–10 years old) were allocated to two groups and fed diets differing only in total sodium chloride content: CONTROL (CON) = 0.13 g sodium /100 kcal ME, or High Sodium Diet (HNA) = 0.41 g sodium/100 kcal ME. Dogs were fed their

respective diet for 6 months. During this period, indirect systolic blood pressure was recorded monthly. Overall health, cardiac function evaluated by echocardiography (performed by boarded veterinary cardiologist), plasma B-type natriuretic peptide (BNP), serum biochemistry and urinalyses were assessed at 0 and 6 months on test. Body composition was analyzed by dual energy X-ray absorptiometry (DEXA) at 0, 3 and 6 months. Treatment effects were analyzed using t-test and Generalized Linear Mixed Models.

Overall, dietary sodium level had no significant effect on blood pressure, cardiac function, plasma BNP, serum biochemical profiles or urinalyses. There were no dietary effects or diet by time interactions on bone mineral content or body composition. In conclusion, consumption of a diet containing 0.41 g sodium/100 kcal ME had no adverse effects on blood pressure or cardiac function in healthy adult dogs over a 6-month period.

NM05

DETERMINING THE LACTATE AND GLUCOSE THRESHOLDS AND THE ACID-BASE IMBALANCES IN BEAGLES DOGS. Alejandro Zamora Restan, Evandro Zacché, Jaislaine Braz, Raphaela Marques Canola, Samara Beretta, Antonio Queiroz-Neto, Guilherme Camargo Ferraz, Aparecido Antonio Camacho. Universidade Estadual Paulista – UNESP, Jaboticabal, Brazil

The determination of lactate and glucose threshold in dogs submitted to incremental exercise test (IET) is scarce. This study aimed (1) to determine the lactate and glucose thresholds (2) to investigate the acid-base responses of untrained dogs submitted to incremental exercise test. Fourteen Beagle dogs, 1–2 years old, healthy and untrained were used for determination of lactate and glucose thresholds and submitted to IET using treadmill, with slop from 5 to 7.5% and increasing speeds of 0.5 m/s every 5 minutes. The test was concluded when dogs presented fatigue signals. Blood samples were collected after 90 seconds of each effort step, though venous catheter placed in the jugular vein. The samples used for determination of acid-base alterations were collected in the moments before of IET, immediately and five minutes after the fatigue. Data were submitted to Shapiro-Wilk test, and the Pearson was used to correlate the running velocities corresponding to the lactate and glucose threshold and analysis between moments of assessment was performed by using repeated measures one-way ANOVA ($P < 0.05$). It was found high correlation between lactate and glucose threshold velocities, r = 0.84 ($P < 0.01$), and for pH, PCO_2, HCO_3^- strong ions difference (SID) were found moment effect ($P < 0.001$). The basal values of pH increased ($P < 0.001$) in fatigue (7.341 ± 0.02 to 7.390 ± 0.04, respectively), and the PCO_2 presented reduction ($P < 0.001$) in the fatigue and 5 minutes after fatigue (31.74 ± 5.9, and 37.01 ± 5.8), in comparison with basal value (41.4 ± 2.2). The HCO_3^- decreased in fatigue and 5 minutes after IET (18.2 ± 2.3 and 19.1 ± 2.5) when compared with basal (21.9 ± 1.1). The values of SID showed decreased in fatigue compared to values before exercise (36,2 ± 1,7 e 36,6 ± 2.1) respectively. Running velocities corresponding to the lactate and glucose threshold showed accordance between them, what has been already shown in horses and humans, besides results showed respiratory alkalosis and metabolic acidosis, which is considered a change due to hyperventilation and strenuous anaerobic activity, decreasing PCO_2 concentrations, which leads to increase pH through the increase of the relation of HCO_3^- and PCO_2. SID showed a metabolic acidosis possibly from lactate values increase. Results of this study suggest that glucose threshold can be used as a marker of aerobics capacity in dogs as it is used in humans and horses, besides, maximum exercises in dogs cause acid basic alterations.

NM06

WHEN FED FOODS WITH SIMILAR PALATABILITY, CATS CHOOSE 30%, DOGS 23% OF CALORIES AS PROTEIN. Dennis Jewell[1], Jodi Vondran[1], Melissa Vanchina[1], Jean Hall[2]. [1]Hill's Pet Nutrition, Inc., Topeka, KS, USA, [2]Oregon State University, Corvallis, OR, USA

Dogs and cats have specific taste preferences that are influenced by macronutrient composition and physical characteristics of food, as well as presence or absence of specific taste imparting compounds (palatability enhancers). This study investigated the relationship of food choice on macronutrient composition and that choice on subsequent plasma amino acid concentrations. Experimental foods for dogs and cats were individually prepared to have similar palatability through manipulations of factors independent of macronutrients. Foods with similar palatability preference for their respective populations as a whole were then investigated for intake choice using 20 dogs with varying body fat composition (mean, 3.9 kg; SD, 0.9 kg; range, 2.4–6.2 kg;) and 27 cats (mean body fat composition, 1.4 kg; SD, 0.9 kg; range 0.3–2.2 kg). Subsequent effects of food choice on serum amino acids were also determined. Four completely balanced foods were available to dogs and cats at all times. The four varied in macronutrient content: Food 1: high protein (33% of calories from protein for dogs; 42% for cats); Food 2: high fat (54% of calories from fat for dogs; 44% for cats); Food 3: high carbohydrates (56% of calories from carbohydrate for dogs; 52% for cats); and Food 4: blended macronutrients. Dogs on average chose to consume 23.0% calories from protein (SD, 1.5%; range 20–26%) whereas cats chose 30.3% of calories from protein (SD, 4%; range 24–38%). Body composition influenced their choice of calories from protein. In dogs, there was a positive relationship between body fat and percent calories consumed as protein ($r = 0.56$; $P = 0.02$). There was no relationship between lean body mass and percent calories consumed as protein ($r = 0.06$; $P = 0.82$). Overall, dogs with high body fat showed the highest preference for dietary calories from protein. In cats, both body fat and lean body mass were negatively associated with calories consumed as protein ($P = 0.04$; $P = 0.02$, respectively), with a positive association for the interaction ($P = 0.02$). Overall, cats with high body fat and high lean body mass showed the highest preference for dietary calories from protein. Dogs had higher circulating amino acid concentrations of threonine, methionine, isoleucine, and valine ($P < 0.05$) compared with cats, the later which had higher circulating amino acid concentrations of isoleucine, valine, tyrosine, phenylalanine, and histidine ($P < 0.05$). In both dogs and cats there was no effect of protein intake on circulating essential amino acid concentrations. Increased intake of calories as protein was associated with increasing plasma ornithine (dogs only) and citrulline (dogs and cats) concentrations ($P < 0.05$). In summary, given the opportunity to choose between foods with similar palatability, cats chose to consume 30.3% and dogs 23.0% of their calories as protein. Although there were species differences between circulating amino acid concentrations indicating metabolic shifts associated with protein intake, there were no changes in circulating essential amino acid concentrations.

NM07

A DOUBLE MASKED CLINICAL TRIAL OF A THERAPEUTIC FOOD IN THE MANAGEMENT OF CANINE ATOPY. Jennifer Macleay, Heidi Schiefelbein, Kathy Gross. Hill's Pet Nutrition Inc., Topeka, KS, USA

The purpose of this double masked, controlled multicenter clinical trial was to determine the impact of a food with ingredients designed to have skin and coat health benefits and immune modulating effects on clinical signs of seasonal atopic dermatitis versus a control food. Dogs with a history of seasonal dermatitis, but without current clinical signs consistent with atopy (CADESI and pruritis scores = 0) were recruited in the spring from 11 general practices in the United States. Dogs currently on foods designed for adverse food reactions were excluded. Eligible dogs were randomly assigned to Test or Control groups and evaluated by their veterinarian at 0, 4, 8, 12 and 16 weeks. Consistent with accepted standards of care, treatment was not withheld, therefore, prescribed medications and dosing information was collected; only oclacitinib which was not uniformly available to all practices was disallowed. Veterinarians graded skin lesions using a modified CADESI (0:absent - 4:severe for erythema, alopecia, excoriations and lichenification for 27 body sites, max score of 432) and a pruritis score (0:absent - 4:severe).

Forty-four adult dogs (22 Test, 22 Control) were enrolled and completed the study. During the course of the study 18% of all dogs had no reported dermatological clinical signs whereas 82%

developed a positive CADESI and/or pruritis score. CADESI and pruritis scores rose significantly from 0 at all subsequent time points but were generally low. Mean ± SEM CADESI scores at weeks 4, 8, 12 and 16 were; 2.6 ± 0.98, 5.8 ± 2.47, 8.6 ± 3.31 and 6.6 ± 1.91 respectively. Mean ± SEM pruritis scores were 0.5 ± 0.16 at week 4 and were highest at week 16; 1.2 ± 0.16. Incidence, location and types of clinical signs manifested were not different between groups. Medications were only dispensed to dogs with a positive CADESI and/or pruritis score, which was 66% of all dogs (73% of Control versus 59% of Test). Medications dispensed were categorized as a topical containing a glucocorticoid, a systemic antihistamine and/or a systemically administered glucocorticoid. An event history analysis revealed that time until prescribing topical medications was highly similar between groups (Test 98 ± 7.0 versus Control 100 ± 5.2 days) but was less so for antihistamines (Test 62 ± 4.1 versus Control 55 ± 3.3 days) and systemic glucocorticoids (Test 105 ± 4.4 versus Control 90 ± 6.4 days).

This study is the first to describe the recurrence rate of seasonal atopy in dogs from general practices as part of a grade 1, multicenter clinical trial. Onset of clinical signs resulted in medical intervention in the majority of dogs. Statistical power in this study was likely influenced by the generally low CADESI and pruritis scores combined with medical intervention. In addition, the interval to medical intervention was likely influenced by the study schedule which promoted frequent evaluations and case management. This data suggests that dogs with seasonal atopy my benefit from frequent evaluations. This data also suggests altered management of dogs fed the Test food as medication use was lower and the interval prior to the onset of systemic antihistamines and/or glucocorticoids was longer. A food designed for skin and coat health benefits and immune modulating effects can be part of a proactive atopy management program and may result in less medication needed.

NU01

LONGITUDINAL EVALUATION OF SERUM SYMMETRIC DIMETHYLARGININE (SDMA) AND CREATININE (SCR) IN DOGS WITH EARLY CKD.

Sarah Guess[1], Maha Yerramilli[2], Edward Obare[2], Greg Grauer[1,2]. [1]Kansas State University, Manhattan, KS, USA, [2]IDEXX Laboratories, Westbrook, ME, USA

Retrospective feline studies have shown SDMA is more sensitive than sCr for early CKD detection. Our objective was to compare SDMA and sCr for detection of early CKD in a prospective, longitudinal study of older dogs.

SDMA and traditional serum and urine clinicopathologic tests were measured biannually for four years in 43 dogs with an initial median age of 8.9 years. Persistent increases in UPC, sCr, or SDMA or decreases in USG triggered renal ultrasound (US) and GFR (plasma iohexol clearance) evaluations. Necropsies were performed whenever possible when dogs died or were euthanized.

CKD was documented in 23 dogs (53%) by US abnormalities (n = 13), decreased GFR (> 40% reduction) (n = 13), persistent renal proteinuria (UPC ≥ 0.5) (n = 6), or renal histology (n = 6) (12 dogs had multiple abnormalities).

9 of 23 dogs had increased SDMA (≥ 14 µg/dl) without hypersthenuria concurrent or subsequent to CKD diagnosis. One dog had a single (endpoint) increased SDMA and 8 had persistent/multiple SDMA increases. Conversely, only 2 of the 23 dogs had increased sCr (> 1.8 mg/dl) at any point and both of these dogs had concurrent/prior SDMA increases. In the 13 dogs with decreased GFR, sCr and SDMA were increased in 1 and 7 dogs, respectively. There were no persistent increases in SDMA without hypersthenuria without CKD. Increased SDMA without hypersthenuria had 39% sensitivity and 100% specificity for CKD whereas increased sCr without hypersthenuria had 9% sensitivity but 100% specificity ($P = 0.024$).

SDMA is a sensitive biomarker for early canine CKD.

NU02

SDMA CORRELATES BETTER WITH CREATININE THAN HIGH THROUGHPUT IMMUNOTURBIDOMETRIC CYSTATIN C ASSAY IN FELINE SERUM.

Polina Prusevich, Julie Cross. IDEXX Laboratories, Westbrook, ME, USA

The purpose of this study was to determine the correlation of SDMA and Cystatin C with creatinine in felines, to assess the utility of these markers together in diagnosing chronic kidney disease (CKD).

Fifty-six feline serum samples were sourced from samples submitted to IDEXX Reference Laboratories for general chemistry testing. Cystatin C, SDMA and creatinine were assayed using a Beckman AU5800 clinical chemistry analyzer. All three methods were calibrated appropriately on the day of assay, and QC was performed prior to analysis of the feline serum samples. Fifty-six feline serum samples were assayed in duplicate for all three analytes on the same day.

SDMA is validated in cats, with values above 14 µg/dL indicating that CKD is likely present. In this study, SDMA correlated well with serum creatinine. All but one of the samples above the feline creatinine cut-off of 2.5 mg/dL was also above the SDMA cut-off (19 of the 20 elevated creatinine samples). This one sample was very near the cut-offs for both SDMA and creatinine. Samples with normal creatinine values were distributed between normal SDMA values (22 of 36 samples with normal creatinine values) and elevated SDMA values (14 of 36 samples with normal creatinine values) consistent with previous data showing that SDMA can be elevated earlier than creatinine in some cases of CKD.

However, Cystatin C did not effectively differentiate between felines with normal or abnormal creatinine levels, with 25% (5 of 20 samples) of the feline samples with abnormal creatinine levels showing normal Cystatin C, using the previously published Cystatin C cut-off of 1.95 mg/dL, which was determined in cats. For cats with normal creatinine levels, half of the Cystatin C results were discrepant, with 50% (18 of 36 samples) having abnormal Cystatin C levels.

In this study, there was no alternative cut-off value that could be determined for this Cystatin C assay, which would significantly increase its diagnostic value.

SDMA has previously been shown to correlate well to GFR, and can be elevated prior to creatinine in some animals, making it an excellent marker for CKD in felines. However, this high-throughput immunoturbidometric Cystatin C assay did not differentiate between animals with elevated creatinine, and cannot be recommended for aid in diagnosing CKD in felines.

NU03

FIBROBLAST GROWTH FACTOR-23 IN CANINE CHRONIC KIDNEY DISEASE.

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In humans and cats with chronic kidney disease (CKD), fibroblast growth factor-23 (FGF-23) has been associated with disease progression and development of renal secondary hyperparathyroidism (RHPT). In humans, elevations in FGF-23 often precede hyperphosphatemia and RHPT. The objectives of this study were to measure plasma FGF-23 concentrations in healthy dogs and dogs with CKD and to determine its association with serum creatinine, phosphorus, and parathyroid hormone (PTH) concentrations.

Thirty-four dogs with CKD and 10 healthy dogs were included. Dogs with CKD were staged according to International Renal Interest Society (IRIS) guidelines. A human FGF-23 ELISA was validated for canine samples, showing linearity and intra- and interassay coefficients of variation <7%. Values are presented as median (range). Plasma FGF-23 concentrations in healthy dogs and dogs with IRIS stages 3 and 4 CKD were 315 pg/mL (211–449), 2,302 (455–24,409) and 7,733 (2,520–41,265), respectively ($P < 0.01$).

Plasma FGF-23 concentrations were positively correlated with creatinine ($r = 0.86$, $P < 0.01$), phosphorus ($r = 0.69$, $P < 0.01$), and PTH ($r = 0.72$, $P < 0.01$) concentrations in all study dogs. Nineteen (56%) CKD dogs had FGF-23 concentrations above the

upper range of normal dogs. Based on healthy dog phosphorus concentrations, 11 (32%) CKD dogs had elevated phosphorus concentrations. Only eight (24%) CKD dogs had hyperparathyroidism.

These findings suggest that FGF-23 concentrations rise in canine CKD before other traditional markers of RHPT do. Consequently, FGF-23 concentrations should be considered as an early indicator of disease progression, RHPT, and potential target for therapeutic intervention.

NU04
THE USE OF DARBEPOETIN ALFA TO STIMULATE ERYTHROPOIESIS IN DOGS WITH CHRONIC KIDNEY DISEASE.

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Erythropoiesis-stimulating agents are currently recommended for veterinary patients with anemia secondary to chronic kidney disease (CKD). Darbepoetin alfa (darbepoetin) has replaced epoetin due to a three-fold longer half-life allowing for less frequent dosing. Also, darbepoetin is perceived to be less likely to elicit formation of anti-erythropoietin antibodies resulting in pure red cell aplasia (PRCA). A previous study examined the efficacy of darbepoetin in cats with CKD; 14 of 25 cats responded, with adverse events observed in 12 cats, including two with possible PRCA. This retrospective study evaluated response to darbepoetin therapy, protocols for administration, and potential adverse events in dogs with CKD.

Thirty-four dogs with IRIS stage 3 or 4 CKD met the inclusion criteria and their medical records were reviewed. Data recorded included CBC, serum biochemistry panel, reticulocyte count, serum iron parameters, systolic blood pressure, packed red blood cell (pRBC) transfusion, iron supplementation, darbepoetin dose and frequency of administration, comorbidities, adverse events, and survival.

Starting HCT ranged from 9.6–28.9% (mean of 19.7%). The starting dose of darbepoetin ranged from 0.4 - 2.08 mcg/kg (median 0.5 mcg/kg). The initial dosing frequency was every 7 days in 31/34 dogs. Nine of 34 dogs received a pRBC transfusion and 27/34 dogs received iron supplementation in the form of a pRBC transfusion, intramuscular iron dextran, oral supplementation, or a combination thereof at the start of darbepoetin therapy. Sixteen of 34 dogs received on-going iron supplementation throughout treatment.

Survival following initiation of darbepoetin therapy ranged from 30 – 546 days (median 118 days). An estimated 442 doses of darbepoetin were administered over that course of treatment; the range of doses administered per dog was 2 - 61 (median 11.5 doses per dog). Dogs were considered responders if they achieved a HCT of 30% over the course of treatment. Twenty-nine of 34 dogs were responders. Time to response ranged from 6 - 106 days (median 28 days). There was no association between starting HCT, dose or frequency of administration of darbepoetin, or initial iron supplementation between responders and non-responders. Dogs that received a pRBC transfusion at the onset of treatment were more likely to be non-responders ($P = 0.013$). Twenty-two dogs were transitioned to an extended dosing interval following response, and 15 of those dogs subsequently required the dosing interval to be shortened to maintain target HCT. No dog sustained a response at a dosing interval greater than every 21 days. Potential adverse events were documented in 19/34 dogs and included increased systolic blood pressure requiring treatment (n = 12), seizures (n = 5), vomiting (n = 3), diarrhea (n = 3), hypersensitivity reaction to iron injection (n = 1), and possible PRCA (1). Development of potential adverse events resulted in discontinuation of darbepoetin only with respect to possible PRCA; in all other cases, adverse events were more likely attributed to underlying disease.

Darbepoetin is an effective treatment for anemia secondary to CKD. A dosing interval of >21 days is not effective at maintaining a response to therapy. Further studies are needed to define the most effective dose. PRCA was a possible adverse event in 1/34 dogs (2.9%).

NU05
VITAMIN D-BINDING PROTEIN – EARLY MARKER OF TUBULAR INJURY IN DOGS WITH CHRONIC KIDNEY DISEASE.

Fernanda Chacar[1], Márcia Kogika[1], Talita Sanches[2], Douglas Caragelasco[1], Cinthia Martorelli[1], Camila Rodrigues[2], José Capcha[2], Lúcia Andrade[2]. [1]School of Veterinary Medicine and Animal Science, University of Sao Paulo, SP, Brazil, [2]School of Medicine, University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil

Vitamin D-binding protein (VDBP) is the main carrier of vitamin D metabolites into circulation, and it is freely filtrated through glomerulus, and the complex VDBP-25-OH-vitamin D is uptaken by megalin-cubilin receptors in proximal tubules. Once the presence of VDBP in urine, it may indicate proximal tubular dysfunction/injury as well as interstitial tubular fibrosis. Also it still remains to be proven whether loss of VDBP in urine may lead to vitamin D deficiency and its contribution to the progression of chronic kidney disease (CKD) in dogs. The hypothesis and the aim of this study were to investigate whether VDBP could be previously detected in urine of dogs with CKD regardless of urinary-to-protein ratio (UPC) values, and it will be an early and specific marker of proximal tubular injury. Forty dogs according to IRIS staging of CKD (stage 1 n = 10; stage 2 n = 10; stage 3 n = 10; stage 4 n = 10) were enrolled, various breeds and age, and with no proteinuric-associated disease. Nine clinically healthy dogs, different breeds and age, composed the control group. Dogs with CKD were classified according to IRIS guidelines based on UPC as non-proteinuric (UPC<0.2), borderline proteinuric (UPC= 0.2 to 0.5) and proteinuric (UPC>0.5). Western blotting was performed to investigate VDBP in canine urine (Anti-Vitamin D Binding Protein antibody, ab95469, Abcam© 1:500). No urinary VDBP was detected in control dogs and UPC was 0.14 ± 0.04 (mean±SEM) and min= 0.028 and max= 0.41. In CKD dogs, urinary VDBP was observed since the early stages of CKD, stages 1 and 2, and the UPC was 0.39 ± 0.29; 0.02–3.01 and 0.33 ± 0.12; 0.14–1.14 (mean ±SEM; min - max), respectively. In stage 1 CKD dogs, urinary VDBP was detected in 7 out of 10 dogs and in 4 of those 7 dogs, UPC was into the non-proteinuric range, 2 dogs in borderline range and only one was proteinuric. In stage 2 CKD dogs, VDBP in urine was noticed in 9 out of 10 CKD dogs and 5 of them had UPC < 0.2, one dog was in borderline (UPC= 0.42) and 3 dogs were slightly proteinuric (UPC of 0.67, 0.72 and 1.14). In CKD dogs in stage 3, UPC was 1.51 ± 0.54 (0.07–4.57) and VDBP in urine was immunodetected in 8 out of 10 dogs and in 3 of those 8 dogs, UPC was ≤ 0.28 and the left 5 dogs were proteinuric. All CKD dogs in stage 4 were proteinuric (UPC = 4.37 ± 0.47; 1.4–6.94) and showed VDBP in urine. In conclusion, the immunodetection of VDBP in urine noticed in the early stages of CKD in dogs, mainly in stages 1 or 2, associated with UPC into non or borderline proteinuric range, it reinforces that urinary VDBP could be a potential early marker of kidney injury as well as to detect the specific segment (proximal) of the nephron affected.

NU06
ASSESSMENT OF REPEATED ADMINISTRATION OF A FELINE FVRCP VACCINE AS A MODEL FOR INTERSTITIAL NEPHRITIS.

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Progressive interstitial nephritis (IN) is the primary cause of feline chronic kidney disease which is considered to be the leading cause of death in adult cats. The Crandell Rees feline kidney (CRFK) cell line is commonly used to grow feline herpesvirus 1 (FHV-1), calicivirus, and panleukopenia virus used in (FVRCP) vaccine production. Previous studies have shown that cats

administered FVRCP vaccines parenterally develop antibodies against CRFK lysates and alpha enolase (glycolytic pathway enzyme in all mammalian cells) and these antibodies can bind to feline renal cell lysates. In addition, three of six cats that were hyperinoculated (11 injections) with CRFK lysates over two years had IN on renal biopsy collected two weeks after the last booster. The primary objective of this study was to determine whether IN could be induced over 16 weeks by repeatedly administering a market leading parenteral FVRCP vaccine to potentially use as a short term model to study biomarkers associated with IN in cats.

A total of six (three male; three female) one-year-old purpose bred cats were included in the IACUC approved study. The cats were previously maintained as unvaccinated controls in a FHV-1 study at five months of age and none of the cats had clinical signs of FHV-1 when enrolled in the current study. On Week 0, blood, serum, urine were collected for biomarker assays and a wedge kidney biopsy for histopathological evaluation and alpha-enolase immunohistochemistry was obtained. After sample collection on Week 0 and again on Weeks 2, 4, 6, 8, 10, 12, and 14, all cats were administered a commercially available FVRCP vaccine shown previously to induce anti-CRFK and anti-enolase antibodies and samples were collected for biomarker assays. After sample collection on Week 16, similarly sized renal biopsies were made, avoiding the previous biopsy sites. Haematoxylin and eosin stained sections were provided to two board-certified pathologists that were masked to the timing of the biopsies. Anti-CRFK and anti-enolase antibodies levels in serum were determined by ELISAs.

All 6 cats developed progressively increasing anti-enolase and anti-CRFK serum antibodies.

Mean and SD of ELISA absorbance values of 6 cats administered an FVRCP vaccine

Antibody	Week 0	Week 4	Week 8	Week 12	Week 16
Enolase	0.57 ± 0.16	2.00 ± 0.74	3.48 ± 0.72	3.82 ± 0.47	3.88 ± 0.43
CRFK	0.28 ± 0.03	0.66 ± 0.23	1.38 ± 0.39	1.63 ± 0.37	1.81 ± 0.4

Histological evidence of interstitial nephritis was not detected by light microscopy in any of the tissue biopsies. Significant biochemical or urinalysis changes during the study were not detected and the cats were adopted to private homes for long term monitoring.

While anti-CRFK and anti-enolase antibodies were induced in this model, biochemical or histopathology abnormalities were not detected. Results of selected biomarkers and enolase immunohistochemical staining will be used to further evaluate this potential IN model.

NU07
COMPARISON OF VISUAL AND AUTOMATED INTERPRETATION OF URINARY DIPSTICKS WITH GLUCOSE:CREATININE RATIO AND GLUCOSE CONCENTRATION.

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The accuracy of Bayer Multistix with visual reading for determination of the glucose concentration in canine urine is only 59% (Behrend et al, *J Vet Int Med*, 2009). The correlation between visual and automated assessment has not been investigated. Furthermore, urine glucose:creatinine ratio (UGCR) may be a useful method in quantifying glucosuria similar to urine protein:creatinine ratio but has not been assessed. The purpose of this study was to compare automated readings, visual readings and UGCR for quantification of glucosuria in canine urine.

Urine samples submitted to the Auburn University Clinical Pathology Laboratory for urinalysis and that were naturally glucosuric (n = 39) were included. Multistix (Bayer) were used according to manufacturer instructions and were read visually by a single trained investigator ("visual reading") and by the CLINITEK 50 analyzer (Bayer; "automated reading"). Urine glucose and urine creatinine concentrations were measured using a Hitachi 911 Chemistry Analyzer (Boehringer Mannheim Corp.); the glucose measurements were considered to be the true concentration (gold standard). Spearman's rank order correlation was used to evaluate the relationship between: 1. glucose concentration and automated readings; 2. glucose concentration and visual reading; 3. automated and visual readings; 4. automated readings and UGCR; and 5. visual readings and UGCR. Significance was set at $P < 0.05$. Correlation was classified as excellent for r = 0.93–0.99, good for r = 0.8–0.92, fair for r = 0.59–0.79 and poor for r < 0.59. Reference intervals that correlated with the colors on the test strip were devised based on the package insert (trace=76–175 mg/dL; 1 + = 176–375 mg/dL; 2 + = 376–750 mg/dL; 3 + = 751–1500 mg/dL; 4 + = >1500 mg/dL).

Significant correlation was detected between both automated and visual readings and glucose concentration ($P < 0.0001$). The correlation for automated readings was good (r = 0.834) but fair for visual readings (r = 0.761). Overall, the underestimations and overestimations of glucose concentration for the automated readings was 56.4% and 15.4%, respectively. For the visual readings, they were 13.2% and 36.8%, respectively. Significant correlation was detected between both automated and visual readings and UGCR ($P < 0.0001$). However, correlation for automated readings was classified as good (r = 0.801) but for visual readings was fair (r = 0.719). Furthermore, automated and visual readings had a significant ($P < 0.0001$) and good correlation (r = 0.817).

Our results indicate that despite a good correlation between automated and visual reading of Bayer Multistix, automated reading may be better. Interestingly the automated readings tend to underestimate glucose concentration while with visual reading, overestimations are more common. The UGCR is a valid way to assess urinary glucose excretion; however, it offers no advantage over measurement of glucose concentration in urine.

NU08
IMPACT OF CANINE PANCREAS-SPECIFIC LIPASE ON THE OUTCOME OF DOGS WITH HEMODIALYSIS-DEPENDENT ACUTE KIDNEY INJURY.

Kanae Takada, Carrie Palm, Steven Epstein, Larry Cowgill. University of California-Davis Veterinary Medical Teaching Hospital, Davis, CA, USA

Acute pancreatitis (AP) and acute kidney injury (AKI) are common co-morbidities in both dogs and humans. In human patients the presence of AP with AKI is a negative prognostic factor; however, a similar relationship has not been evaluated in dogs. When considering hemodialysis for management of AKI in dogs, this prognostic information could be important for therapeutic decisions. Canine pancreas-specific lipase (SPEC) is used commonly to support the diagnosis of AP in dogs. The purpose of this study was to investigate elevated SPEC on the outcome of dogs with AKI treated with hemodialysis.

Medical records were evaluated retrospectively from August 2011 to June 2015 to identify dogs presented to the UC Davis VMTH that received intermittent hemodialysis for management of AKI and also had a SPEC (Spec cPL™, IDEXX Laboratories) measured during the course of therapy. Outcome was assessed at 30 days from admission, and dogs were classified as died/euthanized or alive. Surviving dogs were stratified further into non dialysis-dependent or dialysis-dependent. An elevated SPEC was defined as ≥ 400 mcg/L. Median values of SPEC were compared with a Mann-Whitney U test for each outcome category, above. Categorical data were compared with a Fisher's exact test. A *P*-value of <0.05 was considered significant. Data are presented as median [range].

Forty-three client-owned dogs were included. Median initial serum creatinine (iSCr) was 8.5 [3.6 - 16.6] mg/dl and median age at presentation was 5.6 [0.70 - 13.5] years. Eighteen were male neutered, 14 were female spayed, 4 were intact male, and 7 were intact female dogs. At 30 days, 30/43 dogs (70%) were alive (iSCr, 8.6 [4.7 - 16.6] mg/dl) and 13/43 (30%) had died or been euthanized (iSCr, 8.0 [3.6 - 15.3] mg/dl). Nine of 30 dogs (30%) were still dialysis dependent at 30 days (iSCr, 10.9 [6.1 - 15.4] mg/dl), and 21/30 (70%) were non dialysis-dependent (iSCr, 8.3 [4.7 - 16.6] mg/dl). SPEC was not significantly different between dogs alive at 30 days and dogs that had died or were euthanized (SPEC 627.5 [29 -

2001] mcg/L versus 455.0 [30 - 2001] mcg/L, $P = 0.50$). SPEC in dialysis-dependent dogs was significantly higher than non dialysis-dependent dogs (SPEC 1001.0 [177 - 2001] mcg/L versus 379.0 [29 - 1001] mcg/L, $P = 0.008$). The proportion of dogs alive at 30 days with SPEC ≥ 400 mcg/L during the treatment course was not different from alive dogs with SPEC < 400 mcg/L (71% versus 68%, $P = 1.0$). In addition, the proportion of the non dialysis-dependent dogs with SPEC ≥ 400 mcg/L during the treatment course was not different from non dialysis-dependent dogs with SPEC < 400 mcg/L (42% versus 58%, $P = 0.36$).

These results suggest the presence of pancreatitis assessed as SPEC ≥ 400 mcg/L did not affect survival outcome at 30 days. However, pancreatitis may be a more common co-morbidity in dogs with more severe (dialysis-dependent) AKI and it may influence renal recovery.

NU09

CHARACTERIZATION OF SUBCLINICAL BACTERIURIA, URINARY TRACT INFECTION, AND PYELONEPHRITIS IN DOGS WITH CHRONIC KIDNEY DISEASE. Jonathan Foster, Harathi Krishnan, Stephen Cole. University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA, USA

Bacterial urinary tract infection (UTI) is common in dogs, however the prevalence of subclinical bacteriuria (SBU) in healthy dogs has recently been demonstrated to be substantially lower. Following standards created by human infectious disease specialists, there has been more initiative put towards identifying and characterizing SBU in dogs. Although it has been considered a risk factor for UTI, the prevalence of UTI and SBU in dogs with chronic kidney disease (CKD) is not known. The objectives of this study were to retrospectively evaluate the prevalence of positive urine cultures in dogs with CKD, and to further categorize these patients as having SBU, clinical UTI, or pyelonephritis.

Medical records were reviewed retrospectively from 1/2010–7/2015 for dogs with a diagnosis of CKD who had a urine culture submitted. The diagnosis of CKD was based on International Renal Interest Society (IRIS) guidelines. Patients were excluded if they had another reason to develop UTI, namely endocrine disease (hyperadrenocorticism, diabetes mellitus), urolithiasis, urinary incontinence, urinary bladder neoplasia, had a nidus for infection such as ureteral stent or subcutaneous ureteral bypass, or were receiving systemic immunosuppression. Based on previously reported definitions, patients were placed into one of the following three categories based on the review of their medial record: SBU, pyelonephritis, or UTI. The frequency of negative culture, SBU, pyelonephritis, and UTI were compared across IRIS stages using Fisher's exact analysis. $P < 0.05$ was considered significant for all comparisons.

Two hundred and eighty-two cultures were submitted on 195 patients during the study time period. Forty-seven cultures performed on 13 patients were excluded due to the presence of ureteral stents (5 patients), chemotherapy (2 patients), diabetes mellitus (2 patients), transitional cell carcinoma (2 patients), urinary incontinence and cystic calculi (1 patient each). A total of 235 cultures submitted on 182 patients were included in the final analysis. There were 8 intact males, 52 castrated males, 5 intact females, and 117 spayed females. There were 40 positive urine cultures (17.0% of all cultures) obtained on 33 patients (18.1% of all dogs). Of all positive cultures, the most frequently determined diagnosis was SBU (18 cultures, 45%), followed by pyelonephritis (16 cultures, 40%), then UTI (6 cultures, 15%). There were no statistically significant observed relationships between any IRIS stage and diagnosis ($P = 0.635$). The number of positive cultures, regardless of patient diagnosis, was not statistically significant between IRIS stages ($P = 0.432$). *Escherichia coli* was the most frequently observed isolate (29/40 cultures, 72.5%).

In this population of dogs with CKD the prevalence of dogs with positive urine culture was 18.1%. The most frequently observed diagnosis associated with a positive culture was SBU. Increasing IRIS stage of CKD was not found to be associated with a higher frequency of any particular diagnosis. *E. coli* was the most commonly observed isolate. Despite the rigorous criteria for defining SBU, pyelonephritis, and UTI in the current study, it is possible that some patients were incorrectly categorized. Prospective research is needed to determine if antibiotic therapy is indicated in dogs with CKD and SBU. Additionally, renal biomarkers may allow for better characterization of patients with SBU and occult pyelonephritis and improve antibiotic stewardship.

NU10

INITIAL OUTCOMES AND COMPLICATIONS OF THE SUBCUTANEOUS URETERAL BYPASS PROCEDURE AT TWO UNIVERSITY HOSPITALS (2012–2015). Ewan D.S. Wolff[1], Roswitha Dorsch[2], Julia Knebel[2], Daniel J. Duffy[1], Lynetta J. Freeman[1], Lynn Guptill[1], Larry G. Adams[1]. [1]Purdue University, West Lafayette, IN, USA, [2]Ludwig Maximilians University Munchen, Munich, Bavaria, Germany

The aim of this study was to report combined experience of two institutions placing subcutaneous ureteral bypass systems (SUBs). We compared our data to historical data from cats with ureteral stents for ureteral obstruction. Hypotheses included 1) Patients with SUBs would have the same or better 7–14 day and 3–4 month post-operative creatinine as historic ureteral stent patients, 2) SUB patients would experience the same or improved peri-operative, post-operative and recurrent urinary tract infection rates as ureteral stent cats, 3) clinical signs related to implants would be similar or better with the SUB system versus ureteral stenting.

Adult cats were enrolled retrospectively, subsequent to the 2012 alteration to SUB placement procedure. All cats were managed per clinician preference pre-operatively. Flushes of SUB systems were performed every 3–6 months for the majority of patients.

Records from 19 cats from Purdue and Munich Universities were reviewed. Median age was 9 years at Purdue and 7.7 years at Munich. There were 12 spayed female and 7 castrated male cats; 13 domestic short-haired cats, 2 domestic longhaired cats, and three other breeds. 17% of cats had preexisting IRIS Stage II CKD. Lethargy, vomiting and inappetence were the most common clinical signs (>25% of patients). Median renal pelvic diameter in affected kidneys was >0.5 cm at both institutions. Median creatinine immediately post-operatively was 2.6 mg/dL (0.9–2.3 mg/dL) at Purdue and 1.43 mg/dL at Munich. Major peri-operative complications were uncommon; one cat had cardiac arrest and another was transfusion dependent. Post-operative complications included 2 cats with fluid overload and pleural effusion. 8/19 cats required a blood transfusion in the peri- or post-operative period. The median creatinine was 3.6 mg/dL at 3 months and 1.8 mg/dL at 6 months. 3 patients had repeat surgeries; median time to repeat surgery was 225 days. Causes of repeated surgery were SUB port obstruction or encrustation of SUB tubing. Total mortality was 21% with a median disease free interval of 180 days and a 1 year survival of 83%.

No cats had active urine sediment or culture prior to SUB placement, but post-operative and follow up urinary tract infections occurred. 21% of patients had urinary tract infection within 10 days of surgery. Two patients had recurrent multi-drug resistant *E. coli* infection, one was cultured as a biofilm. Another patient developed encrustation 10 months post-procedure. Mandelic acid, EDTA, antibiotic infusions into SUBs failed to resolve UTI in 2 cats.

Comparing SUB outcomes to ureteral stenting, 6 month post-procedural creatinine was better in the SUB group (1.8 mg/dL) than that reported for stents (2.6 mg/dL). One year survival was similar for stent cats (86%) and SUB cats (83%). Long-term infection rates were similar (around 31%). Only one SUB patient experienced dysuria beyond the post-operative period whereas this is more common in cats with stents.

This study suggests that the SUB system carries similar potential complications of re-obstruction, encrustation and infection to ureteral stenting. Recurrent and resistant infections and the potential for biofilms is a significant concern in SUB patients. Protocols for treatment of resistant UTIs utilizing local instillation and indwelling bladder treatment should be developed in a prospective fashion. Further work with more cases could explore risk factors for UTI and obstruction.

NU11

OCCULT URINARY TRACT INFECTION IN CATS: PREVALENCE AND FINDINGS ON CONTEMPORANEOUS URINALYSIS. Melanie Puchot[1], Audrey Cook[1], Caitlin Pohlit[2]. [1]Texas A&M University, College Station, TX, USA, [2]Michigan State University, East Lansing, MI, USA

Cats are frequently screened for bacterial urinary tract infection (UTI) in the absence of clinical signs of lower urinary tract disease (LUTD) when managing conditions such as chronic kidney disease and diabetes mellitus. The purposes of this study were to determine the prevalence of occult UTI in cats and to describe results of the contemporaneous urinalysis (UA). We hypothesized that a positive quantified urine culture (QUC) could be predicted by findings on microscopic sediment examination (MSE) such as bacturia, pyuria (≥3 white blood cells/hpf) and hematuria (≥10 red blood cells/hpf).

An electronic search identified all QUCs performed on cats at a university teaching hospital between 2009 and 2015. Results were included in the study if there were no signs of LUTD and the QUC was not performed as follow up to a previous UTI. A QUC was classified as positive if growth >1,000 cfu/mL was reported. For each positive, five controls were randomly selected from samples with a negative QUC. Medical records for both groups were reviewed.

In all, 31/500 (6.2%) samples were positive. Most infections were a single organism (n = 27); four contained multiple organisms. *E. coli* was the most common species (58% of isolates) followed by *Enterococcus* species (25% of isolates). Positive specimens were more likely to be from female cats (n = 24) versus male (n = 7) ($P = 0.0054$). Patient age and body weight were not associated with increased risk of occult UTI. Median urine specific gravity was similar for the positive group (1.022) and controls (1.024). The prevalence of proteinuria on dipstick testing was also similar (84% v 80%). Occult UTIs were strongly associated with bacteriuria (60% v 6%; odds ratio 24.3 [CI: 9.0–65.7]; $P < 0.0001$) or pyuria (67 v 19%; odds ratio 8.7 [CI: 3.7–20.5]; $P < 0.0001$) but not microscopic hematuria. Positive specimens were significantly more likely to have an abnormal sediment exam (odds ratio 13.5 [CI 3.9–45.5]; $P < 0.0001$).

Occult UTI appears to be uncommon in cats and can usually be predicted on the basis on an abnormal MSE (bacturia, pyuria). Based on this study, there is little indication to perform a QUC in a cat with no clinical signs of LUTD and an unremarkable MSE.

NU12

DIAGNOSTIC PERFORMANCE OF URINARY CANINE CALGRANULINS IN DOGS WITH LOWER URINARY TRACT CARCINOMA. Romy Heilmann[1], Elizabeth McNeil[2], David Lanerie[1], Niels Grützner[3], Jan Suchodolski[1], Joerg Steiner[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA, [2]Cummings School of Veterinary Medicine, Tufts University, Boston, MA, USA, [3]Farm Animal Clinic, Vetsuisse University Bern, Bern, BE, Switzerland

Aside from its association with several inflammatory diseases, the S100A8/A9 calgranulin complex was shown to be expressed by epithelial cells after malignant transformation. In humans, S100A9 or S100A8 have been reported to be up-regulated in patients with prostate carcinoma (PCA) or transitional cell carcinoma (TCC), respectively. Results of the expression of the S100A12 calgranulin in TCC/PCA in humans are ambiguous. Pilot data by our group suggest that the urine canine S100A8/A9-to-S100A12 ratio (uCalR) may have potential as a marker for TCC/PCA in dogs. Further evaluation of urinary S100A8/A9, S100A12, and uCalR in dogs is needed to determine its specificity for a diagnosis of TCC/PCA. Thus, this study investigated urinary S100A8/A9, S100A12, and uCalR in dogs with TCC/PCA, other urinary tract diseases, or other malignancies.

Urine samples from dogs with TCC/PCA that were treatment-naïve (n = 22) or under treatment (n = 40), dogs with non-neoplastic diseases of the urinary tract (n = 22) or other neoplasms (n = 35), dogs with a urinary tract infection (UTI, n = 45), and a group of healthy control dogs (n = 75) were used. Urinary canine S100A8/A9 and S100A12 were measured in all samples using in-house radioimmunoassays and the uCalR was calculated. All parameters were tested among the different groups of dogs using non-parametric multiple-/two-group comparisons. Sensitivities and specificities were determined for the optimal cut-off values, and positive (PPV) and negative predictive values (NPV) were calculated. Significance was set at $P < 0.05$.

Dogs with TCC/PCA had significantly higher urinary S100A8/A9 and S100A12 concentrations than all other groups of dogs (all $P < 0.016$) except for dogs with UTI, whereas uCalR was significantly higher in TCC/PCA dogs compared to only those with a UTI ($P < 0.001$). Treatment-naïve TCC/PCA patients and UTI dogs were best distinguished by a uCalR of ≥9.1 (sensitivity: 91%, specificity: 60%). A urine canine S100A8/A9 concentration (normalized against the urine specific gravity [USG]) of ≥109.9 had the highest sensitivity (96%) and specificity (66%) to diagnose treatment-naïve TCC/PCA dogs from all other groups of dogs, and the specificity increased to 75% if a UTI had been excluded. Using a urine S100A8/A9 concentration (normalized against USG) of ≥109.9 to screen dogs ≥6 years of age for TCC/PCA (estimated prevalence: <1%) yielded a PPV of 4% and a NPV of 100%.

The results of this study show that urine S100A8/A9 and the uCalR have utility for the diagnosis of TCC/PCA in dogs. The high sensitivity and NPV of urine S100A8/A9 also suggest that S100A8/A9 may be a good screening test for TCC/PCA in dogs. Further studies are warranted to validate our findings in a larger cohort of dogs and to evaluate the source of S100A8/A9 and S100A12 expression in canine TCC/PCA.

NU13

SURVEY OF SUBCUTANEOUS FLUID PRACTICES IN CATS WITH CHRONIC KIDNEY DISEASE. Crystal Cooley[1], Sarah Caney[2], Liberty Sieberg[1], Jessica Quimby[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]Vet Professionals, Roslin, Midlothian, UK

Chronic kidney disease (CKD) is common in elderly cats. The purpose of this study was to describe subcutaneous fluid (SQ) administration practices of owners of CKD cats to help more owners successfully give SQ fluids to their cat.

An anonymous web-based survey was advertised via list serves, websites and social media. Owners of 468 cats with CKD participated. 87% of cats were 10 years or older. Cats were IRIS stage I (1%), II (20%), III (37%), IV (17%), and unknown (25%).

95% of owners said they discussed giving fluids with their veterinarian. 399 respondents stated they gave SQ fluids, 57 did not, and 12 tried but could not. Only 42% of owners were given additional educational resources. 79% said the process was ok/easy to learn. Once experienced, 15% said it was still somewhat/highly stressful on them, and 11% said it was somewhat/highly stressful for the cat. To improve tolerance 57% used food for positive reinforcement with 59% stating this improved tolerance, 60% warmed the fluids and 83% felt warming fluids increased tolerance. 74% felt that length of time it took to administer fluids affected tolerance. 82% said needle size affected tolerance. 40% of owners checked hydration status daily or twice daily and 18% of owners did not know how. 43% said they skipped/added fluids based on hydration assessment.

The majority of owners were successful in administering fluids but additional education materials could be provided. Variables such as needle size, warming fluids, and length of time of administration may improve tolerance.

NU14

QUANTIFYING URINE ELIMINATION BEHAVIORS IN CATS USING A VIDEO RECORDING SYSTEM. Ryan Dulaney, John Kruger, Marie Hopfensperger, Rob Malinowski, Joe Hauptman, Brenna Lashbrook. Michigan State University, East Lansing, MI, USA

Feline lower urinary tract disorders often rely on subjective caregiver quantification of clinical signs to establish a diagnosis

and monitor therapeutic outcomes. The objective of this study was to investigate use of a video recording system (VRS) to more reliably assess and quantify urination behaviors in cats. Litter box urination behaviors were quantified in 11 healthy cats and 8 cats with abnormal urination patterns using a VRS for 14 days and compared to daily caregiver observations. Video recordings were analyzed using a behavior analysis software program.

The mean number of urinations per day detected by VRS (2.5 ± 0.2) was significantly greater compared to caregiver observations (0.6 ± 0.2; $P < 0.0001$). Five of 19 cats were never observed by their caregivers in the litter box. The mean number of urinations per day detected by VRS was significantly higher for abnormal cats (2.9 ± 0.3) compared to healthy cats (2.1 ± 0.2; $P = 0.02$); there were no apparent differences in frequency between healthy and abnormal cats reported by caregivers (0.7 ± 0.3 and 0.5 ± 0.3 respectively). There were no differences in mean urination time between normal and abnormal cats determined by VRS or caregivers. Mean cover-up time determined by VRS was significantly longer in normal cats (23 ± 4 seconds/urination compared to abnormal cats (9 ± 5 seconds/urination; $P = 0.03$); differences in cover-up time were not detected by caregiver observations.

In conclusion, there was considerable disparity between caregiver and video observations with caregivers commonly underestimating urination frequency. VRS appears to facilitate objective assessment of urination behavior and could be of value in future clinical studies of feline urinary disorders.

NU15

EVALUATION OF THE EFFECT OF URINE DIP VERSUS URINE DRIP ON MULTITEST STRIP RESULTS. Alisdair Boag, Craig Breheny, Adam Gow. Royal (Dick) School of Veterinary Studies, Edinburgh, UK

Standard operating procedures (SOPs) have been established for in-clinic laboratory tests, such as the World Health Organisation guidelines for packed cell volume. No independent evidence based guidelines exist for dipstick urinalysis, however manufacturer's instructions state to dip the stick into urine. In veterinary medicine, occasionally urine samples of such small volume are obtained that this is impractical and dripping urine on the stick from a pipette or syringe is performed. This also has the advantage of conserving the sample for additional tests.

The hypothesis of this study was that the method of drip or drip would have no effect on the test results. Siemens Multistix 10SG strips were used. To standardise the method of strip analysis, a Siemens Clinitek Status+ analyser was used to read the strips; controlling time of reading the results and user variability in interpretation of colourimetric changes. Three individuals tested 25 aliquots of urine from dogs with a range of disease processes by both the dip and drip method. Results were compared for each variable between drip and dip using Wilcoxon-signed rank test.

Across the nine variables assessed (SG was not), a significant difference between methods was found for one variable, bilirubin ($P = 0.046$). This difference was accounted for by one dog who tested negative for bilirubin for all three users using a dip method and had a trace value for all three users using a drip methodology. No other significant differences were found between variables.

This study demonstrated a significant difference between dipping and dripping for bilirubin, caused by one dog with the same level of variability for all three users. This difference is, in the authors' opinion, clinically insignificant. This study therefore supports the use of either a dipping or dripping approach to urinalysis. Further studies are required to more fully assess the role of individual variation when performing the test including level of experience.

NU16

POSITIVE IMPACT OF NUTRITIONAL INTERVENTIONS IN CLIENT-OWNED DOGS WITH IRIS STAGE-1 CHRONIC KIDNEY DISEASE. Jean Hall[1], Dale Fritsch[2], Maha Yerramilli[3], Edward Obare[3], Murthy Yerramilli[3], Dennis Jewell[2]. [1]Oregon State University, Corvallis, OR, USA, [2]Hill's Pet Nutrition, Inc., Topeka, KS, USA, [3]IDEXX Laboratories, Inc., Westbrook, ME, USA

A prospective 12-month clinical trial was performed in client-owned dogs with IRIS stage 1 chronic kidney disease (CKD) to measure their ease of transition to a commercial renal protective food and to assess the effects of food on renal biomarkers and quality of life attributes. Dogs with IRIS stage 1 CKD ($n = 36$) were transitioned over 1 week from various grocery brand foods to a modified protein, low phosphorus, antioxidant enriched test food (Prescription Diet® k/d®, Hill's Pet Nutrition, Inc.). At months 0, 3, 6, 9, and 12 owners completed a questionnaire to assess their pet's acceptance of the test food and their dog's quality of life. Renal biomarkers, including serum creatinine (Cr), blood urea nitrogen (BUN), and symmetric dimethylarginine (SDMA), and urinalysis parameters, including urine specific gravity (USG) and urine protein:creatinine ratio (UPC), were measured. Of the 36 dogs initially enrolled, 35 (97%) transitioned to the test food. Pets moderately or extremely liked the test food 88% of the time, ate most or all of the food 84% of the time, and were moderately or extremely enthusiastic while eating 76% of the time. Dogs consuming test food showed a decrease in serum Cr and BUN concentrations across time (both $P = 0.01$) and a decrease in serum SDMA concentration and USG across time (both $P = 0.09$). All serum renal biomarkers (Cr, BUN, SDMA) were decreased ($P \leq 0.05$) from baseline at 3 months, and remained decreased from baseline at 12 months in dogs completing the study ($n = 20$). This study shows that dogs with IRIS stage 1 CKD readily transition to a commercial renal food, and decreasing serum biomarker concentrations suggest improvement in kidney function. In addition, owners reported improvement in a consortium of overall health and quality of life attributes ($P < 0.01$) and hair and coat quality attributes ($P < 0.01$).

NU17

FIBROBLAST GROWTH FACTOR 23 (FGF-23) IN DOGS WITH NATURALLY OCCURRING CHRONIC KIDNEY DISEASE. Marcia Kogika[1], Cinthia Martorelli[1], Douglas Caragelasco[1], Fernanda Chacar[1], Luciano Giovaninni[2], Dennis Chew[3], Ramiro Toribio[3], Katarzyna Dembek[3], Camila Rodrigues[4], Talita Sanches[4], Lucia Andrade[4]. [1]School of Veterinary Medicine, University of São Paulo, SP, Brazil, [2]Sao Paulo, SP, Brazil, [3]College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, [4]School of Medicine, University of São Paulo, Sao Paulo, SP, Brazil

Decreased glomerular filtration rate in chronic kidney disease (CKD) reduces renal phosphorus excretion, leading to increased total body phosphorus retention and eventually hyperphosphatemia. In addition of promoting parathyroid hormone synthesis, hyperphosphatemia stimulates the synthesis of fibroblast growth factor 23 (FGF-23), a phosphaturic/hypophosphatemic hormone. There are no reports on serum FGF-23 in healthy dogs or in dogs with CKD. The goal of this study was to measure serum FGF-23 concentrations in healthy dogs and in dogs with CKD, and to determine its association with serum phosphorus concentrations and severity of renal disease based on IRIS staging of CKD. We hypothesized that serum FGF-23 will be increased in dogs with CKD, that its concentrations will be proportional to CKD severity, and that it will be an early marker of altered phosphorus metabolism.

A total of 17 dogs of various breeds and age with CKD ($n = 6$ in IRIS stage 2; $n = 11$ in IRIS stage 3) that were being fed a renal diet, and 15 healthy dogs of different breeds and age, being fed a maintenance diet, were included. Dogs with CKD were followed for up to 12 months or until death. A human-specific FGF-23 ELISA was validated for this study, showing linearity and 2.8% and 8.8% intra and inter-assay coefficients of variation, respectively. Serum FGF-23 concentrations were higher in CKD

dogs in stages 2 and 3 on presentation (2,605.4 ± 872.0 pg/mL; mean±SEM) compared to healthy dogs (258.7 ± 21.2 pg/mL); significant difference was detected between healthy versus CKD dogs in stage 3 ($P < 0.01$;one-way ANOVA), and stage 2 versus stage 3 ($P < 0.05$). On presentation, all dogs in stage 2 CKD were normophosphatemic (3.8 ± 0.2 mg/dL), serum FGF-23 was 499.1 ± 135.8 and 3/6 dogs showed increased FGF-23 concentrations (505.5 – 1,065.5 pg/mL), and there was no correlation between FGF-23 and phosphorus concentrations on admission and follow up ($r = 0.286$; $P = 0.1185$).

In stage 3 CKD dogs, on presentation, 7 were normophosphatemic (2.5 – 5.0 mg/dL) and 4 were hyperphosphatemic (5.3 – 6.6 mg/dL), but all (n = 11) had increased serum FGF-23 concentrations (3,754.3 ± 1,225.2 pg/mL; 957.6–14,193.0 pg/mL) that remained elevated or increased during follow up (843.9–184,581.0 pg/mL). Of the normophosphatemic dogs in stage 3 CKD (n = 7), 5 developed hyperphosphatemia (5.6 – 16.0 mg/dL) after 6 months of follow up. Serum phosphorus was positively correlated with FGF-23 in these dogs ($r = 0.71$; $P < 0.0001$). In the study period, 7/11 dogs in stage 3 died.

In conclusion, serum FGF-23 could be used as an early marker of phosphorus dysregulation in dogs with CKD as increased FGF-23 concentrations were found in normophosphatemic CKD dogs in stages 2 and 3. More marked elevated serum levels of FGF-23 were found in advanced renal disease – IRIS stage 3. It is likely that increased concentrations of FGF-23 contribute to disturbed calcium and phosphorus homeostasis and altered vitamin D metabolism and parathyroid gland function. Further studies at targeted control of serum phosphorous and FGF23 concentrations in dogs with CKD are warranted.

NU18

SERUM CYTOKINE PROFILES OF CATS WITH IDIOPATHIC CYSTITIS. Maciej Parys, Vilma Yuzbasiyan-Gurkan, John Kruger. Department of Small Animal Clinical Sciences, Michigan State University, East Lansing, USA

Idiopathic cystitis (IC) is a common lower urinary tract disorder in cats that resembles painful bladder syndrome/interstitial cystitis in humans. In both disorders, the etiology is unknown and specific therapy is not available. Diagnosis is based on clinical signs and exclusion of other disorders due to lack of characteristic pathologic findings, well-defined phenotypes, or objective biomarkers. Cytokines can serve as noninvasive biomarkers to define the presence, severity, and progression of disease, and response to treatment. The objective of this pilot study was to determine concentrations of selected cytokines in serum from healthy cats and cats with IC. Thirteen serum samples from healthy cats and 13 from cats with IC were evaluated. Multiplex analysis of 19 cytokines (sFas, TNF-α, SDF-1, SCF, RANTES, PDGF-BB, MCP-1, KC, IL-18, IL-13, IL-12 (p40), IL-8, IL-6, IL-4, IL-2, IL-1β, IFN-γ, GM-CSF, Flt-3L) was performed with a multiple antigen Luminex bead-based assay according to manufacturer recommendations. Results were then analyzed for normality of data with D'Agostino-Pearson test and groups were compared using Mann-Whitney test.

Significantly increased mean concentrations of IL12(p40) (1191 ± 484.9 versus 219.6 ± 393.3 pg/mL; $P < 0.0001$), SDF1 (2015 ± 1131 versus 528.7 ± 1002 pg/mL; $P = 0.002$), and IL18 (483.8 ± 468.8 versus 92.03 ± 272.6 pg/mL; $P = 0.02$) were detected in the serum of cats with IC compared to unaffected cats. Further studies are necessary to evaluate the diagnostic and prognostic utility of serum cytokine biomarkers in cats with IC.

NU19

CYSTOLITH DISSOLUTION IN CATS USING A COMMERCIALLY AVAILABLE DIET. Camille Torres-Henderson[1], Jamie Bunkers[1], Elena Contreras[1], Deborah Greco[2], Emily Cross[2], Michael Lappin[1]. [1]Colorado State University, Fort Collins, CO, USA, [2]Nestle Purina PetCare, St. Louis, MO, USA

The most common radiopaque cystoliths in cats are composed of struvite or calcium oxalate minerals. Struvite cystoliths have been shown to dissolve with dietary management whereas calcium oxalate cystoliths must be removed surgically. The commercially available diet, Purina® Pro Plan® Veterinary Diets UR Urinary® St/Ox®, has been formulated for the dissolution of struvite cystoliths and to lessen the recurrence of both struvite and oxalate cystoliths. The purpose of this study was to describe the clinical and laboratory findings in cats with radiopaque cystoliths fed this commercially available diet.

The study was announced to northern Colorado animals shelters by list serve. Cats with clinical signs of lower urinary tract disease and cystoliths confirmed via radiographs were enrolled in this IACUC approved study. Qualifying cats were transferred and maintained at the research study site to assure that only the test diet was fed. Complete blood cell count, serum biochemistry profile, abdominal ultrasound, and urinalysis with aerobic bacterial culture and antimicrobial sensitivity were performed on entry to and exit from the study. The cats were housed in a gang room, fed the study diet ad libitum, and assessed by abdominal radiographs weekly. Cats with cystoliths that resolved based on two sequential weekly radiographs and confirmatory ultrasound examination were considered diet successes. Cats with no change in cystolith size after four weeks underwent cystotomy for stone removal, aerobic culture and antimicrobial susceptibility testing, and analysis at the University of Minnesota Urolith Center. The cats were then adopted to private homes and when possible, the study diet was continued and abdominal radiographs were obtained to evaluate for recurrence.

To date, five cats between the ages of four and eight years of age have completed the study. All cats accepted the study diet, weight loss was not noted over the course of the study in any cat, serum biochemical data were normal for all cats, and urine culture was negative for all cats when exiting the study. Total cystolith dissolution was achieved by Week 2 for three cats and two cats still had radiographic evidence of cystoliths by Week 4. One cat with persistent cystoliths had a single ammonium urate cystolith with a struvite nidus (normal pre and post-bile acids) and the other cat had multiple calcium oxalate cystoliths; cultures were negative for both cats. Recheck radiographs at two months for two cats with stone resolution on diet and the cat with calcium oxalate cystoliths (three month post-cystotomy) showed no evidence of cystolith recurrence.

While larger case numbers are needed, the preliminary results suggest that feeding Purina Veterinary Diet UR Urinary® St/Ox® can successfully dissolve cystoliths that are likely struvite and may lessen risk of recurrence of struvite and calcium oxalate cystoliths.

OT01

EFFECT OF THREE RESUSCITATIVE FLUID PROTOCOLS ON N-TERMINAL PROHORMONE BRAIN NATRIURETIC PEPTIDE IN HEALTHY DOGS. Alison Khoo, Matthew Gaunt, Anthony Carr, Cheryl Waldner. University of Saskatchewan, Saskatoon, SK, Canada

The purpose of this crossover design study was to determine if 3 different resuscitative fluid therapy protocols would increase N-terminal proBNP (NT proBNP) levels in healthy dogs. The protocols included a balanced isotonic replacement crystalloid solution (Normosol R) administered at 90 mL/kg/h over one hour, a 7.2% hypertonic saline bolus administered at 5 mL/kg over 15 minutes, and a 6% hydroxyethyl starch fluid bolus (Voluven ®) administered at 20 mL/kg over 30 minutes. Serum NT-proBNP concentrations were measured in 6 healthy purpose bred dogs prior to fluid administration and then 0.5, 1, 1.5, 2, 4, 8, 12, 18, 24, 36 and 48 hours after receiving one of the three fluid therapy protocols. Each dog was randomly assigned to receive a different fluid protocol each week for 3 weeks, allowing a 5-day washout period between treatment protocols.

Serum NT-proBNP concentration was significantly more elevated from baseline in dogs receiving 90 mL/kg/hr of crystalloid solution compared to dogs receiving hypertonic saline or hydroxyethyl starch boluses. None of the fluid therapy protocols caused elevations of serum NT-proBNP above the previously reported cut-off concentration used to discriminate between dogs with congestive heart failure and dogs with primary respiratory disease.

Resuscitative fluid therapy does increase serum NT-proBNP concentrations but does not impact the ability to use serum NT-proBNP concentration to discriminate between cardiac failure and respiratory disease in healthy dogs.

OT02

DEXMEDETOMIDINE OROMUCOSAL GEL FOR ALLEVIATION OF ACUTE ANXIETY AND FEAR ASSOCIATED WITH NOISE IN DOGS. Mira Korpivaara[1], Kaisa Laapas[1], Mirja Huhtinen[1], Barbara Schöning[2], Karen Overall[3]. [1]Orion Corporation, Espoo, Finland, [2]Veterinary Specialty Practice for Behavior, Hamburg, Germany, [3]Biology Department, University of Pennsylvania, Philadelphia, PA, USA

Distress, fear, and anxiety during exposure to loud noises (e.g., storms, fireworks) are behavioral and welfare concerns for dogs, and affect up to 50% of dogs over their lifetime. Our objective was to confirm that a sub-sedative dose of dexmedetomidine, an alpha-2 adrenoceptor agonist, alleviates acute anxiety and fear associated with noise in dogs.

This randomized, double-blind, placebo-controlled, parallel-group study was conducted at 17 sites in 2 European countries on New Year's Eve. 182 dogs with a history of anxiety or fear due to fireworks received 125 $\mu g/m^2$ dexmedetomidine as an oromucosal gel (89 dogs) or placebo (93 dogs). Owners applied the first dose before or at the onset of distress associated with fireworks. Dosing could be repeated up to 4 times, every 2 hours. Owners assessed treatment effect and dog's behavior. They also reported oral mucosal color, observational and functional alertness, adverse events, and ease of use.

An excellent or good treatment effect was seen in 64 (72%) dexmedetomidine-treated dogs and in 34 (37%) placebo-treated dogs. The overall treatment effect was superior with dexmedetomidine ($P < 0.0001$). Dogs on dexmedetomidine displayed less panting, trembling, and hiding. Transient pale mucous membranes were observed more frequently with dexmedetomidine, but no mucosal irritation was reported. Most dogs (82/89, 92%) remained functional and no adverse events of concern were observed. Most owners (154/182, 85%) considered the product very easy or quite easy to use.

This study confirms that oromucosal dexmedetomidine at a sub-sedative dose alleviates acute anxiety and fear associated with noise in dogs.

OT03

PROGNOSTIC INDICATORS IN CATS WITH SEPTIC PERITONITIS: 44 CASES (2002–2015). Katherine Scotti[1], James Barr[1], Micah Bishop[2], Michael Kato[3], Matthew Mellema[3], Medora Pashmakova[1]. [1]Texas A&M, College Station, TX, USA, [2]Animal Specialty Hospital of Florida, Naples, FL, USA, [3]UC Davis, Davis, CA, USA

Septic peritonitis (SP) in cats carries significant mortality (12–70%) and etiologies may be indeterminate. The aim of this study was to identify etiologies and prognostic factors in cats with SP.

Medical records of cats hospitalized for SP between 2002 and 2015 at Texas A&M University and University of California (Davis) were reviewed. Septic peritonitis was diagnosed by intracellular bacteria on cytology or positive bacterial culture. Data was checked for normality and compared using an unpaired t-test and a Mann-Whitney test. Fisher's exact test and Odds ratios were used to compare variables. Alpha was set at 0.05.

Seventy-nine cats were identified and 55 met inclusion criteria. The source of SP was undetermined in 28/55 cats. Gastrointestinal perforation was the cause of SP in 16/55 cats. Thirty of 44 (68.18%) cats that were surgically managed and 5/11 (45.45%) that were medically managed survived to discharge. In this population, there was no association between leukocytosis, leukopenia, anemia, hypoalbuminemia, hyperlactatemia, ionized hypocalcemia, or band neutrophilia and survival. Cats with increased creatinine were 4.68 times more likely to die (95%CI: 1.40–15.69; P = 0.012). Hypothermic cats were 4.67 more likely to die (95% CI: 1.36–15.98; P = 0.014), while febrile cats were 5.409 times more likely to live (95% CI: 1.58–18.58; P = 0.0063).

The origin of SP was undetermined in half of the cats in this study. Survival was in agreement with previous reports. Azotemia and hypothermia, which may reflect persistence of shock in this species, should be closely monitored in septic cats.

OT04

RELIABILITY OF THERMOMETER PROTECTIVE SHEATHS FOR MEASUREMENT OF RECTAL TEMPERATURE IN DOGS. Franck Jolivet[1], Maud Pic[1], Didier Concordet[2], Olivier Dossin[3]. [1]Department of Small Animal Clinical Sciences, INP – National Veterinary School and Federal University of Toulouse Midi Pyrénées, Toulouse, France, [2]UMR1331 Toxalim, INP - National Veterinary School and Federal University of Toulouse Midi Pyrénées, Toulouse, France, [3]Department of Small Animal Clinical Sciences and IRSD INSERM 1220, INP – National Veterinary School and Federal University of Toulouse Midi Pyrénées, Toulouse, France

Rectal temperature is commonly used in clinical evaluation of canine patients. Single-use lubricated protective thermometer sheaths are largely used in human medicine but this practice is not routinely reported in veterinary medicine. Therefore the aim of our study was to compare rectal temperature measured with the same thermometer with and without protective sheaths in a large canine population.

Three hundred and eighty two dogs were included in the study. Rectal temperature was assessed in a standardized manner by two persons (FJ and MP) with two identical digital rectal thermometers. The thermometers were validated for accuracy and repeatability (within-day variability) with a high precision thermometer. The thermometers validation was performed with and without protective sheaths. The rectal temperature was measured consecutively in each dog with and without protective sheaths in a randomized order. The difference of temperature measured with the two methods was analyzed with a generalized linear model with order of temperature measurement, age, body condition score (underweight, normal and overweight), format (stratified as mini, medium and large) and conscience level (conscious, sedated and anesthetized) as explanatory variables. Results are presented as mean ± SD.

The accuracy of the study thermometers was excellent with average temperature differences of 0.067 ± 0.064 and 0.066 ± 0.054 and ranging from −0.28 to +0.15 and from −0.22 to +0.1 with and without sheath respectively. In the field study, the difference of the temperatures measured with and without sheaths were within the −0.1 and +0.1 °C range for 80% of the measurements. Moreover, these differences were ≤ -0.5°C and $\geq$ +0.5°C for 3 and 5 dogs respectively. Except for the body condition score none of the explanatory variables studied were significantly associated with the differences measured suggesting that the differences were more important in overweight dogs that in normal dogs with average differences of −0.087 and −0.009°C respectively ($P = 0.013$).

This study shows that thermometer protective sheaths can be used reliably to measure rectal temperature in dogs in a clinical setting. Therefore, it is strongly advised to use this hygienic protective measure in daily clinical practice because even in overweight dogs the differences between the two methods are clinically non significant.

OT05
ISOLATION AND IDENTIFICATION OF MICRORNA FROM THE MATURE FELINE ERYTHROCYTE: A PILOT STUDY. Katrina Stewart, Pierre Deshuillers, Joanne Messick. Purdue University, West Lafayette, IN, USA

MicroRNAs have been identified as sensitive, non-invasive diagnostic markers for early detection of human malignancies. To date there have been very few studies of microRNA expression in the serum of domestic animals with no known analysis of the microRNA present in mature erythrocytes. Feline erythrocytes are known to be sensitive to oxidative damage and we are interested in their potential use as a model for the study of systemic oxidative stress. Identification of specific microRNA which are dysregulated under these conditions may be sensitive biomarkers of oxidative stress.

This pilot study was aimed at identifying the microRNAs expressed in mature feline erythrocytes. This specifically involved isolation of a purified population of mature erythrocytes from cat blood; extraction of high quality microRNA from the purified population of mature erythrocytes and sequencing of the microRNA population to form a library from which to facilitate further study.

We successfully purified mature feline erythrocytes from whole blood with a combination of centrifugation and leukoreduction with a specialized filter. This removed a majority of the reticulocytes, white blood cells and platelets, however a final purification step utilizing magnetic beads coated with antibodies was subsequently used to ensure a pure mature population of erythrocytes. For this immune-depletion step, antibodies were initially tested for reactivity in the feline, then biotinylated for use within the columns. The antibodies used in this study were: anti-CD71 (anti-transferrin receptor to remove reticulocytes); anti-CD18 (anti-beta2 integrin to remove leukocytes) and anti-CD61 (anti-beta 3 integrin to remove platelets). Assessment of the mature red blood cell preparation purity was performed with an automated flow cytometry-based hematology analyzer to identify the presence of leukocytes, platelets and reticulocytes.

Isolation of total RNA was performed with a modified TRIzol phase separation method with concentration and quality of the RNA assessed prior to library construction. A small RNA library was constructed with cluster generation and sequencing followed by quality trimming and mapping of reads to the *Felis catus* genome.

With this study we have successfully isolated mature feline erythrocytes from whole blood samples, extracted quality microRNA from the isolated erythrocytes and developed a library of microRNA present in these cells in a normal population of cats. Further work will establish comparison of the microRNA profile between this normal population of cats and those at risk of or with evidence of oxidative damage. This will help to identify new and potentially informative biomarkers for systemic redox status and complications associated with oxidative stress in cats.

OT06
EFFECTS OF SEDATION AND ANESTHESIA ON CANINE HEMATOLOGIC AND SERUM BIOCHEMICAL ANALYSES DURING PREVENTIVE HEALTHCARE. Laura McPhee, Xuemei Si, Carolyn Cupp. Nestle Purina Research, St. Louis, MO, USA

This study aims to determine the effects of sedation and anesthesia on complete blood count (CBC) and serum chemistry results in dogs undergoing routine health and wellness care. Ten healthy adult dogs were selected based on physical exams and the need to undergo anesthesia for routine dental prophylaxis. Blood was collected by venipuncture at a series of timepoints: a) before receiving a premedication, b) prior to induction of anesthesia, c) 5 minutes after induction of anesthesia, d) 15 minutes after induction of anesthesia, and e) 45 minutes after induction of anesthesia. Premedication was a combination of acepromazine (0.025 mg/kg) and butorphanol (0.2 mg/kg) given as a single subcutaneous injection 20 minutes prior to induction with propofol. Propofol was calculated at 4 mg/kg and administered intravenously to effect. Anesthesia was maintained with inhalation of isoflurane gas via endotracheal intubation. Generalized linear mixed models were conducted to compare parameters between various time points to account for the correlated data (the same dog participated in multiple time points). P-values less than 0.05 are considered as statistically significant. When compared to blood results prior to premedication, statistically significant changes were observed in average hematocrit, hemoglobin, mean cell volume, red blood cell, albumin, cholesterol, and protein values after premedication alone. After induction with propofol, variation was additionally observed with white blood cell count, alkaline phosphatase, calcium, globulin, potassium, triglycerides, and whole blood DNA concentration. After 15 minutes of maintenance on isoflurane gas, mean cell hemoglobin concentration, monocytes, phosphorus, and bilirubin values were additionally affected. This study highlights the significant effect that sedation and anesthesia have on blood analyses and subsequent interpretation and clinical relevance.

OT07
DOES FINE NEEDLE ASPIRATION AFFECT MANAGEMENT OF DOGS WITH INCIDENTAL SPLENIC NODULES OR HETEROGENEOUS PARENCHYMA?. Igor Yankin, Sarah Nemanic, Silvia Funes, Helio de Morais. Oregon State University, Corvallis, OR, USA

Splenic nodules and heterogeneous splenic parenchyma are relatively common findings in older dogs undergoing abdominal ultrasound. The impact of fine needle aspiration and cytology in the management of those patients is not clear.

The aim of this retrospective study was to determine how often results of ultrasound-guided splenic fine-needle aspirates impact case management in adult dogs with splenic nodules or heterogeneous splenic parenchyma.

Medical records from adult dogs in which ultrasound-guided splenic fine-needle aspirates were performed at the Oregon State University Veterinary Teaching Hospital from June 2011 through July 2015 were retrospectively reviewed. Dogs were included if splenic nodules or heterogeneous splenic parenchyma was incidentally found under ultrasonographic examination. Only patients with splenic fine-needle aspirates performed on the same day of the ultrasonographic examination were included. Dogs were not included if a disease associated with splenic abnormalities was known to be present before the ultrasound.

One hundred and twenty six adult client-owned dogs met the inclusion criteria. Fine-needle aspiration changed the clinical management of 25 of 126 dogs (20%). Those included patients diagnosed with malignant neoplasia or suppurative inflammation: lymphoma (n = 10), sarcoma (n = 3), malignant histiocytosis (n = 3), suppurative inflammation (n = 3), epithelial neoplasm (n = 2), melanoma (n = 1), hemangiosarcoma (n = 1), carcinoma (n = 1), and spindle cell tumor (n = 1). In the remaining 101 dogs (80%), fine-needle aspiration of the spleen did not change the management of the case. Those include patients with extramedullary hematopoiesis (n = 79), lymphoid reactivity/hyperplasia (n = 11), normal spleen (n = 9), hyperplastic nodule (n = 1), and non-diagnostic sample (n = 1).

Splenic fine-needle aspirate is a minimally invasive diagnostic modality providing information that changed case management in 1/5 adult dogs with splenic nodules or heterogeneous splenic parenchyma, and should be considered in this population of patients.

P01
ITRACONAZOLE ABSORPTION FROM PROPRIETARY AND COMPOUNDED FORMULATIONS IN HEALTHY CATS. Dianne Mawby[1], Leanne Fowler[1], Mark Papich[2], Jacqueline Whittemore[1]. [1]University of Tennessee, Knoxville, TN, USA, [2]North Carolina State University, Raleigh, NC, USA

Itraconazole is available in FDA-approved oral capsules and solution (Sporanox®), as well as compounded formulations. The purpose of this study was to determine absorption of proprietary and compounded itraconazole in cats.

After a 12 hour fast, 8 cats received 50 mg (~12.7 mg/kg) of itraconazole (Sporanox® capsule, Sporanox® solution, compounded capsule, and compounded suspension) in a randomized crossover with a 21-day washout. Capsules were administered with a small meal whereas solution was not. Plasma was collected at pre-determined intervals for analysis using high pressure liquid chromatography. Compartmental and non-compartmental pharmacokinetic analyses were performed using Phoenix® software (Certara) on the proprietary Sporanox® and compounded formulation data, respectively.

For the Sporanox® capsule, the peak concentration (C_{MAX}) and half-life (t½) were 0.54 μg/mL (37% CV) and 18 hours (21% CV), respectively. These values for Sporanox® solution were 1.82 μg/mL (50% CV) and 23.7 hours (42%CV), respectively. Absorption of the Sporanox® solution was 4.2 times greater than for the Sporanox® capsule based on AUC ratios, and C_{MAX} was 3.4 times higher for the solution. Compounded formulations were absorbed inconsistently, commonly resulting in undetectable concentrations. Complete pharmacokinetic results were obtained from only 3 and 1 cats in the compounded capsule and suspension groups, respectively. Relative absorption of compounded itraconazole was 8% from capsule and 2% from suspension.

The long half-life of itraconazole in cats allows once daily oral dosing. Due to much higher absorption of Sporanox® solution, versus capsules, the oral solution dose can be substantially reduced. Compounded itraconazole should not be used in cats because of poor oral absorption.

P02

BIOAVAILABILITY OF A NOVEL FORMULATION OF S-ADENOSYLMETHIOINE GIVEN WITH FOOD IN BEAGLE DOGS. David Griffin, Carolyn Warner. Nutramax Laboratories Veterinary Sciences, Inc., Lancaster, SC, USA

S-adenosylmethionine (SAMe) is used to support liver function both in companion animals and humans, and is also used in humans for mental and joint health. Previous studies have shown that when SAMe is administered with food there is a substantial decrease in SAMe bioavability (Table 1). In a blinded crossover study a new Denamarin® (Nutramax Laboratories Veterinary Sciences, Inc.) chewable tablet containing a novel formulation of SAMe salt (NMXSS75Atm) was administered to beagle dogs to assess its bioavailability relative to administration with food. Six beagles were dosed after an overnight fast with 225 mg SAMe ion (mean 21.7 mg/kg) in one formulation of the supplement, followed by the other formulation 7 days later administered with food. Plasma values for SAMe before dosing and at 0.5, 1, 2, 4, 6, 8, and 24 hours were determined using an HPLC-ESI-MS/MS assay. With both supplements SAMe plasma levels increased substantially above baseline values (21–38 ng/mL) and returned to baseline after 24 hours. Mean values for C_{max} ,T_{max}, AUC_{0-24} and $t_{1/2}$ were comparable for both formulations with no statistically significant differences (Table 2). The data indicate that this novel SAMe salt may be used in dogs with equivalent bioavailability when administered with food which may increase owner compliance.

Table 1 Effects of Food Consumption on SAMe Tosylate Absorption.

Grams of Food	Cmax	± Std
0	1,736	907
100	713	350
225	562	512

Table 2 Effects of Novel SAMe Salt with Food Consumption Compared to SAMe Tosylate Fasted.

	SAMe Tosylate Chewable Tablet (Mean ± Std)	Novel Salt NMXSS75A™ Chewable Tablet (Mean ± Std)
	Fasted (n=6)	**Fed** (n=6)
C_{max} (ng/ml)	1,202 ± 365	1,157 ± 683
T_{max} (hr)	1.5 ± 0.5	1.9 ± 2.0
AUC_{0-24} (ng•hr/ml)	7,060 ± 1,431	4,579 ± 1,454
$t_{1/2}$ (hr)	9.03 ± 3.39	10.78 ± 4.57

P03

POSACONAZOLE PHARMACOKINETICS IN CATS AFTER ORAL AND IV ADMINISTRATION. Dianne Mawby[1], Leanne Fowler[1], Mark Papich[2], Jacqueline Whittemore[1]. [1]University of Tennessee, Knoxville, TN, USA, [2]North Carolina State University, Raleigh, NC, USA

Posaconazole is the most active available azole antifungal drug, but absorption and pharmacokinetics are not available to guide dosing regimens in cats. The purpose of this study was to determine pharmacokinetics of oral suspension and IV formulations of posaconazole in cats to determine the feasibility of oral administration for treatment of fungal infections.

After a 12 hour fast, each of 6 healthy cats received 15 mg/kg of posaconasole (Noxafil®) oral suspension with food. Four cats also received 3 mg/kg IV posaconazole after a 7-day washout period. Plasma was collected at predetermined intervals for analysis using high pressure liquid chromatography (HPLC). Concentration data were analyzed using a two-compartment pharmacokinetic analysis for IV data and a one-compartment analysis with first-order input for oral data using Phoenix® software (Certara).

After IV dosing, volume of distribution (V_{SS}) was 1.9 (0.3) L/kg (mean, std. deviation), terminal half-life (t½) was 39.4 (15.9) hours, and clearance was 28.1 (17.3) mL/kg/hr. After oral dosing, peak concentration (C_{MAX}) was 1.2 (0.5) μg/mL and terminal t½ was 38.1 (15.0) hours. Bioavailability from oral administration was 15.9% (8.6). No adverse effects were observed from either route of administration.

Despite low oral absorption, these data allow for simulation of oral dosage regimens that could be explored in clinical studies. Two regimens can be considered to maintain targeted trough concentrations of 0.5–0.7 μg/mL: 1) 30 mg/kg PO loading dose followed by 15 mg/kg q 48 hours, or 2) 15 mg/kg PO loading dose followed by 7.5 mg/kg q 24 hours.

P04

EVALUATION OF INTRAVENOUS MYCOPHENOLATE MOFETIL USE IN HEALTHY CATS. JE Slovak, SM Rivera, JK Hwang, MH Court, N Villarino. Washington State University, Pullman, WA, USA

Mycophenolic acid (MPA) is the active moiety of the prodrug mycophenolate mofetil (MMF). It is a selective non-competitive inhibitor of inosine-5′-monophosphate dehydrogenase. MPA is an attractive immunosuppressant option in veterinary medicine due to its rapid onset and commercial availability in multiple formulations. Although MPA is used routinely in humans and canines, there is a paucity of literature supporting MPA's use in feline patients, likely from cats' limited ability to glucuronidate certain medications. Our goal for this study was to evaluate the pharmacokinetics, pharmacodynamics, and safety of MPA in 9 healthy cats.

The pharmacokinetics of MPA was evaluated following an intravenous infusion of 5 mg/kg (n = 2) and 10 mg/kg (n = 1) of MMF (CellCept® intravenous, Roche Lab Inc.) Surprisingly, the plasma concentration of MPA in cats at these doses were remarkably low compared with the plasma levels consistent with immunosuppressive levels in humans. Therefore, we evaluated the PK of MPA using 20 mg/kg of MMF administered twice 12 hs apart (n = 6). Following the infusion, all cats metabolized MMF into MPA. The maximum observed MPA plasma concentration ranged between 3.4 and 22 μg/mL after the first dose and between 6.7 and 20 μg/mL, following the second dose. The evaluation of the concentration time profile of MPA in plasma revealed additional peaks of MPA which could correspond to enterohepatic recycling of MPA.

Mycophenolic acid is eliminated by liver biotransformation. Unexpectedly, the concentration of MPA in plasma decayed quickly (terminal half-life ~1.5 hs) in all the cats, suggesting a fast rate of biotransformation, explaining the higher dose of MMF. The metabolite MPA phenol glucuronide was quantifiable in 2 out of 6 cats while acyl glucuronide was not detected in any of the cats. In contrast, the metabolite MPA phenol glucoside was quantifiable in plasma from all the cats, suggesting that glucosidation of MPA is a key route for MPA elimination in cats.

The administration of MMF showed a rapid effect on immune cells. After the second dose of MMF, there was a reduction of the peripheral blood mononuclear cells (PBMC) by ~24%. The PBMCs recovered to baseline levels ~36 hs after stopping the infusion of MMF. This indicates that repeated administrations of MMF are required to maintain a constant suppression of immune cells in cats. Importantly, there were neither serum biochemistry changes nor significant adverse events associated with the administration of MMF in any of the cats.

This study is the first step towards improving treatment of various autoimmune diseases in cats. The results of this study suggest that the infusion of MMF once at 20 mg/kg 12 hs apart, provides a safe and rapid immunosuppressive effect in cats. Future studies will evaluate the pharmacokinetics and the effect of MPA on target lymphocyte subpopulations ($CD25^+CD8^+$ and $CD25^+CD4^+$ T cells) following multi-day intravenous and oral dosing of MMF.

P05

THE ACCURACY, PRECISION AND STABILITY OF COMPOUNDED MILBEMYCIN OXIME IN AQUEOUS SUSPENSION. Zachary Cochrane, Darren Berger, Dwayne Schrunk, Johann (Hans) Coetzee. Iowa State University College of Veterinary Medicine, Ames, IA, USA

Production of a veterinary proprietary product solely containing milbemycin oxime (MO) for use in the United States was temporally ceased in 2011, resulting in use of compounded MO formulations for the treatment of canine generalized demodicosis. Despite the return of a labeled single agent product to the veterinary marketplace, compounded formulations are still available through national compounding pharmacies. This study describes the accuracy, precision and stability of MO strength when compounded as an aqueous suspension (20 mg/mL).

Preparation choice reflected current prescribing practices and routinely utilized concentration. Samples were acquired by prescription from two national veterinary compounding pharmacies at three time points spanning a 30-day period. Two different storage conditions were evaluated and sampled at four time points from the order date (Day 7, 14, 21, and 28). MO recovery was performed by solid-phase extraction and concentration strength measured via high-performance liquid chromatography.

Overall, accuracy ranged from 67.5 to 135.5% with pharmacy A having a range of 67.5 to 91.5% and pharmacy B 78.5 to 135.5%. Of the initial Day 7 samples, only 3 of 12 were within the United States Pharmacopeia's guideline defining acceptable range of 90 to 110% compared with labeled strength. The precision for pharmacy A was 1.0 to 16.9% while for pharmacy B it was 3.9 to 32.8%. Storage condition did not affect stability in the tested aqueous suspensions. The average decrease in MO concentration in pharmacy A's samples was 21.8% (13.3 to 26.1%) while from pharmacy B it was 18.6% (9.6 to 27.7%).

Compounded MO suspension deviated by more than 10% from their labeled strength, which could lead to potential over-dosage and severe neurologic toxicity in predisposed breeds. Bioavailability and clinical efficacy of compounded MO suspensions still remain unknown, and the use of these products should be discouraged at this time.

P06

PHARMACOKINETICS OF INTRAVENOUS AND SUBCUTANEOUS DOLASETRON AND PHARMACODYNAMICS OF SUBCUTANEOUS DOLASETRON IN PURPOSE-BRED CATS. Andrea Herndon, Liberty Sieberg, Leigh Davis, Amber Caress, Ryan Hansen, Luke Wittenburg, Daniel Gustafson, Jessica Quimby. Colorado State University, Fort Collins, CO, USA

Dolasetron is a 5-HT_3 receptor antagonist anti-emetic and is typically dosed at 0.5–1 mg/kg IV or SQ once daily. Pharmacokinetic (PK) and pharmacodynamic (PD) studies in cats have not been previously performed. The purpose of this study was to evaluate these parameters in purpose-bred cats.

Five purpose-bred cats with unremarkable complete blood count, serum biochemistry and urinalyses were utilized. PK study: Each cat received 0.8 mg/kg subcutaneous (SQ) and intravenous (IV) dolasetron in a cross-over manner. Serum samples were obtained via jugular catheter at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 hours after administration of dolasetron. Dolasetron and the active metabolite hydrodolasetron were measured using liquid chromatography/tandem mass spectrometry. Non-compartmental pharmacokinetic analysis was performed. PD study: Subcutaneous dolasetron (0.8 mg/kg or 1.0 mg/kg) or placebo was administered 30 minutes prior to intramuscular administration of 0.44 mg/kg xylazine in a randomized crossover manner. Number of emetic events, lip licks, time to onset of vomiting or visual nausea score were scored by a blinded observer.

PK: Dolasetron was quickly metabolized to hydrodolasetron with only two cats in each administration route having measurable concentrations beyond 2 hours, limiting the assessment of dolasetron PK parameters. There was no significant difference in exposure to the active metabolite between the two routes of administration. Pharmacokinetic parameters are summarized below.

	IV administration		SQ administration	
Parameter	Dolasetron	Hydrodolasetron	Dolasetron	Hydrodolasetron
C_{max} (ng/ml)	761.4 ± 518.9	140.6 ± 106.0	196.6 ± 253.1	78.3 ± 26.1
T_{max} (hr)	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.63 ± 0.3
T_{last} (hr)	5 ± 4.8	13.6 ± 6.1	3.8 ± 3.1	18 ± 6.9
$T_{1/2}$ λ (hr)	NP	4.2 ± 1.8	NP	4.0 ± 1.0
AUC_{0-T} (hr*ng/mL)	325.0 ± 210.0	314.0 ± 130.9	92.0 ± 86.7	429.4 ± 165.4

PD: When dolasetron or placebo was administered prior to xylazine, there was no significant difference in the mean number of emetic events, lip licks, time to onset of vomiting or visual nausea score when compared to placebo.

At 0.8 mg/kg dolasetron does not maintain serum concentrations for 24 hours and does not adequately control xylazine-induced vomiting when given SQ even at 1 mg/kg. Additional dose studies are needed to determine if a higher dose is more effective.

P07

INVESTIGATION OF THE PHARMACOKINETICS OF TRANSDERMAL ONDANSETRON IN NORMAL PURPOSE-BRED CATS. Lara Zajic, Andrea Herndon, Liberty Sieberg, Amber Caress, Ryan Hansen, Daniel Gustafson, Jessica Quimby. Colorado State University, Fort Collins, CO, USA

Ondansetron is a 5-HT3 receptor antagonist used as an anti-emetic in ill cats. Ondansetron can be dosed orally, IV, or

subcutaneously but has previously been demonstrated to have poor oral bioavailability and a short elimination half-life requiring frequent dosing (every 6–8 hours). Because of the impracticality of dosing (>3 doses/day) at home, it is often resigned to in-hospital administration. Ondansetron is a candidate for a transdermal medication because it is small in size (294 Daltons) and is moderately lipophilic (log p ~ 2.1). The purpose of this study was to assess the pharmacokinetics of transdermal ondansetron administration in healthy, purpose-bred cats.

Five purpose-bred cats with unremarkable CBC, biochemistry and urinalysis were utilized. 2 mg transdermal ondansetron Lipoderm gel was applied once to the internal ear pinna (total volume of 0.1 mL). Blood samples were collected via jugular catheter over a 48 hour period following administration (0, 15 minutes, 30 minutes, 1, 2, 4, 8, 12, 24 and 48 hours). Serum was separated and frozen prior to analysis. Ondansetron was measured via liquid chromatography coupled to tandem mass spectrometry.

Analysis revealed no appreciable drug was present in serum after transdermal administration of 2 mg ondansetron, indicating that this is not an acceptable method of drug delivery despite the characteristics of the drug that imply that it would adequately pass the skin barrier.

This study highlights the importance of assessing the potential of each medication for transdermal administration.

R01

COMPARISON OF BRONCHOALVEOLAR LAVAGE TECHNIQUES FOR SAMPLING LOWER AIRWAYS IN CATS. Kimberly Hooi, Alice Defarges, Andrea Sanchez, Stephanie Nykamp, Anthony Abrams-Ogg, Scott Weese, Dorothee Bienzle. Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

The purpose of this study was to compare bronchoscopic bronchoalveolar lavage (B-BAL) and non-bronchoscopic bronchoalveolar lavage (NB-BAL) in clinically healthy cats.

Two bronchoalveolar lavages (BAL) were performed 2 weeks apart in twelve cats free of respiratory disease. BALs were performed in a randomized order under general anesthesia by wedging a 2.9 mm fiber-optic bronchoscope (B-BAL) or an 8Fr red rubber catheter (NB-BAL). Ten mL aliquots of saline were infused into the left and right caudal lung fields and aspirated with a 20 mL syringe. Proportion of fluid retrieved, depth of wedging and anesthetic complications were recorded. Total nucleated cell count, differential cell count and semi-quantitative evaluation of cytologic quality were performed on all BALF samples. Results were compared using ANOVA and Wilcoxon signed-rank tests.

Proportion of BALF retrieved and depth of wedging was significantly greater for B-BAL than NB-BAL. Differential cell counts and cytologic quality did not differ significantly between techniques. Results were [median (range)]:

		NB-BAL	B-BAL	P-value
Proportion of BALF retrieved (%)		55.0 (20.0-80.0)	70.0 (10.0-100.0)	0.008*
Depth of wedging (mm)		81.6 (67.0-93.0)	90.3 (72.8-122.0)	<0.001*
Differential cell count (%)	Macrophages	68.5 (24.0-90.0)	70.0 (41.0-91.0)	0.851
	Eosinophils	13.0 (0.0-73.0)	10.0 (0.0-28.0)	0.451
	Lymphocytes	5.0 (1.0-17.0)	5.5 (1.0-23.0)	0.853
	Neutrophils	8.5 (0.0-36.0)	9.5 (2.0-36.0)	0.270

* p<0.05

Complications included transient hemoglobin desaturation (24/24 BALs) and prolonged anesthetic recovery time (4/24 BALs). However, anesthetic recovery scores did not differ significantly between techniques.

The results suggest that NB-BAL procedure provided BALF samples of equivalent quality to B-BAL procedure in cats clinically free of respiratory disease.

R02

CHARACTERIZATION OF THE FELINE RESPIRATORY MICROBIOME. Aida I. Vientos-Plotts[1], Aaron Ericsson[2], Hans Rindt[1], Megan Grobman[1], Amber Graham[1], Kaitlin Bishop[1], Leah Cohn[1], Carol Reinero[1]. [1]University of Missouri, College of Veterinary Medicine Comparative Internal Medicine Laboratory, Columbia, MO, USA, [2]University of Missouri Metagenomics Center, Columbia, MO, USA

Although lower airways have been considered sterile by culture, sequencing 16S rRNA amplicons has revealed diverse bacterial species in human lungs. Using healthy research cats in a stable environment, we hypothesized that 16S rRNA amplicon sequencing of DNA from bronchoalveolar lavage (BAL), oropharyngeal and fecal swabs, and blood, would reveal distinct microbiomes from each site.

Six healthy research cats <1 year old had BAL, oropharyngeal and fecal samples collected at day 0, week 3 and 8; and blood week 8. Extracted DNA underwent PCR of the 16S rRNA gene. Once sequenced, number of reads/sample, richness and relative abundance of representative operational taxonomic units (OTUs) were determined. Principle component analysis (PCA) described relatedness of samples.

Fecal and oropharyngeal swabs yielded a mean±SD of 65,652 ± 26,069 and 20,632 ± 18,499 reads/sample, respectively. BAL and blood yielded lower coverage at 1,489 ± 1,822 and 269 ± 43 reads/sample, respectively. Oropharyngeal and fecal swabs were significantly richer than BAL (mean number OTUs 93, 88 and 36, respectively; $P < 0.001$) with no significant difference ($P = 0.180$) in richness between time points. *Fusobacterium sp*., *Campylobacter sp*., and *Bacteroides sp*. were predominant fecal taxa. *Pseudomonas sp*. was most abundant in the oropharynx (day 0) and *Pasteurellaceae* and *Porphyromonas sp*. were most abundant weeks 3&8. In BAL, *Pseudomonas sp*., *Bradyrhizobiaceae,* and *Sphingobacteriaceae* predominated day 0 and week 3 with abundance of *Pseudomonas sp*. declining week 8. *Bradyrhizobiaceae* and *Sphingobacteriaceae* were predominant taxa in blood. PCA revealed distinct microbiomes in each site; however, blood had marked compositional similarity with BAL. Samples clustered more by time than by individual, with oropharyngeal swabs having subjectively greater variation than other samples.

Healthy cats have a rich and distinct airway microbiome with dynamic bacterial populations likely depending on age and environment. Further data is necessary to determine how the distinct feline microbiomes from the upper and lower airways, feces and blood are established and evolve. These data are relevant for comparisons between healthy cats and cats with respiratory disease.

E01

PULMONARY DISPOSITION AND PHARMACOKINETICS OF ORAL MINOCYCLINE IN THE ADULT HORSE. Kate Echeverria[1], Kara Lascola[1], Steve Giguere[2], Jon Foreman[1], Scott Austin[1], Pamela Wilkins[1]. [1]University of Illinois, College of Veterinary Medicine, Urbana, IL, USA, [2]University of Georgia, College of Veterinary Medicine, Athens, GA, USA

The objective of this study was to determine the pharmacokinetics and pulmonary disposition of minocycline in adult horses after multiple intragastric doses.

Minocycline (4 mg/kg) was administered via the intragastric route to 6 healthy adult horses every 12 hours for a total of 5 doses. Minocycline concentrations were measured in plasma, pulmonary epithelial lining fluid (PELF), and bronchoalveolar lavage (BAL) cells via liquid chromatography mass spectrometry at multiple time points over a 96 h time period. Pharmacokinetic variables were determined after the last dose using a noncompartmental approach. Comparison of minocycline concentrations between sampling sites was done using a Friedman repeated measures ANOVA and significance was set at $P < 0.05$.

After the last dose, the mean + SD maximum plasma concentration (Cmax) of minocycline was 2.3 + 1.3 μg/mL and the terminal half-life (T1/2) was 11.8 + 0.5 hours. The median (25th and 75th percentiles) time to peak concentration (Tmax) was 1.3 (1.0 – 1.5) hrs. The Cmax and Tmax of minocycline in the PELF were 10.5 + 12.8 μg/mL and 9.0 (5.5 – 12.0) hrs, respectively. The Cmax and Tmax for BAL cells were 0.24 + 0.1 μg/mL and 6.0 (0.0 – 6.0) hrs, respectively. Peak and trough minocycline

concentrations in PELF were significantly higher than in plasma whereas concentrations in BAL cells were significantly lower.

Orally administered minocycline distributes into the PELF and BAL cells of the horse. Knowledge of specific minimum inhibitory concentrations is required to predict efficacy of minocycline for the treatment of bacterial pneumonia in adult horses.

E02
INTRAVENOUS ADMINISTRATION OF COBALT CHLORIDE IS ASSOCIATED WITH HEMODYNAMIC ALTERATIONS IN HORSES.

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Cobalt is a substance of abuse in humans and animals performing in strenuous athletic competitions. When administered at pharmacologic doses, it has been associated with induction of erythropoietin release, increased hematopoiesis, which is thought to confer competitive advantage. Cobalt chloride ($CoCl_2$) is reportedly given to racehorses to enhance performance, and recently, allowable limits for cobalt have been set by several racing jurisdictions for post-race illicit substance testing of blood and urine. While preliminary single-dose pharmacokinetic data have been published for $CoCl_2$ in horses, information regarding the effects of repeated dosing (which is how the substance is reportedly used illicitly in performance horses) is unavailable. Even fewer data have been published describing the pharmacodynamic effects of $CoCl_2$ administration in horses, particularly at high doses. The purpose of this pilot study was to describe the physiologic and biochemical effects of weekly intravenous doses of $CoCl_2$ to Standardbred horses. This report describes the hemodynamic effects of $CoCl_2$ in a dose escalation study.

Five Standardbred mares (12–13 years-old; 460–530 kg) were randomly assigned to receive one of 5 doses of $CoCl_2$ (4, 2, 1, 0.5, or 0.25 mg/kg) as an intravenous bolus (infused over 1 minute) once weekly for 5 weeks. Prior to each dose, animals were instrumented with pulmonary artery and right atrial catheters, a transverse facial artery catheter, two external jugular venous catheters, an indwelling urinary catheter, and electrocardiography leads. Physical examination parameters, blood pressure (systolic arterial pressure [SAP], diastolic arterial pressure [DAP], and mean arterial pressure [MAP]), cardiac output, and qualitative ECG assessment were evaluated every 5–10 minutes for 4 hours immediately after administration of the first and fifth weekly doses of $CoCl_2$.

All mares were subjectively anxious (nostril flaring, muscular tremor/fasciculation, pawing, straining) by 5 minutes following the $CoCl_2$ infusion; this persisted for ~60 minutes in mares receiving higher doses (4, 2, and 1 mg/kg). Mares receiving 4, 2, or 1 mg/kg doses developed tachycardia immediately after dosing (HR 60–126 bpm), but this was not observed in mares receiving 0.5 or 0.25 mg/kg (HR 36–52). Paroxysmal ventricular tachycardia was noted in the first 10 minutes post-administration in the mare receiving the 4 mg/kg dose. Elevations in SAP, DAP, and MAP were noted following drug administration at most doses; while profound hypertension was observed at 4 mg/kg (SAP/DAP, MAP [mmHg] = 291–300/163–213, 218–279), all mares became hypertensive in the 30–45 minutes following $CoCl_2$ administration. Mares receiving 4 and 2 mg/kg developed conspicuous oral mucous membrane congestion that persisted for 20 minutes post-dosing and subsequently resolved. At all doses, cardiovascular parameters returned to baseline by 1–2 hours post-administration.

Results of this preliminary study document significant, repeatable hemodynamic alterations associated with intravenous $CoCl_2$ administration to horses. Further, the degree of hypertension observed following infusion raises humane and human safety concerns if doses of >1 mg/kg are used.

E03
THE PHARMACOKINETICS OF INTRAVENOUS GENTAMICIN IN HEALTHY YOUNG-ADULT VERSUS GERIATRIC HORSES.

Andrew Gestrich[1], Daniela Bedenice[1], Michelle Ceresia[2], Iman Zaghloul[2], Nisanne Ghonem[2], Mary Rose Paradis[1]. [1]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA, [2]School of Pharmacy, MCPHS University, Boston, MA, USA

The study objective was to evaluate the effects of patient age on aminoglycoside pharmacokinetics in young-adult (5–10 years) versus geriatric (≥25 years) healthy horses, receiving a single 6.6 mg/kg intravenous injection of gentamicin (Henry Schein®). Heparinized plasma samples were obtained at designated time-points following drug administration and frozen at −80°C until assayed by a validated immunoassay (QMS®). Gentamicin plasma concentrations versus time plots were analyzed using a two-compartment model and commercial software (WinNonlin-v6.4). Data were compared between age-groups using independent samples t-test; $P < 0.05$ considered significant.

Baseline physical examination and hematological parameters were not significantly different between young (median age [range]: 7 [6–8] years) and aged (26 [25–41] years) horses, aside from a lower mean bodyweight in the geriatric group (402 ± 56 versus 477 ± 38 kg). Similarly, pharmacokinetic parameters were not statistically different between age groups (mean ±SD; $P > 0.05$):

	C_0 (μg/mL)	$AUC_{0-\infty}$ (μg*h/mL)	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	V_{ss} (L)	Cl (mL/h/kg)
Young-adult (n=8)	86.2±25.9	75.6±12.9	0.1±0.03	1.22±0.2	69.2±12.9	89.3 ±13.8
Geriatric (n=8)	86.6±21.8	82.7±10.3	0.11±0.04	1.42±0.33	60.2±12.2	80.8 ±9.4

C_0–Initial plasma concentration; AUC–Area under the curve; $T_{1/2}$ α and β–Distribution and elimination half-life; V_{ss}–Volume of distribution at steady state; Cl–Clearance

Peak plasma concentrations (C_{pk}) at the end of the distribution phase were 31.7 ± 4.4 μg/mL in young and 32 ± 2.2 μg/mL in aged horses ($P = 0.901$), while gentamicin trough levels reached 2 μg/mL (common MIC for equine pathogens) at 6.1 ± 1.2 and 7.3 ± 1.5 hours, respectively ($P = 0.09$). Considering an expected post-antibiotic effect of ≤8 hours, dosing adjustments are likely required to meet therapeutic needs in both study groups.

E04
ENDOTOXIN-INDUCED MICRORNA EXPRESSION IN EQUINE PERIPHERAL BLOOD MONONUCLEAR CELLS.

Nicholas Parkinson, Virginia Buechner-Maxwell, Sharon Witonsky, R. Scott Pleasant, Ansar Ahmed. Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

The innate immune response to bacterial lipopolysaccharide (LPS) mediated by toll-like receptor 4 (TLR4) contributes substantially to the morbidity of a number of important equine disease syndromes including gastrointestinal disease and neonatal sepsis, and contributes to other chronic pro-inflammatory states. MicroRNAs (miRNAs) are small non-coding RNA molecules 17–25 nucleotides in length that function as post-transcriptional regulators of gene expression. miRNAs have recently been found to have a central role in the modulation of the TLR4 signaling cascade in other species, and altered expression has been implicated in the pathogenesis of inflammatory diseases. Limited information is available on miRNA expression in horses, and there is no published data on expression in equine inflammatory cells. This objective of this study was to use next generation sequencing to characterize the basal miRNA transcriptome in isolated equine peripheral blood mononuclear cells, and to test the hypothesis that LPS would induce differential expression of miRNAs.

Peripheral blood mononuclear cells were isolated from four healthy adult horses. Cells were cultured with LPS (10 ng/mL) or control for 0, 2 and 4 hours. Tumor necrosis factor alpha (TNFα) was measured in culture supernatants by ELISA. RNA was extracted from the cells and the miRNA transcriptome was sequenced using an Illumina HiSeq 2500 analyzer after size

selection. High quality sequence data were mapped to the equine genome and annotated using the miRNA database miRBase 21. Differential expression was analyzed with generalized linear models, using the Benjamini-Hochberg procedure to control the false discovery rate at 0.1.

327 mature miRNAs were detected, with 191 present in all samples. Basal expression was highly consistent between horses. The most abundant miRNAs in baseline samples were miR-21, let-7 g and miR-150, miRNAs associated with cell cycle regulation and both innate and adaptive immunity in other species. TNFα expression was significantly higher in the supernatants from LPS-treated cells than controls at both 2 and 4 hours ($P = 0.016$ and 0.0003 respectively). After correction for multiple comparisons, only miR-155 was significantly upregulated by LPS ($P = 0.00018$, 1.5 to 1.6 fold change versus controls). 9 miRNAs showed statistically significant expression changes with time. These included miR-146a and miR-146b, which are induced by LPS in other species but had non-significant upregulation by LPS here. MiR-155 expression was significantly correlated to supernatant TNFα (R^2=0.78).

The basal expression characterized by these data provide a foundation for future research into the roles of specific miRNAs in equine inflammatory responses. miR-155 is the principal LPS-induced miRNA in horses, as in humans and mice. This miRNA has documented roles in TLR4 signaling regulation, including enhancement of TNFα translation and suppression of intermediate signal transduction proteins. It is thus likely to influence the magnitude and nature of the acute inflammatory response to LPS in the horse, and could be a target for immune modulatory interventions. It may also prove a useful marker of TLR activation. Further research will be necessary to validate expression changes in a wider sample set, investigate targets and functions of these miRNAs in horses, and to establish roles in naturally occurring disease.

E05

SEVERE EQUINE ASTHMA (HEAVES) IS ASSOCIATED WITH AN INCREASED NUMBER OF CIRCULATING LOW-DENSITY GRANULOCYTES. Nicolas Herteman, Amandine Vargas, Jean-Pierre Lavoie. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada

Airway neutrophilia is a characteristic finding of severe equine asthma (heaves), a common and incurable condition of horses. However, how polymorphonuclear neutrophils (PMNs) contribute to the disease remains poorly understood. "Low-Density Granulocytes" (LDGs), a subpopulation of PMNs, have been suggested to have strong pro-inflammatory properties, which can promote the recruitment of PMNs in an autocrine manner and thus amplifying the inflammatory response. We therefore hypothesized that LDGs are increased in the blood of heaves-affected horses compared to healthy subjects.

We studied 8 horses with heaves during disease exacerbation and remission, and 6 healthy animals. LDGs and normal-density neutrophils were isolated from peripheral blood using Ficoll. Quantification and morphological characterization of LDGs were performed blindly using an optical microscope. LDGs were further characterized by flow cytometry using anti-CD90 and anti-myeloperoxidase (MPO) antibodies.

The number of LDGs was significantly elevated in horses with heaves during both exacerbation and remission compared to healthy horses ($P = 0.005$ and $P = 0.029$, respectively). No significant difference was found between disease exacerbation and remission ($P > 0.1$). LDGs displayed a significantly greater percentage of normally segmented than hyposegmented nuclei in heaves-affected and control horses ($P = 0.0002$ and $P = 0.0022$, respectively). MPO's mean fluorescence intensity of LDGs was significantly lower during heaves exacerbation when compared to control horses ($P = 0.0442$). PMNs counts in BAL and LDG levels in blood of horses with heaves were not correlated ($P > 0.1$ for both disease exacerbation and remission).

This study describes for the first time an association between an increased subpopulation of PMNs, namely the LDGs, and an inflammatory disease in horses. LDGs appear to be mostly constituted of mature cells in both healthy and heaves-affected horses. The significance of these findings remains to be ascertained.

E06

CAN LEVAMISOLE UPREGULATE THE EQUINE CELL-MEDIATED IMMUNE RESPONSE IN VITRO?. Amy Santonastaso[1], Bettina Wagner[2], Siobhan Ellison[3], Virginia Maxwell[4], Scott Pleasant[1], David Lindsay[4], Sharon Witonsky[1]. [1]Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, USA, [2]College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, [3]Pathogenes, Inc., Reddick, FL, USA, [4]Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, USA

Equine Protozoal Myeloencephalitis (EPM) is arguably the most common and costly equine neurologic diseases in the United States. The national seroprevalence is >50%, but only 0.5–1% of all horses develops disease during their lifetimes. Some EPM affected horses have decreased immune responses. A cell-mediated immune response appears to be protective for development of EPM after infection with *S. neurona* in mouse models. Therefore, in addition to specific anti-protozoal medications, levamisole has been proposed as an adjunctive therapy to upregulate the immune response in EPM affected horses. Studies have been performed in other species to address whether levamisole alters immune function in vitro or in vivo, but there are very limited studies in equids. We hypothesized that We hypothesized that levamisole can upregulate a cell mediated macrophage (M1) dendritic cell (DC1) CD4 T-helper 1 (Th1) CD8 Tc1 immune response in vitro.

The first aim was to determine both the optimal conditions and effects of levamisole on cellular proliferation. Equine PBMCs were harvested from ten horses seronegative for *S. neurona*. Cells were cultured alone, or with each of the following mitogens: concanavalin A (conA), phorbol 12-myristate 13-acetate and ionomycin (PMA/I), or with a combination of the above mitogens and levamisole at several conditions. Cellular proliferation was assessed using a colorimetric bromodeoxyuridine ELISA assay (Roche Life Sciences, 11647229001).

The second aim was to determine the ability of levamisole, under optimized conditions, to upregulate the M1 DC1 CD4Th1 CD8 Tc1 response in vitro, based on activation and function.

PBMCs from the same 10 horses were cultured with each of the following, no stimulation, conA, and levamisole with and without conA. To determine proliferation of each specific subset, cells were labelled with a fluorescent dye (CellTrace Violet, ThermoFisher Scientific, C34557). Proliferation of each subset was determined based on dye dilution using flow cytometry (FacsAria Flow Cytometer). To determine the ability of levamisole to upregulate or alter the immune response, immune subsets were identified (CD4, CD8, CD21, CD172a, CD14) using fluorescent labelled antibodies. Activation was assessed for macrophages and DCs using MHC class II and CD86 expression. Induction of T-regs was based on CD4, foxp3 expression. Specific immune phenotypes were determined based on intracellular cytokine expression of specific subsets (M1 DC1 CD4 Th1 CD8: IFN-gamma) versus (M2 DC2 CD4 Th2: IL4) versus (CD4 T-reg: IL-10). Significant differences in response were determined using a mixed model ANOVA with significance set at $P < 0.05$.

Study results indicated that cells cultured with levamisole alone did not alter PBMC proliferation compared to the response of unstimulated cells. Cells cultured with either conA or PMA/I resulted in a statistically significant ($P < .05$) increase in proliferation compared to unstimulated cells. Cells cultured with conA and levamisole at 1 μg/mL resulted in a significant ($P < .05$) decrease in proliferation compared with cells cultured with conA alone. Flow cytometry data analysis results to assess how levamisole directs the immune phenotype are currently pending.

These data demonstrate that, under these conditions, levamisole downregulates conA stimulated PBMC proliferation. Based on these in vitro results, further studies to determine the effectiveness of levamisole on modulating the equine immune system in vivo are warranted.

E07

NOVEL PHARMACOLOGICAL TREATMENT REGIMES IN EQUINE ATRIAL FIBRILLATION. Helena Carstensen[1], Eva Z. Hesselkilde[1], Jørgen K. Kanters[2], Steen Pehrson[3], Maria Mathilde Haugaard[1], Thomas Jespersen[2], Rikke Buhl[1]. [1]Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Taastrup, Denmark, [2]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [3]Department of Cardiology, The Heart Centre, Copenhagen University Hospital, Copenhagen, Denmark

Atrial fibrillation (AF) is the most common pathological arrhythmia diagnosed in horses. Due to the adverse effects encountered when treating with quinidine sulphate we wanted to improve the medical treatment of AF. In this study we aimed to evaluate the effects of the antiarrhythmic compounds dofetilide and ranolazine in the equine heart. Furthermore, we hypothesized that the use of a combination of two antiarrhythmic compounds with distinct mechanisms would allow us to use lower doses of each compound and thereby minimize the risk of adverse effects.

Three horses were included in a safety study testing administration of intravenous dofetilide (8.0 μg/kg BWT), ranolazine (2.4 mg/kg BWT) and a combination of the two using similar doses. The horses were monitored clinically for adverse reactions and Holter monitor ECG recordings, as well as blood pressure measurements, were obtained throughout the study. Following the safety study, nine horses were included in an electrophysiological study testing the effects of dofetilide and ranolazine on electrical induced AF. By placing two intraatrial electrodes we were able to record atrial electrograms and perform atrial pacing for measurements of atrial effective refractory periods (aERPs) and induction of AF. All horses went through four procedures (dofetilide and ranolazine respectively, combination of the two drugs using lower dosages and a control procedure where only saline was administered) with 7 days wash out periods between treatments.

All horses tolerated both drugs and the combination well without development of any clinical signs of discomfort or pain and neither of the drugs resulted in alterations in blood pressures. Plasma levels of cardiac Troponin I and creatinine kinase fraction MB did not increase due to intraatrial pacing or any of the treatments given. Ranolazine alone and the combination therapy terminated AF faster than in control procedures ($P = 0.0178$). The combination therapy resulted in decreased AF duration ($P = 0.0469$) and AF vulnerability ($P = 0.0213$) but neither of the treatments altered the aERP significantly. Dofetilide significantly increased the QT_c-interval at multiple time points. This was, however, not the case when lower doses of dofetilide were administered in the combination procedures.

In this study dofetilide and ranolazine were safe to administer in healthy horses and none of the compounds or the electrophysiological procedure caused myocardial damage in the horses. When dofetilide was given at a high dose the QT- interval prolonged indicating altered ventricular repolarization. Ranolazine showed an antiarrhythmic effect against acutely induced AF in horses. By combining dofetilide and ranolazine antiarrhythmic effects were obtained at low doses that would have been ineffective when administered separately. These findings lead us to believe that combination therapy may be a future treatment regime in equine AF.

E08

CAN EXERCISING ELECTROCARDIOGRAPHY PREDICT PERFORMANCE IN YOUNG STANDARDBRED HORSES AT THE START OF TRAINING?. Linda Frellstedt, Gareth Fitch, Chris Rogers. Massey University, Palmerston North, New Zealand

The aim of this study was to describe the occurrence of cardiac arrhythmias in young Standardbred horses that have recently entered training, to determine whether cardiac arrhythmias are consistently present during several training sessions and to evaluate if performance can be predicted based on the type and frequency of observed arrhythmias.

Fifty-one Standardbred horses (aged 2–4 years, 16 F, 32 G, 2 M) were examined during multiple training sessions over a three month period. Resting and exercising electrocardiograms (Holter monitor, Televet®) as well as exercising speeds (GPS, Polar®) were recorded. Descriptive statistics were used to analyze the data.

In total 120 training sessions were recorded. Thirty horses completed 3 training sessions, 8 horses completed 2 training sessions and 13 horses completed one training session. Single or multiple arrhythmias were observed during 42/120 (35%) sessions in a total of 30/51 horses. Secondary AV-block at rest was observed during 15/120 sessions. Ventricular premature contractions (VPCs) were recorded during 20/120, supraventricular premature contractions (SVPCs) during 4/120, and sinus arrhythmias (SAs) during 9/120 sessions. Thirteen horses dropped out of training after their first training session, 7/13 (54%) of these had arrhythmias, 3/7 had VPCs, 2/7 has SVPCs and 2/7 has SAs. Three horses dropped out of training after two sessions, all of them had arrhythmias (100%), 2/3 had VPCs and 1/3 had SA. In 5/51 (9.8%) horses, arrhythmias were consistently recorded during several training sessions. Horses that dropped out of training had >5 VPCs per session (mean of 11.14 ± 7.66).

Cardiac arrhythmias are commonly observed in young Standardbred horses that have recently entered training. Horses with cardiac arrhythmias observed during multiple training sessions and with more significant abnormalities may be less likely to continue training and to reach their first trials.

E09

IS ECG IN HORSES ONLY FOR DYSRHYTHMIA DIAGNOSIS? INTRODUCING A NEW METHOD FOR 12-LEAD ECG. Eva Z. Hesselkilde[1], Helena Carstensen[1], Bettina Vandsø[1], Thomas Jespersen[2], Jørgen Kanters[2], Rikke Buhl[1]. [1]Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [2]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

ECG is an invaluable diagnostic tool in human cardiology where conditions such as left ventricular hypertrophy (LVH) and bundle branch block can be diagnosed based on the 12-lead ECG. Guidelines for 12-lead ECG in horses exist. These guidelines have imitated the human electrode placement with the limb leads recorded from the extremities and the precordial leads on the thorax. However, the different orientation of the equine heart alters Einthoven's triangle and Wilson's Central Terminal (WCT) and thereby changing the basis of the recordings.

The aim of this study was to evaluate if a different electrode placement based on the physiological ideas of the human Einthoven's triangle and WCT may provide better information about the equine atria and ventricles compared to the standard extremity leads.

Development of the new method for 12-lead ECG recordings was performed by placing the limb electrodes at right and left scapula and in the midline caudal to the xiphoid process. This will place WCT in the middle of the heart and Einthoven's triangle will be nearly perpendicular to the main cardiac depolarization vector. By placing the precordial leads between the superficial pectoral muscles (V1), on the ventral part of both the left and right triceps muscle (V2 and V6), in the 6st intercostal space, in level of the shoulder joint of the left side (V3) and in level of the elbow joint on both sides (V4 and V5), information from all heart chambers would be recorded. 35 Standardbred horses (14 mares, 15 geldings and 6 stallions. Mean age: 5.8 years range 2–14) had ECGs recorded using both the existing method and the new modified method. All horses had an echocardiographic examination performed to measure atrial (left atrium area (LAA) and left atrium diameter (LAD)) and ventricular size (including calculation of LV mass). All waves from each lead were measured and all data were blinded before analyses.

Comparing the various leads of the ECGs from the two methods showed a significant increase in both P wave amplitude and duration in the new modified method ($P < 0.0005$). Significant correlation between P wave duration and LAA and LAD was found for the new modified method ($P < 0.0005$, $R^2=0.32$ and $P < 0.005$ $R^2=0.27$, respectively). When amplitudes of R and S wave was added, significant correlation to LV mass was found for the new modified method ($P = 0.0005$, $R^2=0.31$) while the existing method did not ($P = 0.08$, $R^2=0.01$). Also, significant correlation between

mean QRS duration and LV mass were found for the new modified method ($P < 0.005$, R^2=0.22) but not for the existing method ($P = 0.54$, R^2=0.01).

This study shows that the new method were better correlated with left atrial size and LV mass suggesting that the correction of Einthoven's triangle WCT gives better results when obtaining 12-lead ECGs in horses.

E10
HUMORAL HYPERCALCEMIA OF MALIGNANCY IN HORSES: A RETROSPECTIVE STUDY (2010–2015).

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The reported frequency of neoplasia in the horse is low but is likely under-reported due to the difficulty of establishing a definitive diagnosis. Clinical signs may be vague, and neoplasia is often identified only at post-mortem examination. Delays in diagnosis may lead to increased morbidity whereas definitive diagnoses in terminal cases provide objective evidence in electing for humane euthanasia. Humoral hypercalcemia of malignancy is a common paraneoplastic syndrome in other species, and could be utilized to aid in the identification of equine neoplasia. The objectives of this study were to identify the prevalence of hypercalcemia among horses with neoplasia, and to compare calcium, albumin, and the relationship between these values in horses with neoplasia, systemically ill horses without neoplasia, and healthy animals.

An electronic medical record search was performed to identify all horses over 6 months of age on which serum total calcium and albumin were measured. Eligible cases grouped by diagnosis: neoplasia (n = 43), sick non-neoplasia (n = 466), and healthy/orthopedic (n = 55). The sick non-neoplasia group was further sub-divided based on the body system affected by disease for additional comparisons. Exclusion criteria included lack of definitive diagnosis or a diagnosis of renal disease or rhabdomyolysis.

Preliminary data identified higher calcium and lower albumin in the neoplasia group when compared to the sick non-neoplasia group. The calcium:albumin ratio was higher in the neoplasia group than in the healthy/orthopedic group, and lower than the sick non-neoplasia group. This data suggests that hypercalcemia may support a diagnosis of neoplasia in equine patients.

E11
DEVELOPMENT OF A TECHNIQUE FOR DETERMINATION OF PULMONARY ARTERY PULSE WAVE VELOCITY IN HORSES.

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Calcification of the tunica media of the main pulmonary arteries has been observed in a large proportion of young racehorses. In humans, medial calcification is the most important cause of increased arterial stiffness, and has been implicated in the pathogenesis of cerebral and renal microvascular diseases. Pulse wave velocity (PWV) is a marker of arterial stiffness. This study aimed to develop a technique for determination of pulse wave velocity of the main pulmonary arteries of horses.

A convenience sample of 9 adult horses with no history of respiratory or cardiovascular disease were used. The horses were sedated for catheter placement, and continuously monitored with electrocardiography during the procedure. The pulmonary artery (PA) trunk was cannulated via right heart catheterization, with a catheter introducer sheath (9Fr x 100 cm). Introducer placement was guided with echocardiography. A custom-made dual pressure sensor catheter (PSC) (7Fr x 170 cm) was inserted through the introducer sheath, and into one of the main branches of the PA. The position of the PSC in one of the main branches of the PA was confirmed with thoracic radiography. The catheter was withdrawn in 5 cm steps and pressure measurements were recorded at each location. The time delay of the pulse waves between the two sensors was used to calculate PWV. Histology of the PA trunk and main branches was performed to investigate the presence of medial lesions and calcification.

The PSC placement was successfully achieved in all horses (9/9), without significant complications, aside from transient arrhythmias. The catheter was more commonly located on the left PA (8/9). At the time of pressure measurements, the level of sedation was variable between horses. The mean (±SD) PWV, was 2.3 ± 0.7 m/s in the proximal PA trunk and 1.1 ± 0.1 m/s further distal (20 cm), in a main PA branch. The mean (±SD) of mean arterial pressures in the proximal PA trunk was 30.1 ± 5.2 mmHg, and 22.0 ± 6.0 mmHg further distal (20 cm), in a main PA branch. The mean (±SD) pulse pressure in the proximal PA trunk was 15.0 ± 4.7 mmHg, and 13.5 ± 3.3 mmHg further distal (20 cm), in a main PA branch. Five out of nine horses presented moderate to severe lesions and calcification of the tunica media of the PA trunk and/or main branches.

This study demonstrated the feasibility of a technique to determine PA-PWV in standing horses. The technique developed may allow further investigation of the effect of calcification of large pulmonary arteries in the development of pulmonary microvascular disorders in horses.

E12
REPEATED MEASUREMENTS OF AUTONOMIC TONE MARKERS OVER A TRAINING SEASON IN EVENTING AND ENDURANCE HORSES.

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The purpose of this study was to describe normal values and changes over a competition season of markers of autonomic tone in competing eventing (EV) and endurance (EN) horses, in conjunction with previously reported variables to monitor training and compare these to non-competitive breed-matched controls (EVc and ENc).

26 EV, 13 EVc, 11 EN and 7ENc started the project. Heart rate variability (HRV), non-invasive blood pressure (NIBP), splenic volume, pre- and post-exercise hematocrit and cortisol, standardized exercise tests (SETs) and muscle enzyme activities, were measured pre-season (T1), mid-season (T2) and at the peak/end of the competition season (T3).

HRV was lower ($P < 0.05$) in EV than in EVc at all times and post-exercise cortisol lower at T3. There were no significant differences in EN versus ENc. HRV and post-exercise cortisol did not change over the season in any group. EV had higher fitness markers during SETs compared to EVc, but there was no difference between EN and ENc. NIBP, splenic volume, hematocrit, pre-exercise cortisol, muscle enzyme activities, and weight were not significantly different between groups and did not change over the season.

In conclusion, competing eventing horses showed higher sympathetic tone and lower post-exercise cortisol than controls. NIBP, splenic volume and resting or post-exercise hematocrit did not detect changes in autonomic tone in this population. The studied markers of autonomic tone did not change significantly throughout the season in any group and did not detect changes in competing endurance horses compared to controls.

E13
EPIDEMIOLOGICAL CHARACTERISTICS OF HORSES WITH HYPERINSULINEMIA IN A LARGE POPULATION OF HORSES.

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Pituitary pars intermedia dysfunction (PPID) and Equine Metabolic Syndrome (EMS) have been described as the most common

endocrinologic disorders in horses. Few large studies exist that describe the epidemiological characteristics of horses with these endocrine diseases. The purpose of this study was to obtain information that included age, breed, sex, clinical signs, and ACTH/insulin/glucose status at initial diagnosis of potential endocrine cases (new cases) to determine the epidemiological characteristics of horses with hyperinsulinemia.

Horses of any age, breed, and sex from the continental US were eligible for study enrollment as long as they were documented to be exhibiting one or more of the following clinical signs: generalized or regional hypertrichosis, muscle wasting, abnormal fat distribution, lethargy, laminitis (unknown etiology), polyuria, polydipsia, susceptibility to infections, abnormal sweating, and/or inappropriate lactation. Normal horses were excluded from the study. At initial visit, demographic data, signalment, a physical examination was conducted. Clinical signs were documented and blood was drawn for basal ACTH, fasting insulin, and glucose. Blood samples were processed and shipped overnight to the Animal Health Diagnostic Center, Cornell University, Ithaca, NY for analysis. The association between hyperinsulinemia (based on fasting insulin results), clinical signs and glucose were statistically evaluated individually using the Pearson chi-square test. Odds ratios for significant predictors of hyperinsulinemia were computed using corresponding 95% confidence intervals when applying multiple logistic regression analysis.

Four-hundred eighteen of nine-hundred eighty two horses with complete epidemiological information were included in the final data analysis. Of the 418 horses, 221 (52.8%) were HI^+. Further, horses were stratified into 1 of 3 groups based on ACTH and insulin laboratory results ($PPID^+/HI^+$), ($PPID^-/HI^+$), and ($PPID^-/HI^-$). Of the 418 horses, 115 (27.5%) were $PPID^+/HI^+$, 106 (25.3%) were $PPID^-/HI^+$, and 197 (47.1%) were ($PPID^-/HI^-$). Of the 418 horses evaluated, 9.6% of horses <15 years of age were $PPID^+HI^+$, 23.5% of horses 15 to 19.9 years of age were $PPID^+HI^+$, 27.8% of horses 20 to 24.9 years of age were $PPID^+HI^+$, and 39.1% of horses >25 years of age were $PPID^+HI^+$. Further, 47.3% of horses <15 years of age were HI^+, 61.2% of horses 15 to 19.9 years of age were HI^+, 46.7% of horses 20 to 24.9 years of age were HI^+, and 44.8% of horses >25 years of age were HI^+. One-hundred ninety seven of 418 horses were $PPID^-HI^-$.

Analysis for PPID+/HI+ and PPID-/HI+ horses was evaluated. Based on the data for clinical signs only in the PPID-/HI+ horses, HI was significantly ($P < 0.000$) greater among horses with cresty neck and laminitis present. Although not statistically significant ($P > 0.025$), HI was greater in horses with 2 other clinical signs; abnormal sweating and pot belly/weight gain. Based on clinical signs data, the odds of HI in horses with cresty neck was more than twice (2.3X) that for horses without this clinical sign. Similarly for horses with laminitis, the odds ratio of HI was more than twice (2.2X) that for horses without this clinical sign.

Of the 418 enrolled horses, 52.8% were diagnosed with hyperinsulinemia. Based on the combined data of clinical signs (11 interpretable), glucose levels and fasting status, cresty neck, laminitis and high glucose were significant predictors of HI. Specifically, horses with high glucose levels were seen to have almost four times the odds (3.5X) of HI compared to those with normal glucose levels. Therefore, when evaluating horses with suspected endocrine disease, at a minimum, ACTH, insulin and glucose should be evaluated. Long term studies need to be conducted in large populations of horses to further evaluate endocrinopathies in horses.

E14

MANAGEMENT OF EARLY PPID IN HORSES. John Haffner[1], Christine Cocquyt[2], Dwana Neal[3], Steven Grubbs[3], Thomas Keefe[4], [1]Middle Tennessee State University, Murfreesboro, TN, USA, [2]Tennessee Equine Hospital, Thompson Station, TN, USA, [3]Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA, [4]Colorado State University, Fort Collins, CO, USA

Pituitary Pars Intermedia Dysfunction (PPID) has previously been diagnosed and managed as an older horse disease with advanced clinical signs. However, in early PPID, the clinical signs are typically more subtle. Clinical signs of early PPID include: decreased athletic performance, change in attitude/lethargy, delayed hair coat shedding (subtle), regional hypertrichosis, loss of epaxial muscle mass (topline), regional adiposity, and laminitis. One of the major limitations of diagnostic testing for PPID is the sensitivity of available diagnostic assays. Thyrotropin-releasing hormone (TRH) stimulation test has been shown to have an increased sensitivity of detecting horses with early PPID compared to resting ACTH. Few studies exist concerning endocrinologic testing and follow-up monitoring the clinical progression and diagnostic assay results following treatment in horses with early PPID. The purpose of this study was to identify cases of early PPID based on clinical signs and laboratory testing then determine improvements in ACTH levels using resting ACTH, TRH stimulation test, and clinical signs over time.

Sixteen horses were enrolled (January) with at least one clinical sign of early PPID listed above and diagnostically confirmed by the TRH stimulation test measuring ACTH at 0 (T0ACTH; pre-TRH) and 10 (T10ACTH) min following 1 mg i.v. TRH administration. Fasting insulin and glucose was also determined pre-treatment and at each follow-up visit. Each horse was administered Prascend® (pergolide mesylate) orally at a starting dosage of 2 mcg/kg once daily, dosage adjusted to effect if required, but not to exceed 4 mcg/kg daily, per manufacturers label recommendations. A history, physical examination, and TRH stimulation test evaluating T0ACTH and T10ACTH was conducted for 5 months (February through June) following initial treatment administration. The comparative changes to the baseline ACTH levels over time were evaluated using descriptive statistics. The statistical significance of the change in ACTH levels to be evaluated using a confidence interval of 95%.

At initial examination, the most common clinical signs at initial diagnosis were regional hypertrichosis (9/16), delayed shedding (5/16) and muscle wasting (4/16). The baseline (arithmetic mean) for T0ACTH and T10ACTH for all horses was 44.6 pg/mL and 360.5 pg/mL, respectively. Following six-months of treatment, the resting T0ACTH in 12 of 16 (75%) horses were less than normal reference range (35 pg/mL), whereas 7 of 16 (44%) of horses had T10ACTH less than the recommended T10ACTH reference range (110 pg/mL). In June, the decrease from baseline for T0ACTH was 7.1%; whereas the decrease from baseline for T10ACTH was 49.1%. All horses with regional hypertrichosis and delayed shedding were considered improved or resolved, whereas body condition and muscle wasting was slightly improved. Four adverse events not related to treatment were reported and considered resolved within 24 hours.

All horses had improvements in clinical signs following treatment. No significant changes from baseline were observed in insulin or glucose parameters. Following 5 months of treatment, 12 of 16 (75%) horses T0ACTH decreased below normal range compared to 7/16 (44%) T10ACTH that decreased below the recommended reference range. Although all horses responded clinically, the T10ACTH in 9/16 (54%) horses remained positive. Even though T10ACTH did not decrease below reference range, the option to hold the treatment dosage at the same level is justifiable based on clinical sign improvement. Further long term studies in large numbers of horses should be conducted in horses with early PPID.

E15

DEVELOPMENT OF AN OCTREOTIDE RESPONSE TEST FOR DETECTION OF INSULIN DYSREGULATION IN HORSES. Nicholas Frank[1], Pilar Hermida[1], Alfredo Sanchez-Londono[1], Cassandra Uricchio[2], Ranee Singh[3]. [1]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA, [2]Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA, USA, [3]Veterinary Medicine Faculty, Khon Kaen University, Khon Kaen, Thailand

Octreotide is a somatostatin analog that suppresses insulin secretion and we hypothesized that an octreotide response test (ORT) could be developed to detect insulin dysregulation (ID).

Twelve Morgan horses were included and insulin status was assessed with the oral sugar test (OST). An ORT was then performed by fasting horses overnight and administering octreotide IV at a dosage of 1 μg/kg. Blood was collected at 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 minutes, and 2, 3, 4, 6, 8, 12, and 24 h.

Mean AUCg did not differ significantly between normal (n = 5) and ID (n = 7) groups, but mean glucose concentrations at 75, 180, 240, and 360 minutes were significantly ($P < 0.05$) higher in the ID group, when compared with t-tests. Mean AUCi was significantly higher ($P = 0.003$) in the ID group when compared with the normal group. Mean plasma insulin concentrations differed significantly between groups at 180, 240, 360, and 480 minutes. A significant time effect ($P < 0.001$) was detected and there was a trend towards higher insulin concentrations over time (group × time; $P = 0.091$).

Results show that insulin concentrations decrease within 60 minutes of octreotide administration and hyperglycemia develops as a result. As the effects of octreotide wane, insulin concentrations increase above the pre-injection baseline as more insulin is secreted to regain glycemic control. The magnitude of the increase in insulin concentrations reflects insulin sensitivity, as evidenced by the higher AUCi values detected in horses from the ID group.

E16

INSULIN AND INCRETIN HORMONE CONCENTRATIONS IN HORSES DURING AN ORAL SUGAR TEST AND PASTURE CHALLENGE.

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An oral sugar test (OST) is used to assess insulin status in horses and this study was undertaken to test the hypothesis that test results are closely correlated with blood insulin responses to grazing on pasture grass. Correlations among insulin and incretin hormone concentrations were also assessed to better understand mechanisms of hyperinsulinemia in horses.

Eight Morgan horses from the University of Massachusetts herd were fasted overnight and then subjected to an OST over 180 minutes, immediately followed by turnout on pasture grass. Blood samples were collected at 0, 30, 60, 75, 90, 120, 150, 180, 240, 300, 360, 420, and 540 minutes. Plasma active and total glucagon-like peptide-1 (aGLP-1 and tGLP-1, respectively) and glucose-dependent insulinotropic polypeptide (GIP) concentrations were measured, along with glucose and insulin concentrations. Peak concentrations and area under the curve values were compared between the OST (0 to 180 minutes) and pasture challenge (180 to 540 minutes).

Peak insulin concentration ($r_s = 0.83$; $P = 0.015$) and area under the insulin curve values ($r_s = 0.95$; $P = 0.001$) were positively correlated between the OST and pasture challenge. Area under the tGLP-1 curve (0–540 minutes) and area under the GIP curve were correlated with one another ($r_s = 0.76$; $P = 0.037$), but did not correlate with area under the insulin curve. Active GLP-1 concentrations are pending.

Our hypothesis was supported and we conclude that the OST can be used to predict insulin responses to grazing on pasture grass.

E17

ASSOCIATION OF ANDROGENS AND PREGNANES RESPONSE TO ACTH STIMULATION WITH ADRENAL DYSFUNCTION IN HOSPITALIZED FOALS.

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Sepsis continues to cause high mortality in human and equine neonates. The hypothalamic-pituitary-adrenal axis is activated during sepsis to increase cortisol concentrations; however, the secretion of cortisol can be insufficient for the severity of disease in a number of sick foals. Our group and others have shown that relative adrenal insufficiency (RAI), characterized by a poor cortisol response to adrenocorticotropin (ACTH), is associated with mortality and severity of disease in foals. Most studies investigating RAI in equine patients have been focused on cortisol, while other adrenocortical steroids have been overlooked.

We hypothesized that a high dose ACTH stimulation test will stimulate the release of multiple adrenocortical steroids; however, RAI in septic foals will be characterized by a weak glucocorticoid and mineralocorticoid response, but normal androgen and pregnane response when compared to healthy foals and sick foals with normal adrenocortical function. We also proposed that the steroid response to ACTH will be associated with severity of disease.

Foals <1 day of age (n = 25) were categorized into 3 groups: septic (n = 5), sick non-septic (SNS; n = 10) and healthy (n = 10). After baseline blood sample collection on admission (time 0), foals received ACTH (100 μg, IV). Additional samples were collected at 30 and 90 minutes post-ACTH. Hormone concentrations were determined by immunoassays. The delta hormone was defined as the percent concentration change between time 0 and 30 minutes ($Delta_{0–30}$).

Septic and SNS foals had higher pregnenolone, progesterone, 17α-OH-progesterone and estradiol compared to healthy foals at all 3 time points ($P < 0.05$). Cortisol, aldosterone and androstenedione concentrations were higher in septic and SNS than healthy foals at time 0 and 90 minutes ($P < 0.05$). The $Delta_{0–30}$ was lower in septic compared to healthy foals for cortisol and aldosterone ($P < 0.05$). Septic foals had higher dehydroepiandrosterone (DHEA) $Delta_{0–30}$ than healthy foals ($P < 0.05$).

This study shows that an impaired response to ACTH in septic foals involves multiple adrenocortical steroids. The increased DHEA response to ACTH stimulation might be a good indicator of RAI in septic foals.

E18

ASSOCIATION OF OXYTOCIN AND NEUROSTEROIDS WITH NEONATAL MALADJUSTMENT SYNDROME (NMS) IN HOSPITALIZED FOALS.

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Neonatal maladjustment syndrome (NMS) is a common illness of newborn foals. Neuroactive steroids (pregnanes, androgens) participate in neurological development; imbalances have been implicated in human disorders, and more recently in the pathogenesis of NMS. Oxytocin regulates uterine contraction, lactation, and social functioning. Oxytocin modulates neurosteroid actions and neurosteroids (pregnanes) can stimulate oxytocin release in growing animals. Oxytocin release may be altered in human patients with a variety of neurological disorders; however, the role of oxytocin in pathologies of newborn foals, including sepsis and NMS, has not been investigated.

We hypothesized that foals with NMS and sepsis will have higher oxytocin and neurosteroids concentrations compared to healthy foals. We also proposed that concentrations of neuroactive steroids and oxytocin will be associated with outcome.

Blood samples were collected on admission from 23 foals with NMS, 37 foals with other neonatal diseases and 15 healthy foals of <3 days of age. Blood concentrations of steroids and oxytocin were determined by immunoassays.

Oxytocin concentrations were higher in foals with NMS compared to healthy foals but lower in foals with other disorders including sepsis compared to healthy and NMS foals ($P < 0.05$). Sick foals (NMS and other diseases) had increased cortisol, aldosterone, 17α-OH-progesterone, progesterone and pregnenolone concentrations compared to healthy foals ($P < 0.05$). There were no differences in steroid concentrations between foals with NMS and other diseases. Progesterone concentration was higher in sick non-surviving foals compared to survivors ($P < 0.05$). Androstenedione and dehydepiandrosterone concentrations were not different between groups of foals.

Due to its neuronal modulatory actions, our study suggests that oxytocin concentration may be involved in the pathogenesis of NMS and other disorders of the equine neonate. Hyperprogesteronemia was associated with NMS, sepsis, and poor prognosis for survival in hospitalized foals.

E19

IDENTIFICATION OF GENETIC LOCI UNDERLYING EQUINE METABOLIC SYNDROME AND LAMINITIS RISK IN WELSH PONIES. Elaine Norton[1], Nichol Schultz[1], James Mickelson[1], Ray Geor[2], Molly McCue[1]. [1]University of Minnesota, Saint Paul, MN, USA, [2]Massey University Colllege of Science, Palmerston North, New Zealand

Equine metabolic syndrome (EMS), a clustering of clinical signs including insulin resistance and dyslipidemia, is the most common cause of laminitis, a painful and life-threatening disease of the horse's hoof. In a large across-breeds study of metabolic variation in horses, our lab demonstrated that EMS phenotypic variability in metabolic traits is influenced by genetic and environmental factors. Further, we demonstrated that certain features of the EMS phenotype are different between breeds. The objective of this project was to identify genomic regions contributing to EMS by performing a genome-wide association study (GWAS) in a cohort of 232 Welsh ponies phenotyped for 11 metabolic traits. Individuals were genotyped on one of two SNP arrays (670,000 to 1,800,000 SNPs); the software program Beagle was used to generate a uniform set of makers across both platforms (~1.8 million SNPs). GWAS was performed using a mixed linear regression model that included a random polygenic term determined from a genomic relationship matrix calculated from select trait associated SNPs, random herd effect, and fixed covariates sex and age. Significant loci were identified for several EMS traits. Specific examples include loci on ECA1 (P = 5.04e-08) and ECA15 (P = 5.43e-12) for fasting adiponectin and triglyceride levels, respectively. For fasting insulin levels, significant loci were identified on ECA18 (P = 4.77e-10) and ECA6 (P = 4.33e-09). Candidate genes in these regions include a gene associated with obesity in humans on ECA18, and a gene associated with height and insulin resistance on ECA6. Future directions include haplotype analysis and interrogation of these regions through whole genome sequencing.

E20

THE FIBROBLAST GROWTH FACTOR-23/KLOTHO AXIS IN HEALTHY AND HOSPITALIZED FOALS. Ahmed Kamr[1], Katarzyna Dembek[1], Blake Hildreth III1?^!"number(boolean(following-ibling::degrees))",[1], Stephen Reed[2], Nathan Slovis[3], Bonnie Barr[2], Ahmed Zaghawa[4], Ramiro Toribio[1]. [1]College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, [2]Rood and Riddle Equine Hospital, Lexington, KY, USA, [3]Hagyard Equine Medical Institute, Lexington, KY, USA, [4]Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt

Hyperphosphatemia, hypocalcemia, increased parathyroid hormone (PTH), and reduced vitamin D metabolite concentrations were recently documented in critically ill foals by our group. However, information on fibroblast growth factor-23 (FGF-23) and klotho, two factors that regulate vitamin D activation and PTH secretion, and their association with calcium, phosphorus, PTH, and vitamin D concentrations in healthy and hospitalized foals is lacking. FGF-23 is released by osteocytes in response to increased $1,25(OH)_2D_3$, PTH, and phosphorus concentrations. FGF-23 suppresses renal 1α-hydroxylase activity and PTH synthesis. Klotho is secreted by the kidneys and acts as a co-receptor for FGF-23. The goal of this study was to determine the relevance of FGF-23 and klotho in calcium, phosphorus, PTH, and vitamin D regulation, including their association with clinical and laboratorial findings, disease severity and outcome in hospitalized foals. We hypothesized that elevated FGF-23 and reduced klotho concentrations will be frequent, associated with calcium and phosphorus dysregulation, PTH and vitamin D metabolite ($25(OH)D_3$, $1,25(OH)_2D_3$) concentrations, severity of illness, and mortality in critically ill foals.

One hundred newborn foals ≤ 3 days old divided into hospitalized (n = 83; 59 septic, 24 sick non-septic [SNS]) and healthy (n = 17) groups were included. Blood samples were collected on admission. Serum FGF-23, klotho, PTH, and vitamin D metabolites were measured by immunoassays. Data were analyzed by non-parametric methods and logistic regression.

Serum FGF-23 concentrations were significantly higher while klotho, $25(OH)D_3$, and $1,25(OH)_2D_3$ concentrations were significantly lower in septic and SNS compared to healthy foals ($P < 0.01$). Septic foals had higher phosphorus and PTH, and low calcium concentrations than SNS and healthy foals ($P < 0.05$). In hospitalized and septic foals, serum FGF-23 concentrations were associated with phosphorus and PTH ($P < 0.05$), but not with calcium and vitamin D metabolite concentrations ($P > 0.05$). In septic foals, serum klotho concentrations were positively associated with $1,25(OH)_2D_3$ concentrations (rs =0.42; P = 0.01). Hospitalized foals with the highest FGF-23 and lowest klotho concentrations were more likely to die (OR= 3.1; 95% CI = 1.2–9.1; OR=3.6; 95% CI=1.4–10.0, respectively).

High FGF-23 and low klotho concentrations in the presence of elevated phosphorus and PTH concentrations suggests that FGF-23 resistance may be involved in the pathogenesis of mineral and endocrine disorders of critically ill foals.

E21

EFFECT OF RRR-ALPHA-TOCOPHEROL FORMULATION ON SERUM AND CSF ALPHA-TOCOPHEROL CONCENTRATIONS IN VITAMIN E DEFICIENT HORSES. Jennifer C. Brown[1], Stephanie J. Valberg[1], Mattie Hogg[1], Carrie J. Finno[2]. [1]University of Minnesota, College of Veterinary Medicine, St Paul, MN, USA, [2]University of California, School of Veterinary Medicine, Davis, CA, USA

Alpha-tocopherol (α-TP) deficiency in horses can lead to nutritional myodegeneration, neuraxonal dystrophy, vitamin E deficient myopathy or motor neuron disease depending on its temporal occurrence and individual susceptibility. In the absence of adequate grass pasture, α-TP supplementation is becoming increasingly important and, due to better bioavailability, the "natural" isoform of α-TP, RRR-α-TP, is increasingly being used. RRR-α-TP is available in two formulations, a water-dispersible liquid and an acetate powder, with a more rapid increase in serum α-TP occurring with the more expensive liquid RRR-α-TP. RRR-α-TP acetate is often used for long-term supplementation however, due to the higher cost of liquid RRR-α-T.

The objective of this eight-week study was to compare serum and cerebrospinal fluid (CSF) α-TP concentrations in three groups of horses that had serum α-TP concentrations of <2 μg/mL. Groups were 1) no α-TP (n = 5), 2) 5000 IU daily of RRR-α-TP acetate (n = 7) and 3) 5000 IU daily of RRR-α-TP liquid, transitioning to 5000 IU daily of acetate (n = 7). The transition was; liquid RRR- α-TP (2 weeks), transition (4 weeks) and RRR-α-TP acetate (2 weeks). Horses appeared healthy and were fed the same diet of hay cubes in a dry lot. Serum samples were obtained weekly and atlanto-occipital CSF fluid was obtained before and after the eight-week supplementation period. A GLM ANOVA with post hoc Bonferroni pair wise comparisons ($P < 0.05$) was performed.

Beginning in week 1, serum α-TP was significantly higher in the liquid to acetate RRR-α-TP transition group than in the acetate and the no supplement groups. Serum α-TP increased more gradually in the RRR-α-TP acetate group and was significantly higher than in the no supplement group. CSF α-TP increased significantly between pre and post supplementation in the liquid to acetate RRR-α-TP group only.

In conclusion, for horses deficient in α-TP, the fastest and highest increase in serum α-TP was obtained using water-dispersible RRR-α-TP. A regimen of RRR-α-TP water-dispersible product, tapered to RRR-α-TP acetate, provided an effective means to rapidly and sustainably increase α-TP in serum and CSF over an eight-week period.

E22

DIFFERENTIAL GENE EXPRESSION IN EQUINE SUBCUTANEOUS AND INTERNAL ADIPOSE DEPOTS. Ann Kemper, James Mickelson, Molly McCue. University of Minnesota, St Paul, MN, USA

Equine metabolic syndrome is characterized by regional adiposity, insulin resistance, predisposition to laminitis, and activated

inflammation. Regional adiposity in horses is characterized by subcutaneous fat deposits at the nuchal ligament, behind the shoulder, and at the tail head, and increased neck circumference is associated with insulin resistance. In humans and mice, visceral fat is associated with insulin resistance and development of diabetes, and is more metabolically active than other fat depots. In contrast, candidate gene expression studies in horses have suggested that nuchal fat may be more metabolically active than visceral fat. We have collected samples from six adipose tissue depots within the horse; nuchal, shoulder, tail head, (subcutaneous, SQ) and visceral, omental, and retroperitoneal (internal) in six horses of varying age, breed, and sex. To quantify the differences in gene expression in the various depots, an average of 19,085,946 paired-end RNA sequencing reads per sample were generated on the Illumina HiSeq platform. Limma-voom and linear modeling identified 4,252 genes that were differentially expressed between SQ and internal adipose tissue depots. Interestingly, several pathways, including PPAR and PTEN, associated with metabolically active beige/brown fat were highly expressed in SQ compared to internal fat depots. Further, SHOX2 (P = 1.54E-10), a marker for beige fat whose subcutaneous expression levels are also positively correlated with central obesity in humans, was overexpressed in SQ. TBX15 (P = 4.72E-8) and ZIC1 (P = 5.03E-5), genes expressed in brown adipose tissue, were also overexpressed in subcutaneous tissue. Several differences in SQ gene expression were driven by differences in the nuchal fat.

E23

EFFECTS OF A COMMERCIAL ANIONIC SUPPLEMENT ON URINARY ACIDIFICATION IN HORSES. Elizabeth Nelson, Martha Mallicote, Lori Warren, Ariel Robelen, Sarah Reuss. University of Florida, Gainesville, FL, USA

There are multiple clinical applications for the acidification of equine urine, including prevention of reoccurrence of urolithiasis. This study evaluated the efficacy of a commercial anionic supplement (SoyChlor®) for urinary acidification without altering systemic pH. Eight horses were administered SoyChlor® to achieve a DCAD of 0 mEq/kg or −40 mEq/kg or fed a control diet for 14 days in a randomized crossover trial. Urine and plasma pH, plasma strong ion difference, plasma anion gap and fractional excretion of calcium were measured and compared between treatment groups. Mean urine pH across each collection period was significantly greater in control horses compared to either treatment group but there was no difference between treatment groups. Blood pH and anion gap were not affected by treatment, but blood pH decreased and anion gap increased over time. Mean bicarbonate concentration was lower in the 0 mEq/kg group than the control group, but did not differ between the 0 mEq and −40 mEq/kg treatments. Strong ion difference of the 0 mEq treatment was lower than the control group. The mean fractional excretion of calcium over all time points did not differ between treatment groups but there was an effect of time and treatment. Both treatment groups had increased fractional excretion of calcium relative to the control group at trial day 7 only. This study shows SoyChlor supplementation can decrease equine urinary pH. Systemic pH was not affected but an increase in fractional excretion of calcium did occur.

E24

EFFECTS OF ABRUPT CONCENTRATE INCREASE AND PREBIOTIC SUPPLEMENTATION ON EQUINE CECAL PH AND LACTATE. Katherine Williamson[1], Amanda Reeg[2], Teresa Douthit[2], Murali Raghavendra Rao[1], Mary Beth Gordon[1]. [1]Purina Animal Nutrition, LLC, Gray Summit, MO, USA, [2]Kansas State University, Manhattan, KS, USA

Abrupt changes in diet have been shown to cause gastrointestinal upset in horses. In particular, sudden large increases in concentrate intake can lead to hindgut disruption due to acidosis. Various dietary additives have been studied to determine if this response may be ameliorated or blunted by exerting a prebiotic effect. The objective of this study was to test the hypothesis that the supplementation of a proprietary yeast fermentation product would ameliorate the disruptive effects of a sudden increase in concentrate intake on the cecum.

Nine cecally-cannulated Quarter horses (6–7 years, 4 mares, 5 geldings, approximately 500 kg) were divided into control (n = 4) and treatment (n = 5) groups based on gender and body weight and acclimated for 19 d to the diets and housing. The control diet consisted of 1.5% BW/d prairie grass hay (divided into 2 feedings at 0700 & 1900) and 0.5% BW/d of a 12% protein commercially-available concentrate (Omolene 200®, Purina Animal Nutrition) top-dressed with 10 g/45.5 kg BW alfalfa pellet placebo (1 feeding at 0700). The treatment diet was identical but concentrate was top-dressed with 10 g/45.5 kg BW of a pelleted proprietary yeast fermentation product. On d 19, cecal fluid samples were collected 1 h before and after feeding (−1 h, +1 h) and at + 4 h, +8 h, +12 h, +16 h, +20 h and +24 h for 2 additional days to establish baseline levels. On d 22, the morning meal consisted of 1.25% BW concentrate (challenge) followed by 0.75% BW hay. Cecal samples were collected as on previous days. Cecal fluid pH was measured in samples at the time of collection. Lactate was later assessed using colorimetric analysis. A completely randomized split-split-plot design was used to test the effects of the yeast fermentation product. Analysis of variance was done with mixed models (SAS 9.3, SAS Institute Inc., Cary, NC, USA) and least squares means were compared using Fisher's least significant difference ($P < 0.05$).

No adverse clinical effects were noted following dietary challenge. Mean cecal pH was decreased at +8 h and +16 h ($P = 0.017$ & 0.001 respectively) and mean cecal lactate was increased at +1 h, +8 h and +24 h ($P = 0.046$, <0.0001 & 0.031 respectively) after challenge as compared to baseline regardless of treatment. Treatment group horses had increased mean cecal pH at +4 h ($P = 0.042$) and decreased mean cecal lactate at +16 h ($P = 0.026$) compared to control group. There were no significant differences between control and treatment horses' mean cecal pH and lactate at other sampling times.

The sudden increase in concentrate intake, from 0.5% BW to 1.25% BW, induced a significant, transient decrease in cecal pH and increase in cecal lactate which was inconsistently blunted by consumption of a proprietary yeast fermentation product.

E25

EFFECTS OF A SUPPLEMENT (ALFA-LOX FORAGE®) ON EQUINE GASTRIC ULCER SCORES AND GASTRIC JUICE PH. Frank Andrews[1], Pilar Camacho-Luna[1], Kelsey Bailey[1], Isabelle Nesen[2], Michael Keowen[1], Frank Garza Jr1?^!"number(boolean(following-ibling::degrees))",[1], Chin-Chi Liu[1]. [1]Equine Health Studies Program, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA, USA, [2]École Nationale Vétérinaire, Toulouse, France

Gastric ulcers are common in stall-confined horses under intermittent feeding conditions. Alfalfa hay feeding has been shown to buffer stomach contents and decrease gastric ulcer scores. The purpose of this study was to determine the effects of a supplement (ALF; Alfa-Lox Forage, Triple Crown Nutrition, Inc., Wayzata, MN), containing chopped alfalfa hay, mannanoligosaccharides, Omega 3 Fatty Acids, and L-Carnitine, top-dressed on grain on nonglandular gastric ulcer scores and gastric juice pH in horses after omeprazole treatment and after intermittent feeding. Eight healthy Thoroughbreds (7 geldings and 1 mare; ages 2–12 years.) were used in a 2-period crossover study, where horses were fed ALF (2.0 lbs. [0.91 Kg], twice daily, for 56 days) top dressed on grain (2.5 Kg; Omelene® 100, Purina Animal Nutrition, LLC., Grey Summit, MO) or control (grain alone). While being treated the horses had their stomach scoped on day 0 (before treatment with ALF or grain only) and the on days 14, 28, 42, 49 and 56. In addition horses were weighed, body condition score (BCS) recorded and gastric juice pH measured. During the 56 day periods, from days 14 to 28 horses were treated with omeprazole paste (OME; 4.0 mg/kg, P.O., Q.D.; GastroGard®, Merial Ltd., Duluth, GA) and from days 42 to 49 horses underwent an intermittent feeding model.[1] Stomach nonglandular gastric ulcer number (NGN) and severity (NGS) scores were assigned at each scoping by the PI (FMA), who was masked to treatment. Previous forage analysis

data was used to compare nutritional quality between the two periods. Data were analyzed statistically using an ANOVA for repeated measures and when significant differences were found in the main model, a post-hoc Least Squares Means test was performed to determine significant differences at $P < 0.05$. Alfa-Lox Forage® top-dressed on grain was readily eaten by all horses, and did not result in any adverse effects. When period data were pooled, Alfa-Lox Forage® did not have a treatment by day effect on gastric ulcer scores or gastric juice pH during the study period. However, there was an overall period effect on treatment, in that BCS was higher and gastric ulcer scores were lower in the AFL-treated horses during the first period of the study, but no treatment by day effect was seen. Forage analysis showed lower digestible energy in the first period of the study. Other significant effects seen during the study included OME-treatment significantly decreased gastric ulcer scores and increased gastric juice pH in both ALF-treated and control horses and gastric ulcers healed in 6/8 (75%) horses after 14 days of treatment. In addition, gastric ulcer scores significantly increased 2 weeks after OME treatment and after feed-deprivation regardless of treatment. Alfa-Lox Forage® had no effect on gastric ulcers scores after OME treatment or during feed-deprivation when compared to untreated controls. However, when forage had lower digestible energy and higher fiber content, Alfa-Lox® Forage-fed horses had better BCS and fewer ulcers. Thus, Alfa-Lox Forage® might provide gastric support when forage has marginal nutritional value. It should be pointed out that the quantity of Alfa-Lox Forage® fed may not have been adequate to affect gastric ulcer scores or gastric juice pH in horses in this study, but other nutritional benefits might result.

[1]Murray MJ and Eichorn ES. (1996) *Am J Vet Res*. 57 (11):1599–1603.

E26

CHANGES OF THE EQUINE NEONATAL INTESTINAL MICROBIOTA ASSOCIATED WITH AGE AND DIARRHEA. Angelika Schoster[1], Henry Staempfli[2], Mohammad Jalali[3], Luca Guardabassi[4], Scott Weese[3]. [1]University of Zurich, Vetsuisse faculty, Clinic of Equine Internal Medicine, Zurich, Switzerland, [2]University of Guelph, Ontario Veterinary College, Departemnt of Clinical Science, Guelph, ON, Canada, [3]University of Guelph, Ontario Veterinary College, Departmnet of Pathobiology, Guelph, ON, Canada, [4]Departement of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Copenhagen, Denmark

Neonatal diarrhea is an important disease complex in foals. Diarrhea is associated with significant changes in composition and structure of the microbiota in adult horses. The changes associated with diarrhea in neonatal foals are unknown.

The objective was to investigate changes of the equine neonatal microbiota associated with age and occurrence of diarrhea in a field trial.

Fecal samples were collected from 20 foals at 2, 4 and 6 weeks of age and assessed via metagenomic sequencing. Foals were monitored and occurrence of diarrhea was recorded. The Wilcoxon and Steelman-Dwass tests were used to assess differences between age groups and between foals with diarrhea and healthy foals within an age group.

Age had a significant effect on relative abundance of bacterial taxa on the phylum, order and class level. There was a significant increase in richness over time ($P = 0.02$). 9/20 (45%) foals developed diarrhea. The structure of the microbiota was significantly different at Week 2 compared to Week 4 ($P = 0.09$) and Week 6 ($P = 0.02$). Diarrhea had a significant effect on relative abundance of orders and classes. Foals with diarrhea had reduced bacterial richness at four weeks of age ($P = 0.04$). Diarrhea did not have a significant effect on the beta diversity in any age group (all $P > 0.36$).

The composition and richness of the equine neonatal microbiota is changing rapidly after birth. Similar to adults, foals with diarrhea have changes in composition and richness of the microbiota. Further studies are needed to assess whether these changes are the cause or effect of diarrhea.

E27

COMPARISON OF TUBE, GEL, AND IMMUNOCHROMATOGRAPHIC STRIP METHODS FOR EVALUATION OF EQUINE BLOOD TRANSFUSION COMPATIBILITY. Daniela Luethy[1], Sean Owens[2], Urs Giger[3], Darko Stefanovski[4], Rose Nolen-Walston[1]. [1]School of Veterinary Medicine, University of Pennsylvania, Clinical Studies-New Bolton Center, Kennett Square, PA, USA, [2]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA, USA, [3]School of Veterinary Medicine, University of Pennsylvania, Ryan Small Animal Hospital, Philadelphia, PA, USA

Assessment of compatibility is recommended prior to blood transfusion in horses, typically using gross or microscopic tube agglutination and hemolysin crossmatch, or less commonly, as predicted by blood type and alloantibody assay. In contrast, gel column and immunochromatographic strip methods are the preferred techniques for compatibility testing in small animals. The purpose of this study was to determine agreement between novel and standard crossmatch and typing methods. 33 blood typed and alloantibody screened horses were evaluated. TUBE and GEL crossmatches were performed on 144 recipient-donor pairs and compared using non-parametric area under the curve (AUC) receiver operating characteristic (ROC) analysis. With TUBE assigned as the reference variable, TUBE and GEL had excellent agreement for agglutination (AUC ROC = 0.87), but borderline for hemolysis (AUC ROC = 0.64). There was outstanding agreement between TUBE (gross) and TUBE (microscopic) scores for agglutination (AUC ROC = 0.91). The predicted crossmatch compatibility based on blood type and alloantibody assay showed excellent agreement with TUBE and GEL (AUC ROC = 0.84 and 0.89, respectively). However, there were more recipient-donor pairs identified as incompatible by both TUBE and GEL than predicted by blood type and antibody screen, suggesting unidentified alloantibodies. A blood typing STRIP assay was performed on each aliquot and exhibited 100% sensitivity and specificity for 30 Ca+ and 3 Ca- horses. These findings show that gel column crossmatch techniques appear to be a practical and accurate equine blood compatibility test method, and that immunochromatographic strips are accurate at identifying the Ca blood type.

E28

INFLUENZA-SPECIFIC IMMUNE RESPONSES TO A COMBINATION VACCINE IN NAÏVE PONIES. Amanda Adams[1], Stephanie Reedy[1], Melissa Siard[1], Sarah Elzinga[1], Thomas Chambers[1], Steven Grubbs[2]. [1]Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA, [2]Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA

Equids, like humans and swine, are recognized as natural hosts for a variety of common viral pathogens, in particular equine influenza virus (EIV). EIV has resulted in worldwide disease outbreaks. Vaccination is essential to prevent or limit disease spread. Many commercially available vaccines are sold as single pathogen vaccines, however nowadays several are available as multi-pathogen or combination vaccines. A significant amount of equine research has been conducted to measure immune responses to single pathogen vaccines however data is lacking to determine if combination vaccines stimulate the immune system in particular cell-mediated immunity. Thus, the objective of this study was to characterize the cell-mediated immunity (CMI) and humoral immune responses to a combination vaccine in influenza naïve animals. A total of 16 influenza naïve yearling ponies were randomly assigned to receive one of two treatment groups: (Group 1) (n = 8) Vetera® EWT + EIV/EHV (BIVI) vaccination and (Group 2) (n = 8) controls to receive a saline vaccination. All vaccinate ponies received a primary vaccination on day 0, followed by a booster vaccination on day 28. Peripheral blood was collected from all ponies prior to vaccination on day 0 and every 2 weeks following for an 8 week period. Serum and peripheral blood mononuclear cells were isolated to measure influenza specific antibody and cell-mediated immune responses by the following assays: HI, ELISA, interferon-gamma intracellular staining by

flow cytometry, and Real-Time PCR. Statistical analysis was performed using SIGMA Plotv12.0tm (Systat Inc., Richmond, CA) with significance determined at the level of ($P \leq 0.05$). Non-normally distributed data was log transformed prior to statistical analysis. For each variable measured (cell-mediated immunity, etc.), a mixed model two way ANOVA with repeated measures was used. In measuring the humoral immune response to the EIV component of the vaccine, results showed an overall significant ($P < 0.05$) increase in the change of HI antibody titers in vaccinated ponies compared to non-vaccinated saline controls. Further, there was an increase in EIV-specific IgGa and IgGb antibody titers in the vaccinated group of ponies compared to the saline controls, there was no change in IgGT responses in the vaccinated ponies. Measurement of CMI responses by flow cytometry showed a significant increase in EIV-specific interferon-gamma being produced per cell from the vaccinated ponies compared to the saline controls. Measurement of EIV specific CMI responses by Real-Time PCR showed a significant increase in EIV-specific interferon gamma and granzyme B gene expression in the vaccinated ponies compared to controls. There was no significant difference in the gene expression of perforin, IL-2 or IL-18 when comparing the vaccinated ponies to the controls. Overall, the combination killed vaccine induced significant humoral and cell-mediated responses in naïve animals.

E29

WNV-SPECIFIC IMMUNE RESPONSES TO A COMBINATION VACCINE IN NAÏVE PONIES. Amanda Adams[1], Alex Betancourt[1], Day Barker[1], Melissa Siard[1], Sarah Elzinga[1], Stephanie Reedy[1], Steven Grubbs[2]. [1]Department of Veterinary Science, M.H. Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA, [2]Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA

West Nile virus (WNV) mosquito-borne flavivirus and is the leading cause of arbovirus encephalitis in horses in the United States. WNV infection can cause severe acute illness, with devastating clinical signs of illness affecting gait and behavioural abnormalities often times resulting in a high case fatality rate. Thus, WNV vaccination is a recommended core vaccine and is an essential standard of care for all horses in North America as recommended by the AAEP. Many commercially available vaccines are sold as single pathogen vaccines, however nowadays several are available as multi-pathogen or combination vaccines. Little data exists to characterize the immune response to the WNV antigen following a combination vaccination. We hypothesize that a multi-pathogen "combination" vaccine will elicit significant cell-mediated and humoral immune responses in naïve ponies. A total of 16 influenza naïve yearling ponies were randomly assigned to receive one of two treatment groups: (Group 1) (n = 8) Vetera® EWT + EIV + WNV (BIVI) vaccination and (Group 2) (n = 8) controls to receive a saline vaccination. All vaccinate ponies received a primary vaccination on day 0, followed by a booster vaccination on day 28. Peripheral blood was collected from all ponies prior to vaccination on day 0 and every 2 weeks following for an 8 week period. Serum and peripheral blood mononuclear cells were isolated to measure WNV-specific antibody and cell-mediated immune responses by the following assays: ELISA, interferon-gamma intracellular staining by flow cytometry, and Real-Time PCR. Statistical analysis was performed using SIGMA Plotv12.0tm (Systat Inc., Richmond, CA) with significance determined at the level of ($P \leq 0.05$). Non-normally distributed data were log transformed prior to statistical analysis. For each variable measured (cell-mediated immunity, etc.), a mixed model two way ANOVA with repeated measures was used. In measuring the humoral immune response to the WNV component of the vaccine, results showed a significant ($P < 0.05$) increase in WNV IgG titers in vaccinated ponies compared to non-vaccinated saline controls. Measurement of CMI responses by flow cytometry showed a significant ($P < 0.05$) increase in the percentage of WNV-specific interferon-gamma producing cells from the vaccinated ponies compared to the saline controls. Measurement of WNV-specific CMI responses by RT-PCR showed a significant ($P < 0.05$) increase in WNV-specific interferon gamma and IL-2 gene expression in the vaccinated ponies compared to controls. There was no significant ($P > 0.05$) difference in the gene expression of GrzB, IL-6 or IL-18 when comparing the vaccinated ponies to the controls. Overall, the combination killed vaccine induced significant WNV-specific humoral and cell-mediated immune responses in naïve animals.

E30

CHARACTERISTICS OF INFECTION CONTROL PRACTICES AT NORTH AMERICAN VETERINARY TEACHING HOSPITALS. Cristine S. De La Hoz Ulloa[1], Katharine M. Benedict[2], Paul S. Morley[2], Brandy A. Burgess[1]. [1]Virginia Tech, Blacksburg, VA, USA, [2]Colorado State University, Fort Collins, CO, USA

Infection control is crucial in the operation of all veterinary hospitals to not only protect the patients and hospital, but to protect personnel as those in the veterinary field have an increased risk of occupational exposure to zoonotic diseases. In a 2008 study, 50% of Veterinary Teaching Hospitals (VTHs) surveyed reported significant health problems due to zoonotic infections among hospital personnel. *Cryptosporidium parvum* infections accounted for 68% of these infections. The objective of this study was to characterize current infection control practices in place for the prevention of zoonotic disease infection, specifically infection of *C. parvum,* in VTHs.

All VTHs located in North America (n = 35) that had been operational for at least one year were eligible to participate in this study. Phone surveys of biosecurity experts were conducted from July - October 2015 which addressed policies for hygiene, surveillance, patient contact, education, awareness, and enteric infectious disease control.

Among participating VTHs (n = 20/35; 57.1%), greater than half reported significant outbreaks of disease among hospitalized patients in the previous 5 years; most commonly due to *Salmonella enterica* or equine herpesvirus-1. In addition, 50% reported significant health problems in personnel, in the previous 2 years, that most likely resulted from zoonotic infection. Of these, *Cryptosporidium* was identified as the most common agent. The majority of VTHs surveyed had a committee that oversaw biosecurity program activities as well as written biosecurity policy documents. However, only half conducted mandatory training on the biosecurity and infectious disease control program.

The results of this study will help to improve strategies for preventing healthcare-associated infection, including zoonotic diseases, among patients and veterinary personnel; and allow for targeted educational tools to promote a safety culture in veterinary medicine.

E31

ANTI-ENDOTOXIC PROPERTIES OF KETOROLAC TROMETHAMINE AND FLUNIXIN MEGLUMINE IN HORSES. Alexandra Bianco, George Moore, Sandra Taylor. Purdue University, West Lafayette, IN, USA

Non-steroidal anti-inflammatory drugs (NSAIDs) play an integral role in equine medicine due to their combined analgesic and anti-inflammatory properties. Despite their widespread use, there are limited NSAIDs available that demonstrate variable adverse effects and safety. While several NSAIDs have been proven to be adequate analgesics, few have undergone rigorous evaluation for anti-inflammatory efficacy. Ketorolac tromethamine (KT) is a non-selective cyclooxygenase inhibitor that has been used in human patients since 1989. The pharmacokinetic properties of KT have recently been determined in the horse, and have been previously determined in several other species. However, there have been only two previous studies examining KT's anti-inflammatory properties in animals, both of which were *in vivo*. There have been no published studies evaluating the *in vitro* anti-inflammatory effects of KT in any veterinary species. The purpose of this study was to evaluate the anti-inflammatory effects of KT compared to flunixin meglumine using an *in vitro* model of LPS-stimulated equine monocytes.

Equine monocytes were isolated from whole blood from a single horse and incubated with either KT or flunixin meglumine at six concentrations ranging from 2.5 μg/mL to 80 μg/mL for 1 hour.

After the initial incubation, *E. coli* 055:B5 LPS was added to each well at a concentration of 1 μg/mL, which has been shown to consistently activate monocytes. The wells were incubated at 37°C and 5% CO_2 for 4, 8, 12, and 24 hours. Samples were collected at each time point and stored at −80°C until analysis. Equine-specific ELISAs were used to measure the eicosanoids PGE_2 and TXB_2 as well as the cytokines TNF_{α}, IL-6, and IL-8.

Preliminary results demonstrated that flunixin meglumine suppressed PGE_2 production up to 12 hours at concentrations ≥5 μg/mL and up to 24 hours at concentrations ≥40 μg/mL. Flunixin meglumine suppressed TXB_2 production up to 12 hours at all concentrations and up to 24 hours at concentrations ≥20 μg/mL. Ketorolac tromethamine suppressed PGE_2 production up to 12 hours at all concentrations and up to 24 hours at concentrations ≥20 μg/mL. Ketorolac tromethamine also suppressed TXB_2 production up to 12 hours at all concentrations but did not suppress production up to 24 hours at any concentration. Peak eicosanoid concentration in the non-treated samples occurred at 4 hours for PGE_2 and 12 hours for TXB_2.

While *in vitro* results cannot be directly correlated to *in vivo* efficacy, the results thus far indicate both drugs effectively suppress eicosanoid production after LPS stimulation, with an effect of both time and drug concentration. Based on the results of this study, a therapeutic dose of 2.5 μg/mL KT would effectively suppress eicosanoid production in cases of endotoxemia. Further research is needed to correlate *in vitro* results with *in vivo* efficacy.

E32

EFFECTS ON SWEATING OF CHLORAMPHENICOL AND THE MACROLIDE GAMITHROMYCIN: COMPARISON WITH ERYTHROMYCIN. Amy Stieler[1], Chris Sanchez[2], Martha Mallicote[2], Amy Smith[2], Jim Burrow[2], Rob MacKay[2]. [1]University of Georgia, Athens, GA, USA, [2]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, USA

Hyperthermia in foals treated with erythromycin (ERY), clarithromycin, or azithromycin is associated with reduction of sweating by an unknown mechanism. The aims of this study were to evaluate effects on sweating of (1) chloramphenicol (CHL) which, like macrolides, binds to the 50S subunit of the bacterial ribosome and (2) gamithromycin (GAM), a macrolide sub-class 7a azalide which is not yet licensed for treatment of foals. Over 3 experimental periods, 12 foals (1 to 3 months old) were treated with ERY (25 mg/kg PO, 3 times daily for 5 days), GAM (8 mg/kg IM once) and CHL (50 mg/kg PO, 3 times daily for 5 days) according to a masked, duplicated, fully counterbalanced design. Quantitative intradermal terbutaline sweat tests were performed on 3 successive days before treatment (baseline) and on days 1, 2, 5, 9, 24, and 39 after treatment began. Data were analyzed by 1-, 2-, and 3-factor repeated measures ANOVA with *post hoc* pairwise analyses of significant tests. There was significant ($P < 0.05$) suppression of sweating by ERY but not CHL or GAM. Compared with sweating at baseline, values when foals were given ERY were significantly lower on all test days except day 39. Sweating responses in foals given ERY were significantly lower than when given GAM or CHL on days 2, 5 and 9 at every terbutaline concentration except 100 μg/mL. In contrast, there was no difference between CHL and GAM. Results show that (1) ability to bind the bacterial 50S ribosome does not by itself confer potential to induce anhidrosis and (2) unlike the macrolides ERY and clarithromycin and the 9a-azalide azithromycin, the 7a-azalide GAM did not suppress sweating responses.

E33

COMPARISON OF THE CLINICOPATHOLOGIC SIGNATURES OF EQUINE CORONAVIRUS AND *SALMONELLA* ENTEROCOLITIS. Arlie Manship, Johanna Elfenbein. North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA

Recent worldwide outbreaks of Equine Corona Virus (ECoV) have led to an increased awareness of this pathogen as a cause of colitis in adult horses. The objective of this study was to determine whether ECoV enterocolitis has a distinct clinical and/or clinicopathologic signature that can distinguish it from enteric salmonellosis or unknown causes of fever and neutropenia.

Data were collected from medical records from North Carolina State University (2003–2015). Horses >1 year old were divided into 3 groups based on diagnosis: ECoV (fecal PCR), enteric salmonellosis (fecal culture or PCR), or unknown diagnosis by ruling out ECoV and *Salmonella* (3 negative *Salmonella* cultures, negative ECoV and *Salmonella* PCR). Clinical features, diagnostic test results, and outcome were compared between groups. Statistical significance was determined using a Mann-Whitney U test with $P < 0.05$.

Data were obtained from 7 horses with ECoV, 11 horses with *Salmonella*, and 11 horses without a diagnosis. ECoV cases had significantly fewer neutrophils (median 569/uL; range 105-1,029/uL) compared with all other diagnoses (1,310/uL; 337-9,720/uL) but there was no difference in neutrophil count when compared with *Salmonella* or unknown diagnosis. There were no differences in temperature, lymphocyte or platelet counts, fibrinogen, or any biochemical parameter between groups.

The results suggest that disease with ECoV and Salmonella share clinical features and should be included together on a differential list for fever and neutropenia. Further investigation is warranted to determine whether there is an unknown pathogen as the cause of the fever and leukopenia in the group without a diagnosis.

E34

PHENOTYPIC CHARACTERIZATION OF *SARCOCYSTIS NEURONA* LESIONS IN GRAVELY AFFECTED HORSES. JunJie Liu[1], Micheal Dark[1], Lisa Farina[1], Robert MacKay[2], Nancy Denslow[3], Lei Zhou[4], Maureen Long[1]. [1]Department of Infectious Diseases and Pathology, University of Florida, Gainesville, FL, USA, [2]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, USA, [3]Department of Physiology, University of Florida, Gainesville, FL, USA, [4]Department of, University of Florida, Gainesville, FL, USA

The primary goal of this research was to contribute new knowledge to the understanding of *Sarcocystis neurona* (SN) infection in horses. Our goal was to identify SN infected horses and to use different markers to investigate and characterize the infiltration of inflammatory cells and resident cellular changes in EPM-diseased horses by genetic (PCR) and immunohistochemical (IHC) techniques. The hypothesis investigated was that phenotypic characterization the CNS of gravely SN affected horses could define disease pathogenesis. Tissues from 2 groups of humanely euthanized horses with either grave or no neurological symptoms were collected under University of Florida IACUC protocols 3984, 4109, and 4559. Sets of CNS tissues from all levels of brain and spinal cord were fixed for pathology and immunohistochemistry and frozen for nucleic acid extraction. Histopathologically, encephalomyelitis and normal CNS was confirmed in affected and unaffected horses, respectively. SN, *Neospora hughesi*, EHV-1, WNV, and EEEV virus PCR was performed to confirm neuropathogen status in all horses. For RNA expression analysis, the tissues in which SN was detected, the corresponding section of frozen tissue was cut into 30–50 30 μg sized cubes. Relative parasite burden was calculated based on the standard curve generated by the use of cultured SN merozoites. 160 samples were tested and the correlation coefficient (R, Psych package) between $CD3^+$ and TNF-α expression was 0.874. The correlation coefficient between $CD3^+$ expression and parasite burden was 0.8304 (186 samples) and the correlation coefficient between TNF-α and parasite burden was 0.7757 (101 samples). Immunohistochemical phenotypic analysis was conducted for T cells, macrophages, B cells, microglia and astrocytes for C3 + T cells, B cells, macrophage

cells, glial cells, and astrocytes and this was performed on 12/14 EPM cases that had SN+ and adjacent SN- tissue formalin fixed tissue (FFPE) blocks. Eleven normal control horses were used and sections were location matched to lesion sections. All sections were compared for significant differences by ANOVA ($P < 0.05$). The number of $CD3^+$ T cells and microglia/macrophage cells of SN+ tissues were significantly increased compared with its adjacent section and the sections from the normal horses. Although present in extremely limited numbers (< 10 cells per site), macrophage only staining cells had statistically higher cell counts in affected horses compared to normal horses. Because CD3 + cells were the most numerous, differentiation of CD4 + and CD8 + cells was attempted using IHC by several markers, none of which worked on FFPE sections. CD4 + and CD8 + relative expression was investigated by quantitative PCR and the most significant change was that of CD4 + cell marker expression at 144.6 fold (log_2) over controls. The fold change of CD8 + between the lesion group and the normal group was 10.8. In conclusion, the presence of SN was only present within inflammatory nodules and the presence of parasite burden was associated with the presence of CD3 + CD4 + T cell markers compared to that of CD8 + T cells. Based on the presence of inflammatory markers, the presence of SN was not detected outside of aggregates of either resident or infiltrating inflammatory cells.

E35

NORMAL ULTRASONOGRAPHIC PLEURAL THICKNESS IN CLINICALLY HEALTHY ADULT HORSES. Breanna Sheahan, Kara Lascola, Scott Austin. University of Illinois, Veterinary Teaching Hospital, Urbana, IL, USA

The objectives of this study were to establish reference ranges for normal pleural thickness measurements in healthy adult horses and to compare measurements between horses < 15 years of age (YOUNG) and ≥ 15 years of age (OLD). Pleural thickness is assessed as part of a complete thoracic ultrasonographic examination but no normal measurements have previously been reported. We hypothesized that measurements of pleural thickness would be < 2 mm.

18 clinically healthy adult horses (11 YOUNG; 7 OLD) were examined with a curvilinear 3.5–8 mHz probe in eight thoracic locations (four per side): cranioventral (intercostal space (ICS) 6), mid-thoracic (15 cm dorsal to point of shoulder (POS) in ICS 10), caudoventral (ICS 10 at POS), and caudodorsal (ICS 14). All measurements were performed post hoc by two observers on three recorded images from each site. Differences among locations were evaluated with Kruskal-Wallis. Difference between age groups was evaluated with Mann Whitney U. Inter-observer reliability was evaluated using intraclass correlation (ICC). Significance was set at $P < 0.05$.

Pleural thickness median (range) was 1.5 (1.11–2.33) mm in all horses, 1.47 (1.11–2.09) mm in YOUNG horses and 1.55 (1.18–2.33) mm in OLD horses. Significant differences among locations and between age groups were not noted. Mild pleural irregularities were subjectively more common in older horses. ICC was significant for measurements at each location.

Older horses did not have significantly greater pleural thickness measurements. Measurements were reproducible. Measurements above the normal variation should be cause for further investigation of respiratory disease.

E36

SYRINGE VERSUS MECHANICAL SUCTION WITH N-BUTYLSCOPOLAMMONIUM EFFECTS ON BAL PARAMETERS IN HORSES WITH PASTURE RAO. Jacquelyn Bowser, Lais Costa, Christine Lopp, Rachel Wilson, Rebecca Byrne, Melissa Steichen, Santosh Kumar TK, Alba Rodil, Michael Brashier, Cyprianna Swiderski. Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA

Bronchoalveolar lavage (BAL) is frequently employed to evaluate lower airway inflammation in horses with RAO. In these horses, bronchial collapse often limits retrieval of BALF. N-butylscopolammonium bromide (Buscopan®), a parasympatholygic agent, safely produces short term bronchodilation, reversing airway obstruction RAO-affected horses.

Based upon the hypothesis that syringe aspiration would improve BALF retrieval, we performed BAL in the left and right lungs of 8 sedated summer pasture associated RAO-affected horses using a flexible endoscope. Saline (300 cc) was instilled via a polyurethane tube inserted through the biopsy port using a 60 cc syringe and aspirated using gentle manual suction (SS) with a 60 cc syringe or with mechanical suction (MS) using a pressure regulated suction pump. Pressures of 15–75 mm H_2O retrieved BALF without airway collapse. Bilateral BAL was performed twice in each horse with or without Buscopan® (0.3 mg/kg IV) in a crossover fashion with a 21 day washout period. The effects of suction type and Buscopan® administration on volume yield, cellularlity and cell viability were determined.

BAL performed using SS demonstrated higher volume yield, with lower RBC and lower nucleated cell counts. There was a tendency for improved viability with MS. Differences in yield, nucleated cell count and red blood cell count were not identified with Buscopan®, with the exception of horses demonstrating profound collapse.

Use of a modified BAL technique that includes manual syringe suction with Buscopan® administration may provide an advantage in horses with significant airway hyper-reactivity by limiting airway collapse and enhancing the diagnostic utility of BALF obtained.

E37

PREVALENCE OF FUNGI IN RESPIRATORY SAMPLES OF HORSES WITH INFLAMMATORY AIRWAY DISEASE. Julie Dauvillier, Emmanuelle van Erck. Equine Sports Medicine Practice, Waterloo, Belgium

Inflammatory Airway Disease (IAD) is a common cause of respiratory problems and poor performance in horses. It has recently been suggested that IAD and Recurrent Airway Obstruction (RAO) could be two forms of a same inflammatory process and recent evidence tends to establish a close parallel between these equine diseases and asthma in humans. Fungi have been shown to contribute to the inflammatory response of lungs in RAO horses and in some forms of asthma in humans. The role of fungi in IAD has not yet been assessed to the knowledge of the authors.

The purpose of this prospective observational clinical study was (1) to evaluate the prevalence of fungal isolates in the respiratory samples of horses diagnosed with IAD, (2) to describe clinical signs associated with the presence of fungi in respiratory samples of horses diagnosed with IAD and (3) to assess the risk factors associated with IAD and positive fungal culture and proliferation. The study was performed in a population of working sport, race and leisure horses based in France, Belgium and the Netherlands.

A total of 482 cases horses, referred to a specialized ambulatory internal medicine practice in Europe, were assessed between June 2013 and December 2014. The horses were referred either for respiratory problems or loss of performance. For each case, an environmental evaluation, a clinical examination, an airway endoscopy, a tracheal wash (TW) and a bronchoalveolar lavage (BAL) were performed. The TW and the BAL underwent cytologic evaluation and the TW was submitted for bacteriology and mycology. Diagnosis of IAD was established based on BAL fluid cytology, according to the ACVIM consensus statement definition. On both cytologic examinations, the presence of fungal elements (spores, conidiophores, hyphae) was recorded, as well as signs of active proliferation.

In the population studied, a diagnosis of IAD was established in 84% of cases. A positive fungal culture was obtained in 49% of IAD+ cases and in 44% of IAD- cases. The most commonly isolated fungus were *Aspergillus sp, Penicillium* and *Rhizomucor*. No relationship could be established between positive mycology and cytologic evidence of fungal elements or active proliferation of fungi in the airways. However horses with fungal elements on the TW cytology had 3.8 more chances of having IAD than horses with no fungi (OR=3.8; 95% CI 1.8–7.8; $P = 0.0003$). Twenty IAD horses had signs of fungal proliferation in their TW and/or

BAL, but all mycologies remained negative in these cases. Distinctive respiratory clinical signs such as cough, breathlessness or nasal discharge showed low sensitivity and high specificity for IAD diagnosis. Concomitant bacterial infection did not increase the risk of fungal isolation. The degree of inflammation (% inflammatory cells in BAL) was higher when horses were housed indoors, bedded on straw versus shavings (Mann-Whitney test, 25.53 + /-17.14 versus 19.9 + /-17.5; $P < 0.001$) or fed dry hay versus steamed hay (23.2 + /- 17.5 versus 18.8 + /-18.9; $P < 0.001$).

The prevalence of fungal contamination in IAD cases is high in Europe. Horses inhaling aerosolized fungal particles are at a significantly higher risk of developing IAD. The type of bedding and forage as well as other environmental conditions represent significant risk factors for IAD and fungal contamination of equine airways. The identification of proliferating fungi in the airways is a sign of fungal infection and warrants further investigation. In the current study, mycology culture seems to lack sensitivity in the detection of fungi in respiratory samples.

E38

INTRAVENOUS MAGNESIUM SULFATE AS A RESCUE THERAPEUTIC FOR BRONCHOCONSTRICTION IN HORSES. Jacquelyn Bowser, Caitlin Wenzel, Robert Wills, Noel Bondi, Rachel Wilson, Rebecca Byrne, Cyprianna Swiderski. Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA

Horses with RAO exhibit exacerbations of acute airway obstruction that cause respiratory distress resulting from bronchospasm, mucus accumulation, and airway inflammation. Emergency therapeutic options for control and reversal of airway bronchoconstriction in equine airways include parasympatholytic agents which inhibit gastrointestinal motility, β2-adrenergic agonists, as well as systemic corticosteroids, which require 4–6 hours for full effect. Meta-analysis of emergency intervention for asthmatic adults found that adjunctive infusion of magnesium sulfate ($MgSO_4$) improves lung function and reduces hospital admissions. This study examined utility and effectiveness of intravenous $MgSO_4$ as a novel, inexpensive, easily administered bronchodilatory agent for horses with a naturally occurring pasture-associated form of RAO (SPARAO).

Six SPARAO-affected horses exhibiting naturally occurring disease exacerbation (CSRE ≥5, $\Delta Ppl_{max} > 15$ cm H_2O) were treated with three doubling dosages of $MgSO_4$ by rapid IV infusion using three way cross-over design. Horses were instrumented for continuous ECG and blood gas monitoring. Tidal volume (TV), pulmonary resistance (R_L), dynamic compliance (C_{dyn}), and maximum change in pleural pressure (ΔPpl_{max}) were monitored at 5 minute intervals using conventional pulmonary mechanics.

Mixed models were fit for percent change from baseline values for each outcome using PROC MIXED (SAS, SAS Institute. Cary, NC). Significant decreases in ΔPpl_{max}, R_L and significant increases in C_{dyn} were identified at all dosages and were accompanied by increased P_aO_2 and decreased P_aCO_2, substantiating improved ventilation. We conclude that intravenous infusion of $MgSO_4$ has immediate bronchodilatory effects in horses making this therapy a valuable adjunct in horses with signs of respiratory distress referable to bronchoconstriction.

E39

INVESTIGATION OF MISOPROSTOL AS A NOVEL ANTI-INFLAMMATORY IN EQUINE LEUKOCYTES. Emily Medlin Martin, Samuel Jones. North Carolina State University, College of Veterinary Medicine, Raleigh, NC, USA

E-type prostaglandins (PGEs) play diverse roles throughout the body, including regulation of gastrointestinal (GI) homeostasis and modulation of inflammation. To treat inflammation, anti-inflammatory medications including nonsteroidal anti-inflammatory drugs (NSAIDs) are designed to inhibit prostaglandin production. Unfortunately, potential adverse effects of NSAID use in horses include gastric ulceration and right dorsal colitis. To prevent and treat NSAID-associated GI injury, equine practitioners commonly administer the PGE_1 analog, misoprostol. In addition to serving as a gastroprotectant, misoprostol has been shown to have anti-inflammatory effects in cell models, potentially by increasing intracellular cyclic AMP (cAMP). Elevated cAMP is known to dampen leukocyte effector functions that can damage host tissues, including cellular adhesion, tissue infiltration, and cytokine and superoxide production. The effects of misoprostol on equine leukocyte effector functions are undetermined. Thus, the purpose of this study was to determine the effects of misoprostol on equine leukocyte pro-inflammatory cytokine and superoxide production *in vitro*. Equine leukocytes were isolated from whole blood and stimulated with lipopolysaccharide (LPS) in the presence or absence of misoprostol. Misoprostol at concentrations of 1–10 μM significantly inhibited tumor necrosis factor-α, interleukin-1β, and interleukin-6 mRNA production, as measured by qPCR. Misoprostol also elicited concentration-dependent decreases in neutrophil superoxide production in response to granulocyte-monocyte colony-stimulating factor priming and LPS stimulation, as measured by luminol-enhanced chemiluminescence. From this data we conclude that misoprostol produces anti-inflammatory effects in equine leukocytes *in vitro*, paving the way for *in vivo* studies investigating misoprostol as an anti-inflammatory treatment in horses.

E40

DO ENDOCRINE DISRUPTING CHEMICALS PLAY A ROLE IN HORSES WITH EQUINE METABOLIC SYNDROME?. Sian Durward-Akhurst[1], Nichol Schulz[1], Elaine Norton[1], Raymond Geor[2], James Mickelson[1], Molly Mccue[1]. [1]University of Minnesota, MN, USA, [2]Mmassey University, Palmerston North, New Zealand

Equine Metabolic Syndrome (EMS) is characterized by abnormalities in insulin regulation, increased adiposity and laminitis. In a large across-breed study (610 individuals) of 11 morphometric and biochemical metabolic traits, our lab has demonstrated that 51–77% of the phenotypic variability is due to individual factors including age, breed, sex and genetics and 23–49% of the phenotypic variability is the result of shared environment. Despite up to 49% of the variability in EMS phenotype being related to shared environment; only 4–18% of this variability is explained by diet, amount and intensity of exercise and season, suggesting that other environmental factors play a role in EMS development. Recent work has identified associations between Endocrine Disrupting Chemicals (EDCs) and human metabolic syndrome and other endocrine abnormalities. Our preliminary data demonstrated that horses from farms within 30 miles of EDC disposal sites (EPA "Superfund sites") were more likely to have had laminitis ($P = 0.002$) and had higher post oral sugar challenge insulin concentrations (OST INS) ($P = 0.00005$), suggesting EDC exposure is an EMS risk factor. The objective of this study was to determine if plasma EDC concentration is correlated to metabolic measurements. Plasma EDC concentrations were measured using the CALUX-DR bioassay in 158 Morgans and 137 Welsh Ponies from 32 farms. Grams fat extracted from plasma and sex (female) were negatively correlated with EDC concentration. EDC concentration was positively associated with OST INS ($P = 0.03$). The results suggest that some of the unexplained environmental variance identified in individuals with EMS is due to exposure to EDCs.

E41

EVALUATION OF AN ORAL SUGAR TEST FOR DYNAMIC ASSESSMENT OF FIVE EQUINE BREEDS' INSULIN RESPONSE/SENSITIVITY. Jane Manfredi[1], Raymond Geor[2], Patty Sue Weber[1], Bo Norby[1], L Jill McCutcheon[2]. [1]Michigan State University, East Lansing, MI, USA, [2]Massey University, Palmerston North, New Zealand

Veterinarians have identified equine metabolic syndrome (EMS) as the most common cause of laminitis within equine practice. An

oral sugar test (OST) has been used clinically to identify "at risk" horses although it has not been rigorously compared to a "gold standard" frequently sampled intravenous glucose tolerance test (FSIGTT) in a large number of horses or across different breeds.

Eighty-nine horses/ponies of five different breeds (Quarter Horses [QH], Arabians, Morgans, Welsh Ponies, and Thoroughbreds) had both an OST (0.25 mL/kg Karo syrup) and an FSIGTT performed at least 24 hours apart. Optimal time for sampling was determined with a repeated measures ANOVA. A ROC curve used to identify an optimal threshold insulin value for insulin resistant (IR) equids (insulin sensitivity [SI] < 1). Spearman correlations were utilized to examine possible correlations between OST outcome measures (area under the curve for insulin [AUCi] and peak insulin concentration) and SI and the acute insulin response to glucose (AIRg) (the latter two determined by Minimal Model analysis of the FSIGTT).

IR horses had OST insulin but not glucose concentrations from 60–120 minutes that were significantly higher than insulin sensitive horses. In this cohort, a single time point insulin concentration of ≥30.2 □IU/mL was indicative of insulin resistance. Moderate correlations (*rho* = −0.6) to SI and strong correlations to AIRg (*rho*≥0.74) were evident for AUCi and peak insulin concentration. Weak correlations existed between glucose concentrations and SI and AIRg. QHs have significantly lower OST AUCi than all breeds except Morgans.

Different from what is currently recommended in the literature, we advise measuring the insulin concentration of a single blood sample at 60, 75, 90, or 120 minutes post–Karo syrup administration and using a ≥ 30.2 IU/mL threshold. Practitioners should be aware that breed may play a role in insulin concentrations that are achieved during the OST.

E42

BASAL INSULIN CONCENTRATION IN COMPETITION DRAFT HORSES. Harold Schott[1], Lisanne Gallant[1], Elizabeth Tadros[1], Sarah Jacob[1], Jane Woodrow[2], Melissa Hines[2], Susan Ewart[1]. [1]Michigan State University, East Lansing, MI, USA, [2]University of Tennessee, Knoxville, TN, USA

Measurement of basal insulin (INS) concentration is currently advocated as the endocrine test of choice for screening horses for insulin dysregulation. Values >20 μU/mL after overnight fasting and >50 μU/mL in hay fed horses are considered supportive of insulin dysregulation. Although it is well recognized that INS increases with body condition (fat mass), recent evidence suggests that there may also be age and breed differences with regard to basal insulin concentrations. However, little data exists for draft breeds. Consequently, we tested the hypothesis that INS differs in various breeds of draft horses, independent of age and body condition. Age was obtained from owners/trainers and body condition score (BCS) was assessed and basal (resting but not fasting) INS was measured in 164 draft horses participating in an international competition, including 48 Belgians, 52 Clydesdales, and 64 Percherons. INS was measured using a radioimmunoassay validated for equine serum (with average intra- and inter-assays CVs of 3.4% & 6.9% and 4.7% & 7.2% for low and high equine control sera, respectively). None of the horses examined had a history or current evidence of laminitis. Age, BCS, and INS were not normally distributed; thus, median values and 25% and 75% confidence intervals were determined (Table). Data were analyzed using ANOVA on ranks, Chi square analysis, and Spearman rank order correlation.

	Belgian	Clydesdale	Percheron	P value
Age	5 (3-7.5)	6 (3-8.5)	6 (4-9.5)	0.33
BCS	6 (6-7)[a]	6 (5-6)[b]	6 (6-7)[a,b]	**0.007**
Insulin	37.1 (29.5-72.0)	36.7 (23.4-53.5)	38.0 (29.2-62.5)	0.30
# (%) >50 μU/mL	17 (35%)	15 (29%)	39 (47%)	0.07

Median age was not different between horses sampled for each breed. Although the Clydesdale breed had a lower BCS as compared to the Belgian breed, there was no difference in median INS between breeds or the proportion of horses with an elevated INS (>50 μU/mL). Further, there were significant, albeit weak, positive correlations between age and INS (R = 0.32, $P < 0.01$), BCS and INS (R = 0.19, $P < 0.02$), and age and BCS (R = 0.36, $P < 0.01$). However, of greatest interest, one-third to nearly half (varying with breed) of all horses tested had an elevated INS, supportive of insulin dysregulation despite horses being trained for competition and without evidence of laminitis. In conclusion, this convenience sampling study of draft horses provides support that INS may vary between light breeds and draft breeds and that breed needs to be considered when interpreting INS results.

E43

MATRIX METALLOPROTEINASE-2 AND -9 LEVELS IN HORSES WITH EXPERIMENTAL SMALL COLON INTRALUMINAL OBSTRUCTION. Kamila Gravena[1], Beatriz de Assis Pimenta[1], Lina Maria Wehrle Gomide[2], Vinicius Athaydes Canello[1], Nara Saraiva Bernardi[1], Amanda Festa Sabes[1], Guilherme Dias Melo[2], Paulo Ricardo Dell'Armelina Rocha[2], Gisele Fabrino Machado[2], José Corrêa Lacerda-Neto[1]. [1]FCAV – University of Estadual Paulista – Unesp, Jaboticabal, São Paulo, Brazil, [2]FMVA – University of Estadual Paulista, Araçatuba, São Paulo, Brazil

Among different causes of colic, intestinal obstruction has been identified as the major cause of hospitalization and death in horses around the world. In small colon occurs mainly due to fecalomas or enteroliths. During ischemic process, decreases in oxygen tension inside the cell occur, reducing the generation of adenosine triphosphate (ATP) and resulting in cellular homeostasis disorders. Calcium accumulation inside the cell produces significant increase in the phospholipase A_2 activity, which directly damage cell membrane through increased production of arachidonic acid, lysophosphatidylcholine and platelet aggregating factor. The platelet aggregation factor is a potent inflammatory mediator, which has an important effect on vascular permeability and on neutrophil activity. Neutrophils adhesion to endothelium may also cause damage to endothelial cells, thus amplifying the reaction. This occurs due to release of reactive oxygen species (ROS), proteoglycans enzymes such as matrix metalloproteinases (MMPs) and elastase from lysosomes during cell degeneration. Therefore, the aim of this study was to evaluate neutrophil migration and matrix metalloproteinase-2 and -9 activity in horses with experimental intraluminal obstruction.

For this purpose, eight healthy adult mixed-breed horses were subjected to small colon distension using a surgically implanted latex ball in the lumen (Ethical approval: CEBEA- Protocol #007568-09). Blood and peritoneal fluid samples were obtained before intestinal distension (M0), after 4 hours of distension (M4 - when the ball was deflated and removed) and 72 hours after decompression (M72). Twelve hours after the ball removal was also collected peritoneal fluid sample for neutrophils count. Data were submitted to Friedman's test followed by post-hoc Dunn's Multiple Comparison Test.

This model induced alterations in neutrophils migration into peritoneal fluid, the concentration ($x10^3/\mu L$) at M12 and M72 was significantly higher when compared to M0 (M0 = 0.55 ± 0.14; M4 = 3.21 ± 1.24; M12 = 133.18 ± 35.80; M72 = 35.07 ± 6.08). There was also alterations in plasma proenzyme form of MMP-2 (pro-MMP-2) at M4 and in peritoneal fluid pro-MMP-2 at M72 and pro-MMP-9 was significantly increase in M4 when compared to M0. Mean (± standard error) in arbitrary units of plasma pro-MMP-2 was M0 = 94.25 ± 6.14; M4 = 63.72 ± 4.57 and M72 = 89.52 ± 6.70. Peritoneal fluid Pro-MMP-2 was M0 = 53.07 ± 10.90; M4 = 89.42 ± 6.23 and M72 = 128.13 ± 10.86. Plasma Pro-MMP-9 was M0 = 46.75 ± 6.77; M4 = 50.65 ± 6.45 and M72 = 44.92 ± 4.74. Peritoneal fluid Pro-MMP-9 was M0 = 16.70 ± 5.10; M4 = 155.77 ± 5.77 and M72 = 90.21 ± 13.14.

Knowing that multiple proteinases are present in the granules of neutrophils, it was conclude that increase in pro-enzyme form after small colon decompression may be associated with neutrophil migration.

E44

EFFECTS OF COLLAGEN HYDROLYSATES ON EQUINE GASTRIC ULCER SCORES AND GASTRIC JUICE PH IN HORSES. Michael Keowen[1], Pilar Camacho-Luna[1], Lisa Micheau[2], Frank Garza Jr1?^!"number(boolean(following-ibling::degrees))",[1], Jos Olijve[3], Brian Lamp[3], Chin-Chi Liu[1], Frank Andrews[1]. [1]Equine Health Studies Program, Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA, USA, [2]École Nationale Vétérinaire, Toulouse, France, [3]Darling Ingredients International Holding B.V., Son, The Netherlands

Nonglandular (NG) gastric ulcers, as part of the equine gastric ulcer syndrome (EGUS), are common in performance horses and the current FDA-approved pharmaceutical agent, omeprazole is effective in treatment, but expensive and alters gastric juice pH. Recently, there has been interest in natural feed supplements that improve stomach health and can be used in horses competing in drug-free competitions. The purpose of this study was to evaluate the effect of a feed supplement containing porcine collagen hydrolysates on gastric ulcer scores and gastric juice pH in stall-confined horses, treated with omeprazole and undergoing intermittent feeding. Ten Thoroughbreds were used in a two-period crossover study. The supplement (CHL; collagen hydrolysates, 45 grams) was mixed with sweet feed (Omelene 100, Purina Animal Nutrition, LLC, Gray Summit, MO) or sweet feed only (Control group) twice daily for two 56-day periods. From days 14 to 28, both treatment groups were administered omeprazole (OME; 4.0 mg/kg, P.O., Q.D.; Gastrogard®, Merial Limited, Duluth, GA) and from days 42 to 49, horses underwent an alternating feed-deprivation period, to induce or worsen ulcers.[1] Gastroscopies were performed on all horses on days −1 (before treatment) and days14, 28, 42, 49 and 56. Gastric juice was aspirated pH measured at each scoping. Nonglandular gastric ulcer number (NGN) and severity (NGS) scores were assigned at each examination by the PI (FMA), who was masked to treatment. Data were analyzed using an ANOVA for repeated measures and when significant differences were found in the main model, a post-hoc Tukey's test was used to determine significant differences of $P < 0.05$. The CHL-supplement in powder form, mixed with the grain, was readily consumed by all horses and no adverse effects were seen. Mean NGN and NGS gastric ulcer scores were lower at each gastroscopy examination and a significant treatment effect was seen on day 56 of the study. In addition, gastric ulcer scores were significantly lower in both groups on day 28, compared to the other days, due to omeprazole treatment. By day 42, two weeks after omeprazole was discontinued, ulcers reoccurred in both groups of horses. On day 49, gastric ulcer scores were significantly increased due to intermittent feeding, but no treatment effect was observed. Gastric juice pH values were low and variable throughout the trial period, except on day 28 when pH was significantly higher in CHL-supplement treated group compared to control group. Omeprazole treatment was effective in increasing gastric juice pH and significantly decreasing gastric ulcers scores. In addition, the collagen hydrolysate supplement significantly increased gastric juice pH while horses were on omeprazole treatment.

Conclusion: Collagen hydrolysates ameliorated the severity of gastric ulcers in stall-confined horses undergoing feed stress after 56 days of feeding.

[1]Murray MJ and Eichorn ES. (1996) Am J Vet Res. 57 (11):1599–1603.

E45

SAFETY, HUMORAL IMMUNE RESPONSE AND FECAL SHEDDING OF MODIFIED-LIVE BOVINE CORONAVIRUS VACCINES GIVEN TO HORSES. James Prutton[1], Samantha Mapes[2], Nicola Pusterla[2]. [1]Liphook Equine Hospital, Liphook, UK, [2]University of California, Davis, USA

Equine coronavirus (ECoV) is considered an emerging enteric virus with reported morbidity rates ranging from 10–83% and a fatality rates ranging from 7–27% in adult horses.

The objectives of the study were to assess the safety, humoral response and viral shedding of a commercially available modified-live bovine coronavirus (BCoV) vaccine given to healthy adult horses. Twelve confirmed healthy adult horses were vaccinated twice, 3 weeks apart, either orally, intra-nasally or intra-rectally. Two healthy unvaccinated horses served as sentinel controls. Following each vaccine administration, horses were monitored daily for physical abnormalities and onset and duration of BCoV shedding determined by quantitative PCR (qPCR) in nasal secretions and feces. Whole blood was collected every 3 weeks to determine BCoV-specific antibodies.

With the exception of transient and self-limiting changes in fecal character observed in 7 vaccinated and 1 control horse, no additional abnormal clinical findings were found in the study horses. Following the first and second vaccine administration, two and one horse, respectively, tested qPCR-positive for BCoV in nasal secretions 1 d post intra-nasal vaccination. No vaccinated horses tested qPCR positive for BCoV in feces following each vaccine administration. One of the two horses that shed BCoV seroconverted to BCoV after the first vaccine administration and an additional 2 vaccinated horses (oral and intra-rectal) seroconverted to BCoV after the second vaccine administration.

In conclusion, the results show that the modified-live BCoV is safe to administer to horses via various routes, causes minimal shedding and detectable antibodies to BCoV in 25% of the vaccinates.

E46

DO HORSES WITH EQUINE METABOLIC SYNDROME HAVE REDUCED IMMUNE RESPONSES TO VACCINATION?. Sarah Elzinga, Stephanie Reedy, Day Barker, Tom Chambers, Amanda Adams. University of Kentucky, Lexington, KY, USA

Recent reports indicate the percentage of overweight horses may range anywhere from 20.6–41%. Obesity is a key component of Equine Metabolic Syndrome (EMS), which is typically defined by criteria set in a 2010 ACVIM consensus statement as; regional (neck crest, rump, etc.) or general obesity, hyperinsulinemia or insulin resistance, and a history of or predisposition towards laminitis. Obese humans and diet-induced obese mice have been shown to have reduced immune responses to vaccination. However, the immune response of EMS horses to vaccination is unknown. Therefore, the objective of this study was to determine the effect of EMS on immune responses to routine vaccination. We hypothesized that, similar to other species, EMS horses would have a reduced response to a routine influenza vaccine compared to non-EMS age matched controls.

To establish this, 25 horses from a pre-existing herd were used for this study. Of these, 13 were classified as EMS by the 2010 AVCIM consensus statement criteria, and 12 were non-EMS age matched controls. Within each group, 4 served as saline controls and the remaining horses served as vaccinates. A low-dose dexamethasone suppression test and TRH stimulation test were performed on all horses prior to the start of the study to insure no horses tested positive for pituitary pars intermedia dysfunction (PPID). Venous blood was drawn weekly for 6 consecutive weeks, with vaccination or saline administered intramuscularly on weeks 0 and 3. Horses were vaccinated with an inactivated influenza vaccine (Fluvac Innovator®, Zoetis). Blood samples were used to determine humoral and cell mediated immune responses. Humoral immune responses against KY/97 were measured by hemagglutination assay for total Ig antibody responses and ELISA for IgGa, IgGb, and IgGt antibody isotype responses. Cell mediated immune measures were determined via PBMC isolation, EIV-stimulation, and subsequent flow cytometry and RT-PCR analysis for cytokine protein and gene expression, respectively. Humoral immune data were log transformed and analyzed using a repeated measures design with protected LSD, adjusting for age. All analyses were completed using PROC MIXED, SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Results are expressed as least square means ± standard error of the mean. Data were considered statistically significant when $P \leq 0.05$.

Overall, all horses responded to vaccination with a significant increase ($P < 0.05$) in HI titers following initial vaccination compared to saline controls. Significant increases ($P < 0.001$) in IgGa and IgGb, but not IgGt EIV specific antibody responses to vaccination were also observed. EMS did not significantly affect ($P > 0.05$) humoral immune responses as measured by HI titers or IgG isotypes to influenza vaccination, however more work is needed to explore possible differences in CMI responses. It is possible that obesity as compared to insulin resistance plays a larger role in modulating the immune process considering that while EMS horses were insulin resistant and had significantly greater regional obesity

(cresty neck score) compared to non-EMS controls, they had moderate general obesity (body condition score), which may have not been sufficient to impact immune response to vaccination.

E47
IMMUNOLOGICAL COMPARISONS OF AGED HORSES WITH VERSUS WITHOUT PITUITARY PARS INTERMEDIA DYSFUNCTION. Melissa H. Siard, Virgina D. Barker, Stephanie E. Reedy, Amanda A. Adams. Gluck Equine Research Center, Lexington, KY, USA

Pituitary pars intermedia dysfunction (PPID) is an endocrinopathy affecting 15–30% of the aged equid population. PPID is characterized by various clinical manifestations, the hallmark of which is hypertrichosis. Additionally, PPID horses exhibit immunosuppression, making them more susceptible to opportunistic infections. Our lab has established that aged horses exhibit both immunosenescence and inflamm-aging, the low-grade chronic inflammation occurring systemically with aging. To determine whether these age-related changes in cell-mediated immune responses may be affected by the presence of PPID, peripheral blood mononuclear cell (PBMC) proliferation and cytokine production were compared in age-matched PPID and non-PPID horses. PPID status was determined using thyrotropin releasing hormone (TRH) stimulation testing.

Sixteen aged horses (18–27 yrs), including n = 8 PPID horses (mean = 24.5 ± 2.2 yrs) and n = 8 non-PPID horses (mean = 23.1 ± 3.1 yrs) of mixed breeds and sex, were sampled to determine immune function. Heparinized blood was collected aseptically, and PBMCs were isolated, stimulated with Phorbol 12-myristate 13-acetate (PMA), and stained intracellularly for pro-inflammatory cytokines interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα). Flow cytometry was performed to determine the percent of lymphocytes producing IFNγ and TNFα. Real-time polymerase chain reaction (RT-PCR) was used to determine PBMC gene expression of cytokines interleukin (IL)-2, IL-4, IL-6, IFNγ, and TNFα. PBMC proliferation was also determined using carboxyfluorescein succinimidyl ester (CFSE) staining and stimulation with various amount of concanavalin A (Con A; 2.5, 5, and 10 □L/mL). TRH stimulation testing was performed, in which adrenocorticotropin hormone (ACTH) levels were measured in plasma 10 minutes post (T-10) intravenous administration of TRH (1 mg/mL saline/horse).

Data was analyzed using the statistical software program SigmaPlot version 12.3. To analyze flow cytometry and RT-PCR data for differences between PPID and non-PPID horses, t-tests were performed. To analyze lymphocyte proliferation data at various concentrations of Con A stimulation, two-way analysis of variance with repeated measures was performed. No significant differences between PPID and non-PPID horses were found for any of the immune measures examined including flow cytometry, RT-PCR, and proliferation data ($P > 0.05$). These results indicate that the immunosuppression associated with PPID does not appear to be due to differences in PBMC proliferation or cytokine production when comparing aged PPID horses with aged non-PPID horses. Thus, more research is warranted to further understand the immunological mechanisms responsible for immunosuppression and susceptibility to opportunistic infections in the PPID horse.

E48
***CORYNEBACTERIUM PSEUDOTUBERCULOSIS* ANTIBODY DETECTION IN HORSES: SYNERGISTIC HEMOLYSIS INHIBITION TEST AND SMALL RUMINANT ELISA COMPARISON.** Marta Barba[1], Anne A. Wooldridge[1], Robert Glass[2], Thomas Passler[1], Allison J. Stewart[1]. [1]Auburn University, Auburn, AL, USA, [2]Pan American Veterinary Laboratories, Hutto, TX, USA

False positive results may occur when testing horses for antibodies against *Corynebacterium pseudotuberculosis* by synergistic hemolysis inhibition (SHI). In small ruminants, ELISA tests based on exotoxin and cell wall antigens have greater accuracy than SHI in diagnosis of caseous lymphadenitis. The purpose of this study was to compare the detection of *C. pseudotuberculosis* antibodies in equine serum by SHI and a small ruminant ELISA test that uses exotoxin and cell wall antigens. Sera from 7 ponies experimentally infected with *C. pseudotuberculosis* were analyzed by both tests. Correlation and agreement were calculated by Pearson and Kappa coefficients, respectively. Receiver operating characteristic analysis was used to obtain the optimal cut-off value for the calculated ELISA score (Patient optical density/Negative control optical density x 100). Antigen reactivity to the sera was evaluated by immunoblotting. When SHI titers ≥1:128 were considered a positive result, the optimal ELISA score cut-off to determine positive status was 106%, with 73% sensitivity and 72% specificity with respect to SHI. Correlation between both tests was strong [r = 0.904; (95%CI 0.472–0.986, P = 0.005)]. Agreement in determining positive status was poor (Kappa=0.439; (95%CI 0.226–0.652). Correlation and agreement were strong in 3/7 ponies, but weak in 4/7. Immunoblot showed a band 13.4 times more intense in infected compared to pre-infected sera corresponding with the exotoxin antigen, but only non-specific reactivity with the cell wall antigen. The use of this ELISA test in horses is not recommended. Development of an ELISA test with specific antigens from *C. pseudotuberculosis* biovar *equi* is needed.

E49
EFFECTS OF ERYTHROMYCIN ON RESPONSES INDUCED IN FOALS BY INTRAVENOUS EPINEPHRINE. Amy Stieler[1], Chris Sanchez[2], Martha Mallicote[2], Ashley Coxen[2], Jim Burrow[2], Rob MacKay[2]. [1]University of Georgia, Athens, GA, USA, [2]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, USA

Macrolide-induced anhidrosis is a concern in foals treated for *Rhodococcus equi* pneumonia. Because sweating in horses is caused by activation of β2-adrenergic receptors on sweat glands, it was of interest to evaluate responses to IV epinephrine in foals given erythromycin. Four pony-cross foals were treated for 3 days prior to epinephrine infusion with either erythromycin (25 mg/kg orally, 3 times daily) or a control preparation according to a 2-period randomized masked design. Epinephrine was given by CRI over 10 minutes at 0.5 μg/kg/minute and the following data were collected before and at intervals for 180 minutes after onset of infusion: sweat (absorbed into absorbent pads), heart and respiratory rates, systemic arterial BP, pupil size, PCV, plasma protein, and blood glucose concentration. Cardiac rhythm was monitored continuously by ECG. Univariate and 2-factor repeated measures ANOVA were used to analyze data. Friedman tests were used for data sets that failed assumptions for ANOVA. *Post hoc* pairwise analyses were performed after ANOVA indicated significant ($P < 0.05$) effect. In foals given either treatment, there were significant ($P < 0.05$) effects of epinephrine on heart rate, pupil size, PCV and blood glucose. Sweating and arterial BP also increased significantly in control- but not erythromycin-treated foals. Significant treatment x time interaction was found only for sweating; pairwise comparisons were significant at every point after time 0. These results suggest that erythromycin does not cause generalized suppression of α- and β-adrenergic responses. Anhidrosis may be a tissue-specific receptor or post-receptor effect of erythromycin and other macrolides.

E50
EQUINE VECTOR-BORNE DISEASES DETERMINED BY SEROLOGICAL AND MOLECULAR DIAGNSOTICS. Barbara Qurollo[1], Barbara Hegarty[1], Jeffrey Tyrrell[1], Edward Breitschwerdt[1], Susan Tornquist[2], Kathryn Schlaich[2], Jennifer Kelsey[2], Melissa Beall[3], Ramaswamy Chandrashekar[3]. [1]Intracelluar Pathogens Research Laboratory, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA, [2]Oregon State University, College of Veterinary Medicine, Corvallis, OR, USA, [3]IDEXX Laboratories, Inc., Westbrook, Maine, USA

Vector-borne disease impacts the health and welfare of horses, worldwide. Recognized vector-borne diseases among horses

include Anaplasmosis (*Anaplasma phagocytophilum*), Lyme Disease (*Borrelia burgdorferi*), Potomac Horse Fever (*Neorickettsia risticii*) and Piroplasmosis (*Babesia caballi* /*Theleria equi*). Additionally, *Bartonella*, *Ehrlichia*, *Rickettsia*, and hemotropic *Mycoplasma* species have been detected in horses with clinical disease spectrums ranging from nonclinical to fatal. The goal of this study is to investigate serological and molecular (PCR) tests that will detect vector-borne disease (VBD) pathogens in horses and apply a broad diagnostic panel to gain a more definitive picture of vector-borne equine pathogens, including novel species and co-infections. Three populations of horses were used to test serological and molecular diagnostic tests. Group I (n = 16): EDTA-whole blood (WB) and serum (S) sets from clinically healthy horses pastured in North Carolina with no clinical suspicion of VBD; Group II (n = 20): WB and S sets from horses submitted for diagnostic testing to the VBDDL, where presumably the clinician suspected vector-borne disease; Group III (n = 15): WB from horses in Mérida, Nicaragua with a high degree of tick exposure. Serologic and PCR testing were performed retrospectively. DNA was extracted from WB, and qPCR assays were performed to test samples for infection by bacterial species within the following genera: *Anaplasma, Babesia, Bartonella, Ehrlichia, Mycoplasma, Neorickettsia, Rickettsia* and *Theilera*. For samples with positive results, the infectious agent was speciated by species-specific qPCR assay of the sample and/or DNA sequencing of the PCR amplicon. Seroreactivity to *Babesia*, *Bartonella*, *Neorickettsia* and *Rickettsia* antigens was determined using IFA, and seroreactivity to 3 *Ehrlichia species*, 2 *Anaplasma species* and *B. burgdorferi* antigens was determined using both a commercially available SNAP®4DX®Plus ELISA and a research-based SNAP® MA ELISA. The combined use of serological and molecular assays in this study gave evidence of infection (by PCR) or exposure (by seroreactivity) to *Anaplasma*, *Babesia*, *Bartonella*, *Borrelia*, *Ehrlichia spp*., *Theileria,* and *Rickettsia* spp. None of the samples contained DNA from Mycoplasma spp. and two samples were positive for infection by *Rickettsia felis*, constituting the first reported instances of equine infection with *R. felis* detected by PCR. A novel Equine *Ehrlichia* species, reported in horses from Nicaragua, was detected in additional Nicaraguan horse samples from this study by both PCR, IFA and ELISA. This study highlights the wide-range of exposure to and infection with vector-borne pathogens in horses.

E51

SELENIUM DEFICIENCY ASSOCIATED WITH THE DEATHS OF FIFTEEN ADULT HORSES. Andrew Allen, Debra Sellon, Fairfield Bain, Danielle Nelson. Washington State University, Pullman, WA, USA

Nutritional myopathy due to selenium (Se) deficiency typically presents itself in young rapidly growing calves, lambs, kids, and foals. Reports of myodegeneration secondary to selenium deficiency in adult horses are less frequent. We investigated 2 herds with nutritional myopathy with generalized skeletal muscle degeneration and significant death loss in adult horses. In 2014, two American Paint horses from the same farm presented to our diagnostic laboratory for post mortem evaluation. Significant necropsy and histopathology findings included myonecrosis of the cardiac and skeletal muscles, widespread subcutaneous edema, pleural effusion and ulcerative glossitis. An on farm investigation was performed where it was discovered that 13 horses, including the two presented horses, had died on this farm with similar clinical signs over the last year. Complete blood counts and serum chemistry panels were performed on 4 of the 12 remaining horses and physical examinations were performed on the entire herd. All four horses had elevated creatinine kinase activity (CK) and aspartate aminotransferase (AST) levels, the herd had an overall low body condition score, 3 horses had elevated heart rates, and one horse had pitting ventral edema. All of the horses had extremely low blood selenium levels, no box elder trees were found on the farm and gastric contents of the dead horses and feed supplements were found to be free of ionophores. An on farm echocardiographic study was performed 6 months post initial investigation on three of the most severely affected horses. All findings appeared within normal limits. In 2015 a second investigation of two dead horses revealed a very similar scenario and herd wide myodegeneration secondary to selenium deficiency. Although unusual, selenium deficiency should be considered a rule out with adult horses showing signs of ventral edema, weakness, and abrupt death.

E52

EFFECTS OF ALFA-LOX FORAGE® ON BLOOD GLUCOSE AND INSULIN ACTIVITY AFTER GRAIN FEEDING IN HORSES. Frank Garza Jr[1], Pilar Camacho-Luna[1], Kelsey Bailey[1], Isabelle Nesen[2], Michael Keowen[1], Liu Chin-Chi[1], Frank Andrews[1]. [1]Equine Health Studies Program, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA, USA, [2]Ecole Nationale Veterinaire, Toulouse, France

Processed concentrate feeds contain a high concentration of sugars and in some cases non-structural carbohydrates (NSC) exceed 35%. These feeds are commonly fed to stall-confined horses, especially those in competition. Grains, high in NSC, can overwhelm glucose receptors in the proximal small intestine and increase delivery of sugars to the hindgut leading to fermentation and colonic acidosis. Feed supplements, that might enhance glucose absorption in the small intestine, would provide more energy substrate for exercise and decrease delivery of sugars to the hindgut. The purpose of this study was to determine effects of a feed supplement (ALF; Alfa-Lox Forage, Triple Crown Nutrition, Inc., Wayzata, MN), containing chopped alfalfa hay, mannanoligosaccharides, Omega 3 Fatty Acids, and L-Carnitine, top-dressed on grain, on plasma glucose concentration and serum insulin activity in horses before and after grain feeding. Eight healthy Thoroughbreds (7 geldings and 1 mare; ages 2–12 yrs.) were used in a 2-period cross over study, where horses were fed ALF (2.0 lbs. [0.91 Kg], twice daily) top dressed on a sweet feed grain (2.5 Kg; Omelene® 100, Purina Animal Nutrition, LLC., Grey Summit, MO) or control (grain alone) for 56 days, as part of another study. On Days 41 and 48, after withdrawing feed overnight, blood samples were drawn at 8 am, before the morning grain feeding, with or without ALF, and then again 2 hours after ALF was top-dressed on grain (treated) or grain alone (control) was fed. Plasma and serum were separated within 30 minutes of collection and samples frozen at −70°C for shipment to the Diagnostic Center for Population Animal Health (DCPAH; Michigan State University, East Lansing, MI). Plasma glucose and serum insulin were analyzed using an automated chemistry analyzer. Data were analyzed statistically using an ANOVA (PROC Mixed; SAS, Cary, NC) for repeated measures and when significant differences ($P < 0.05$) were found in the main model, post-hoc t-tests were performed to determine significant differences. Alfa-Lox Forage® top-dressed on grain was readily eaten by all horses, and did not result in any adverse effects. When period data were pooled, on day 42, fasting mean (SEM) serum insulin activity significantly increased from 65.1 (3.5) pmol/L and 61.9 (5.1) pmol/L in control and ALF-treated horses, respectively, to 126.3 (24.2) pmol/L and 203.1 (30.1) pmol/L, respectively, 2 hours after feeding sweet feed. In addition, on day 48, mean serum insulin activity significantly increased from mean (SEM) serum insulin concentrations of 76.3 (4.3) pmol/L and 69.9 (3.8) pmol/L in control and ALF-treated horses, respectively to 194.6 pmol/L and 290.0 pmol/L, respectively, two hours after feeding sweet feed. Mean serum insulin activity was significantly higher in ALF-treated horses compared to control horses 2 hours after feeding sweet feed. Mean plasma glucose concentrations did not significantly increase 2 hours after feeding. Alfa-Lox® Forage top-dressed on grain resulted in higher blood insulin values, but not significantly increased blood glucose concentrations 2 hours after feeding sweet feed. Alfa-Lox Forage® resulted in increased absorption of sugars from the small intestine and more efficient utilization for glucose for energy, which likely decreased the amount of sugars delivered to the large colon.

E53

VENOUS BLOOD GAS, ELECTROLYTE AND METABOLITE FINDINGS IN HEALTHY NEONATAL FOALS RECEIVING SODIUM LACTATE INFUSIONS. Breanna Sheahan[1], Pamela Wilkins[1], Ray Boston[2], Kara Lascola[1], Igor Canisso[1], Kevin Kline[3], Scott Austin[1], Levent Dirikolu[1], Brian Aldridge[1]. [1]University of Illinois College of Veterinary Medicine, Urbana, IL, USA, [2]University of Pennsylvania School of Veterinary Medicine, New Bolton Center, Kennett Square, USA, [3]University of Illinois Department of Animal Science, Urbana, IL, USA

Exogenous sodium L-lactate (LAC) (1 mmol/kg BWT over 15 minutes) was administered to 6 healthy neonatal foals to determine LAC clearance variables at 12 hours (12Hr) and again at 60 hours (60Hr) of age. We hypothesized there would be differences in some venous blood gas (VBG) variables associated with both age (12Hr versus 60Hr) and sample time.

Venous blood was collected into heparinized syringes via a catheter in the right jugular vein prior to lactate infusion for blood gas (BG), electrolyte and metabolite analyses and repeated at 0, 15, and 60 minutes after infusion completion using the NOVA Critical Care Express. Statistical comparisons were by Friedman analysis between time points of the same infusion, and Wilcoxon Rank Sum between age groups, $P < 0.05$. Two-way ANOVA by general linear model was also used to assess differences and interactions associated with age at testing and sample time.

There were no appreciable deleterious clinical effects of infusion. Sodium LAC infusion in normal neonatal foals resulted in clinically unimportant changes in VBG measures. HCT, O2CT, Cl-, LAC, creatinine and BUN were significantly greater in 12Hr foals. pH, O2SAT, HCO3-, BEecf, K+ and Ca++ were significantly greater in 60Hr foals. Only LAC was different related to sample time, being largest at end infusion. No differences were found for pCO2, pO2, Mg++, glucose, TCO2, Gap, or osmolarity.

Infusion of sodium LAC does not importantly impact VBG, electrolyte or metabolite measures in healthy 12Hr and 60Hr foals, with the identified differences primarily due to age.

E54

GROWTH AND FUNCTION OF EQUINE ENDOTHELIAL PROGENITOR CELLS LABELED WITH SEMICONDUCTOR QUANTUM DOTS. Randolph L. Winter[1], Wen Seeto[2], Yuan Tian[2], Fred J. Caldwell[1], Elizabeth A. Lipke[2], Anne. A Wooldridge[1]. [1]Department of Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn, AL, USA, [2]Department of Chemical Engineering, Auburn University, Auburn, AL, USA

Endothelial progenitor cells (EPCs) contribute to neovascularization and vascular repair in vivo and are attractive for clinical use in ischemic disease. EPC tracking is essential to determine engraftment after administration. Semiconductor quantum dots (QD) are promising for cell labeling due to ease of uptake by many cell lines and their continued presence after many cell generations. The purpose of this study was to evaluate function and growth of equine EPCs after QD label.

Equine EPCs were incubated with QD (2 – 20 nM) for 12-hr or 24-hr, and intensity of label was assessed with fluorescent microscopy. Cell proliferation of EPCs labeled with QD for optimum time and concentration was then assessed by comparing the number of cell doublings per day (NCD) and the population doubling time (PDT) in labeled and unlabeled cells. EPC function was assessed by comparing uptake of acetylated low-density lipoprotein (DiO-Ac-LDL) and in vitro tubule formation in labeled and unlabeled cells.

Equine EPCs readily labeled with QD, showing maximum fluorescence using 20 nM QD, with a 24-hr incubation time. NCD and PDT were unchanged with QD label ($P = 0.95$, $P = 0.99$). Uptake of DiO-Ac-LDL by EPCs was not affected by the presence of QD label (97.9% labeled cells; 97.0% unlabeled cells). Tubule formation on Matrigel was not affected by the presence of QD label.

Equine EPCs are effectively labeled with QD, and QD concentrations up to 20 nM do not affect cell growth or function. The use of QD labeling with equine EPCs may help track engraftment of EPCs in clinical applications.

F01

PREVALENCE OF COAGULASE-NEGATIVE STAPHYLOCOCCI SPECIES IN INTRAMAMMARY INFECTION IN DAIRY GOATS. Véronique Bernier Gosselin, Pamela R.F. Adkins, John R. Middleton. University of Missouri, Columbia, MO, USA

Coagulase negative staphylococci (CNS) are the most prevalent pathogens isolated from intramammary infection in goats, with more than 15 different species being identified in goat milk. Unlike cows, the relationship between CNS infection, somatic cell count and milk loss in goats is less clear. The conflicting results between studies on CNS mastitis in goats may be explained by the variation between studies in regard to distribution of CNS species, varying pathogenicity between species, the presence of confounding factors affecting outcome measures, and variation between studies in regard to speciation methods. Phenotypic speciation was used in most studies; however genotyping is considered more reliable than phenotyping for identification of CNS species in goats. The objective of this study was to estimate the prevalence of species-specific CNS intramammary infections in a large dairy goat herd in Missouri.

Composite milk samples were aseptically collected from all 940 lactating goats and aerobic bacteriological culture was performed. A culture yielding 100 CFU/mL CNS was considered positive. Isolates from milk samples yielding a single colony morphology identified as CNS were used to prepare lysates for PCR amplification. Species identification was based on PCR amplification and sequencing of either the *rpoB*, *tuf* gene, or 16S rRNA. Sequences were compared with the GenBank database using the NCBI Nucleotide-BLAST algorithm. For *rpoB*, species identification was assigned with $\geq 97\%$ identity and $\geq 5\%$ separation between different species. For *tuf*, species identification was assigned with $\geq 98\%$ identity and $> 0.8\%$ separation between different species. For 16S, species identification was assigned with $\geq 99\%$ identity and $> 0.8\%$ separation between different species. PCR amplification and sequencing of *rpoB*, *tuf* and 16S were performed sequentially until identification was assigned. For instance, *tuf* was performed on isolates for which *rpoB* either yielded unsuccessful amplification, or criterion for identification with sequence analysis were not met, followed by 16S if *tuf* amplification or sequence analysis failed.

From 940 milk samples cultured, 214 yielded growth of CNS of a single colony morphology. Of these 214 isolates, 184 were successfully speciated, of which 157 were identified with *rpoB*, 26 with *tuf*, and 1 with 16S. The remaining 30 isolates were unable to be speciated based on the criteria listed above. Overall, 13 different species were identified (Table 1). Among isolates that could not be assigned an identification, 3 met the criterion for identity but insufficient separation between the species (*S. warneri* and *S. pasteuri*) with both *rpoB* and *tuf* sequencing.

Table 1. Species-specific prevalence among 184 CNS isolates and number of isolates successfully speciated by each gene, per species.

Species	Number of isolates	%	*rpoB*	*tuf*	16S
S. simulans	69	37.5	65	4	0
S. caprae	31	16.8	30	1	0
S. chromogenes	26	14.1	25	0	1
S. epidermidis	19	10.3	17	2	0
S. xylosus	16	8.7	12	4	0
S. equorum	7	3.8	0	7	0
S. cohnii	6	3.3	3	3	0
S. lentus	5	2.7	1	4	0
S. arlettae	1	0.5	1	0	0
S. aureus	1	0.5	1	0	0
S. auricularis	1	0.5	1	0	0
S. gallinarum	1	0.5	1	0	0
S. sciuri	1	0.5	0	1	0
Total	184	100	157	26	0

Typeability of CNS isolates from goat milk in this study using gene sequencing was 86%. Typeability with *rpoB* gene sequencing alone was 73%, which was lower than expected. This may result from underrepresentation of isolates of caprine origin in the reference database. Unsuccessful PCR amplification in a proportion of isolates could reflect imperfect match between the primer and the target sequence. Relative prevalence of different species was similar to that of previous studies using genotyping or phenotyping.

F02

ASSESSMENT OF AN ANTIMICROBIAL-USE ALGORITHM FOR TREATMENT OF DIARRHEA IN DAIRY CALVES. Diego E. Gomez[1], Luis G. Arroyo[2], Laurent Viel[2], J. Scott Weese[1]. [1]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

The objective of this study was to evaluate the impact of implementing an antimicrobial-use algorithm on antimicrobial (AB) treatment and mortality rates of diarrheic calves.

Three commercial dairy farms (F1, F2, F3) were enrolled. Farm records were obtained to collect data about the incidence of diarrhea, AB treatment and mortality rates for the preceding 18 months. Then, an antimicrobial-use algorithm for farm personnel based on the presence of three clinical signs (diarrhea, fever and decreased milk intake) was developed to direct AB therapy in diarrheic calves (<30 day-old) and the same farm data were collected from the same records systems for 5, 2 and 2 months for F1, F2 and F3, respectively.

Treatment records of 1208 (F1), 1051 (F2) and 296 (F3) calves were available for the pre-intervention period. The incidence of diarrhea was 86, 90 and 57%, AB were administered to 99, 95 and 33% of diarrheic calves and mortality rates attributed to diarrhea were 3, 3 and 2% in F1, F2 and F3, respectively. After the algorithm implementation, data were available for 105 (F1), 282 (F2) and 31(F3) calves. Diarrhea developed in 74, 89 and 67% calves on the three farms, but treatment rates were significantly lower; 39, 14 and 0% ($P < 0.0001$), with no impact on mortality (1.3, 0.8 and 0%, respectively, $P > 0.05$).

The use of a simple and practical antimicrobial-use algorithm can provided a significant reduction in the use of antimicrobials, decreasing treatment costs and improving antimicrobial stewardship with no negative impacts on calf health.

F03

PREVALENCE OF BOVINE CORONAVIRUS IN FECES OF HEALTHY AND DIARRHEIC CALVES. Diego E. Gomez[1], Luis G. Arroyo[2], Laurent Viel[2], J. Scott Weese[1]. [1]Department of Pathobiology, Ontario Veterinary College, University of Guelph., Guelph, Ontario, Canada, [2]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Bovine coronavirus (BoCV) is an endemic pathogen that can be associated with enteric disease, especially in calves. This study was conducted to assess the prevalence of BCoV in healthy and diarrheic calves and to determine whether the presence of BCoV in feces is associated with the age and health status of the calf.

This was a prospective case-control study. Fecal samples from 89 diarrheic calves from three different commercial dairy farms were tested for BCoV using a PCR assay. For each study case one age-matched control calf from the same farm was enrolled. Categorical variables were analyzed using X^2 and univariable logistic regression analyses. Multivariable analysis using a mixed effect logistic regression model (with farm as a random effect) was performed to determine significant association between testing positive for BCoV in feces with the age and the presence of diarrhea. Age of the calf was categorized using a cut-off of 10 days.

The age of healthy and diarrheic calves was 8.5 ± 3 and 9.4 ± 3.9 days, respectively ($P = 0.06$). There was a significant difference in the prevalence of BCoV between healthy (54%) and diarrheic (69%) calves ($P = 0.039$). Multivariable logistic regression revealed that calves positive for BCoV were more likely to be older than 10 days of age (OR: 2.2, CI, 1.21 to 3.95; $P = 0.009$) and have diarrhea (OR: 1.73, CI, 1.07 to 2.81; $P = 0.025$).

This study determined that BCoV can be detected in both healthy and diarrheic calves; however, the likelihood of BCoV is higher in diarrheic calves. Future studies investigating whether BCoV strains detected in healthy and diarrheic calves are genetically different are warranted.

F04

USE OF AN ALIVECOR HEART MONITOR FOR HEART RATE AND RHYTHM EVALUATION IN DOMESTIC GOATS. Joe Smith[1], Meera Heller[2], Fauna Smith[2], Mikaela Mueller[3], Jessica Ward[1]. [1]Iowa State University, Ames, IA, USA, [2]University of California Davis, Davis, California, USA, [3]Blue Pearl Veterinary Partners, Seattle, WA, USA

The AliveCor Heart Monitor, a smartphone-based electrocardiogram (ECG) recording device, is a promising new diagnostic tool in veterinary medicine. While this device has been validated for canine, feline, and equine species, its clinical utility in domestic goats has not yet been evaluated.

Eight healthy adult dairy breed and eight healthy adult meat breed goats had simultaneous AliveCor and standard base apex ECGs performed in a standing position. The AliveCor ECG was recorded using two different device positions (over the left cardiac apex and over the brisket region). Paper speed was 25 mm/sec and amplitude was 10 mm/mV. All ECGs were evaluated unpaired and independently by a data-blinded veterinary cardiologist. The average heart rate was determined using 6 seconds of ECG recording, rhythm diagnosis was recorded for each tracing, and each ECG was given a quality score (0–3). The findings were then reviewed to determine if the AliveCor device was comparable to the base apex ECG for rate and rhythm diagnosis via a paired t-test. Differences in quality scores between the readings for each goat were assessed via a Mann-Whitney U-test. Quality score differences were assessed with a Mann-Whitney U-test between breeds and location of AliveCor ECG. Rhythm agreement was assessed by comparing the blinded rhythm diagnoses made by the cardiologist.

No significant differences were found between heart rates collected via AliveCor or standard base apex ECGs ($P = 0.8151$). Quality scores were significantly higher for AliveCor tracings ($1.85 + /- 0.66$) compared to standard base apex tracings ($0.94 + /- 0.78$) ($P < 0.0001$). No significant difference in AliveCor quality score was noted between meat and dairy breeds ($P = 0.094$). No significant differences were noted between quality scores of AliveCor readings taken from the left cardiac apex or sternal region ($P = 1.000$). Normal sinus rhythm was present in all goats, and could be diagnosed via the AliveCor tracings in 93.75% of cases. Based on this study, the AliveCor ECG was clinically useful for heart rate determination and diagnosis of normal sinus rhythm in goats. Interestingly, AliveCor ECGs had higher quality scores compared to standard base apex ECGs.

F05

LEFT DISPLACEMENT OF THE ABOMASUM IN FOUR BEEF CALVES. Rachel Oman[1], Robert Streeter[1], Emily Reppert[2], Clemence Chako[3]. [1]Oklahoma State University, Stillwater, OK, USA, [2]Kansas State University, Manhattan, KS, USA, [3]Midwestern University, Glendale, AZ, USA

Left displaced abomasum (LDA) is an uncommon condition in beef cattle compared to dairy cattle and is rarely described in sucking beef calves. The true prevalence of LDA in beef calves is unknown and difficult to gauge based on the few reported cases. There remains a paucity of information regarding the diagnosis and treatment of LDA in beef calves.

Four beef calves (two mixed breed beef, one Beefmaster, and one Angus) between two weeks and three months of age were

treated for LDA between November 2013 and March 2015. All four calves had a history of decreased appetite and left-sided abdominal distention; two had recently been treated for necrotic laryngitis and one calf was being treated for suspected clostridial abomasitis. A high-pitched ping and splash were ausculted with percussion and succussion of the left abdomen. Ultrasonography confirmed the abomasum to be displaced between the rumen and the left body wall in all four calves. Blood gas analysis revealed hypokalemic, hypochloremic metabolic alkalosis in three of the calves.

All four calves were initially treated by rolling to correct the left abomasal displacement, and facilitate correction of metabolic abnormalities. Following rolling, the abomasum re-displaced in three of the calves within one hour to 11 days; one calf became acutely colicky as a result of a mesenteric volvulus. A right paramedian abomasopexy was preformed in all cases. Three of four calves grew well and remained in the herd six to 18 months later; one calf was euthanized 10 days after surgery due to complications associated with necrotic laryngitis.

Based on these findings, LDA is likely more common in beef calves than many veterinarians recognize and it should be considered as a differential for calves that present with abdominal distention. Concurrent laryngeal dysfunction has not been previously described in beef calves with LDA and may be a contributing factor in the pathogenesis of this disorder. Ultrasonography is a widely available chute-side diagnostic technique that can rapidly confirm the diagnosis in beef calves. Rolling is a useful adjunctive measure for allowing temporary improvement in cases with severe metabolic alterations, but may be associated with catastrophic intestinal complications in calves as has been reported in adult cows. Therefore, surgical correction is recommended, and right paramedian abomasopexy under sedation and local block is a feasible method of correction of LDA in beef calves.

F06

APPARENT EFFICIENCY OF COLOSTRAL IMMUNOGLOBULIN ABSORPTION IN HOLSTEIN HEIFERS. Jennifer Halleran[1,2], Derek Foster[1,2]. [1]Oklahoma State University, Stillwater, OK, USA, [2]North Carolina State University, Raleigh, NC, USA

Adequate absorption of bovine colostrum correlates with improved neonatal health. The apparent efficiency of absorption (AEA) of immunoglobulins can be measured using a mathematical equation based on serum and colostral IgG concentration levels, as well as the volume of colostrum being fed and the body weight of the calf. While commonly measured in research projects, little information is available on the normal AEA across a large group of healthy calves on multiple farms. The purpose of this study was to observe how contributing factors (volume of feeding, birthweight and time of feeding) can alter AEA, and to establish a normal range of AEA in healthy calves.

One hundred and two Holstein heifer calves from 5 different dairies in North Carolina and Colorado were sampled. After a normal calving, an aliquot of colostrum being fed to the heifer was saved and the heifer received either 4 liters or 5.6 liters of colostrum within 6 hours of birth and a blood sample was collected between 24 and 36 hours after birth. Birthweights were measured using the same weight tape on each farm. Radial immunodiffusion assay was performed to obtain IgG concentrations in the colostrum and serum samples. From this data, the AEA was calculated.

AEA ranged from 7.7% to 59.9% with mean of 28.1% ± 9.5 and median of 27.5%. AEA of 69% (70/102) of the calves fell between 21–40%. There was a significant correlation ($P = 0.033$, $r = 0.21$) between birthweight and AEA and volume fed and AEA ($P = 0.030$, $r = 0.22$). There was no correlation between time of feeding and AEA or total IgG mass fed and AEA.

AEA varies widely between calves even when feeding is standardized. There appears to be opportunities to improve the serum IgG concentration in calves by feeding increased volumes of colostrum, as in this study we were unable to saturate the absorption process. With the wide range of AEA values obtained, there may be opportunities to improve AEA through genetic selection.

F07

CONCENTRATIONS OF CHLORTETRACYCLINE IN FETAL TISSUES FOLLOWING ORAL ADMINISTRATION IN THE EWE. Kevin Washburn[1], Virginia Fajt[1], Hans Coetzee[2], Shannon Washburn[1]. [1]Texas A&M University, College Station, USA, [2]Iowa State University, Ames, IA, USA

The objective of this study was to determine the disposition of chlortetracycline (CTC) in pregnant ewes and their unborn fetuses following oral administration of 500 mg/head/day during the last trimester of gestation.

Five pregnant sheep were administered 500 mg of CTC in divided doses for 8 days. On day 7, the fetus was surgically implanted with venous lines for sampling, and placenta and amniotic fluid were harvested. Fetal plasma was sampled for 36 hours after the last dose of CTC. Subsequently, ewes were sacrificed and samples of amniotic fluid, placenta, fetal kidneys, fetal liver, and fetal stomach contents were collected.

Observed CTC concentrations were below the limit of quantification for all fetal tissues with the exception of liver and kidney in all but two fetuses. Fetal liver concentrations ranged from 4.6 to125.9 ng/mL, while fetal kidney concentrations ranged from 8.1 to 17.4 ng/mL 36 hours after the last dose of CTC.

Although our study indicated that CTC crosses the placental barrier based on accumulation in the fetal kidney and liver, concentrations achieved were far below the minimum inhibitory concentrations (MIC) reported in the literature for isolates from abortion outbreaks and below our limit of quantification in amniotic fluid, fetal stomach contents and placenta. No studies demonstrate the concentrations of CTC that are preventative for abortion from *Campylobacter* spp.; our findings suggest that either the dosage used in this study is not high enough or that the pharmacodynamic parameter relating preventive dose to pathogen MICs is yet to be determined.

F08

HYPERKETONEMIA AS A TOOL TO PREDICT MORTALITY IN DAIRY GOAT DURING LAST MONTH OF PREGNANCY. Vincent Dore[1], Jocelyn Dubuc[2], Anne-Marie Belanger[2], Sebastien Buczinski[2]. [1]North Carolina State University, Raleigh, North Carolina, USA, [2]Universite de Montreal, Saint-Hyacinthe, QC, Canada

Increased ketone bodies concentration in blood has been used to monitor goat during last month of pregnancy to diagnose animal with subclinical and/or clinical cases pregnancy toxemia (PT). Since clinical cases of PT are highly fatal, early diagnostic tool to predict mortality on farm would be useful to producer to save money on animal with lower chance of survival or to initiate more aggressive therapy depending on the value of the animal and its fetuses.

The objective of this study was to determine if increased ketone bodies concentration were associated with mortality in goats from herds with a history of pregnancy toxemia.

A prospective cohort study was performed on 1242 lactating goats from 10 commercial herds in Québec, Canada. Each goat was followed weekly during the 5 last weeks of pregnancy or until parturition. During each farm visit all pregnant goats were sampled until more than 95% of the group had kidded. Blood samples from jugular vessel were collected and were analyzed directly on farm using an electronic on-farm test for the quantification of blood β-hydroxybutyrate acid (BHBA) concentration (Precision Xtra, Abbott Diabetes Care, Saint-Laurent, Canada). Producers evaluated each goat for presence of PT based on a 4 degrees scale (absence, low, moderate, or strong suspicion of PT) using a standardized definition. Number of fetuses, mortality, difficulty of kidding, and presence of treatment, anorexia, lethargy and isolation from the rest of the herd were evaluated during the first week following parturition.

Pregnancy toxemia was diagnosis in 108 goats. Prevalence of mortality was 5.0% during prepartum and first week of lactation period. There were 26 cases of mortality during prepartum period (96.2% associated with PT) and 28 cases of mortality during the first week of lactation (71.4% associated with PT). Critical cut-points for predicting mortality during last month of pregnancy

and first week of lactation were determined based on the highest sum of sensitivity and specificity every week. Blood concentration above 0.6 mmol/L during week 4 before kidding (sensitivity (Se): 44.7%; specificity (Sp): 86.7%), 3 (Se: 65.4% ; Sp: 82.7%), and 2 (Se: 70.8%; Sp: 76.1%) before kidding, above 1.4 mmol/L during week 1 before kidding (Se: 51.3% ; Sp: 93.5%) and above 1.7 for the first week of lactation (Se: 28.6% ; Sp: 95.0%) were selected as the optimal cutpoints in this study. Presence of treatment during the last month of pregnancy was associated with increased mortality in dairy goat (OR: 11.79, $P < 0.01$)

In conclusion, even if hyperketonemia is associated with low to moderate sensitivity from week to week, the test shows good specificity with relatively low prevalence of mortality. For this reason hyperketonemia appeared as a cheap test to assess the risk of mortality during the last month of pregnancy. However, others parameters should be evaluated to access prognosis in goat with PT.

F09

EFFICACY AND PHARMACOKINETICS OF INTRAVENOUS FAMOTIDINE IN ADULT STEERS. Christie Balcomb[1], Meera Heller[1], Heather Kynch[1], Munashe Chigerwe[1], Allison Meyer[2]. [1]University of California, Davis, Davis, CA, USA, [2]University of Missouri, Columbia, MO, USA

The objectives were to measure the pH of abomasal outflow fluid as an indicator of abomasal pH in adult cattle after administration of the H_2-receptor agonist famotidine as a single dose of 0.4 mg/kg IV or as three doses of 0.4 mg/kg IV every eight hours to mimic clinical application and secondly to describe the pharmacokinetic behavior of intravenous famotidine in adult cattle.

A prospective random two-way crossover study design was implemented using 4 healthy adult Angus steers previously fitted with duodenal cannulae located orad to the bile and pancreatic ducts. The steers were fed alfalfa hay *ad-lib* and whole corn and soybean hull meal pellets at 4 lbs/head 12 hours apart. On Day 1, steers were administered either 0.4 mg/kg famotidine or an equal volume of saline via jugular catheters. Whole blood and abomasal outflow samples were obtained at intervals for a 12-hour period. On Day 2, steers were administered either 0.4 mg/kg famotidine or saline IV at time 0, 8, and 16 hours. After a 24-hour washout period, the opposite treatments were administered and the study repeated. Whole blood and abomasal outflow samples were obtained at intervals for a 24-hour period. Abomasal outflow fluid was tested for pH using a commercial bench top pH meter within 5 minutes of sampling. Fluid pH was analysed using a mixed model in SAS 9.4 with treatment, sampling hour, and treatment*hour as fixed effects and steer and treatment period as random effects. Least square means were separated using least significant difference. Serum was separated and stored at −20°C until pharmacokinetic analysis. Serum famotidine analysis was performed using tandem liquid chromatography mass spectrometry and pharmacokinetic analysis was performed using commercial software.

The treatment*sampling hour interaction affected abomasal outflow pH for both a single dose and multiple doses of famotidine ($P < 0.001$). A single dose of intravenous famotidine resulted in a significant increase of abomasal outflow pH as compared to the control group for 4 hours after administration ($P < 0.05$). When administered every 8 hours, the abomasal outflow pH of the famotidine treated group was greater than the control group for 4 hours after the first dose ($P < 0.05$), 2 hours after the second dose, and 1 hour after the third dose ($P < 0.05$). Pharmacokinetic analysis demonstrated that famotidine had a terminal half-life of 3.33 (3.21 – 3.54; median and range) hours, a volume of distribution of 0.042 (0.014 – 1.89) L/kg and a clearance of 1.26 (0.625 – 11.5) mL/min/kg.

In conclusion, an H_2-antagonist, famotidine increases gastric pH by reducing acid production by parietal cells. This study showed that a single intravenous dose of famotidine administered at 0.4 mg/kg is effective at increasing the abomasal outflow pH of adult cattle for up to 4 hours as compared to saline control. When administered every 8 hours, to mimic clinical therapeutic recommendation, the abomasal outflow pH was significantly higher for 4 hours after administration of the first dose; however, the effect seemed to decrease after each subsequent dose. Results of this study indicate that famotidine administered at 0.4 mg/kg IV is effective at raising the abomasal pH and may be an effective treatment for abomasal ulceration in adult cattle. However, the efficacy of treatment may decrease over time or may require more frequent dosing to sustain elevated abomasal pH.

F10

THE PHARMACOKINETICS OF INTRAVENOUS GENTAMICIN IN HEALTHY YOUNG-ADULT VERSUS AGED ALPACAS. Andrew Gestrich[1], Daniela Bedenice[1], Michelle Ceresia[2], Cheryl Stockman[1], Iman Zaghloul[2]. [1]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA, [2]School of Pharmacy, MCPHS University, Boston, MA, USA

The study objective was to evaluate the effects of patient age on aminoglycoside pharmacokinetics in young-adult (≤5 years) and aged (≥14 years) healthy alpacas, receiving a single 6.6 mg/kg intravenous injection of gentamicin (Henry Schein®). Heparinized plasma samples were obtained at designated time-points following drug administration and frozen at −80°C until assayed by a validated immunoassay (QMS®). Gentamicin plasma concentrations versus time plots were analyzed using a two-compartment model and commercial software (WinNonlin-v6.4). Data were compared between age-groups using independent samples t-test; $P < 0.05$ considered significant.

Baseline physical and hematological parameters were not significantly different between young (median age [range]: 2.6 [1.7–3.7] years) and aged (15.5 [14.6–18] years) alpacas, aside from a lower mean heart rate in older animals (50 ± 13 versus 79 ± 15 beats/minute). Laboratory results did not differ pre and post-treatment.

Comparative pharmacokinetic parameters are displayed below (mean ±SD; $^{\dagger}P < 0.05$):

Both the peak gentamicin concentration at the end of the distribution phase (23.8 ± 2.1 versus 26.1 ± 2 μg/mL, $P = 0.04$) and the mean time-point at which gentamicin trough levels reached 2 μg/mL (common MIC for large-animal pathogens) were significantly lower in young-adult versus older animals (6.7 ± 1 versus 8.7 ± 1.9 hours, $P = 0.03$). These data indicate reduced renal drug clearance in aged alpacas.

	C_0 (μg/mL)	$AUC_{0-\infty}$ † (μg*h/mL)	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	V_{ss} (L/kg)	Cl † (mL/h/kg)
Young-adult (n=8)	54.6±6.5	70.4±10.5	0.13±0.02	1.7±0.02	0.21±0.02	95.5±13.4
Aged (n=8)	65.2±13.2	90.4±17.6	0.12±0.04	2.1±0.5	0.21±0.02	75.6±16.1

C_0–Initial plasma concentration; AUC–Area under the curve; $T_{1/2}$ α and β–Distribution and elimination half-life; V_{ss}–Volume of distribution at steady state; Cl–Clearance

F11

PROGNOSIS ASSOCIATED WITH CEREBROSPINAL FLUID ANALYSIS RESULTS IN RECUMBENT DAIRY CATTLE: RETROSPECTIVE STUDY (2006–2014). Marie-Ève Bilodeau, David Francoz, Damien Achard, André Desrochers, Sylvain Nichols, Marie Babkine, Hélène Lardé, Carolyn Grimes, Gilles Fecteau. Université de Montréal, Saint-Hyacinthe, QC, Canada

In recumbent dairy cows, the analysis of the cerebrospinal fluid (CSF) is an ancillary test frequently used at our referral center. Interpretation of the CSF analysis results in cattle is challenging, in part, because reported intervals for this species differ. In a previous retrospective study, we demonstrated that recumbent cows with a CSF total nucleated cells count (TNCC) ≥ 4.5 cells/μL and/or a protein concentration ≥ 0.40 g/L had a spinal cord lesion on post mortem examination. However, CSF results below those threshold values did not exclude the presence of such a lesion.

The objective of this study was to determine the short-term and long-term prognosis in recumbent adult dairy cows who underwent

CSF analysis. The previously mentioned threshold values for TNCC and protein concentration were used. We hypothesized that recumbent cows with CSF values higher than the pre-determined threshold values would have a less favorable short-term and long-term prognosis. All dairy cattle, of 2 years or more of age, that were recumbent and who have had a CSF analysis were included in the study (n = 214 cases). CSF analysis was performed by board certified pathologists, and only CSF analysis which contained both the TNCC and protein concentration were included in the study. A successful short-term prognosis was based on the return to the farm. A successful long-term prognosis was based on the completion of at least one lactation using the Canadian Dairy Network database. Statistical analysis was performed using the Chi-squared test to compare numbers of cows in each group.

Age of patients ranged from 2.0 to 14.5 years (median 5.6 years). Two hundred and eight cows were Holstein, 4 were Ayrshire and 2 were Jersey. The duration of hospitalization ranged from 1 to 48 days (median 7 days). In cows for which the information was available, days in milk ranged from 1 to 516 days (median 25 days) and 18% of the cows (23/130) were dried. Of the 214 cases studied, 167 cows had CSF values for TNCC and protein concentration below the threshold values, and 47 cows had CSF values above the pre-determined threshold values.

The results showed that TNCC $\geq$ 4.5 cells/μL and/or a protein concentration $\geq$ 0.40 g/L was associated with a worse short-term prognosis in recumbent dairy cattle. Indeed, 53% of cows with CSF values below the pre-determined threshold values were discharged from the hospital, which is comparable to the survival rate of all recumbent adult dairy cows that present to our referral center (survival rate of 48% between 1994 and 2014, n = 1271). The short-term survival rate was 30% in cows where only the TNCC value was above the threshold ($P = 0.035$), 28% if only the protein concentration was above the threshold ($P = 0.012$), and 34% if both CSF parameters were above the thresholds ($P = 0.024$). However, in cows discharged from the hospital (n = 104), the long-term prognosis was comparable amongst groups; 60% (53/88) of cows with CSF values below the thresholds, and 75% (12/16) of cows with one or more CSF value above the thresholds completed at least one lactation after their return to the farm ($P = 0.37$).

Based on this study, we conclude that the short-term prognosis of recumbent adult dairy cattle with CSF TNCC $\geq$ 4.5 cells/μL and/or a protein concentration $\geq$0.40 g/L is lower than that of cows with CSF values below those thresholds. However, some cattle with CSF values above the thresholds survive and their long-term prognosis seems to be acceptable suggesting that these cows may have a spinal cord lesion that is reversible.