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2018 ACVIM Forum Research Abstract Program

2018 ACVIM Forum Research Abstract Program
Seattle, Washington, June 14 - 15, 2018

Index of Abstracts

ORAL PRESENTATIONS - THURSDAY, JUNE 14

Time	#	Presenting Author	Abstract Title
CARDIOLOGY			
2:45 PM	C01	Daniel Newhard	Pharmacodynamics of Entresto® (Sacubitril/Valsartan) Versus Placebo in Dogs with Preclinical Myxomatous Mitral Valve Disease (ACVIM Resident Research Award & Cardiology Research Abstract Award Eligible)
3:00 PM	C02	Emily Karlin	Trimethylamine-N-Oxide and its Precursors in Dogs with Degenerative Mitral Valve Disease (ACVIM Resident Research Award & Cardiology Research Abstract Award Eligible)
3:15 PM	C03	Brianna Potter	Comparison Between the Effects of Torsemide and Furosemide on the Renin-Angiotensin-Aldosterone System of Normal Dogs (ACVIM Resident Research Award & Cardiology Research Abstract Award Eligible)
4:15 PM	C04	Bruno Boutet	Transvenous Echocardiography in Conscious Sedated Horses (ACVIM Resident Research Award & Cardiology Research Abstract Award Eligible)
4:30 PM	C05	Julia Treseder	Inotropic and Chronotropic Effects of Sotalol in Healthy Dogs (ACVIM Resident Research Award & Cardiology Research Abstract Award Eligible)
4:45 PM	C06	Christopher Whipp	Complications and Outcomes of Multi-Institution Transvenous Pacemaker Implantation (ACVIM Resident Research Award & Cardiology Research Abstract Award Eligible)
5:00 PM	C07	Nicole LeBlanc	Pharmacokinetics of Transdermal Atenolol Administration in Healthy Cats
5:15 PM	C08	Sonya Wesselowski	Anatomy, Baseline Characteristics and Procedural Outcome in German Shepherd Dogs with Patent Ductus Arteriosus
5:30 PM	C09	Marisa Ames	Effect of Loop Diuretics on Novel Components of the Renin-Angiotensin-Aldosterone System of Healthy Dogs
5:45 PM	C10	Ryan Fries	Precision and Repeatability of Transthoracic Two-Dimensional and Three-Dimensional Echocardiography in Normal Conscious Dogs
6:00 PM	C11	Darcy Adin	Echocardiographic Phenotype of Canine Dilated Cardiomyopathy Differs Based on Diet
NEUROLOGY			
10:15 AM	N01	Susan Arnold	Nanofiber Lined Catheter Treatment of Intracranial Gliomas in Dogs (ACVIM Resident Research Award Eligible)
10:30 AM	N02	Marios Charalambous	Repetitive Transcranial Magnetic Stimulation for the Treatment of Drug-resistant Canine Idiopathic Epilepsy (ACVIM Resident Research Award Eligible)
10:45 AM	N03	Erik Johnson	Pharmacokinetics of Approved and Compounded Extended Release Levetiracetam After Single Oral Dose Administration in Cats (ACVIM Resident Research Award Eligible)
11:00 AM	N04	Amanda Jurkoshek	Spatial Resolution of 3D Printed Stereolithographic Models of the Canine Thoracolumbar Vertebral Column (ACVIM Resident Research Award Eligible)
11:15 AM	N05	Jeanie Lau	Clinical Characteristics of Steroid Responsive Meningitis-Arteritis in a Population of Dogs in North America (ACVIM Resident Research Award Eligible)
11:30 AM	N06	Casey Smith	Serum Levetiracetam Concentrations Following Chronic Administration of Three Times Daily Transdermal Levetiracetam in Six Cats (ACVIM Resident Research Award Eligible)

11:45 AM	N07	Georgina Stewart	Clinical Safety and Magnetic Resonance Imaging Characteristics of Brain Implanted Nanofiber-Lined Catheters in Healthy Dogs (ACVIM Resident Research Award Eligible)
12:00 PM	N08	R. Timothy Bentley	Microsurgery and intratumoral Concentration and Safety of Metronomic Chlorambucil for Spontaneous Canine Glioma
12:15 PM	N09	R. Timothy Bentley	Development of an Intraventricular Hemorrhage Model of Obstructive Hydrocephalus in Pigs
1:30 PM	N10	Amanda Brenna	Pharmacokinetics of Fenbendazole in Canine CSF and Plasma: A Pilot Study
1:45 PM	N11	Aude Castel	Risk Factors for Progressive Myelomalacia in Dogs with Complete Sensorimotor Loss Following Intervertebral disc Extrusion
2:00 PM	N12	Thomas Flegel	Accuracy of a Novel Magnetic Resonance Imaging-Based Patient-Individual Stereotactic Brain Biopsy Device in the Dog
2:15 PM	N13	Sarah Kappel	Can Neuronavigation Aid in Pituitary Removal in Horses?
2:30 PM	N14	Susan Knowler	Morphometric Analysis of Brachycephalic Features Identified by Machine Learning Technique in Dogs with/without Syringomyelia
2:45 PM	N15	Courtney Sparks	Morphometric Analysis of Spinal Cord Termination in Cavalier King Charles spaniels
3:00 PM	N16	Go Togawa	A Comparison of Erythrocyte Membrane Fatty Acids Between Shiba and Non-Asian Dogs
3:15 PM	N17	Yoshihiko Yu	Genetic Association Analyses of Familial Spontaneous Epileptic Cats

ONCOLOGY

1:30 PM	O01	Emily Gould	Acid Suppressants Modulate in Vitro Mast Cell Structure, Degranulation, and Viability (ACVIM Resident Research Award Eligible)
1:45 PM	O02	Rachel Kovac	Plasma Cytokeratin-18 Levels as Non-invasive Biomarker of Early Gastrointestinal Toxicity in Dogs Receiving Toceranib (ACVIM Resident Research Award Eligible)
2:00 PM	O03	Hajime Asada	Comprehensive Analysis of Gene Mutations in Canine Histiocytic Sarcoma by Whole Exome Sequencing
2:15 PM	O04	Michael Childress	Predicting the Response of Canine B-Cell Lymphoma to CHOP Chemotherapy with Ex Vivo Biodynamic Imaging
2:30 PM	O05	Michael Coyne	The Association between Symmetric Dimethylarginine Concentrations and Neoplasia in Dogs and Cats
2:45 PM	O06	Linda Matthewman	Treatment with Enterococcus faecium NCIMB 10415 Does Not Affect the Outcome of Chemotherapy-Induced Diarrhea
3:00 PM	O07	Alicia McLuckie	Felis catus Gammaherpesvirus 1 Associated with Shorter Survival but not Risk of Lymphoma in Cats
3:15 PM	O08	Ryo Miyamoto	YM155 Inhibits the Growth of Canine Squamous Cell Carcinoma Cells with High Expression of Survivin
4:15 PM	O09	Lisa Parshley	Nanoparticle and Laser Thermal Ablation in Canine Low Grade Mast Cell Tumors
4:30 PM	O10	Andrew Poon	Amiloride Sensitizes Canine Osteosarcoma Cells to Doxorubicin Chemotherapy
4:45 PM	O11	Kosei Sakai	Antitumor Effect of Lapatinib in Canine Transitional Cell Carcinoma Cell Lines
5:00 PM	O12	Enrico Spugnini	Adjuvant Electrochemotherapy with Bleomycin and Cisplatin Combination for the Canine Soft Tissue Sarcoma: 30 Cases
5:15 PM	O13	Hiroyuki Tani	Treatment of Histiocytic Sarcoma with Nimustine in Dogs: 9 Cases
5:30 PM	O14	Jennifer Lenz	Generation of Myeloid Derived Suppressor Cells by Tumor Exosomes (VCS Award Winner)
5:45 PM	O15	Leanne Magestro	Comparison of Various Imaging Modalities for Setup Verification Prior to Delivery of Stereotactic Radiation Therapy for Head and Neck Cancers in Veterinary Patients (VCS Award Winner)

SMALL ANIMAL INTERNAL MEDICINE - ENDOCRINOLGY

10:15 AM	EN01	Ellen Behrend	Effect of Sample Type on Measurement of Cortisol and T4 by Immulite in Dogs
10:30 AM	EN02	Arnon Gal	Isolated Renal Glycosuria Does Not Lead to Polyuria in a Feline Model
10:45 AM	EN03	Lune Geurts	Transcription Factors Involved in the Development and Reprogramming of the Endocrine Pancreas in Diabetic Cats
11:00 AM	EN04	Chen Gilor	Comparison of Pharmacodynamics Between Insulin Degludec and Insulin Glargine 300 U/mL in Healthy Cats
11:15 AM	EN05	Jean-Sébastien Palerme	Thyroid Status in Dogs with Renal Proteinuria
11:30 AM	EN06	Jennifer Wakeling	Diagnosis of Hypothyroidism following Thyroid Stimulating Hormone Stimulation Testing of Radio-Iodine-Treated Hyperthyroid Cats
11:45 AM	EN07	Jennifer Wakeling	Effect of Illness on Response of Euthyroid cats to Thyroid Stimulating Hormone Stimulation Test

12:00 PM	EN08	Eric Zini	Oxidative Stress of Erythrocytes in Cats with Diabetes Mellitus
1:30 PM	EN09	Karin Sanders	Molecular Prognostic Markers in Canine Cortisol-Secreting Adrenocortical Tumours (ESVE Award Winner)

SMALL ANIMAL INTERNAL MEDICINE - GASTROENTEROLOGY

10:15 AM	GI01	Betty Chow	Comprehensive Comparison of Upper and Lower Endoscopic Small Intestinal Biopsies in Cats with Chronic Enteropathy (ACVIM Resident Research Award Eligible)
10:30 AM	GI02	Madeline Fujishiro	Effects of a Combination Anthelmintic (Febantel, Pyrantel, Praziquantel) on the Fecal Microbiota of Dogs Infected with Giardia and Cryptosporidium (ACVIM Resident Research Award Eligible)
10:45 AM	GI03	Ju-Hyun An	TNF-inducible Gene/Protein-6 (TSG-6) Released by Canine Mesenchymal Stem cells Alleviate Inflammatory Bowel Disease in Mice
11:00 AM	GI04	Agostino Buono	Serum IL-2, IL-6, IL-8, and TNF-α Concentrations in Dogs with Chronic Enteropathies
11:15 AM	GI05	Rachel Dickson	Enterococcus Inhibit Growth and Adhesion of Feline Tritrichomonas foetus
11:30 AM	GI06	Romy Heilmann	Association between Serum Calprotectin Concentrations and Insulin Resistance in Miniature Schnauzers with Idiopathic Hyperlipidemia
11:45 AM	GI07	Tracy Hill	Assessment of Gastrointestinal Injury in Racing Alaskan Sled Dogs
12:00 PM	GI08	Roman Husnik	Effect of Metoclopramide, Erythromycin and Exenatide on Solid Phase Gastric Emptying in Healthy Cats
1:30 PM	GI09	Francesco Lotti	Esophageal Lumen Ph and Lavage in Dogs with Gastroesophageal Reflux
1:45 PM	GI10	Janne Lyngby	Contrast Videofluoroscopy Can Help Manage Dogs with Congenital Idiopathic Megaesophagus
2:00 PM	GI11	Kasey Mabry	Utility of Capsule Endoscopy in the Assessment of Microcytosis in Dogs
2:15 PM	GI12	Sina Marsilio	Histopathology, Immunohistochemistry, and Clonality of Intestinal Biopsies from Healthy Cats
2:30 PM	GI13	Sina Marsilio	Characterization of the Intestinal Proteome of Cats with Inflammatory Bowel Disease or Alimentary Lymphoma
2:45 PM	GI14	Kate Spies	Effects of Gastrointestinal Diets on Feline Fecal Occult Blood Testing
3:00 PM	GI15	Rachel Pilla	Fecal Metabolites from the Tryptophan-Serotonin-Indole Pathway in Dogs with Intestinal Disease
3:15 PM	GI16	Rachel Pilla	Longitudinal Characterization of the Fecal Microbiome in Dogs with Idiopathic Inflammatory Bowel Disease
4:15 PM	GI17	Fatima Sarwar	Prevalence of Clostridium perfringens Encoding netF Gene in Dogs with Acute and Chronic Gastrointestinal Diseases
4:30 PM	GI18	Linda Matthewman	Microbiota analysis-detected dysbiosis in dogs with cancer is unaffected by Enterococcus faecium NCIMB 10415 therapy
4:45 PM	GI19	Joerg Steiner	Linearity, Precision, and Reproducibility of the VetScan cPL Rapid Test
5:00 PM	GI20	Aarti Kathrani	Effects of Malnutrition on the Mortality Rate in Dogs with Protein-losing Enteropathy

SMALL ANIMAL INTERNAL MEDICINE - IMMUNOLOGY

5:30 PM	IM02	Nadine Vogt	Efficacy of the Canine Lyme Vaccine in North America: A Systematic Review and Meta-analysis

SMALL ANIMAL INTERNAL MEDICINE - INFECTIOUS DISEASE

3:15 PM	ID01	Krystle Reagan	Risk Factors for Candida Urinary Tract Infections in Dogs and Cats (ACVIM Resident Research Award Eligible)
4:15 PM	ID02	Rachel Acciacca	Clinical Evaluation of a Commercial Hyperimmune Plasma Product in Dogs with Parvoviral Enteritis
4:30 PM	ID03	Nanelle Barash	Prevalence of Babesia spp. and Clinical Characteristics of Babesia Microti-Like Infections in North American Dogs
4:45 PM	ID04	Moria Borys	Differences in Clinicopathologic Variables between Borrelia C6 Antigen Seroreactive and Borrelia C6 Seronegative Canine Glomerulopathy
5:00 PM	ID05	Sarah Caddy	Primary-Care Practice Clinical Trial Provides Evidence That Reduced Antibiotic Use Does Not Affect Clinical Outcome
5:15 PM	ID06	Charlotte Edinboro	Feline Retroviral Prevalence in Feral Cats on the San Francisco Peninsula, 2001-2003, 2005-2007, and 2014-2016
5:30 PM	ID07	Michelle Evason	Canadian K9 Lifetime (Lyme) Study: Descriptive analyses of Year 1 Data
5:45 PM	ID08	Jennifer Ogeer	Investigation of the Association Between Lyme Seroreactivity and Chronic Kidney Disease in Dogs
6:00 PM	ID09	Barbara Qurollo	Feline Vector-borne Disease in Cats with Acute-Onset Fever

SMALL ANIMAL INTERNAL MEDICINE - NUTRITION / METABOLISM

1:45 PM	NM01	JD Foster	Prospective Evaluation of a Novel Esophagostomy Feeding Tube Placement Device in Dogs
2:00 PM	NM02	Yuet-Ming Leung	Metabolic and Immunological Effects of Intermittent Fasting in Healthy Dogs Fed a High Fat Diet
2:15 PM	NM03	Yuanlong Pan	Effects of Continuous and Intermittent Caloric Restriction Regimens on Body Fat Loss in Obese Dogs
2:30 PM	NM04	Alexandra Rankovic	Effect of Different Carbohydrate Sources on Glycemic Index and Satiety-Related Gut Hormones in Dogs
2:45 PM	NM05	Josefin Söder	Differences in Postprandial Metabolites related to Lipid Metabolism between Lean and Overweight Labrador Retriever Dogs
3:00 PM	NM06	Brian Zanghi	Nutrient-Enriched Water Enhances total Water Intake and Hydration in Dogs

SMALL ANIMAL INTERNAL MEDICINE - OTHER

5:45 PM	OT01	Bryan Brown	The Relationship Between Pet-Owner Satisfaction and Loyalty: the Mediating Role of Communication
6:00 PM	OT02	Colleen Tansey	The Effect of Client Complaints on Small Animal Veterinary Internists

EQUINE

10:15 AM	E01	Rosemary Bayless	Equine Urinary N-Acetyl-β-D-Glucosaminidase Assay Validation and Correlation with Other Markers of Kidney Injury (ACVIM Resident Research Award Eligible)
10:30 AM	E02	Daniel Jean	Urinary Tract Infections: Retrospective Study in an Equine Hospital between 1995 to 2016
10:45 AM	E03	Vicky Savage	Characterization of Acute Kidney Injury in Hospitalized Horses
11:00 AM	E04	Hayley Chidlow	Comparison of Two Collection Methods for Cerebrospinal Fluid Analysis from the Standing, Sedate Adult Horse (ACVIM Resident Research Award Eligible)
11:15 AM	E05	Demia de Tonnerre	Eastern Equine Encephalitis in Horses – 104 cases (1979 – 2017)
11:30 AM	E06	Zoë Williams	Response of Warmbloods with Type 2 Polysaccharide Storage Myopathy to Diet and Exercise
11:45 AM	E07	Daniela Luethy	Cyclophosphamide Dose Escalation during Equine Chemotherapy (ACVIM Resident Research Award Eligible)
12:00 PM	E08	Daniela Luethy	Retrospective Evaluation of Clinical Outcome following Chemotherapy for Lymphoma in 11 Horses (ACVIM Resident Research Award Eligible)
1:30 PM	E09	Emma Gorenberg	Medical and Surgical Treatment of Primary Hyperparathyroidism in 17 Equids (1999-2016) (ACVIM Resident Research Award Eligible)
1:45 PM	E10	Rebecca Legere	Pioglitazone in Equids Increases High-Molecular-Weight Adiponectin Concentrations and Decreases Insulin Response After Oral Sugar (ACVIM Resident Research Award Eligible)
2:00 PM	E11	Valentina Ragno	Investigation of Novel Biomarkers for Early Detection of Equine Metabolic Syndrome (ACVIM Resident Research Award Eligible)
2:15 PM	E12	Lindsey Rings	Enteroinsular Axis Response to Fasting and Dextrose in Healthy Neonatal Foals (ACVIM Resident Research Award Eligible)
2:30 PM	E13	Sanna Lindåse	Evaluation of an Oral Sugar Test to Diagnose Insulin Dysregulation in Horses
2:45 PM	E14	Alexandra Meier	Prevention of Laminitis in Ponies Using Velagliflozin, a Novel Treatment for Insulin Dysregulation
3:00 PM	E15	Tobias Warnken	The Impact of Different Glucose Dosages in Oral Glucose Test for Assessment of Insulin Dysregulation
3:15 PM	E16	Heidi Banse	Comparison of Insulin and Glucose Response in Horses Using Two Different Formulations of Corn Syrup
4:15 PM	E17	Remona Horn	Combination of TRH Stimulation Test and 2-Step Insulin Sensitivity Test to Diagnose PPID and EMS
4:30 PM	E18	Emma Stapley	Effect of Using Corn Syrup with Fructose on Equine Oral Sugar Test Results
4:45 PM	E19	Callum Donnelly	Serum and Cerebrospinal Fluid Alpha-Tocopherol Concentration in Adult Horses Supplemented with Subcutaneous Alpha-Tocopherol (ACVIM Resident Research Award Eligible)
5:00 PM	E20	Laura Dunbar	Serum Phosphorus, Magnesium, and Potassium Concentrations in Small Equids with Dyslipidemias
5:15 PM	E21	Lucas Nolazco Sassot	Lipidome of Thoroughbred Horses Before and After Supra-Maximal Exercise Using an Untargeted Lipidomics Approach
5:30 PM	E22	Christina Eberhardt	Quantification of Left-Atrial Stunning in Horses After Cardioversion of Atrial Fibrillation Using Two-Dimensional Speckle Tracking (ACVIM Resident Research Award Eligible)
5:45 PM	E23	Clémentine Gy	Acute, Subacute and Chronic Assessment of Horses Exposed to Lethal and Sublethal Doses of Monensin (ACVIM Resident Research Award Eligible)
6:00 PM	E24	Natalia Rodriguez	Comparison of Non-invasive, Invasive Central and Invasive Peripheral Blood Pressure in the Standing Horse

FOOD ANIMAL

10:15 AM	F01	Maria Puerto-Parada	Risk Factors Associated with Mycobacterium avium Subsp. paratuberculosis Herd Status in Québec Dairy Herds (ACVIM Resident Research Award Eligible)
10:30 AM	F02	Emily John	Risk Factors Associated with Bovine Leukemia Virus Infection in Dairy Herds in Atlantic Canada (ACVIM Resident Research Award Eligible)
10:45 AM	F03	Fabienne Uehlinger	The Nemabiome: A New Tool for the Small Ruminant Clinician
11:00 AM	F04	Sarah Raabis	The Nasopharyngeal Microbiota of Pre-Weaned Dairy Calves with and without Ultrasonographic Lung Lesions
11:15 AM	F05	Brandy Burgess	Evaluation of a Rapid Immunoassay for Detection of Cryptosporidium parvum in Bovine Calf Fecal Samples
11:30 AM	F06	Eloi Guarnieri	Abomasitis in Calves: Retrospective Study of 20 Cases (2006-2016) (ACVIM Resident Research Award Eligible)
11:45 AM	F07	Diego Gomez-Nieto	Investigation of the Fecal Virome of Neonatal Dairy Calves with Diarrhea
12:00 PM	F08	Rachel Oman	Use of a Digital Brix Refractometer to Estimate Serum Immunoglobulin in Goat Kids

ORAL PRESENTATIONS - FRIDAY, JUNE 15

Time	#	Presenting Author	Abstract Title
CARDIOLOGY			
1:30 PM	C12	Mariko Yata	Effects of BNP-32 in Dogs with Congestive Heart Failure Due to Myxomatous Mitral Valve Disease
1:45 PM	C13	Lee-Jae Guo	Natural History of Cardiomyopathy in Adult Dogs with Golden Retriever Muscular Dystrophy
2:00 PM	C14	Mengmeng Liu	Acute Phase Proteins in Cats with Congestive Heart Failure Due to Primary Cardiomyopathy
2:15 PM	C15	Laetitia Duler	Pancreatic Injury in Cats with Primary Cardiomyopathy
2:30 PM	C16	Laetitia Duler	Left Atrial Size and Volume in Cats with Cardiomyopathy with and without Congestive Heart Failure
2:45 PM	C17	Jenny Wilshaw	Is Progression of Murmur Intensity Associated with the Rate of Remodelling in Mitral Valve Disease?
3:00 PM	C18	Osman Safa Terzi	Speckle Tracking Echocardiography in Adult Turkish Kangal Dogs
3:15 PM	C19	Ingrid van Hoek	Associations Between Echocardiography, Cardiac Biomarkers, Insulin Metabolism, Morphology and Inflammation In Feline Asymptomatic Hypertrophic Cardiomyopathy
4:15 PM	C20	Nobuyuki Kanno	Outcome of Dogs with Mitral Valve Disease with Medical Therapy or Surgical Repair
4:30 PM	C21	Kazuya Mamada	Phrenic Nerve Palsy Following Mitral Valve Repair in Dogs
4:45 PM	C22	Kaitlin Abbott-Johnson	Evidence of Endothelial-to-Mesenchymal Transition In Canine Degenerative Mitral Valves
5:00 PM	C23	Alina Teslenko	Comparison of In-Hospital Non-Recordable Telemetry Versus Recordable Holter Monitoring in Dogs with Arrhythmias
SMALL ANIMAL INTERNAL MEDICINE - HEMATOLOGY			
8:00 AM	HM01	Megan McClosky	Prevalence of Naturally Occurring non-AB Red Blood Cell Alloantibodies in Cats (ACVIM Resident Research Award Eligible)
8:15 AM	HM02	Jason Bestwick	The Use of High-Dose IgM-Enriched Human Immunoglobulin in Canine Immune-Mediated Hemolytic Anemia
8:30 AM	HM03	Jillian Haines	Educating Veterinary Students in an Intensive Care Unit: Impact of a Transfusion Reaction Learning Module
8:45 AM	HM04	Anne Hale	Evaluation of Two Apheresis Techniques for Plateletpheresis in the Dog
9:00 AM	HM05	Maria Lyraki	Comparison of Haemoglobin and Haematocrit Measurements Using Ear Prick and Venous Blood Samples in Dogs
SMALL ANIMAL INTERNAL MEDICINE - HEPATOLOGY			
4:45 PM	HP01	Ashley Hartley	Bacterial Community Composition of Bile from Healthy Dogs and Dogs with Suspected Cholangitis or Mucocele (ACVIM Resident Research Award Eligible)
5:00 PM	HP02	Chantel Raghu	Potential Serum Biomarkers of Hepatic Fibrosis and Necroinflammatory Activity in Dogs with Liver Disease (ACVIM Resident Research Award Eligible)
5:15 PM	HP03	Tarini Ullal	The Evaluation of Cyclosporine in the Treatment of Chronic Hepatitis in Dogs (ACVIM Resident Research Award Eligible)
5:30 PM	HP04	Cynthia Webster	Serum Vitamin D Status in Cats with Cholestatic Hepatobiliary Disease
5:45 PM	HP05	Gonçalo Serrano	Treatment of Canine Congenital Extrahepatic Portosystemic Shunts – A Systematic Review and Meta-Analysis
6:00 PM	HP06	Chung-In Wang	Is the Common Bile Duct Truly Obstructed or Not?

SMALL ANIMAL INTERNAL MEDICINE - NEPHROLOGY / UROLOGY

10:15 AM	NU01	Masahiko Befu	Increase in Serum Symmetric Dimethylarginine Level Following Anesthesia in Dogs
10:30 AM	NU02	Felipe dos Santos Muniz	Evaluation of Serum Symmetric Dimethylarginine in Dogs Naturally Infected by Leishmania infantum
10:45 AM	NU03	Graham Bilbrough	Validation of an Point-of-Care Immunoassay for Measurement of Symmetric Dimethylarginine in Feline Serum
11:00 AM	NU04	Timothy McCarthy	Complications of Transurethral Ectopic Ureter Correction
11:15 AM	NU05	Henk van den Broek	Serum Calcification Propensity In Cats with Chronic Kidney Disease
11:30 AM	NU06	Joao de Brito Galvao	Prospective Evaluation of the Incidence of Canine Hypercalciuria and iCa Concentration with Calcium Oxalate Urolithiasis
11:45 AM	NU07	Sarah Lorbach	Clinical Presentation and Prognosis of 77 Dogs Diagnosed with Focal Segmental Glomerulosclerosis by Renal Biopsy
1:30 PM	NU08	Susan Carr	Pre and Postprandial Urine Calcium-to-Creatinine Ratio to Identify Calcium Oxalate Urolithiasis in Miniature Schnauzers (ACVIM Resident Research Award Eligible)
1:45 PM	NU09	Zachary Kern	Population Characteristics and Clinical Presentation of Dogs with Biofilm-forming Escherichia coli Urinary Tract Infection (ACVIM Resident Research Award Eligible)
2:00 PM	NU10	Stacie Summers	The Fecal Microbiome, Indoxyl Sulfate, and P-Cresol Sulfate in Cats with Stable Chronic Kidney Disease (ACVIM Resident Research Award Eligible)
2:15 PM	NU11	Kendall Wilson	Assessment of Symmetric Dimethylarginine and Creatinine Concentrations in Cats with Urethral Obstructions (ACVIM Resident Research Award Eligible)
2:30 PM	NU12	Adrienne Cheney	A Multi-Institutional Retrospective Study of 17 Cases of Histopathologically Confirmed Feline Pyelonephritis
2:45 PM	NU13	Allyson Berent	Use of a Subcutaneous Ureteral Bypass Device for Treatment of Benign Ureteral Obstructions in Dogs
3:00 PM	NU14	Bianca Lourenco	Characterization of Hypoxia-Induced, Profibrotic Pathways in an ischemic Model of Feline Chronic Kidney Disease
3:15 PM	NU15	Carrie Palm	Early Clinical Evaluation of Urethral Thermoplasty In The Treatment of Urinary Incontinence in Dogs
4:15 PM	NU16	Alexander Saver	Calcium Oxalate Urolithiasis in Juvenile Dogs
4:30 PM	NU17	Sarah Steinbach	Transcutaneous Assessment of Renal Function in Hyperthyroid Cats pre and post Radioiodine Treatment

SMALL ANIMAL INTERNAL MEDICINE - OTHER

9:15 AM	OT03	Alyssa Sullivant	Strategies to Improve Case Outcome When Referral is Not Affordable
11:45 AM	OT04	James Warland	An Investigation into Multi-Organ Fibrosis in the Cavalier King Charles Spaniel
12:00 PM	OT05	Nevra Keskin	Hereditary Deafness by BEAR Testing in Purebred Angora Cats

SMALL ANIMAL INTERNAL MEDICINE - PHARMACOLOGY

10:15 AM	P01	Matthew Miller	Localization and Quantification of Cannabinoid Receptors in Canine Tissue
10:30 AM	P02	Jennifer Reinhart	Effect of Glutathione on Itraconazole-Induced Cytotoxicity in Canine Hepatocytes
10:45 AM	P03	Jennifer Slovak	Pharmacodynamics of Mycophenolate Mofetil after Multi-Dose Oral Administration in 10 Healthy Cats
11:00 AM	P04	Kamoltip Thungrat	Genetic Polymorphisms in CYP3A12 and Clinical Outcomes of Vinblastine Chemotherapy in Dogs with Mastocytoma
11:15 AM	P05	Kamoltip Thungrat	Therapeutic Drug Monitoring and Population Analysis of Cyclosporine in Dogs with Immune-Mediated Diseases
11:30 AM	P06	Nicolas Villarino	Is SDMA a Wonder Biomarker for Detecting Kidney Disease in Cats?

SMALL ANIMAL INTERNAL MEDICINE - RESPIRATORY

8:00 AM	R01	Aida Vientós-Plotts	Blood Cultures As Minimally Invasive Surrogates in Diagnosis of Canine Bacterial Pneumonia: A Pilot Study (ACVIM Resident Research Award Eligible)
8:15 AM	R02	Kaitlyn Belanger	Development of Novel Thoracic Radiographic Measurements for Discrimination of Feline Mediastinal Masses versus Pleural Effusion
8:30 AM	R03	Morgane Canonne	Comparison of Two Aerosolized Gentamicin Protocols for Bordetella Bronchiseptica Lower Airway Infection in Dogs
8:45 AM	R04	Megan Grobman	Aerodigestive Disorders Identified in Dogs Evaluated for Cough Using Videofluoroscopic Swallow Studies
9:00 AM	R05	Nathan Squire	Tracheal Stent Following Tracheal Rings for Management of Tracheal Collapse 9 Cases: (2010-2017)
9:15 AM	R06	A. Taylor	Epiglottic Retroversion: Concurrent Diseases, Management, and Outcome in 13 Cases (2012-2017)

EQUINE

1:30 PM	E25	Valentina Ragno	A Comparison of the Stability of Equine Blood D-Lactate in Sodium Fluoride and Serum Tubes (ACVIM Resident Research Award Eligible)
1:45 PM	E26	Katja Roscher	Activated Platelets and Platelet-Leukocyte-Aggregates in Equine Systemic Inflammatory Response Syndrome
2:00 PM	E27	Marcha Badenhorst	First Detection and Frequent Occurrence of Equine Hepacivirus in Horses on the African Continent
2:15 PM	E28	Emma Gorenberg	Validation of an In-Clinic Enzyme-Linked Immunosorbent Assay for Diagnosis of Leptospirosis in Horses (ACVIM Resident Research Award Eligible)
2:30 PM	E29	Amanda Trimble	Prevalence of Equine Leptospiral Shedding using Urine Polymerase Chain Reaction and Serum Microscopic Agglutination Testing (ACVIM Resident Research Award Eligible)
2:45 PM	E30	Diego Gomez-Nieto	Nasal Bacterial Microbiota of Adult Horses Shedding Equine Herpes Virus 1
3:00 PM	E31	Katherine Delph	Very High Streptococcus equi Subspecies equi M Protein Titers (≥1:12,800) with and without Complications Post-Outbreak
3:15 PM	E32	Fe ter Woort	Medical Treatment of Dorsal Displacement of the Soft Palate in Sport Horses
4:15 PM	E33	Emily Berryhill	Pharmacokinetics of Maropitant Citrate in Horses
4:30 PM	E34	Alexandra Carlson	Effect of Soluble Epoxide Hydrolase Inhibitor, t-TUCB, on Recovery of Ischemic Injured Porcine Jejunum
4:45 PM	E35	Jennifer Davis	Pharmacokinetics of 4-Methylaminoantipyrine, the Active Metabolite of Dipyrone, in the Horse
5:00 PM	E36	Sonia Gonzalez-Medina	Susceptibility of Horses and Sheep to Hypoglycin A Intoxication: In/Ex Vivo Bioavailability and Cellular Effects
5:15 PM	E37	Angelika Schoster	Effect of Different Antimicrobial Treatment Regimes on Occurrence of Fecal Extended- Spectrum β-Lactamase-Producing Enterobacteriaceae
5:30 PM	E38	Jennifer Bauquier	Inflammatory Effect of Mitochondrial Fragments in Equine Whole Blood

FOOD ANIMAL

1:30 PM	F09	Marie-Eve Bilodeau	Prognostic Indicators in Downer Cows Presented to a Hospital: Retrospective Study (1472 cases) (ACVIM Resident Research Award Eligible)
1:45 PM	F10	Evelyn MacKay	Pharmacokinetics of Tulathromycin in the Fetal and Maternal Compartments of Sheep (ACVIM Resident Research Award Eligible)
2:00 PM	F11	Lisa Gamsjaeger	Sodium Iodide as a Preventative Strategy against Respiratory Disease in Pre-Weaned Dairy Calves (ACVIM Resident Research Award Eligible)
2:15 PM	F12	Julie Berman	Accuracy of Systematic Thoracic Ultrasound for Diagnosis of Active Pneumonia in Pre-Weaned Calves

POSTER PRESENTATIONS - THURSDAY, JUNE 14

Time	#	Presenting Author	Abstract Title
CARDIOLOGY			
9:35 AM	C24	Alyssa Pinkos	Treatment of Pulmonary Hypertension with Sildenafil Monotherapy versus Combination Therapy with Sildenafil and Pimobendan (ACVIM Resident Research Award & Cardiology Research Abstract Award Eligible)
9:50 AM	C25	Rebecca Saunders	Relationship Between Serotonin and ALK5 Function on Development of Myxomatous Valvular Degeneration in Rat Model (ACVIM Resident Research Award & Cardiology Research Abstract Award Eligible)
12:15 PM	C26	Juliana Araújo	Cardiac Evaluation of Naturally Infected Distemper Dogs
12:30 PM	C27	Rosangela Carvalho	Omega 3 Fatty Acids and Mitral Valvar Disease: B2 and C Stages
12:45 PM	C28	Rosangela Carvalho	Omega 3 Fatty Acids in Dogs with Mitral Valvar Disease without Myocardial Remodeling
1:00 PM	C29	I Ping Chan	Prognostic Value of Right Pulmonary Artery Distensibility Index in Dogs with Pulmonary Hypertension
1:15 PM	C30	Hyeong il Choi	Comparative Efficacy Analysis of Anti-Hypertensive Drugs in Severe Hypertensive Dogs
3:35 PM	C31	Rodrigo DeLaValle	Case Series: Myocarditis in 6 Dogs with Dirofilaria immitis
3:50 PM	C32	Luis Dos Santos	Long-term Outcomes of Pulmonary Artery Banding for Ventricular Septal Defects
9:35 AM	C33	Kathryn Taggart	Role of the Pyruvate Dehydrogenase Kinase-4 (PDK4) Gene Mutation in Mitochondrial Metabolism in Doberman Pinschers
9:50 AM	C34	Wendy Ware	Aortoseptal Angle and Systolic Murmur Occurrence in Normal Cats and Cats with Cardiomyopathy
12:15 PM	C35	Akane Yoshikawa	Investigation of Transfusion-Free Mitral Valve Plasty in Dogs

ONCOLOGY

9:35 AM	O16	Arata Matsuyama	Efficacy of a Doxorubicin and Cytarabine Chemotherapy Protocol in 6 Dogs with Acute Myeloid Leukemia (ACVIM Resident Research Award Eligible)
9:50 AM	O17	Soomin Ahn	Clinical and Immunological Anti-Cancer Effects of Canine IL-15 with Metronomic Cyclophosphamide in Dogs with Cancer
12:15 PM	O18	Chad Johannes	Ecological-Level Analysis of Canine and Feline Primary Lung Tumors and Environmental Radon Levels
12:30 PM	O19	Valter Winkel	Immunohistochemical Expression of Lung Resistance-Related Protein (LRP) in Dogs with Cutaneous Lymphoma
12:45 PM	O20	Meg Musser	Retrospective Evaluation of Palladia® Use in the Treatment of inoperable, Metastatic, Or Recurrent Canine Pheochromocytomas
1:00 PM	O21	Shinichi Namba	Effect of Glucose Transporter Inhibition on Cell Growth in Canine Melanoma
1:15 PM	O22	Austin Viall	Intratumoral Expression of Serotonergic System Genes htr1b, htr2a, htr2b, tph1, and slc6a4 in Canine Osteosarcoma
3:35 PM	O23	Shirley Chu	Unravelling the Chaotic Genomic Landscape of Canine Osteosarcoma with Current Sequencing Technologies and Bioinformatic Approaches (VCS Award Winner)
3:50 PM	O24	Briana Hallman	Incidence and Risk Factors Associated with the Development of Symptomatic Cardiotoxicity in Dogs Receiving Doxorubicin (VCS Award Winner)

SMALL ANIMAL INTERNAL MEDICINE - ENDOCRINOLGY

9:35 AM	EN10	Jodie Anderson	Use of Lispro Insulin for Treatment of Diabetic Ketoacidosis in Cats: A Pilot Study (ACVIM Resident Research Award Eligible)
9:50 AM	EN11	Aubrey Hirsch	Effects of EDTA on Chemiluminescent Immunoassay Measurement of ACTH, Cortisol, and Thyroid Hormones in Dogs (ACVIM Resident Research Award Eligible)
12:15 PM	EN12	Amy Oberstadt	Investigating the Effect of Thyroid Stimulating Hormone Administration on Radioactive Iodine Uptake in Hyperthyroid Cats (ACVIM Resident Research Award Eligible)
12:30 PM	EN13	Allison Rowland	Comparison of Urine Cortisol:Creatinine Ratio and Basal Cortisol for the Diagnosis of Canine Hypoadrenocorticism (ACVIM Resident Research Award Eligible)
12:45 PM	EN14	Ellen Behrend	Accuracy Over Time of Bayer Diastix® Strips for Detection of Glucosuria in Cat Urine
1:00 PM	EN15	Juliette Bouillon	Dexmedetomidine's Effect on Glucose Homeostasis in Healthy Cats
1:15 PM	EN16	Arnon Gal	Changes in Serum total Thyroxin Concentrations in Hyperthyroid Cats Do Not Affect Serum Glucose Concentrations
3:35 PM	EN17	Michelle Giuffrida	Perioperative Characteristics, Histological Diagnosis and Outcome in Cats Undergoing Surgical Treatment of Primary Hyperparathyroidism
3:50 PM	EN18	Ji-Soo Kim	Serum Amino Acid Concentrations in Dogs with Naturally Occurring Pituitary-Dependent Hyperadrenocorticism

SMALL ANIMAL INTERNAL MEDICINE - GASTROENTEROLOGY

9:35 AM	GI21	Jenny Stiller	Usefulness of Blood Urea Nitrogen/Creatinine Ratio (BUN/Cr) in Confirming and Localizing Gastrointestinal Bleeding in Dogs (ACVIM Resident Research Award Eligible)
9:50 AM	GI22	Julia Honneffer	Increased Fecal Fatty Acid Concentrations in Dogs with Chronic Enteropathy Normalize with Treatment
12:15 PM	GI23	Hirakata Igarashi	A Retrospective Study of Granulomatous Gastritis in Miniature Dachshunds: 11 Cases
12:30 PM	GI24	Hirakata Igarashi	Distribution of Regulatory T Cells in Inflammatory Colorectal Polyps in Miniature Dachshunds
12:45 PM	GI25	Michael Lappin	Feeding a High Fiber Diet for Management of Acute Large Bowel Diarrhea in Shelter Dogs
1:00 PM	GI26	Sina Marsilio	Metabolomic Markers in Fecal Samples from Cats with Chronic Enteropathy
1:15 PM	GI27	Khoirun Nisa	Evaluation of Duodenal Perfusion by Contrast-Enhanced Ultrasonography in Dogs with Chronic Enteropathy and Intestinal Lymphoma
3:35 PM	GI28	Yasushi Minamoto	Dysbiosis in Dogs with Acute Diarrhea Treated with a Fecal Microbial Transplantation or an Antibiotic
3:50 PM	GI29	Yasushi Minamoto	Serum Fatty Acid Binding Protein 2 and 6 Concentrations in Dogs with Chronic Enteropathy

SMALL ANIMAL INTERNAL MEDICINE - HEMATOLOGY

9:35 AM	HM06	Junwoo Bae	Therapeutic Monitoring of Rivaroxaban in Dogs Using Thromboelastography and Prothrombin Time
9:50 AM	HM07	Macy Drinkhouse	Influence of Canine Donor Plasma Hemostatic Protein Concentration on Quality of Cryoprecipitate
12:30 PM	HM09	George Lubas	Nucleated Erythrocytes and Anemia in Dogs with Systemic Inflammatory Response Syndrome: Could they Affect Outcome?

12:45 PM	HM10	Keitaro Morishita	Rapid Decrease in Prednisolone Dosage Can Cause Early Recurrence of Immune-Mediated Thrombocytopenia in Dogs
1:00 PM	HM11	Sarah Shropshire	Comparison of Fibrinolysis via Thromboelastography in Greyhounds versus Non-Greyhounds
1:15 PM	HM12	Sarah Shropshire	Viability of Two Platelet Agonist Reagents in Whole Blood Impedance Platelet Aggregometry in Dogs
3:35 PM	HM13	Hayoung Yang	Effects of Irradiation and Leukoreduction on Down-regulation of CXCL-8 and Storage Lesion in Canine Blood

SMALL ANIMAL INTERNAL MEDICINE - HEPATOLOGY

3:35 PM	HP07	Rommaneeya Leela-arporn	Demographic Features, Characteristics and Risk Factors in a Retrospective Study of Hepatocellular Carcinoma in Dogs
3:50 PM	HP08	Jonathan Lidbury	Serum Gastrin Concentrations in Dogs with Hepatic Disease

SMALL ANIMAL INTERNAL MEDICINE - IMMUNOLOGY

12:45 PM	IM03	Anri Celliers	Neutrophil Myeloperoxidase Index in Dogs with Babesiosis
1:00 PM	IM04	Marshal Covin	Analytical Validation of an Immunoturbidimetric Assay for the Measurement of Serum CRP Concentrations in Dogs
1:15 PM	IM05	Sarah Shropshire	Validation of a Clinically Applicable Flow Cytometric Assay for Detection of Immunoglobulin-Associated Platelets in Dogs
3:35 PM	IM06	Dahlia Tesfamichael	Comparing RNA Quality and Quantity Extracted from Canine Blood Using Commercially Available RNA Extraction Kits

SMALL ANIMAL INTERNAL MEDICINE - NEPHROLOGY / UROLOGY

9:35 AM	NU18	Kellyi Benson	Owner Survey of Amoxicillin-Clavulanic Acid Side Effects in Cats With and Without Azotemic CKD (ACVIM Resident Research Award Eligible)
9:50 AM	NU19	Kaitlin Lonc	Feline Urinary Incontinence: A Retrospective Study of 39 Cases (2006-2017) (ACVIM Resident Research Award Eligible)
12:15 PM	NU20	Melisa Rosenthal	Outcome in Dogs with Non-Steroidal Anti-Inflammatory Drug Toxicity Treated by Therapeutic Plasma Exchange (ACVIM Resident Research Award Eligible)
12:30 PM	NU21	Marcia Kogika	Vitamin D Metabolites in Chronic Kidney Disease Cats with Nephrolithiasis
12:45 PM	NU22	Cook English	Evaluation of an Herbal Compound Used to Manage Lower Urinary Tract Disease in Healthy Cats
1:00 PM	NU23	Po-Yao Huang	The Prognostic Factors in Cats with Big Kidney Little Kidney Syndrome
1:15 PM	NU24	Hwei Jing	Expression Profile of Matrix Metalloproteinase-9, Neutrophil Gelatinase-Associated Lipocalin and Hemojuvelin in Cat with Urinary Diseases
3:35 PM	NU25	Denise Kelley	Hyperammonemia in Cats with Renal Azotemia
3:50 PM	NU26	Andre Le Sueur	Symmetric Dimethylarginine Concentrations in Dogs with IRIS Stage 4 Chronic Kidney Disease Undergoing Intermittent Hemodialysis

SMALL ANIMAL INTERNAL MEDICINE - OTHER

12:15 PM	OT06	Scott Secrest	Computed Tomographic Appearance of Abdominal Lymph Nodes in Healthy Cats
12:30 PM	OT07	Michael Verschoor-Kirss	Intravenous Fluid Prescribing Rates for Dogs at a Veterinary Teaching Hospital
12:45 PM	OT08	Woosun Kim	The Effects of 3% Hydroxyethyl Starch-Hypertonic Saline on Resuscitation of Dogs in Controlled Hemorrhagic Shock
1:00 PM	OT09	Andrew Woolcock	Urinary F2-Isoprostanes: A Comparison of Two Methods for Measurement in Cats
1:15 PM	OT10	Woo-Jin Song	Canine Mesenchymal Stem Cells Pre-Treated with TNF-α/IFN-γ Enhance Anti-Inflammatory Effects by Up-Regulating the COX-2/PGE2 Pathway

SMALL ANIMAL INTERNAL MEDICINE - PHARMACOLOGY

9:50 AM	P07	Kate KuKanich	Pharmacokinetics of Oral Fluconazole in a Clinical Population of Dogs and Cats

SMALL ANIMAL INTERNAL MEDICINE - RESPIRATORY

9:35 AM	R07	Po-ching (Lydia) Pan	Comparison of Propofol and Alfaxalone Induction Anesthesia for Evaluation of Laryngeal Function in Healthy Dogs (ACVIM Resident Research Award Eligible)

EQUINE

9:35 AM	E39	Maria Lucia Lourenço	Electrocardiographic Parameters Pregnant Donkeys (Equus Asinus) of the Pega Breed
9:50 AM	E40	Melissa Schott	Expression of Micropeptides that Regulate Intracellular Calcium Concentrations in Horse Skeletal and Cardiac Muscles

12:15 PM	E41	Melissa Mercer	Pharmacokinetics and Safety of Repeated Oral Dosing of Acetaminophen in Adult Horses (ACVIM Resident Research Award Eligible)
12:30 PM	E42	Ronan Chapuis	Pharmacodynamics of Doxycycline in a Streptococcus equi Subsp. zooepidemicus Infection Model in Horses
12:45 PM	E43	Andrew Woodward	Pharmacokinetics and Pharmacodynamics of Subcutaneous Dexmedetomidine in Anaesthetized Horses
1:00 PM	E44	Jennifer Bauquier	Retrospective Evaluation of Plasma Phosphate and Calcium Concentrations as Biomarkers in Horses with Gastrointestinal Disease
1:15 PM	E45	Laura Dunbar	Gastric Outflow Obstruction in Four Foals Associated with an Outbreak of Rotaviral Enteritis
3:35 PM	E46	Stephanie Vijan	Association of Progestogens with Inflammation and Immunity in Critically Ill Foals
3:50 PM	E47	Emily Sundman	Proof of Concept Study of Subcutaneously Administered Anti-TNF Antibody in Neonatal Foals with Sepsis

FOOD ANIMAL

9:35 AM	F13	Jayoung Jang	Gut Microbiota in Diarrheic Calf with Bovine Rotavirus infection in Korea
9:50 AM	F14	Jennifer Halleran	Regression of Squamous Cell Carcinoma in a Mature Bucking Bull and Longhorn Cow Using Immunocidin© (ACVIM Resident Research Award Eligible)
12:30 PM	F16	Aleksandar Masic	Efficacy of Mycobacterium Cell-Wall Fraction on Influx of Polymorphonuclear Cells in Uterus of Dairy Cows
12:45 PM	F17	Andrew Woodward	Physiologically-Based Population analysis for Determination of Milk Discard Times for Intra-Mammary Drugs
1:00 PM	F18	Lisa Gamsjaeger	Effect of Oral Iodide Supplementation on Iodine Concentration in Respiratory Fluids of Pre-weaned Dairy Calves (ACVIM Resident Research Award Eligible)
1:15 PM	F19	Diego Gomez-Nieto	Pharyngeal Trauma and Perforation in Dairy Cattle: 27 Cases

POSTER PRESENTATIONS - FRIDAY, JUNE 15

Time	#	Presenting Author	Abstract Title
CARDIOLOGY			
9:50 AM	C37	Masayuki Enokizono	Changes of Blood Gas Characteristics During Open Heart Surgery
10:05 AM	C38	Katsuhiro Matsuura	Effect of Hemodialysis during Cardiopulmonary Bypass in Dogs with Azotemia Undergoing Mitral Valve Plasty
12:15 PM	C39	Nobuyuki Kanno	Thyroid Hormone and Cardiopulmonary Bypass in Dogs
12:30 PM	C40	Imal Khelik	Clinicopathologic, Hemodynamic, and Echocardiographic Effects of Anti-Inflammatory Glucocorticoids in Systemically Healthy Cats
12:45 PM	C41	Zi Ping Leong	Reversal Effect of Toceranib Versus Sorafenib on Monocrotaline-Induced Pulmonary Arterial Hypertension in Rats
1:00 PM	C42	Maria Lucia Lourenço	The Effects of Obesity on Cardiovascular Parameters in Cats
1:15 PM	C43	Maria Lucia Lourenço	Heart Rate and Heart Rate Variability in Pregnant Dairy Cows and in Fetuses
3:35 PM	C44	Maria Lucia Lourenço	Heart Rate Variability Assessment in Foals Undergoing Hoof Trimming with Use of Equine Maternal Pheromones
3:50 PM	C45	Maria Lucia Lourenço	Heart Rate and Heart Rate Variability in Pregnant American Miniature Horse Mares
9:35 AM	C46	Mengmeng Liu	Cardiovascular-Renal Axis Disorder in Cats with Congestive Heart Failure Due to Primary Cardiomyopathy
9:50 AM	C47	Kathryn Meurs	Familial Ventricular Arrhythmias in the Rhodesian Ridgeback
12:15 PM	C48	Takeshi Mizuno	Platelet Function in Dogs with Myxomatous Mitral Valve Disease
12:30 PM	C49	Michelle Oranges	N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP) in Feline Arterial Thromboembolism
12:45 PM	C50	Guilherme Pereira	Clinical Outcome and Echocardiographic Prediction of Congestive Heart Failure in Dogs with Left Atrial Rupture
1:00 PM	C51	Alan Poppl	Blood Pressure, Serum Fructosamine Concentration, and Insulin Dose Correlations in Diabetic Dogs
1:15 PM	C52	Kevin Render	Elevated Thyroid Stimulating Hormone Levels Following Administration of Amiodarone for Tachyarrhythmias in 10 Dogs
3:35 PM	C53	Haruhiko Suzuki	Residual Pulmonary Hypertension After Mitral Valve Plasty in Dogs With Mitral Valve Disease

NEUROLOGY

9:50 AM	N18	Kelly Cummings	Radiographic Indices for the Diagnosis of Atlantoaxial Instability in Dogs (ACVIM Resident Research Award Eligible)
10:05 AM	N19	Georgina Stewart	Non-Invasive Vagal Nerve Stimulation for Refractory Epilepsy in Dogs (ACVIM Resident Research Award Eligible)
12:15 PM	N20	Daniella Vansteenkiste	Long-Term Computed Tomography Follow-Up in Great Danes with or Without Signs of Cervical Spondylomyelopathy (ACVIM Resident Research Award Eligible)
12:30 PM	N21	Hyunkee Cho	Nerve Growth Factor Gene Therapy Increases the Plasticity in Pyridoxine Induced Neuropathic Dogs
12:45 PM	N22	Ronaldo da Costa	Magnetic Resonance Imaging Characterization of Combined Osseous and Disc-associated Cervical Spondylomyelopathy in Dogs
1:00 PM	N23	Dong-In Jung	Expression of PDGFR-α/-ß, VEGFR-2, c-Abl, and c-Kit in Canine Granulomatous Meningoencephalitis and Necrotizing Meningoencephalitis
1:15 PM	N24	Austin Kerns	Interobserver Agreement of Mechanical Sensory Thresholds in Normal Dogs
3:35 PM	N25	Kazuyoshi Sasaoka	Trace Elemental Profiles of Serum and Cerebrospinal Fluid in Dogs with Idiopathic Epilepsy
3:50 PM	N26	Richard Shinn	Magnetization Transfer and Diffusion Tensor Imaging in Dogs with Spinal Cord Injury

SMALL ANIMAL INTERNAL MEDICINE - ENDOCRINOLGY

10:05 AM	EN20	Allison O'Kell	Untargeted Metabolomic Analysis in Non-Fasted Diabetic Dogs
12:15 PM	EN21	Alan Poppl	Canine Hyperadrenocorticism Enviromental Risk Factors: a Case-Control Study
12:30 PM	EN22	Scott Secrest	Assessment of the Pancreas in Cats with Diabetes Mellitus using Computed Tomographic Angiography
12:45 PM	EN23	Woo-Jin Song	Canine Mesenchymal Stem Cells Improve Insulin Resistance by Secreting Fibroblast Growth Factor-1 In Vitro
1:00 PM	EN24	Albert Jergens	Microbiota-Related Changes in Fecal Bile Acid Metabolism are associated with Diabetes Mellitus in Dogs

SMALL ANIMAL INTERNAL MEDICINE - GASTROENTEROLOGY

9:50 AM	GI30	Ran Nivy	Retrospective Study of 157 Cats with Pancreatitis Requiring Hospitalization: Clinicopathological Findings, Prognostic Markers and Outcome
10:05 AM	GI31	Jean-Sébastien Palerme	Effect of Fat-Loading on Gastrointestinal Transit Time and Mucosal Appearance as assessed by Capsule Endoscopy
12:15 PM	GI32	Rafael Santisteban-Arenas	Immunolocalization of S100A12 and its association with Helicobacter spp colonization in canine gastric mucosa
12:30 PM	GI33	Sirikul Soontararak	Increased Immunoglobulin Binding Fecal Bacteria: Role of Response to Dysbiosis in Dog Inflammatory Bowel Disease
12:45 PM	GI34	Amanda Blake	Altered Fecal Fatty Acid, Sterol, and Bile Acid Metabolism in Dogs with Acute Diarrhea
1:00 PM	GI35	Karin Allenspach	Characterization of Paneth-like Cells in the Canine Small Intestine
1:15 PM	GI36	Karin Allenspach	Sequencing of Chromosome 11 Identifies SNPs Associated with Inflammatory Bowel Disease in German Shepherd Dogs

SMALL ANIMAL INTERNAL MEDICINE - INFECTIOUS DISEASE

3:35 PM	ID10	Kaylyn McDaniel	Three-Year Longitudinal Seroprevalence of Vector-borne Pathogens in Mid-Missouri Dogs
3:50 PM	ID11	Janelle Scott	Clinical and Laboratory Findings in Cats Fed Toxoplasma gondii Sporulated Oocysts
9:50 AM	ID12	Emily Coffey	Comparison of Immediate versus Delayed Streak Plate Inoculation of Urine Bacterial Culture and Sensitivity Testing (ACVIM Resident Research Award Eligible)
10:05 AM	ID13	Franzsiska Sonderegger	Prospective Observational Study: Serum Intercellular Adhesion Molecule 1 (sI-CAM-1) in Canine Leptospiral Pulmonary Hemorrhage Syndrome (ACVIM Resident Research Award Eligible)
12:15 PM	ID14	Cheryl Adler	Antimicrobial Resistance: Comparison of Canine and Pediatric Escherichia coli Urinary Isolates in Washington State
12:30 PM	ID15	Jesse Buch	Distribution of the Feline Lungworm Aelurostrongylus abstrusus in the United States Based on Fecal Testing
12:45 PM	ID16	Jesse Buch	ELISA Seroreactivity to Three Bartonella Species in Dogs in the United States
1:00 PM	ID17	Jesse Buch	Highly Specific Screening Tests Maintain Actionable Positive Predictive Values in Areas with Low Heartworm Prevalence
1:15 PM	ID18	Louana Cheung	Experimental Infection of Dogs with H3N2
3:35 PM	ID19	Ian DeStefano	Use of Vancomycin in Life-Threatening Infection in Dogs and Cats, 41 Cases (2003-2017)

3:50 PM	ID20	Bernhard Gerber	Interleukin (IL)-2, IL-6, IL-8, IL-10 and Tumor Necrosis Factor-Alpha in Dogs Infected with Angiostrongylus vasorum
9:50 AM	ID21	Michael Lappin	Assessment for associations Between Bartonella Spp. and Select Complete Blood Cell Count Abnormalities
10:05 AM	ID22	Michael Lappin	Pradofloxacin for Treatment of Bartonella henselae in Experimentally Inoculated Cats
3:35 PM	ID23	Ashley Hopkins	Efficacy and Safety of Novel isonitrile Compounds for Treatment of Staphylococcus pseudintermedius
3:50 PM	ID24	Megan Jacob	Genetic Characterization of Canine Urinary Pathogenic Escherichia coli

SMALL ANIMAL INTERNAL MEDICINE - NEPHROLOGY / UROLOGY

9:50 AM	NU27	John Kruger	Evaluation of Commercial ELISA Kits for Quantification of Selected Cytokines in Feline Urine
10:05 AM	NU28	John Kruger	Investigating Urinary Bladder Transcriptome Alterations in Feline Idiopathic Cystitis Using RNA Sequence Analysis
12:15 PM	NU29	Marcia Kogika	Serum Creatinine vs Serum Symmetric Dimethylarginine on Follow-Up of Dogs with Chronic Kidney Disease
12:30 PM	NU30	Marcia Kogika	Urinary Protein to Creatinine Ratio, Albumin and Retinol-Vitamin D-Binding Proteins in Chronic Kidney Disease Dogs
12:45 PM	NU31	Marcia Kogika	Fibroblast Growth Factor-23 association with Vitamin D Metabolites in Dogs with Spontaneous Chronic Kidney Disease
1:00 PM	NU32	Maria Lucia Lourenço	Electrocardiographic Parameters, P and QT Dispersions in Dogs with Chronic Kidney Disease Undergoing Intermittent Hemodialysis
1:15 PM	NU33	Jennifer MacLeay	Dietary Sodium Increases Calcium Excretion and Induced Calcium Oxalate Precipitation in Cats
3:35 PM	NU34	Timothy McCarthy	Continuous Flow Cystoscopy: The Next Step
3:50 PM	NU35	Kakanang Piyarungsri	Prevalence and Risk Factors of Feline Lower Urinary Tract Disease in Chiang Mai, Thailand
9:50 AM	NU36	Jessica Quimby	Retrospective Evaluation of the Relationship Between Symmetric Dimethylarginine, Creatinine and Body Weight in Hyperthyroid Cats
10:05 AM	NU37	Ashley Shaw	Evaluation of an Herbal Compound Used to Manage Lower Urinary Tract Disease in Healthy Dogs
12:15 PM	NU38	Sarah Shropshire	Platelet Aggregometry and Measurement of Clopidogrel Metabolite Concentrations in Dogs with Protein-Losing Nephropathy
12:30 PM	NU39	Chan-Joo Sung	Alterations in Oral and Fecal Microbiomes in Dogs with Chronic Kidney Disease
12:45 PM	NU40	Jean-Sébastien Palerme	Serum Lipoprotein Profiles in Cats with Chronic Kidney Disease
1:00 PM	NU41	Brittany Vester Boler	Urine is Not Sterile: Evidence of a Urinary Microbiome in Healthy Adult Cats
1:15 PM	NU42	Michael Wood	Daily Oral Chondroitin Sulfate Administration Increases Urinary Chondroitin Sulfate Concentrations in Dogs
3:35 PM	NU43	Yoshihiko Yu	Assessment of Indicators for Disease Progression in Feline Autosomal Dominant Polycystic Kidney Disease
3:50 PM	NU44	Brian Zanghi	Creatinine and GFR Measurements using Jaffe and Enzyme-Based Methods in Cats

SMALL ANIMAL INTERNAL MEDICINE - NUTRITION / METABOLISM

12:15 PM	NM07	Marcio Brunetto	Pea and Barley as a Starch Source Impact on Hyperlipidemia of Diabetic Dogs
12:30 PM	NM08	Sarah Dodd	Changes in Pet Feeding Practices Over the Past Decade
12:45 PM	NM09	Hannah Klein	Correlation between Dietary Macronutrients and Gut Microbiota Bacterial Groups
1:00 PM	NM10	Sol Rivera	Feline Plasma and Urine Lipidome: A Novel Source of Biomarker Candidates for Feline Medicine
1:15 PM	NM11	Sol Rivera	Protein Carbonyl Content as an Indicator of AGEs Formation in Diabetic Dogs

EQUINE

9:50 AM	E48	Johan Bröjer	Comparison Between Euglycemic-Hyperinsulinemic Clamp Measurements and Proxies for Insulin Sensitivity and B-Cell Response in Horses
10:05 AM	E49	John Haffner	Effects of Freezing on Measurement of Plasma Adrenocorticotropic Hormone Concentrations in Horses
12:15 PM	E50	Katarina Nostell	Blood Pressure and Cortisol in Horses with Diet Induced Obesity and Effect of Pasture
12:30 PM	E51	Harold Schott, II	Metacarpal Bone Thickness and Density in Aged Horses with and without Pituitary Pars Intermedia Dysfunction
12:45 PM	E52	Laura Dunbar	Hyperlipemia in Hospitalized Equids: Treatment and Prognosis

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ABSTRACTS

C01

Pharmacodynamics of Entresto® (Sacubitril/Valsartan) Versus Placebo in Dogs with Preclinical Myxomatous Mitral Valve Disease

Daniel K. Newhard, SeungWoo Jung, Randolph Winter, Sue Duran
Auburn University College of Veterinary Medicine, Auburn, AL, USA

Angiotensin converting enzyme inhibitors (ACEi) are standard of care for treatment of congestive heart failure, but aldosterone breakthrough occurs when ACEi are used in both dogs and humans. Entresto®, a combinational angiotensin-receptor blocker/neprilysin inhibitor, showed superiority in reducing mortality in human patients with heart failure compared to enalapril. Pharmacodynamics of Entresto® have been evaluated in healthy dogs, showing efficacy in altering the renin-angiotensin-aldosterone system (RAAS) without causing adverse effects. The aim of this prospective, blinded, randomized, placebo-controlled study was to compare the pharmacodynamic effects of Entresto® to placebo in dogs with preclinical myxomatous mitral valve disease (MMVD) and to evaluate the safety profile of Entresto® in dogs with cardiac disease.

Client-owned dogs weighing 4-15 kg with ACVIM Stage B2 MMVD were enrolled. Dogs with clinically significant pulmonary hypertension or systemic disease were excluded, as were dogs on any medication(s) known to alter the RAAS. All dogs received pimobendan throughout the study period. Each patient was evaluated at three time points (Day 0, Day 7, and Day 30). Echocardiography, thoracic radiographs (CXR), Doppler systemic arterial pressure (SAP), complete blood count, serum biochemical profile, plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) concentration, and urinary aldosterone to creatinine ratio (UAldo:C) were evaluated on Day 0. On Day 0, each dog was randomly allocated to a treatment group (Entresto®; 20 mg/kg BID) or a placebo group by the attending pharmacist. Investigators, veterinary technicians, and owners were blinded to treatment. Serum renal enzyme and electrolyte concentrations and SAP were rechecked on Day 7. Echocardiography, CXR, SAP, renal panel, plasma NT-proBNP concentration, and UAldo:C were rechecked on Day 30.

Thirteen dogs were recruited: Entresto® (n = 7) and placebo (n = 6). The median percent increase in UAldo:C between Day 0 and Day 30 was significantly lower in the Entresto® group (12%; $P = 0.032$) as compared to the placebo group (195%). The median percent decrease of NT-proBNP concentration from Day 0 to Day 30 was not statistically different between groups ($P = 0.68$). No statistical differences were seen in echocardiographic, CXR, SAP, or biochemical profiles measured at any time point between groups. No adverse events were noted by the owners for dogs in either group.

This study suggests that Entresto® efficiently inhibits RAAS in dogs with cardiomegaly secondary to MMVD in comparison to placebo. Entresto® is safe in regards to SAP and renal enzyme and electrolyte concentrations. No adverse effects were noted in any dog taking Entresto®. Future studies comparing Entresto® to ACEi, evaluating the concomitant use of diuretics and Entresto®, and evaluating long-term (> 30 days) effects of Entresto® are warranted.

C02

Trimethylamine-N-Oxide and its Precursors in Dogs with Degenerative Mitral Valve Disease

Emily T. Karlin[1], John Rush[2], Lisa Freeman[2]
[1]Tufts University, Westborough, MA, USA, [2]Tufts University, North Grafton, MA, USA

Trimethylamine N-oxide (TMAO), the oxidized product of trimethylamine, is produced by gastrointestinal microbiota from certain dietary nutrients including choline and L-carnitine. Elevated plasma concentrations of TMAO, choline, and L-carnitine have been shown to be associated with the presence and severity of cardiovascular disease in people and are independent predictors of adverse cardiac events and mortality. This prospective study investigated whether there are differences in concentrations of circulating TMAO, choline, or L-carnitine in dogs with degenerative mitral valve disease (DMVD) compared to healthy controls (Controls).

Thirty client-owned dogs were recruited, including 10 dogs with CHF secondary to DMVD, 10 dogs with asymptomatic DMVD, and 10 Controls. Echocardiography was performed, and fasting plasma concentrations of TMAO, choline, and carnitine fractions were measured. Data were compared among the three groups using ANOVA or Kruskal-Wallis tests.

Trimethylamine N-oxide ($P = 0.034$), total L-carnitine ($P = 0.034$), carnitine esters, and carnitine esters to free carnitine ratio (E/F ratio) were significantly higher in dogs with CHF compared to asymptomatic DMVD. Trimethylamine N-oxide ($P = 0.022$), choline ($P = 0.011$), total L-carnitine ($P = 0.011$), carnitine esters, free carnitine, and E/F ratio were significantly higher in dogs with CHF compared to Controls. No differences were detected between asymptomatic DMVD dogs and Controls.

Dogs with DMVD and CHF had higher concentrations of TMAO compared to both asymptomatic DMVD dogs and Controls. Prospective studies are warranted to determine if TMAO concentrations can be altered with dietary or gut microflora modification, and whether altering TMAO concentrations could impact disease progression.

C03

Comparison Between the Effects of Torsemide and Furosemide on the Renin-Angiotensin-Aldosterone System of Normal Dogs

Brianna M. Potter, Marisa K. Ames, Ann Hess
Colorado State University, Fort Collins, CO, USA

Loop diuretics provide symptomatic relief for congestive heart failure, yet stimulate the renin-angiotensin-aldosterone system (RAAS). We hypothesized that the two potent loop diuretics, torsemide and furosemide, at approximately equipotent dosages (similar diuresis), would have comparable effects on the circulating RAAS.

Six, healthy, middle-aged, male beagles were randomized to receive torsemide (0.1mg/kg PO q12h), furosemide (2.0mg/kg PO q12h), or placebo for 10 days during 3 separate experiments, in a crossover design with a 10-day washout period between experiments. Blood was collected on days 1, 5, and 9 and 24-hour urine collection ended on days 2, 6, and 10. After repeated measures analysis and Bonferonni correction, variables with an adjusted $P < 0.05$ were investigated further, using Tukey';s method.

Twenty-four hour urine production differed significantly between the diuretics only on day 10, with torsemide causing a 38% greater diuresis than furosemide. There was, however, no significant difference in average 3-day diuresis. There were no significant differences between diuretics in the 24-hour urinary excretion of Na^+, Cl^-, or K^+, though furosemide caused less kaliuresis than torsemide. Serum renin, angiotensin II, and aldosterone and the urine aldosterone-to-creatinine ratio were significantly increased in the diuretic groups, as compared to placebo on days 5/6 and 9/10. There were no significant differences in these parameters between diuretics. Creatinine and BUN concentrations rose comparably in the 2 diuretic groups, remaining within reference intervals in all dogs.

At approximately equipotent dosages (20:1), torsemide and furosemide produced comparable RAAS activation. This, and torsemide';s greater kaliuretic effect decrease support for torsemide';s hypothesized mineralocorticoid-receptor blocking capability.

C04

Transvenous Echocardiography in Conscious Sedated Horses

Bruno G. Boutet[1], Sonya G. Gordon[1], Cristobal Navas De Solis[2], Ashley B. Saunders[1], Mauricio Lepiz[2], Sonya R. Wesselowski[1]
[1]Texas A&M University, College Station, TX, USA, [2]Department of Large Animal Clinical Studies, Texas A&M University, College Station, TX, USA., College Station, TX, USA

The objective was to assess the diagnostic utility and feasibility of using a 3-10MHz, 8 French, 90cm intracardiac echocardiography (ICE) catheter (Acuson AcuNav®) to evaluate cardiac structures and function in conscious horses sedated with xylazine.

Ten apparently healthy horses (458-619kg) from the Texas A&M university teaching herd underwent a physical exam, transthoracic echocardiography (TTE) and ICE through a 10 French right jugular introducer placed in the proximal 1/3 of the neck. A continuous ECG was recorded during the procedure using simultaneous ECG telemetry. Three positions are described with the associated 2D, pulse wave Doppler (PW), continuous wave Doppler (CW), color Doppler (CD) and M-mode imaging planes standardized by referencing the intracardiac positions and common landmarks. The positions were confirmed with simultaneous TTE.

The 1st position (cranial right atrium) allowed visualization of the right atrium, right ventricle, tricuspid valve, cranial and caudal vena cava in long-axis. Alignment was adequate for CD, CW and PW evaluation of the tricuspid valve for assessment of regurgitation and inflow. Clockwise rotation allowed visualization of the aortic valve and ascending aorta in long axis, and the pulmonic valve in short axis with adequate CD evaluation. Posterioflexion provided adequate alignment for transaortic PW and CW. The 2nd position (mid right atrium) allowed visualization of the left ventricular outflow tract, aortic valve and left atrium in long-axis. The depth of the imaging plane resulted in suboptimal CD evaluation. Anteroflexion and counterclockwise rotation allowed visualization of the mitral valve, interatrial septum, foramen ovale, left atrium, pulmonary veins and associated ostia in long-axis. CD evaluation was adequate. The 3rdposition (right ventricle) allowed visualization of the mitral valve, interventricular septum and left ventricle in long and short-axis. A diagnostic M-mode of the left ventricle was possible from this positon.

The procedure was well tolerated with only mild, intermittent ventricular and supraventricular arrhythmias that resolved with ICE catheter repositioning.

We conclude that ICE is feasible, safe, and allows for acquisition of repeatable diagnostic imaging planes in conscious, sedated horses. It could potentially be useful in clinical situations where TTE may be technically difficult to perform or poorly tolerated in horses.

C05

Inotropic and Chronotropic Effects of Sotalol in Healthy Dogs

Julia R. Treseder, Nicole L. LeBlanc, Katherine F. Scollan
Oregon State University, Corvallis, OR, USA

Sotalol is a class III anti-arrhythmic drug that is commonly prescribed in the treatment of ventricular tachyarrhythmias in dogs. The anti-arrhythmic effects of sotalol are mediated by an increase in action potential duration and prolongation of atrial and ventricular repolarization via antagonism of the delayed rectifier potassium current. These effects have been demonstrated to be independent of sotalol';s ß-blocking properties. However, ß blockade may result in reduced myocardial contractility and also contribute to slowing of the sinus rate. In patients with existing heart disease, a cardiodepressant drug effect may be clinically relevant, yet the inotropic properties of sotalol are not well-characterized in dogs. The aim of this study was to investigate the inotropic and chronotropic effects of sotalol on healthy, awake dogs.

Ten adult, large-breed dogs were recruited from the veterinary community at the Oregon State University College of Veterinary Medicine. Dogs were considered healthy based on history, physical exam, oscillometric blood pressure measurement, transthoracic echocardiography, and a 10-lead electrocardiogram. Each dog also had a baseline 24 hour Holter monitor performed. Sotalol at a dose of 1-2 mg/kg orally q12h was then administered for 12-16 days, followed by a second evaluation including the same diagnostics tests. Physical exam parameters, blood pressure measurements, Holter data, echocardiographic measurements including three-dimensional (3D) left

ventricular (LV) volumes and 3D strain were measured at each evaluation. Baseline and post-treatment measurements were assessed for normality, and compared statistically with paired t-tests for normally distributed data and Wilcoxon signed rank tests for non-normally distributed data. 3D data were available for 7/10 dogs. No correction was made for multiple comparisons.

Ten dogs were included in the study, with a mean age of 3.4 years (range, 1.1-6.4 years) and mean weight of 26.1 kg (range, 21-35.8 kg). The mean sotalol dose administered was 1.56 ± 0.23 mg/kg. Heart rate on exam was significantly ($p = 0.036$) lower post-treatment (81 ± 23 bpm) than pre-treatment (101 ± 26 bpm). Maximum heart rate on Holter monitor was also significantly ($p = 0.002$) lower on sotalol (195 ± 14 bpm) than at baseline (215 ± 13 bpm). Several echocardiographic indices of systolic function were altered compared to baseline: Fractional shortening (FS) using two-dimensional (2D) and M-mode (MM) measurements was significantly reduced on sotalol (2D 28.9%, interquartile range (IQR) 25.0-30.2; MM 24.9 ± 5.7%) compared to baseline (2D 30.7%, IQR 28.7-33.8; MM 32.5 ± 2.6%) with $p = 0.010$ and $p = 0.004$ for 2D and M-mode measurements, respectively. Similarly, ejection fraction (EF) via Simpson';s method of disks (SMOD) was significantly ($p = 0.002$) lower on sotalol (48 ± 6.8%) than baseline (53.8 ± 4.4%). On sotalol, there was a 12.4 ± 8.2% increase in LV end-systolic dimension on 2D, and a 12.6 ± 12.4% increase on M-mode measurements ($p = 0.001$, $p = 0.010$, respectively). There was no significant difference in the 3D left ventricular volumes, nor in global longitudinal or circumferential strain, twist, or torsion.

The results of this study suggest that sotalol has negative inotropic and chronotropic effects in healthy dogs. Standard echocardiographic measurements of systolic function showed a small but statistically significant decrease after sotalol treatment, with a mean reduction in EF (SMOD) of 5.8%. The lack of significance for 3D imaging may reflect the variability in 3D measurements and the small sample size. The effects of sotalol in dogs with structural heart disease should be prospectively assessed to further elucidate the clinical significance of decreased systolic function, and the implications of this reduction in patients at risk for heart failure.

C06

Complications and Outcomes of Multi-Institution Transvenous Pacemaker Implantation

Christopher Whipp[1], *Christopher Stauthammer*[2], *Lance Visser*[3], *Kelly Flynn*[4], *Heidi Kellihan*[4], *William Byun*[5], *Robert Sanders*[5], *Aaron Rendahl*[2]
[1]*University of Minnesota, St Paul, MN, USA*, [2]*University of Minnesota, Minneapolis, MN, USA*, [3]*University of California, Davis, Davis, CA, USA*, [4]*University of Wisconsin-Madison, Madison, WI, USA*, [5]*Michigan State University, East Lansing, MI, USA*

Transvenous pacemaker implantation is the definitive treatment for pathologic bradyarrhythmias, though reported complication rates have historically been high. The purpose of this study was to determine survival times and complication rates for dogs who underwent transvenous pacemaker implantation in a large number of institutions. In addition, we wished to identify factors that may predispose patients to the development of complications.

A multicenter retrospective analysis of dogs who underwent transvenous pacemaker implantation between January 1st, 2000 and December 31st, 2016 was performed. Arrhythmia type, generator location, number of leads, complication rate and survival times were evaluated. Complications were categorized as major versus minor and by time to development. Survival time was evaluated by Kaplan-Meyer curve.

In total, 595 dogs were identified (221 male, 186 neutered; 374 female, 349 spayed) with a median age of 9.63 years (range, 0.27-17 years). Presenting arrhythmias included 3rd degree AV block (3AVB) (n=324, 54.5%), Sick Sinus Syndrome (SSS) (n=143, 24%), high grade 2nd degree AV block (2AVB) (n=96, 16.1%), atrial standstill (AS) (n=23, 3.9%), or miscellaneous (n=9, 1.5%). Six hundred and three pacemaker implantations were performed with 601 (99.7%) cases surviving the intraoperative period. Patients received single chamber (n=536, 89.2%), dual chamber (n=34, 5.7%) or VDD (n=31, 5.1%) pacing systems. Both active (n=265, 44.5%) and passive (n=331, 55.5%) ventricular fixation leads were used. Pacemaker generators were placed subcutaneously in the cervical region (n=318, 52.9%), or caudal to the scapula (n=283, 47.1%). A total of 594 cases (98.8%) survived to discharge. Overall median survival time was 38.7 months (2AVB, 43.9 months; 3AVB, 43.3 months; AS, 40.4 months; SSS, 34.8 months). There was a significant difference in survival time between 3AVB and SSS ($p = 0.043$).

Major complications (n=130, 21.8%) included lead dislodgement (37 episodes), lead perforation (5 episodes), infection (23 episodes), or other significant complications (65 episodes). Seventeen infections involved the generator (13 cervical placements, 4 caudal to the scapula), which approached but did not reach statistical significance ($p = 0.055$). Minor complications (n=97, 16.3%) included seroma formation (82 episodes) or other minor issues (15 episodes). Pacing complications (n=127, 21.3%) included oversensing (37 episodes), undersensing (25 episodes), intermittent loss of capture (23 episodes), dysfunction requiring a pacing mode switch (18 episodes), noise reversion (13 episodes), or conduction block at high heart rates (5 episodes).

Transvenous pacemaker implantation is associated with a relatively high complication rate. Predisposing factors for specific complications were unable to be identified. 3AVB was associated with a longer survival time compared to SSS, though did not differ from other bradyarrhythmias.

C07

Pharmacokinetics of Transdermal Atenolol Administration in Healthy Cats

Nicole L. LeBlanc, *Sumeia Mohamed*, *Katherine F. Scollan*, *J. Mark Christensen*
Oregon State University, Corvallis, OR, USA

Atenolol is a β_1-receptor antagonist commonly prescribed in cats affected with hypertrophic obstructive cardiomyopathy. The traditional oral dose is 6.25 to 12.5 mg per cat q12-24h. The oral tablet is highly bioavailable (90 ± 9%) with peak atenolol concentration occurring 1-2 hours post administration in cats. However results from several studies indicate owner compliance may be poor with long-term oral treatment, and feline disposition can present specific challenges to owners with chronic oral medication administration. Previous

veterinary studies investigating transdermal atenolol at equivalent oral atenolol dosages have found the majority of cats receiving transdermal atenolol did not have therapeutic plasma atenolol concentrations 2 hours after administration, and none had therapeutic plasma atenolol concentrations 12 hours after administration. These results raise questions regarding the direct substitution of transdermal doses at equivalent oral doses, and the inherent variability of compounded medications. Therefore the goals of this study were to optimize a non-patented transdermal atenolol formulation and assess atenolol dosage amount and frequency in healthy cats using this formulation. We hypothesized that an optimized transdermal atenolol formula at a higher transdermal dosage rate administered q12h will result in therapeutic (> 260 ng/mL) atenolol concentrations in healthy cats.

We collaborated with Oregon State University (OSU) College of Pharmacy researchers to create a transdermal atenolol formulation that had optimal drug release and good permeation in a steady-state manner. Different concentrations of atenolol gels in a hypomellose carrier and carbomer base were evaluated by an in vitro diffusion study using Franz-Chin upright diffusion cells. The samples were subsequently analyzed for atenolol content using a high-performance liquid chromatography. Candidate gels with the highest in vitro perfusion rates through polymer membranes were applied to post-mortem samples obtained from the nape and ears of 8 cats. The samples were placed on Franz-Chin diffusion cells to measure skin permeability of atenolol through the excised skin between the half cells. The results indicated that application of the atenolol gel to the ear gave significantly greater permeation, and one topical formulation suggested optimized performance for transdermal applications *in vivo*. The optimized atenolol formulation with a concentration of 125 mg/mL was then applied to healthy cats enlisted from the OSU veterinary community. Enrolled cats had no abnormalities noted on physical examination, blood pressure measurement, electrocardiogram (ECG), echocardiography, baseline complete blood count and chemistry evaluation. Cats received an escalating dose of transdermal atenolol over 10 days. On the 10^{th} day of treatment, transdermal atenolol was administered and blood samples were collected for atenolol serum concentrations at 3, 6, and 12 hours after topical application. After phlebotomy at 3 and 6 hours post-atenolol administration, an ECG was also performed and average heart rate (HR) measured. Gradual tapering of transdermal atenolol subsequently occurred over 4 days.

All enrolled cats successfully completed the drug trial and no cats experienced any adverse side-effects. Two cats initially received a maximum transdermal dose of 12.5 mg q12h and serum atenolol concentrations were subtherapeutic (< 260 ng/mL) at 3, 6, and 12 hours post-atenolol. The transdermal atenolol dose was increased to 25 mg q12h, and the same 2 cats were re-enrolled in the study using the higher atenolol dosage after a 4 week washout period. Three cats have completed this updated study protocol at the higher atenolol dosage. Two of the 3 cats had therapeutic serum atenolol concentrations 3 hours post-atenolol; the 3rd cat had a near therapeutic concentration of 255 ng/mL (target ≥ 260 ng/mL) at 3 hours post-atenolol. At the 6 hours post-atenolol timepoint, 1 of the 3 cats had a therapeutic serum atenolol concentration and the other 2 cats had near therapeutic serum atenolol concentrations. At 12 hours post-atenolol dosing, 2 of the 3 cats had therapeutic serum atenolol concentrations. For the HR data post-phlebotomy, all cats had a reduction in HR compared to baseline HR at 3 hours post-atenolol (average reduction of 40 bpm), and 2 of the 3 cats had a reduction in HR at 6 hours post-atenolol (average reduction of 25 bpm) compared to baseline HR.

The results of this pilot study suggest this formulation of transdermal atenolol administered at 25 mg q12h provides therapeutic serum atenolol concentrations and attendant HR reduction in most clinically healthy cats. This preliminary data requires validation in a larger cohort of cats.

C08

Anatomy, Baseline Characteristics and Procedural Outcome in German Shepherd Dogs with Patent Ductus Arteriosus

Sonya R. Wesselowski, Ashley B. Saunders, Sonya G. Gordon
Texas A&M University, College Station, TX, USA

German shepherd dogs (GSD) are predisposed to developing patent ductus arteriosus (PDA) and are reportedly prone to type III PDA anatomy. Dogs with type III anatomy are not considered favorable candidates for device-based intervention. The objective of this study was to describe the PDA anatomy, baseline characteristics and procedural outcome of GSD with PDA.

Medical records of 28 GSD that were diagnosed with PDA and underwent surgical ligation or transcatheter device closure between 2007 and 2017 were retrospectively reviewed. German shepherd dogs with PDA often presented with clinical signs (50%), concurrent congenital heart disease (35.7%) and arrhythmias (29%). Dogs were typically mature at presentation (median: 12.1 months) and had a relatively balanced sex distribution (57% female). Patent ductus arteriosus anatomy was classified in 24 of 28 GSD, with type II anatomy being most common (21/24). Three dogs had unusual anatomy (type IV in 1, type V in 2). Median minimal ductal diameter (MDD) in this population was larger than previously reported MDD in a mixed population and ranged between 4.4-4.9 mm depending upon imaging modality. Successful closure was achieved with an Amplatz Canine Duct Occluder (ACDO) in 22 dogs or via surgical ligation in 6 dogs. No cases of type III anatomy were confirmed.

In this population, the majority of GSD had type II PDA anatomy that was amenable to ACDO deployment. Predisposition for large MDD and occasional, unusual PDA anatomy suggests that transesophageal echocardiography may be beneficial for optimal procedural planning in this breed.

C09

Effect of Loop Diuretics on Novel Components of the Renin-Angiotensin-Aldosterone System of Healthy Dogs

Marisa K. Ames, Brianna M. Potter, Ann Hess
Colorado State University, Fort Collins, CO, USA

Loop diuretics activate the 'classical'; circulating renin-angiotensin-aldosterone system (RAAS), increasing renin, angiotensin I and II (AngI; AngII), and aldosterone. Novel RAAS components include Ang1,7, Ang1,5 (counter-regulatory angiotensin metabolites) and AngIII and AngIV (AngII metabolites). We hypothesized that torsemide and furosemide, at equipotent dosages, would significantly and commensurately increase levels of these novel components, mirroring changes in the 'classical'; RAAS.

Six, healthy, middle-aged, male beagles were randomized to receive torsemide (0.1mg/kg PO q12h), furosemide (2.0mg/kg PO q12h), or placebo for 10 days during 3 separate experiments in a cross-over design, each separated by a 10-day washout period. Blood was collected on days 1, 5, and 9 and 24-hour urine samples were collected, ending on days 2, 6, and 10. Serum aldosterone was quantified via liquid chromatography-mass spectrometry (LC-MS)/MS and angiotensin metabolites were quantified with LC-MS/MS and equilibrium analysis (Attoquant Diagnostics, Vienna Austria). Urine aldosterone was quantified via radioimmunoassay (Beckman Coulter) and used to calculate the urine aldosterone-to-creatinine ratio (UAldo:C). After repeated measures analysis and Bonferonni correction, variables with an adjusted P < 0.05 were investigated further, using Tukey';s method.

Serum AngI, AngII, Ang1,7, Ang1,5, AngIV, and aldosterone and the UAldo:C were significantly increased in the diuretic groups, as compared to placebo on days 5/6 and 9/10. Increases in these parameters were greatest in the torsemide group, but failed to reach significance. Serum AngIII increased after diuretic therapy, yet values did not differ significantly from placebo.

Novel components of the circulating RAAS are significantly increased during therapy with both torsemide and furosemide and mirror changes in the 'classical'; RAAS.

C10

Precision and Repeatability of Transthoracic Two-Dimensional and Three-Dimensional Echocardiography in Normal Conscious Dogs

Ryan Fries[1], *Sonya G. Gordon*[2], *Ashley B. Saunders*[2], *David Schaeffer*[3], *Crystal Hariu*[4], *Matthew W. Miller*[5]
[1]*University of Illinois at Urbana-Champaign, Urbana, IL, USA*, [2]*Texas A&M University, College Station, TX, USA*, [3]*University of Illinois Urbana-Champaign, Urbana, IL, USA*, [4]*Idexx, College Station, TX, USA*, [5]*Texas A&M University / VETMED, Phoenix, AZ, USA*

The purpose of this study was to compare the repeatability of selected transthoracic measurements acquired from two-dimensional echocardiography (2DE), 2D guided M-mode, and three-dimensional echocardiography (3DE) in normal conscious dogs and determine the optimal number of repetitions required to maximize precision for each modality.

Five conscious dogs (18-25 kg) underwent transthoracic 2DE and 3DE examinations by four investigators (SG - cardiologist, AS - cardiologist, CH - cardiologist, RF – cardiology resident). Each dog was imaged five times by each investigator. Each investigator was limited to three studies on any single day. Measurements were performed by the investigator that acquired the images off line at a later time. One additional investigator (MM - cardiologist) completed five 2DE and 3DE examinations on one dog over two days and three different investigators (SG, AS, RF) then performed measurements off line and were blinded to each other';s results.

Repeatability, intra-observer, and inter-observer image acquisition variability and measurement variability were quantified by average coefficient of variation (CV). Lower and upper limit bounds on the coefficient of variation were calculated using exact theory and logistic regression of the upper limit against repetitions of 5, 10, 15, and 25 was used to determine the highest precision for each modality.

Evaluation of the left ventricular size by M-mode had the highest repeatability (CV = 8.8%) and lowest intra- and inter-observer variability for both image acquisition (CV = 9.3%) and measurement (CV = 4.3%). Repeatability of 3DE image acquisition (CV = 21.1%) and measurement (CV = 8.6%) was slightly better than 2DE image acquisition (CV = 21.5%) and measurement (CV = 8.9%). The intra- and inter-observer variability of 3DE (CV = 18.9%) was also slightly better than then 2DE (CV = 22.3%). 2DE left atrial to aortic ratio measurements had higher repeatability (CV = 9.0%) and less variability (CV = 9.5%) than volume assessed by 3DE image acquisition (CV = 15.1 %) and measurement (CV = 12.0%). M-mode assessment of left ventricular size achieved acceptable precision after 5 repetitions while left ventricular size assessed by 3DE and 2DE required 10 and 15 repetitions respectively. These data may be useful in the design of study protocols that plan to use echocardiographic assessment as an outcome variable.

C11

Echocardiographic Phenotype of Canine Dilated Cardiomyopathy Differs Based on Diet

Darcy Adin[1], *Teresa DeFrancesco*[1], *Bruce Keene*[1], *Sandra B. Tou*[1], *Kathryn Meurs*[2], *Clarke B. Atkins*[1], *Brent B. Aona*[1], *Kari B. Kurtz*[1], *Lara B. Barron*[1]
[1]*NCSU, Raleigh, NC, USA*, [2]*North Carolina State University, Raleigh, NC, USA*

Taurine and carnitine deficiencies are associated with dilated cardiomyopathy (DCM) in dogs, but little is known about other diet-related etiologies. Recognition of DCM in atypical breeds fed grain-free diets (GFD) prompted this study.

Diet histories and echocardiograms from dogs diagnosed with DCM at NCSU from 2015-2017 were evaluated. Dogs were grouped by diet into GFD and non-grain-free (NGFD) groups. The GFD group was subdivided into dogs fed the most common brand (GFD-1) or other brands (GFD-o). Echocardiographic parameters were compared between groups.

Of 22 dogs with DCM fed GFD, 10 received GFD-1, including 2 pairs of unrelated housemates. No taurine or carnitine deficiencies were identified among the GFD dogs tested (11 taurine, 4 carnitine). Twenty-seven dogs with DCM were fed NGFD. Of these, 5 of 11 tested were taurine deficient (3 vegetarian). Dogs eating GFD-1 weighed less (23.1 ± 11.5kg); had greater normalized LV diameter in diastole (LVIDdN), 2.55 ± 0.26; and systole (LVIDsN) 2.05 ± 0.30; and lower sphericity index (SI) 1.24 ± 0.09 compared to dogs eating GFD-o (weight 35.9 ± 13.0kg, p = 0.03; LVIDdN 2.26 ± 0.33, p = 0.04; LVIDsN 1.79 ± 0.28, p = 0.05; SI 1.38 ± 0.18, p = 0.03) and compared to dogs eating NGFD (weight 33.5 ± 13.7, p = 0.04; LVIDdN 2.13 ± 0.23, p < 0.0001; LVIDsN 1.71 ± 0.21, p = 0.0006; SI 1.43 ± 0.20, p = 0.001). Dogs eating GFD regardless of brand had greater LVIDdN (p = 0.0019), greater LVIDsN (p = 0.012) and lower SI (P = 0.016) than dogs eating NGFD. Prevalence of congestive heart failure was not different between GFD and NGFD groups.

Echocardiograms of dogs with DCM fed GFD, and specifically GFD-1, suggest more advanced disease or a diet-enhanced pathophysiology compared to dogs eating NGFD.

C12

Effects of BNP-32 in Dogs with Congestive Heart Failure Due to Myxomatous Mitral Valve Disease

Mariko Yata[1], Hans S. Kooistra[2], Niek Beijerink[1]
[1]Sydney School of Veterinary Science, Faculty of Science, University of Sydney, The University of Sydney, New South Wales, Australia, [2]Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht University, Utrecht, Netherlands

Synthetic brain natriuretic peptide (BNP-32) causes natriuresis, diuresis, vasodilation, and RAAS antagonism in dogs with experimentally induced congestive heart failure (CHF); however, it is unclear as to whether dogs with naturally occurring CHF would respond similarly. The purpose of this pilot study was to evaluate the efficacy of synthetic canine BNP-32 given subcutaneously in dogs with naturally occurring CHF.

Seven client-owned male dogs with compensated ACVIM Stage C CHF due to myxomatous mitral valve disease received single doses of subcutaneous BNP-32 (5 ug/kg), frusemide (2 mg/kg), and both BNP-32/frusemide (5 ug/kg and 2 mg/kg, respectively) in a cross-over study design with a two-week washout period between each treatment. Heart rate (HR), blood pressure (BP), plasma concentrations of BNP-32 and cGMP, and fractional excretion of sodium (FE_{Na}) and potassium (FE_K) were measured at 0, 1, and 3 h following treatment. Urine output (UOP) and urinary cGMP production (UPcGMP) were measured at 0, 1, 2, and 3 h following treatment. Between- and within-treatment effects were evaluated using linear mixed modelling with restricted maximum likelihood estimation. Significance was taken at $p < 0.05$.

Frusemide and BNP-32/frusemide resulted in a significant increase in UOP, FE_{Na}, and FE_K from 1 – 3 h post treatment. However, there were no differences between these two treatments, and BNP-32 alone did not affect these parameters. No changes in HR or BP were observed with any of the treatments. Plasma concentrations of BNP-32 increased significantly within 1 h of treatment for both treatments containing BNP-32, but fell to baseline by 3 h post treatment. Although no significant differences in plasma concentrations of cGMP were observed, the UPcGMP increased significantly from 1 – 2 h following treatment with BNP-32 and BNP-32/frusemide.

Subcutaneous BNP-32 resulted in an increase in plasma BNP-32 concentrations and UPcGMP. However, there were no detectable biological effects on HR, BP, UOP, FE_{Na}, and FE_K. This is in contrast to data obtained from dogs with experimentally induced CHF. Though further investigations are recommended, it is possible that dogs with naturally occurring CHF may have a reduction in natriuretic peptide responsiveness.

C13

Natural History of Cardiomyopathy in Adult Dogs with Golden Retriever Muscular Dystrophy

Lee-Jae Guo[1], Jonathan H. Soslow[2], Kevin J. Cummings[3], Mark W. Lenox[4], Matthew W. Miller[5], Joe N. Kornegay[1], Christopher F. Spurney[6]
[1]Texas A&M University, College Station, TX, USA, [2]Vanderbilt University, Nashville, TN, USA, [3]Cornell University, Ithaca, NY, USA, [4]Texas A&M University / QT Ultrasound, LLC, Novato, CA, USA, [5]Texas A&M University / VETMED, Phoenix, AZ, USA, [6]Children's National Health System, Washington, DC, USA

Duchenne muscular dystrophy (DMD) is an X-linked genetic disease causing progressive muscle weakness. There is no cure for DMD, with affected boys typically dying due to respiratory or cardiac failure. Golden retriever muscular dystrophy (GRMD) is a genetically homologous model that has been used increasingly to study pathogenesis and potential treatments for DMD. GRMD dogs develop cardiomyopathy similar to DMD, but the disease progression has not been well defined. In this study, we evaluated echocardiography and cardiac MRI in 24 adult GRMD dogs at different ages. Left heart systolic function, wall thickness, and myocardial strain were assessed with echocardiography. Features evaluated with cardiac MRI included LV function, chamber size, myocardial mass, and late gadolinium enhancement (LGE). Separately, ten GRMD dogs were evaluated with circumferential strain twice in a 12-month period. Taken together, our results showed gradual decline of systolic function in GRMD dogs. The decrease of ejection fraction (EF) in echocardiography correlated well with age and identified 31 months as the approximate age at which EF falls below 55%. Circumferential strain appeared to be more sensitive than EF in early disease detection and declined with disease progression. Evidence of LV chamber dilatation provided proof of dilated cardiomyopathy. The LGE imaging showed LV lateral wall lesions and earlier involvement of the anterior septum in GRMD dogs. In conclusion, this study showed the natural history of GRMD cardiomyopathy largely parallels that of DMD. In addition, circumferential strain and EF were good biomarkers of disease progression.

C14

Acute Phase Proteins in Cats with Congestive Heart Failure Due to Primary Cardiomyopathy

Mengmeng Liu[1], Liza Köster[1], Íñigo Sanz González[1], David Eckersall[2], Christopher Chadwick[3], Geoffrey Fosgate[4], Paul Wotton[1], Anne French[1]
[1]Small Animal Hospital, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK, Glasgow, Scotland, United Kingdom, [2]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK, Glasgow, Scotland, United Kingdom, [3]Life Diagnostics Inc., West Chester,Pennsylvania, USA, West Chester, PA, USA, [4]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa, ONDERSTEPOORT, Gauteng, South Africa

Inflammatory responses occur in congestive heart failure both in humans and in dogs. Previous feline cardiomyopathy research suggests that inflammation plays a role in the disease process. The objective of this study was to assess circulating acute phase proteins in feline patients with congestive heart failure (CHF) due to primary cardiomyopathy (CM).

The study population included 15 CHF cats, 9 asymptomatic CM cats and 16 healthy controls. A panel of serum acute phase proteins were measured using Spatial Proximity Analyte Reagent Capture Luminescence (SPARCL) kits. Clinical and echocardiographic data were collated for the CM cats. Survival status of the CHF cats was recorded. One-way analysis of variance (ANOVA), Student';s T tests, chi-square and Fisher exact tests, Spearman';s rho and Cox proportional hazards models were used for statistical analysis.

Cats with CHF had: higher serum leucine-rich alpha-2-glycoprotein1 compared to healthy controls; higher serum amyloid A compared to asymptomatic CM and healthy controls; higher ceruloplasmin compared to asymptomatic CM ($P < 0.05$). In univariate survival analysis models, serum alpha-1-acid glycoprotein (AGP) level was found to be associated with a higher risk of death in CHF cats ($P = 0.007$). Multivariable analysis suggested that serum AGP ($P = 0.009$), body weight ($P = 0.023$) and LA/Ao ratio ($P = 0.013$) were independent prognostic factors in CHF cats.

Findings suggest that systemic inflammatory response occurs in feline congestive heart failure due to primary cardiomyopathies. Acute phase proteins can be used with other clinical parameters or biomarkers for disease monitoring and prognostication in feline cardiomyopathies.

C15

Pancreatic Injury in Cats with Primary Cardiomyopathy

Laetitia Duler, Katherine F. Scollan, Jana Gordon, Duncan S. Russell, Nicole LeBlanc
Oregon State University, Corvallis, OR, USA

Cardiomyopathies (CM) are the most common acquired feline cardiac diseases and frequently result in congestive heart failure (CHF). Low cardiac output and poor peripheral perfusion may cause multisystemic injury such as pancreatitis. Measurement of feline pancreatic lipase immunoreactivity (fPLI) and trypsin-like immunoreactivity (TLI) aid in the assessment of pancreatitis and pancreatic exocrine function. Previous studies have assessed the association between cardiac disease, CHF, and pancreatitis in humans and dogs. The aim of this prospective study was to assess fPLI and TLI values between healthy cats and cats with primary CM with or without CHF. In addition, a retrospective evaluation of pancreatic histology from cats with CM was performed.

Cats were prospectively recruited into three groups: 1) healthy controls (H group), 2) primary CM (CM group), and 3) CM with active or historical CHF determined by a board-certified cardiologist (CHF group). All included cats underwent a physical exam, blood pressure, echocardiogram, fPLI, TLI, complete blood count (CBC), and chemistry panel. Cats with evidence of hypertrophic cardiomyopathy (HCM) also had total T4 measured. Routine pancreatic histopathology from cats affected by primary CM were retrospectively reviewed by a board-certified anatomic pathologist for histologic evidence of pancreatic injury. Differences between groups were assessed by Kruskal-Wallis tests and association assessed by Fisher';s exact tests.

A total of 25 cats were included and classified into the H (n=4), CM (n=10), and CHF (n=11) groups. There were no statistical differences in age, body weight, or body condition score between groups. CM diagnoses included HCM (4 CM, 5 CHF), hypertrophic obstructive cardiomyopathy (5 CM, 2 CHF), unclassified cardiomyopathy (1 CM, 2 CHF), and restrictive cardiomyopathy (2 CHF). In the CHF group, 6/11 cats had active CHF (pulmonary edema or pleural effusion) at the time of blood collection. Gastrointestinal signs included vomiting (2 CM, 2 CHF), anorexia (2 CM, 1 CHF), and weight loss (1 CM, 2 CHF). Median values for the control, CM, and CHF groups did not differ for fPLI (1.6, 2.2, and 1.8 μg/L) or TLI (35, 27.4, and 39.9 μg/L). Five CM and 3 CHF cats had fPLI values greater than the feline reference range, 2 CM cats had increased TLI values, and 1 CM cat had a deceased TLI value. Active CHF was not statistically associated with an abnormal fPLI or TLI result ($P > 0.99$; $P > 0.99$).

Retrospective pancreatic histopathology of 16 cats with CM found a spectrum of morphologic lesions potentially related to pancreatic ischemia. These included exocrine vacuolation (6/18), focal or multifocal coagulation necrosis (2/16), and saponification of pancreas-associated adipose tissue (5/16). Of the 10 cats with possible cardiac-related pancreatic ischemia, 5/10 had active or historical CHF. Other common feline pancreatic lesions were also identified, including islet amyloidosis (6/16), mild to moderate lymphocytic inflammation (8/16), and periductular fibrosis (6/16).

These preliminary data demonstrate no statistically significant differences in pancreatic biomarkers of cardiomyopathic cats with or without CHF. Potentially relevant histopathologic pancreatic changes were identified, suggesting that biomarkers may vary with time or disease severity and not reflect cardiac-induced injury.

C16

Left Atrial Size and Volume in Cats with Cardiomyopathy with and without Congestive Heart Failure

Laetitia Duler, Katherine F. Scollan, Nicole L. LeBlanc
Oregon State University, Corvallis, OR, USA

Myocardial disease is the most prevalent category of acquired cardiac diseases in cats. As a result of ventricular diastolic or systolic dysfunction, elevated left atrial pressure and left atrial enlargement (LAE) frequently precede left-sided congestive heart failure (CHF). In addition, left atrial function may decline with progressive myocardial disease in affected cats. The left atrium may enlarge in various directions, although is conventionally measured on echocardiography in one-dimension using a short-axis right parasternal view indexed to aortic diameter (LA:Ao). The maximal diameter from a right parasternal long-axis view has also been utilized as an assessment of LA size. Two-dimensional (2D) measurements and volume calculations have replaced one-dimensional linear measurements for chamber quantification in humans and canine echocardiographic exams. The aims of this retrospective study were to compare LA size by conventional 1-dimensional linear measurements to calculated 2D LA volumes (LAV) and compare minimal and maximal values. Our hypothesis was that LAV would provide superior sensitivity (Se) and specificity (Sp) for identifying cats with CHF from those without CHF.

Medical records were reviewed for cats with complete echocardiographic exams performed at the Oregon State University Veterinary Teaching Hospital (OSU-VTH) between January 2008 and July 2017. Cats were categorized into three groups: healthy cats (H group) with a normal echocardiogram and clinical diagnosis; cardiomyopathic cats (CM group) with an echocardiogram consistent with primary cardiomyopathy without clinical or radiographic evidence of CHF; and CHF cats (CHF group) with an echocardiogram consistent with primary cardiomyopathy and with clinical or radiographic evidence of CHF. Hyperthyroid and hypertensive cats were excluded. Seven measurements of LA size were made on each included cat: 1) Minimal LA:Ao (LA:Aom) and 2) Maximal LA:Ao (LA:AoM) according to the Hansson';s method; 3) Minimal LAV and 4) Maximal LAV from the right parasternal long-axis view (LAVmRLA and LAVMRLA); 5) Minimal LAV and 6) Maximal LAV from the left apical view (LAVmLAP and LAVMLAP);

and 7) maximal left atrial diameter (LAD) from the right parasternal long-axis view. Volumes were calculated using the monoplane modified Simpson';s method of discs (MOD). Volume measurements were also indexed to body weight (kg). Minimal measurements were made just after the P wave and maximum measurements made just after the T wave on simultaneously recorded ECG. Receiver operator curves were used to assess area under the curve (AUC) and optimal cut-offs with associated Se and Sp to distinguish groups.

A total of 162 cats were included in the study and classified as healthy (n=56), CM (n=62) and CHF (n=44). Healthy cats were younger than both the CM and CHF groups ($p < 0.0001$) and the groups did not differ in body weight. LAV measurements from the RLA and LAP views were statistically different ($p = 0.0005$). The LA:Aom and LAVmLAP resulted in the largest AUCs to distinguish CM from CHF cats (AUC = 0.839 and AUC = 0.824, respectively). Indexing LAV to body weight did not increase the AUC for any volume measurement. All seven measurements had poor AUC results to distinguish H and CM cats. The LA:Aom value with the optimal sensitivity and specificity to distinguish CM and CHF cats was 1.635 (Se 84% and Sp 75%). The LAVmLAP raw and indexed values with optimal sensitivity and specificity to distinguish CM and CHF cats were 1.46 mL (Se 73% and Sp 75%) and 0.3 mL/kg (Se 81% and Sp 73%).

Results of this study indicate that LA volumes were not superior to linear measurements of LA size in distinguishing CM and CHF cats. Interestingly, the minimal LA size and volume resulted in a higher AUC than each corresponding maximum measurement. This is in contrast to the currently recommended time point to measure the LA:Ao. Minimum LA size or volume may be a better discriminatory factor as atrial contractile function declines with worsening myocardial disease and the development of CHF.

C17

Is Progression of Murmur Intensity Associated with the Rate of Remodelling in Mitral Valve Disease?

Jenny Wilshaw[1], *Ruby Chang*[2], *Jonathan Elliott*[2], *Adrian Boswood*[3]
[1]*Royal Veterinary College, Staplehurst, England, United Kingdom,* [2]*Royal Veterinary College, London, England, United Kingdom,* [3]*Royal Veterinary College, Potters Bar, England, United Kingdom*

Canine degenerative mitral valve disease (DMVD) is a chronic and variably progressive condition. In dogs with DMVD, a left apical, systolic murmur is a consistent clinical finding on physical examination. Heart murmur intensity has been significantly and positively associated with echocardiographic measurements of left heart chamber size and disease severity. These findings have not been evaluated longitudinally. This study aimed to evaluate whether changes in heart murmur intensity reflect longitudinal changes in echocardiographic dimensions in a cohort of dogs variably affected by DMVD.

Clinical data were sourced from the records of dogs that had visited a research clinic on more than one occasion. The daily rate of change in the left atrial to aortic root ratio (LA:Ao) and left ventricular internal diameter in diastole normalised to bodyweight (kg) (LVIDDN) were calculated for each inter-visit interval and multiplied by 1000 to aid data handling and interpretation. Separate linear mixed effects models were constructed for the rates of change of LVIDDN and LA:Ao respectively. Murmur intensity, scored using the Levine 6-point scale, was classified according to whether it had "increased", "decreased" or been "maintained" over each interval and was entered as an explanatory variable. Additional explanatory variables that were included in models had been recorded at the first examination in each interval. These were: patient age; breed; sex; weight; murmur grade; LVIDDN; LA:Ao; ACVIM stage; and treatment status with furosemide, pimobendan, angiotensin converting enzyme inhibitors (ACEi) and spironolactone. Post hoc estimated marginal means were calculated for categorical variables that remained significant in multivariable analyses and were entered into pairwise comparisons to determine if between group differences in the coefficient were statistically significant.

Data from 912 visits were entered from a cohort of 167 dogs. A change in murmur intensity was significantly associated with the rate of change of LVIDDN ($P < 0.001$) in a multivariable analysis alongside: murmur grade; patient age; breed; LVIDDN; LA:Ao and treatment status with an ACEi. A change in murmur intensity was also associated with the rate of change of LA:Ao in a separate multivariable analysis ($P < 0.001$) alongside: patient ACVIM stage; LVIDDN; LA:Ao; and treatment status with furosemide. The rate of change of both echocardiographic measurements was greatest when murmur intensity increased (LVIDDN: $b = 0.472 \pm 0.066$; LA:Ao: $b = 0.818 \pm 0.074$), followed by intervals where murmur intensity did not change (LVIDDN: $b = 0.176 \pm 0.055$; LA:Ao: $b = 0.564 \pm 0.060$), and lowest when murmur intensity decreased (LVIDDN: $b = -0.150 \pm 0.077$; LA:Ao: $b = 0.241 \pm 0.089$). All pairwise comparisons of coefficients were highly significant ($P < 0.001$).

The association between changes in heart murmur intensity and the rate of cardiac remodeling provides evidential support to the utility of longitudinally monitoring murmur grade. In clinical practice, an increase in murmur grade may indicate that a more rapid rate of DMVD progression has occurred and could be used to recommend further echocardiographic evaluation.

C18

Speckle Tracking Echocardiography in Adult Turkish Kangal Dogs

Hasan Albasan[1], *Osman Safa Terzi* [2], *Ayjamal Radgohar*[2], *Aziz Arda Sancak*[2], *Mehmet Sahal*[3], *Kadir Sevim*[2], *Yasin Senel*[2], *Aybike Aliustaoglu*[2]
[1]*Department of Internal Medicine, Faculty of Veterinary Medicine, Ankara University, Saint Paul, MN, USA,* [2]*Department of Internal Medicine, Faculty of Veterinary Medicine, Ankara University, Diskapi, Ankara, Turkey,* [3]*Department of Internal Medicine, Faculty of Veterinary Medicine, Ankara University, diskapi, Ankara, Turkey*

Giant breeds including Turkish Kangal dogs are prone to myocardial diseases. Speckle tracking echocardiography (STE) has been used to measure two-dimensional strain in dogs. Measurements from clinically normal dogs of same breed are needed for canine clinical practice. The objective of this prospective study was to obtain STE data in a routine echocardiographic examination in Turkish Kangal dogs for reference values.

Fifty-two healthy adult Turkish Kangal dogs were included to be scanned under normal clinical conditions to obtain standard echocardiographic parameters and STE data. Five dogs were dropped from study due to poor image quality; therefore, a total of 47 dogs (90.3%) were included in the statistical analysis. Of 47 dogs, 31 were females and 16 were males. The mean age of dogs was 39.6 ± 3.0 months-old

(range:10-108). In 47 dogs, STE data was obtained from three scanning planes. The mean peak radial strain at the apex (A-Rs) was 44.7 ± 13.0%, the mean peak radial strain at the papillary muscle level (PM-Rs) was 41.1 ± 14.7%, and the mean peak radial strain at the base (B-Rs) was 34.1 ± 15.7%. The mean peak circumferential strain at the apex (A-Cs) was -16.4 ± 5.3%, the mean peak circumferential strain at the papillary muscle level (PM-Cs) was -14.8 ± 3.4%, and the mean peak circumferential strain at the base (B-Cs) was -15.8 ± 4.2%.

As a conclusion, this technique can be part of normal echocardiographic examinations in Turkish Kangal dogs under clinical conditions. Our results are similar, in part, with the results of avaliable one report which was performed in 44 mature Irish Wolfhound dogs[1]. However, in order to compare the usefulness of STE data reported here, future studies should be designed in larger populations of Turkish Kangal dogs with cardiologic disorders.

1. Westrup, U., McEvoy, F.J., 2013. Speckle tracking echocardiography in mature Irish Wolfhound dogs: technical feasibility, measurement error and reference intervals. *Acta Veterinaria Scandinavica* 55, 41-41.

C19

Associations Between Echocardiography, Cardiac Biomarkers, Insulin Metabolism, Morphology and Inflammation In Feline Asymptomatic Hypertrophic Cardiomyopathy

Ingrid van Hoek[1], *Hannah Hodgkiss-Geere*[2], *Elizabeth Bode*[2], *Paul Motskula*[3], *Valentina Palermo*[3], *Yolanda Martinez-Pereira*[4], *Jo Dukes-McEwan*[2]
[1]*Royal Canin SAS, Aimargues, Languedoc-Roussillon, France,* [2]*Small Animal Teaching Hospital, Department of Small Animal Clinical Sciences, Institute of Veterinary Science, Neston, England, United Kingdom,* [3]*Anderson Moores Veterinary Specialists, Winchester, England, United Kingdom,* [4]*University of Edinburgh, Midlothian, England, United Kingdom*

Insulin and IGF-1 metabolism might play a role in feline hypertrophic cardiomyopathy (HCM), aggravated by inflammation. This study investigated associations between echocardiography, morphology, cardiac and inflammatory markers, insulin and IGF-1 metabolism, in cats with asymptomatic hypertrophic cardiomyopathy (aHCM).

Fifty-one asymptomatic client-owned cats with diastolic interventricular septum (IVSd) and/or left ventricular wall (LVWd) ≥ 6mm were included with informed consent from the owner. Examination of non-sedated, fasted cats included auscultation, bodyweight (BW), body condition score (BCS) and echocardiography (2D- and M-mode (M-)), with IVSd and LVWd measured basal, mid (LVWd, IVSd) and apical (IVSd), noted as maximum (max-), sum- and number of areas ≥ 6mm (n-)). Blood samples were analyzed for NT-proBNP, ultrasensitive troponin-I (c-TnI), serum amyloid A (SAA), insulin, glucose and IGF-1. Statistical analysis ($P < 0.05$) investigated whether measures were significantly increased above cut-off values, effect of left atrial (LA-) remodeling and generalized versus focal hypertrophy, and correlations between measurements.

Cats had increased BCS ($P = 0.013$), insulin ($P < 0.001$), NT-proBNP ($P = 0.001$) and cTn-I ($P < 0.001$). Correlations were present between NT-proBNP and max-IVSd ($P = 0.048$), max-LVWd ($P = 0.009$), sum-LVWd ($P = 0.012$) and LA-remodeling ($P = 0.030$), between c-TnI and sum-IVSd ($P = 0.039$) and LA-remodeling ($P = 0.009$), and between SAA and sum-IVSd ($P = 0.030$) and n-IVSd ($P = 0.048$). Age was correlated with LA max ($P = 0.026$) and glucose ($P = 0.027$). BW and BCS were correlated with n-LVWd ($P = 0.041$), M-LVWd ($P = 0.035$), IGF-1 ($P = 0.001$), and insulin ($P = 0.017$), glucose ($P = 0.041$) and IGF-1 ($P = 0.021$), respectively. Cats with LA remodeling (n = 27) had higher max-LVWd ($P = 0.026$) and sum-LVWd ($P = 0.012$), NT-proBNP ($P = 0.017$) and c-TnI ($P = 0.006$). Cats with generalized hypertrophy (n = 11) had higher max-IVSd ($P = 0.003$), sum-IVSd ($P < 0.001$), max-LVWd ($P = 0.001$), sum-LVWd ($P < 0.001$) and SAA ($P = 0.018$).

The results suggest a role for insulin, IGF-1 metabolism and inflammation in aHCM. Further research on the contribution to aHCM is needed.

C20

Outcome of Dogs with Mitral Valve Disease with Medical Therapy or Surgical Repair

Nobuyuki Kanno[1], *Haruhiko Suzuki*[2], *Masayuki Enokizono*[3], *Katsuhiro Matsuura*[4], *Dai Nagakubo*[5], *Shusaku Yamada*[6], *Kenji Teshima*[7]
[1]*Nihon University, Fujisawa, Kanagawa, Japan,* [2]*Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Nihon University, Sayama, Saitama, Japan,* [3]*Animal Cardiovascular and Thoracic Surgery Center / Japan Small Animal Medical Center, Tokorozawa, Saitama, Japan,* [4]*Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Tokyo University of Agriculture and Technology, Sayamashi, Saitama, Japan,* [5]*The University of Tokyo/Animal Cardiovascular and Thoracic Surgery Center, Bunko-ku, Tokyo, Japan,* [6]*Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan,* [7]*Nihon univ., Fujsawa, Kanagawa, Japan*

The purpose of this study was to determine whether mitral valve plasty (MVP) under cardiopulmonary bypass would better improve the prognosis of dogs with mitral valve disease (MVD) than treatment with internal medicine.

This study was a retrospective review of medical records to identify dogs with MVD that underwent surgical repair or medical management of MVD. In the internal medicine group, 105 dogs were studied. The dogs in this group were treated chronically with ACE inhibitors, pimobendan, diuresis (thiazide, spironoractone, furosemide, and torsemide), vasodilators (amlodipine, nitroglycerin, isosorbide), and sildenafil. In the surgery group, 92 dogs with mitral valve regurgitation, with a mean age of 10 (8.3-11) years and mean body weight of 3.9 (1.9-20.9) kg, underwent mitral valve plasty with cardiopulmonary bypass (CPB) between July 2016 and October 2017.

In the internal medicine group, median survival times were 649 days (stage B2), 220 days (stage C), and 52 days (stage D). In all cases, the dogs died due to heart failure. In the surgical group, the discharge ratios were 100% (19/19), 93.1% (54/58), and 80% (12/15) for stages B2, C, and D, respectively. Median survival times were undefined because almost patients survived. Survival ratios were 94.7% (18/19), 90.0% (52/58), and 73.3% (11/15) for stages B2, C, and D, respectively. Mean survival times were 825 days (stage B2), 771.3 days (stage C), and 733.6 days (stage D). One dog died from renal failure in stage B2 at 605 days. The causes of death in stage C were technical problems (n=2), protamine shock (n=1), residual pulmonary damage (n=1), and unknown (n=2). The causes of death in stage D were heart failure (n=2), residual pulmonary damage (n=1), and renal failure (n=1).

These results suggest that MVP is more effective than medical therapy for the treatment of MVD, and earlier surgery is recommended.

C21

Phrenic Nerve Palsy Following Mitral Valve Repair in Dogs

Kazuya Mamada[1], Kazuki Takamura[1], Masashi Mizuno[1], Takurou Mori[2], Masami Uechi[1]
[1]JASMINE Veterinary Cardiovascular Medical Center, Yokohama, Kanagawa, Japan, [2]JASMINE Veterinary Cardiovascular Center, Yokohama-shi, Kagawa, Japan

Phrenic nerve palsy (PNP) is one of the complications of open chest surgery. The aim of the present retrospective study was to evaluate the incidence of PNP after mitral valve repair in dogs.

In total, 284 dogs underwent mitral valve repair. PNP was diagnosed using inspiratory chest radiographs (dorsal–ventral) on the day after surgery. In these cases, the left diaphragm was raised by more than 1 interval from the right diaphragm, and signs of respiratory distress and effort respiration were also observed. We assessed the incidence of PNP, the recovery time, and the relationship between the occurrence of PNP and the survival rate, occurrence of respiratory failure, the aortic cross-clamping time during surgery.

PNP was diagnosed in 87 cases (30.6%) on the day after surgery, 63 cases (22.2%) at the time of discharge from the hospital at 1 week after surgery, 24 cases (8.5%) at the 1-month postoperative examination, and one case (0.4%) at the 3-month postoperative examination. PNP developed in nine of the 21 patients who died in the hospital and nine of the 10 patients who died because of respiratory insufficiency ($p = 0.001$). The occurrence of PNP was not associated with the aortic cross-clamping time.

Our findings suggest that the incidence of PNP after mitral valve repair is high in dogs, although the recovery time is shorter than that in humans. PNP alone does not affect the prognosis; however, it is a postoperative risk factor in cases exhibiting respiratory disease.

C22

Evidence of Endothelial-to-Mesenchymal Transition In Canine Degenerative Mitral Valves

Kaitlin M. Abbott-Johnson[1], Christopher Orton[1], Derek Mathews[2]
[1]Colorado State University, Fort Collins, CO, USA, [2]Texas A and M University, College Station, TX, USA

Activated myofibroblastic valve interstitial cells (VIC), identified by expression of alpha smooth muscle actin (αSMA), are thought to play a central role in mediating canine degenerative mitral valve disease (DMVD). Endothelial-to-mesenchymal cell transformation (EndMT) is a developmental mechanism active during valvulogenesis whereby endothelial cells migrate into the interstitum and transform to a mesenchymal phenotype. The study objective was to investigate whether post-developmental EndMT could be a source of high cellular density myofibroblastic VIC found in canine DMVD.

Mitral valves were collected post-mortem from 9 dogs (median age 6 years) determined by echocardiography to be free of mitral regurgitation (control group, median Whitney score = 0) and 7 dogs (median age 12 years) with DMVD (degenerative group, median Whitney score = 3). Sequential histologic sections of mitral valves were stained with Movat pentachrome and immunohistochemistry for platelet endothelial cell adhesion molecule 1 (CD31, a marker of endothelial cell phenotype), αSMA, and heparin binding-epidermal growth factor (HB-EGF).

Control and degenerative mitral valves showed positive staining for CD31 of cells on valve surfaces and within vessels of papillary muscles consistent with normal valvular and vascular endothelial cells, respectively. In degenerative mitral valves, CD31-positive cells were also found within the valve interstitum and co-localized to areas of αSMA-positive VIC suggestive of active EndMT in degenerative valves. Co-localization of HB-EGF (a mediator of endothelial cell migration) to focal areas of positive CD31 and αSMA staining was observed in some degenerative mitral valves.

Post-developmental EndMT appears to be active in canine DMVD and could be a source of myofibroblastic VIC that mediate degenerative changes.

C23

Comparison of In-Hospital Non-Recordable Telemetry Versus Recordable Holter Monitoring in Dogs with Arrhythmias

Alina Teslenko[1], Ryan Fries[2], Laura Selmic[1]
[1]University of Illinois College of Veterinary Medicine, Urbana, IL, USA, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA

The purpose of this study was to determine the ability of non-recordable telemetry to estimate arrhythmia severity in hospitalized dogs compared with Holter monitoring.

Ten dogs with arrhythmias were hospitalized and heart rates and rhythms were simultaneously monitored using non-recordable telemetry and Holter monitoring. Telemetry was assessed by trained ICU technicians for one-minute every hour and heart rate and rhythm were recorded. An arrhythmia scale was used to grade the arrhythmia severity: (Grade 0 = sinus, no VPCs; Grade 1 = single VPCs; Grade 2 = accelerated idioventricular; Grade 3 = bigeminy or trigeminy; Grade 4 = couplets or triplets; Grade 5 = ventricular tachycardia or R on T phenomenon). Holter data was analyzed by a board-certified cardiologist and arrhythmia grade was assigned to the same one-minute time period reported by ICU technicians. A one-hour arrhythmia grade was also determined from Holter data and was reported as the highest grade noted during the previous hour.

Cohen';s weighted kappa analysis was used to compare the agreement of ICU and Holter grade during the same one-minute time period. The same analysis was used to compare one-minute Holter grade with the prior hour Holter grade.

Clinically unacceptable agreements were found between ICU reported grade and Holter grade ($\kappa = 0.40$), as well as between one-minute Holter grade and prior hour Holter grade ($\kappa = 0.37$).

These results show that trained ICU technicians often incorrectly assess the severity of arrhythmias and hourly one-minute evaluations inconsistently estimate the severity of an arrhythmia.

C24

Treatment of Pulmonary Hypertension with Sildenafil Monotherapy versus Combination Therapy with Sildenafil and Pimobendan

Alyssa Pinkos, Christopher Stauthammer, Eva Frantz, Aaron Rendahl
University of Minnesota, Minneapolis, MN, USA

Pulmonary hypertension develops secondary to a multitude of disease processes in dogs and results in an abnormal increase in pulmonary artery pressure, dysfunction of the right heart, and pulmonary interstitial and vascular pathology. Sildenafil is the most common medical treatment used in veterinary patients. Pimobendan has been minimally investigated as a potential treatment. The purpose of this retrospective study was to evaluate the effect of sildenafil monotherapy versus combination therapy with sildenafil and pimobendan in dogs with moderate and severe pulmonary hypertension.

Clinic records were reviewed from 2003-2017. Inclusion criteria included dogs with moderate to severe pulmonary hypertension (estimated systolic pulmonary artery pressure greater than 50 mmHg) and/or evidence of significant right heart dysfunction deemed secondary to pulmonary hypertension. Exclusion criteria included lack of follow up examination and pulmonary hypertension secondary to left sided heart disease. The quality of life score pre and post treatment, survival time, time to cardiac event, and estimated systolic pulmonary artery pressure pre and post treatment, were evaluated in both groups.

A total of 91 dogs were enrolled in the study. Combination therapy significantly improved quality of life score ($p = 0.02$). There was no significant difference between groups on long-term survival, time to cardiac event, or estimated systolic pulmonary artery pressure pre and post treatment. Median time to cardiac event for both groups was 4 months ($p = 0.51$). Median survival was 9 months for sildenafil monotherapy and 10 months for combination therapy ($p = 0.49$). In conclusion, combination therapy of sildenafil and pimobendan versus sildenafil monotherapy does not significantly improve survival time, time to cardiac event, or pulmonary pressure gradient, but it does improve patients' quality of life.

C25

Relationship Between Serotonin and ALK5 Function on Development of Myxomatous Valvular Degeneration in Rat Model

Rebecca Saunders, Dan Hogan, Abigail Durkes, Ryan Schafbuch
Purdue University, West Lafayette, IN, USA

Myxomatous mitral valve disease (MMVD) is the most common cardiac disease in dogs; however, the causative mechanism and specific tissue pathways of the disease remain unclear. Animal modeling and evaluation of clinically affected valves in dogs suggest that serotonin and TGF-β signaling play a role in the development of MMVD with evidence of interaction between the two tissue pathways. The hypothesis of this pilot study was that abnormal TGF-β pathway function, through ALK5 inhibition, would result in dysregulated tissue growth from the now uncontrolled serotonin pathway.

The study was designed as a randomized, blinded, controlled experimental trial in which 30 Sprague Dawley rats were assigned equally into six treatment groups: 1) saline (negative control); 2) serotonin (5-HT positive control); 3) ALK5 inhibition (ALK5 positive control); 4) ALK5 inhibition plus serotonin (synergistic); 5) ALK5 inhibitor plus cyproheptadine (serotonin antagonist); and 6) ALK5 inhibitor plus clopidogrel (antiplatelet). Each group was treated for 14 days then sacrificed and the hearts were collected and stained with hematoxylin and eosin (H&E) for histopathologic examination. The results were scored based on a previously published semi-quantitative method for mitral valve pathology where the score ranged from 0 (no changes) to 12 (most severe changes).

The [median (range)] for each treatment group was as follows: **Negative control** [4.5 (0.5 - 6)]; **5-HT positive control** [3 (1.5 - 5)]; **ALK5 positive control** [3.5 (0 - 5)]; **Synergistic** [3 (0 - 6.5)]; **Serotonin antagonist** [2 (0 - 5.5)]; and **Antiplatelet** [3 (0 - 7)]. Kruskal-Wallis tests comparing the distribution of histologic scores across all 6 groups was not statistically significant ($p = 0.864$), likely due to small sample size and significant overlap of histologic scores between groups.

This pilot study did not identify an obvious relationship between abnormal TGF-β pathway function and serotonin in the mitral valves of rats. However, variability in valve orientation affected tissue sectioning and may have precluded the identification of changes between groups. Special stains and immunohistochemistry are pending which may help provide identifiable changes between groups.

C26

Cardiac Evaluation of Naturally Infected Distemper Dogs

Juliana BC Araújo[1], Mayra FC. Lima[1], Mauricio Filippi[2], Maria Lucia Gomes. Lourenço[1], Antônio C. Paes[1]
[1]São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Botucatu, Sao Paulo, Brazil, [2]São Paulo State University, Botucatu, Sao Paulo, Brazil

Distemper is an important infectious disease and in endemic regions, such as Brazil, the number of dog deaths caused by the canine distemper virus (CDV) have been increasing. As a multi-host pathogen, its death toll has also reached a wide range of carnivore species, causing mass mortalities of wildlife worldwide. It has also been considered re-emergent in countries where it has already been controlled. In Finland, where distemper had been previously controlled, a case report of a 3-month-old pup naturally infected with distemper virus was published in 2016. Recently, a letter was published warning colleagues about the risk of re-emergence of canine distemper in the UK.

It has been proven that the virus has important histopathologic effects over the cardiovascular system although that has been scarcely researched. The present study evaluated the virus';s activity on the heart of 9 naturally infected dogs with the help of echocardiography, electrocardiography and Holter examinations. Distemper diagnosis was confirmed with a positive PCR test and data collected was compared to nine healthy dogs. In agreement with the hypothesis that there is influence of the distemper virus over the cardiovascular system, this study found that the CDV affects directly or indirectly the excitatory and specialized conduction system of the heart. As described in Table 1, Holter evaluation of distemper infected dogs revealed a decrease in heart rate variability and absence of arrhythmias. The study revealed delays in conduction of the cardiac impulse and also an autonomic dysfunction characterized by increased sympathetic tone. Echocardiography revealed no dilation of cardiac chambers.

TABLE 1 (Continued)

Table 1. Holter, electrocardiographic and echocardiographic parameters [mean (standard deviation)] from both infected and control groups.

Variable (Unit)	Exam	Control	Infected	P Value
H-VPCs	Holter	0 (0 - 1)*	0 (0 -7)*	0.9398
H - APCs	Holter	0 (0 - 41)*	0 (0 - 0)*	0.4101
H - Pauses	Holter	70.5 (0 - 574)*	290 (0 - 1890)*	0.1476
mean HR (bpm)	Holter	86.0 (13.3)	109.3 (29.2)	**0.0358**
min. HR (bpm)	Holter	36.0 (31-5)*	62 (27-8)*	0.0885
max. HR (bpm)	Holter	250 (245-250)*	197 (115-250)*	**0.0038**
mean NN	Holter	747.0 (117.6)	527.6 (55.4)	**< 0.0001**
SDNN (ms)	Holter	268.5 (74.6)	108 (42.2)	**< 0.0001**
SDANN (ms)	Holter	168.3 (39.1)	58.33 (19.5)	**< 0.0001**
SDNNINDX (ms)	Holter	216.9 (74.2)	79.55 (29.3)	**< 0.0001**
NNs (ms)	Holter	101479.2 (28601.1)	117978.10 (37116.6)	0.2901
RMSSD (ms)	Holter	146.9 (45.5)	78.8 (32.8)	**0.0018**
PNN50 (%)	Holter	55.9 (12.84)	24.6 (12.1)	**< 0.0001**
mean HR (bpm)	ECG	125.5 (16.5)	106.2 (33.9)	0.1273
Dur P (ms)	ECG	49.3 (3.7)	55.4 (7.0)	**0.0272**
Int PR (ms)	ECG	100.6 (15.9)	100.4 (16.5)	0.9835
Dur QRS (ms)	ECG	56.3 (3.8)	77.3 (12.5)	**< 0.0001**
Int QT (ms)	ECG	206.7 (8.8)	217.7 (22.9)	0.174
Amp P (mV)	ECG	0.2 (0.04)	0.2 (0.06)	0.4781
Amp R (mV)	ECG	1.1 (0.1)	1.06 (0.3)	0.4767
ST (mV)	ECG	0.06 (0 - 0.12)*	0 (0 - 0.23)*	0.7656
LA/Ao	Ecocardiography	1.3 (0.1)	1.4 (0.1)	0.1245
LVD-d	Ecocardiography	37.3 (9.0)	31.3 (7.7)	0.1373
LVD-s	Ecocardiography	19.3 (11.7)	22.4 (7.7)	0.6567
FS%	Ecocardiography	34.2 (2.19)	36.1 (1.81)	0.7821
EF%	Ecocardiography	54.89 (19.60)	59.34 (15.9)	0.849
E/A	Ecocardiography	1.4 (0.2)	1.4 (0.2)	0.849

Variables were evaluated with statistical tests of normality (Shapiro-Wilk); kg: kilogram; ms: milliseconds; mV: millivolts; bmp: beats per minute. H-VPCs: Holter ventricular premature complexes; H-APCs: holter atrial premature complexes; H-pauses: holter pauses; HR: heart rate; NN: mean of all RR intervals (ms); SDNN: standard deviation of all RR intervals (ms); SDNNi: average of standard deviations of the measured RR intervals in 5-minute segments (ms); SDANN: standard deviation of RR intervals measured in 5-minute segments (ms); RMSSD: root mean square of successive differences to the frame between adjacent RR intervals (ms); pNN50: percentage difference between successive RR intervals that are > 50 ms (%). Bold letters indicate a significant difference between groups ($p < 0.05$). * variables analyzed with Mann-Whitney

C27

Omega 3 Fatty Acids and Mitral Valvar Disease: B2 and C Stages

Rosangela de Oliveira Alves Carvalho[1], *Priscilla Regina Nasciutti*[1], *Ana Paula Araújo Costa*[1], *Aline Tavares Morais*[2], *Nayara Gonçalves de Melo*[1], *Thaiz Krawczyk Santos*[1], *Bruna Dantas Matos*[1], *Bruno Santos Ferrari*[1], *Karine Kelly Gonçalves Queiroz*[1], *Kelly Carolina Assis Arantes*[1]
[1]*Federal University of Goias, Goiania, Goias, Brazil,* [2]*Federal University of Goias, 74610070, Goias, Brazil*

Mitral valve disease (MVD) is the cardiovascular disease with the highest incidence among small dogs. Some patients with MVD remain asymptomatic, while others develop signs of congestive heart failure (CHF). The treatment is based on specific drugs and diets to control the signs of CHF, thus reducing clinical signs and promoting quality of life for patients. Dietary treatment includes sodium restriction and nutrient supplementation, including omega 3 (ω - 3) fatty acids. Ω - 3 is an essential fatty acid, which within its chain of metabolization, generates the production of substances with anti-inflammatory activity. In addition, increasing their incorporation into cell membranes causes changes in the ionic channels of cardiomyocytes making the cells less susceptible to arrhythmias. Vasodilator, hypolipidemic activity and blood pressure (BP) modulation are also reported. However, there are no clinical studies evaluating the effects of ω-3 supplementation in dogs with MVD.

Given these effects, it was proposed in this prospective, double-blind study to evaluate the possible benefits of dietary supplementation with ω-3 in dogs with DVM. A total of 1058 dogs were evaluated, 326 of which were carriers of MVD, of these 29 dogs staged in stages B2 (asymptomatic with myocardial remodeling) and C (with clinical signs of CHF) were recruited after evaluation of the inclusion criteria, they could not have other diseases. One group (n = 16) received canine dry food with ω-3 (4000 mg / kg EPA and 3000 mg / kg DHA) and the control group (n = 13) received the same food without supplementation with ω-3. The animals were evaluated every three months for a period of 12 months by means of BP measurement, ambulatory electrocardiographic and Holter examination, echocardiographic examination, chest X-rays and NT-proBNP. All dogs received standardized pharmacological treatment according to ACVIM consensus. The qualitative data were submitted to the Fisher exact test and the quantitative data were compared between the treatments using the Mann-Whitney test and between the times using the Kruskal-Wallis test, adopting a 5 % significance level. Under these conditions, supplementation with ω - 3 did not alter BP values, echocardiographic measurements and NT-proBNP levels. In the radiographic evaluation, the values of VHS decreased (vertebral heart size) (T0 = 11.9 vertebrae, T12 = 10,7 vertebrae, p = 0.033), maintaining pulmonary patterns compatible only with senile features. The electrocardiographic evaluation showed a prevalence of respiratory sinus arrhythmia and a lower incidence of atrial and ventricular arrhythmias. The control group was 2.96 times more likely to develop some type of arrhythmia compared to the ω-3 group (chance ratio test, 95% CI = 1,450, 6,063, p = 0.003).

Thus, it was concluded that, supplementation with ω-3 fatty acids decreased the volumetric overload, demonstrated by the reduction of VHS and prevents arrhythmias in dogs with MVD.

C28

Omega 3 Fatty Acids in Dogs with Mitral Valvar Disease without Myocardial Remodeling

Rosangela de Oliveira Alves Carvalho[1], *Priscilla Regina Nasciutti*[1], *Ana Paula Araújo Costa*[1], *Bruna Dantas Matos*[1], *Gabriel Moreno Matos de Andrade*[1], *Thaiz Krawczyk Santos*[1], *Nayara Gonçalves de Melo*[1], *Aline Tavares Morais*[2], *Karine Kelly Gonçalves Queiroz*[1], *Kelly Carolina Assis Arantes*[1], *Bruno Santos Ferrari*[1]
[1]*Federal University of Goias, Goiania, Goias, Brazil,* [2]*Federal University of Goias, 74610070, Goias, Brazil*

Mitral valve disease (MVD) is characterized by the degeneration of the valvar leaflets, promoted by changes in the cellular constituents that lead to valvar insufficiency. The preclinical period is characterized by the absence of myocardial remodeling and pharmacological treatment is not recommended. Currently, specific diets for dogs with cardiac diseases are available commercially that include, in addition to sodium restriction, supplementation with some nutrients, including ω-3 fatty acids. Ω - 3 is an essential fatty acid that produces eicosanoids with anti-inflammatory activity after its metabolism, thus reducing the production of inflammatory substances, being called cardioprotective. The inflammatory activity is directly related to the development of cardiac cachexia syndrome, observed in dogs with chronic MVD. Effects on the modulation of heart rate (HR) and blood pressure (BP), antiarrhythmic and hypolipidemic activity are also reported. However, clinical studies have not yet been performed evaluating its effects in dogs with MVD.

It was proposed in this prospective study to evaluate the possible benefits of dietary supplementation with ω - 3 in dogs with MVD without cardiac remodeling. Thirty-one dogs were recruited, of which 12 were classified in stage B1 (asymptomatic without myocardial remodeling). One group (n = 8) received dry food supplemented with ω-3 (4000 mg / kg EPA and 3000 mg / kg DHA) and the control group (n = 4) received the same feed without supplementation with ω - 3. The animals were evaluated every three months for a period of 12 months, through clinical evaluations, assessment of body condition (BCS) and muscular condition (MCS) scores, BP measurement, electrocardiographic and Holter electrocardiographic examination, echocardiography, thoracic radiographs, hematological, serum biochemistry evaluation and NT-proBNP, inflammatory cytokines (TNF - α, IL - 1β and IL -6) and C-reactive protein (CRP) measurements. The qualitative data were submitted to Fisher's exact test and the quantitative data were compared between the times by means of Kruskal-Wallis test, adopting 5% significance. During the 12 months, maintenance of clinical parameters and BCS and MCS were observed. The supplementation with ω - 3 did not alter BP values, echocardiographic measurements, chest X-rays, hematological parameters or serum biochemistry, NT-proBNP levels, cytokines and CRP also did not change. Only one dog evolved to stage B2 at 12 months.

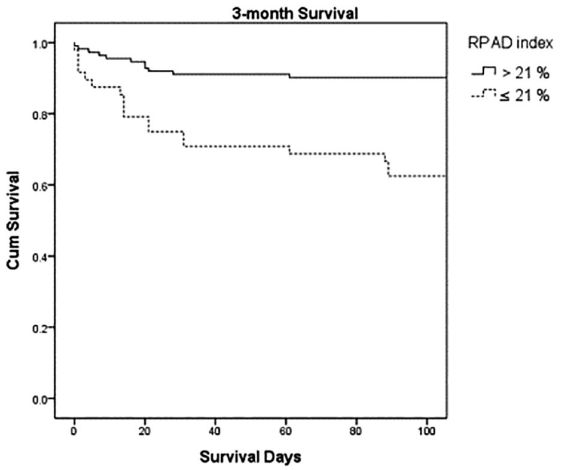
In the electrocardiographic evaluation, there was a predominance of respiratory sinus arrhythmia and low occurrence of atrial arrhythmias. The most frequent heart rhythms were sinus arrest and atrioventricular block (1st and 2nd degrees). Thus, it is concluded that, supplementation with ω - 3 fatty acids don't change clinical, cardiovascular and laboratory parameters of dogs with MVD B1 stage.

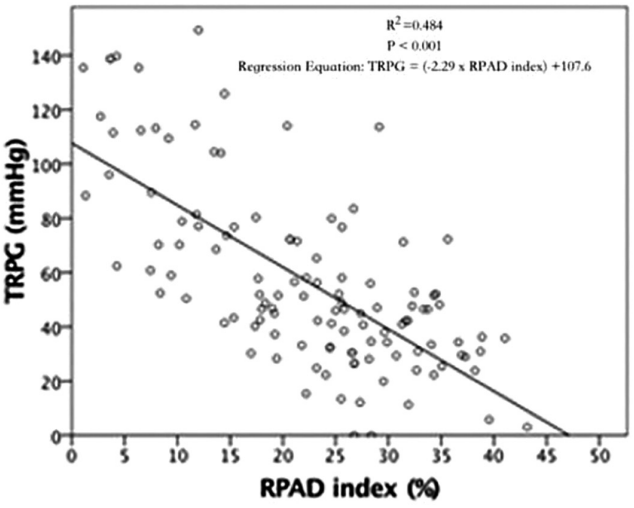
C29

Prognostic Value of Right Pulmonary Artery Distensibility Index in Dogs with Pulmonary Hypertension

I Ping Chan[1], *Min Chieh Weng*[2], *Chung Chun Yang*[1], *Yun Chang Lin*[1], *Shiun Long Lin*[1]
[1]*veterinary medical teaching hospital, National Chung-Hsing University, Taichung, Taichung, Taiwan (Republic of China),* [2]*National Chung-Hsing University, College of Veterinary Medicine, Taichung, Taichung, Taiwan (Republic of China)*

Right pulmonary artery distensibility (RPAD) index has been used in dogs with pulmonary hypertension (PH) secondary to heartworm disease, myxomatous mitral valve disease and patent ductus arteriosus.





Our hypothesis was that RPAD index was correlated with tricuspid regurgitation pressure gradient (TRPG) assessed by echocardiography and it can predict the survival time in dogs with PH secondary to various causes.

Two hundred client-owned dogs at a referral institution were included in the retrospective study.

Medical records were reviewed. Right pulmonary artery distensibility index, acceleration time to peak pulmonary artery flow : ejection time of pulmonary artery flow (AT : ET) were recorded in each dog. Owners were contacted for follow up.

Right pulmonary artery distensibility index (PRAD) was correlated with TRPG (R^2 = 0.484, P < 0.001) and the dogs with RPAD index < 21 % had significantly shorter survival time in 3-month survival group and < 24 % had significantly shorter survival time in one-year survival group.

Right pulmonary artery distensibility index are correlated with TRPG and can predict clinical outcome in dogs with PH caused by various diseases. These indices can be used to evaluate the severity of PH in dogs with absent tricuspid regurgitation.

C30

Comparative Efficacy Analysis of Anti-Hypertensive Drugs in Severe Hypertensive Dogs

Hyeong il Choi[1], Guk hyun Suh[2], Joon young Kim[2], Min young Moon[2], Ha jung Kim[2]

[1]1Department of Internal Medicine, College of Veterinary Medicine, Chonnam National University, Gwangju, Korea, Kwangju, Kwangju-jikhalsi, Republic of Korea, [2]1Department of Internal Medicine, College of Veterinary Medicine, Chonnam National University, Gwangju, Korea, kwangju, Kwangju-jikhalsi, Republic of Korea

Systemic hypertension is a persistent increase in blood pressure which is a health problem associated with target organ damage (TOD). Systolic blood pressure (SBP) is classified based on the risk for future TOD as defined by the American College of Veterinary Internal Medicine (ACVIM) hypertension consensus statement and the International Renal Interest Society (IRIS) staging system, where the following categories for SBP have been defined: < 150 mmHg, minimal risk; 150-159 mmHg, low risk; 160-179 mmHg, moderate risk; > 180 mmHg, high risk. Blood pressure control becomes more difficult to achieve as hypertension progresses. Therefore, early and effective treatment is essential to prevent hypertensive emergencies and to reduce TOD risk. The aims of this retrospective study was to compare short-term SBP variability over seven days in severely hypertensive dogs treated with amlodipine; calcium channel blockers (CCBs), ramipril; angiotensin-converting enzyme inhibitors (ACEIs) and telmisartan; angiotensin receptor blockers (ARBs), either in monotherapy or combination therapy. Medical records between October 2016 and December 2017 were evaluated, and the client-owned dogs that were diagnosed with high-risk systemic hypertension, SBP > 180 mmHg, were included. Systemic hypertension in this study is most commonly associated with hyperadrenocorticism (38%), epilepsy (15%), tumor (12%) and chronic kidney disease (9%). Each patient had their SBP measured by means of an indirect Doppler method, both before and after seven days of treatment with antihypertensive drugs. In the patients treated with CCBs (n = 6), the mean values of SBP before administration were 180 mmHg ± 2.0 mmHg, and after seven days of treatment, the mean SBP had decreased by 18.3 mmHg (p < 0.001), while the mean SBP of two of the six dogs had decreased to < 150 mmHg. In the patients treated with ARBs (n = 6), the mean SBP decreased by 29.2 mmHg (p < 0.005) after one week (day 0: 188.3 ± 13.3 mmHg versus day 7: 159.17 ± 14.3). Two of the six patients receiving ARBs reached target BP; SBP < 150mmHg. In the dogs receiving antihypertensive therapy as a combination of CCBs and ACEIs (n = 14), the mean SBP was reduced by 27.5 mmHg (p < 0.001) after one week (day 0: 190 ± 5.7 mmHg versus day 7: 162 ± 16.3 mmHg), and only six of the dogs had SBP with the minimal risk category. The combination of CCBs and ARBs (n = 10) was associated with a significantly lower SBP: reduced by 46 mmHg (p < 0.001) when compared with the patient';s SBP prior to treatment (day 0: 197.5 ± 17.8 mmHg versus day 7: 151.5 ± 10.0 mmHg). Also, five of ten patients treated with this therapy reached target BP. There was no significant difference between CCBs, ARBs or a combination of CCBs and ACEIs regarding the decrease of SBP in high risk hypertensive patients (p > 0.1). In the dogs treated with a combination of CCBs and ARBs, SBP was significantly decreased when compared with patients receiving other therapies (p < 0.05). Also, after seven days of antihypertensive therapy, 50% (5 of 10) of dogs on CCBs and ARBs achieved target BP, versus 33% (2 of 6) of those on CCBs, 33% (2 of 6) of those on ARBs and 43% (6 of 14) of those on CCBs with ACEIs. In conclusion, antihypertensive drug classes have differentiated effects on short-term blood pressure variability, with a greater reduction in dogs treated with CCBs and ARBs. The combinations of CCBs and ARBs may be the most efficient treatment for lowering SBP in high risk hypertensive patients.

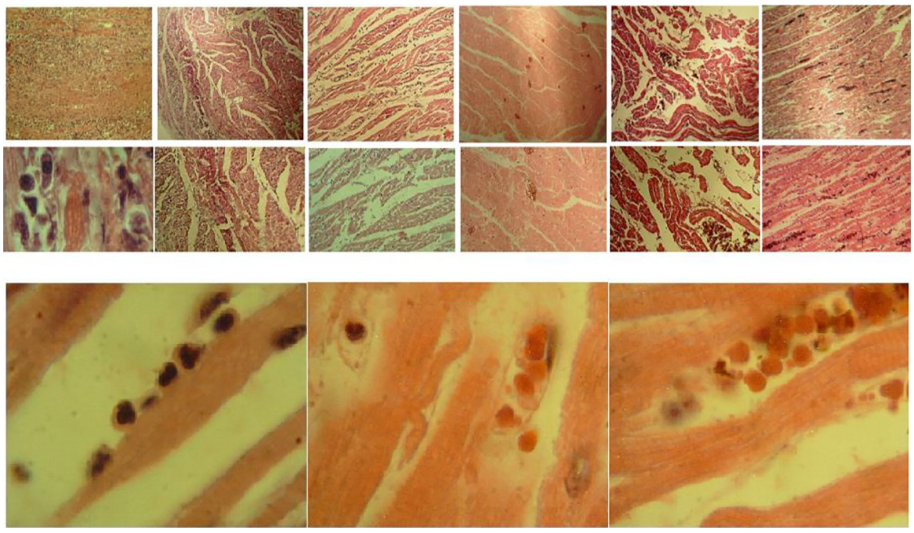
C31

Case Series: Myocarditis in 6 Dogs with Dirofilaria immitis

Rodrigo DeLaValle[1], Alejandra Martinez Cruz[2], Teresa Oviedo Socarral[3]

[1]Universidad de Cordoba, Barranquilla, Atlantico, Colombia, [2]Universidad Nacional de Colombia, Bogota, Cundinamarca, Colombia, [3]Universidad de Cordoba, Monteria, Cordoba, Colombia

The aim of this study is to report the evidence of both macroscopic and histopathological findings compatible with myocarditis in six naturally infected dogs with *Dirofilaria immitis*. The cases were collected retrospectively from a private veterinary clinic located in Barranquilla. To be included in the study, dogs should have complementary tests such as Echocardiography, Electrocardiogram, Troponin I, Immunochromatography test against filaria and necropsy. The heart specimens were taken post-mortem, preserved and stained with haematoxylin and eosin. The echocardiography was normal in 50% of the cases (n= 3), 33% (n=2) of the patients had electrocardiographic abnormalities and elevation of Tropinin I was found in 50% (n=3) of the cases. Macro and microscopic changes were visible in all of the evaluated hearts. The most significant macroscopic finding was diffuse white – yellow areas in all the epicardium, these changes were more severe in the right side of the heart (n=4). Also in 4 of the cases the epicardium had a large area of red color surrounding the left circumflex coronary artery. When the dissection was performed in 4 cases yellow and white spots appeared in the right atrium and the endocardium



presented hemorrhagic zones in the tricuspid valve. In 2 cases endocardial hemorrhagic zones were observed in the left atrium near the mitral valve, additionally in one case at the level of the mitral valve a black spot was observed near the septal leaflet. The histopathological findings included an acute diffuse myocarditis (n=3), acute myocarditis with infarction and parasitic thrombi (n=1), active chronic myocarditis (n=1), necrotizing myocarditis (n=1). This study reports 6 cases of myocarditis in dogs with Dirofilaria immitis were not only the right side of the heart was shown to be affected but also the left side in dogs without pulmonary hypertension or right congestive heart failure.

C32

Long-term Outcomes of Pulmonary Artery Banding for Ventricular Septal Defects

Luis F. Dos Santos, Katherine F. Scollan, Nicole L. LeBlanc, Milan Milovancev, Katy L. Townsend, Michael J. Huber
Oregon State University, Corvallis, OR, USA

Ventricular septal defects (VSD) are common congenital defects in cats and horses. Large defects may cause left-sided volume overload, left-sided congestive heart failure, pulmonary hypertension, and an anticipated poor long-term prognosis. Pulmonary artery banding (PAB) has been reported as a palliative surgical option for hemodynamically consequential VSDs in cats and dogs. PAB surgery results in increased right ventricular systolic pressures, thus decreasing the pressure gradient across the VSD and subsequent shunting volume. There is minimal data in the veterinary literature assessing long-term outcomes following PAB surgery. The aim of this study was to evaluate the long-term outcomes and peri-operative mortality rate of animals who underwent PAB surgery at the Oregon State University Veterinary Teaching Hospital.

Five animals (4 cats and 1 pony) that underwent PAB surgery between 2007 and 2017 were included in this retrospective study. The median age at diagnosis was 5 months (range 4-24 months) and median weight for the cats was 3.55 kg (range 2.1-5.75 kg) and 51.0 kg for the pony. Pre-operative echocardiographic variables indicated left-sided enlargement with a median left atrial to aortic ratio (LA:Ao) of 1.9 (range 1.6-2.4). The median VSD to aortic diameter ratio (VSD:Ao) was 0.32 (range 0.27-0.48) and median VSD flow velocity was 4.81 m/s (range 3.47-6.37 m/s). Two cats were symptomatic with radiographic evidence of left-sided congestive heart failure prior to PAB surgery, and the median pre-surgical VHS was 10.7 (10.4-11) for three cats with available radiographs.

For the surgical procedure, all patients had a left lateral thoracotomy. Umbilical tape was used in the 4 cats for the PAB, while #2 nylon suture was used in the pony. Median surgical time was 2 hours (range 2-4.5 hours) and median total anesthesia time was 3 hours (range 2.5-4.5 hours). No major intra-operative complications were recorded and all animals survived to discharge.

Long-term follow-up was available for all patients with a median of 3.5 years (range 2-9.8 years). Serial echocardiography revealed a median 12.5% reduction in LA:Ao (range 10.5-52.1%) and a median 12.9% reduction in VSD flow velocity (range 2.6-30.2%). No animals were found to have developed left- or right-sided heart failure following PAB surgery, although 2 cats remain symptomatic with exercise intolerance. The 2 cats with heart failure pre-PAB surgery remain alive at 25 and 188 months post-operatively with and without current heart failure therapy, respectively.

The results of this study indicate PAB is a viable palliative treatment option for hemodynamically significant VSDs with the potential for excellent long-term outcomes. The presence of heart failure prior to PAB surgery may not negatively affect the long-term outcome, although a larger study is needed to assess pre-surgical prognostic factors.

C33

Role of the Pyruvate Dehydrogenase Kinase-4 (PDK4) Gene Mutation in Mitochondrial Metabolism in Doberman Pinschers

Kathryn C. Taggart[1], Amara Estrada[1], Patrick Thompson[1], Francisco Lourenco[2], Sara Kirmani[2], Silveli Suzuki-Hatano[3], Christina Pacak[3]
[1]University of Florida College of Veterinary Medicine, Gainesville, FL, USA, [2]University of Florida, Gainesville, FL, USA, [3]University of Florida Child Health Research Institute, Gainesville, FL, USA

Thus far, there are two published studies suggesting specific genetic loci that are associated with the dilated cardiomyopathy (DCM) phenotype in Doberman Pinschers (DP). The pyruvate dehydrogenase

kinase 4 (PDK4) deletion is one of these mutations and is easily tested for using a buccal swab. The PDK4 gene is a mitochondrial protein that controls the shift between glycolysis and oxidative phosphorylation depending on nutrient availability. As cardiomyocytes must produce synchronous, energy-demanding ventricular contractions, oxidative phosphorylation is the preferred method of energy generation because of the higher levels of ATP it yields.

We hypothesize that DPs deficient in PDK4 are unable to switch to oxidative phosphorylation and thus continue to use glycolysis. Over time, the constant reliance upon glycolysis for energy production becomes detrimental, as these hearts are unable to generate sufficient energy for long-term healthy cardiac function. The switch away from fuel sources preferred by the mature heart (fatty acids) and towards glucose which (along with lactate), is a common feature in cardiomyopathies and believed to exacerbate cardiac remodeling and ultimately manifests as ventricular enlargement, arrhythmias, and systolic dysfunction.

For this study, skin biopsies were obtained to isolate dermal fibroblasts from DPs that were pre-screened for DCM with echocardiograms, holter monitoring, and a genetic test for the PDK4 splice site mutation. DPs with phenotypic features of DCM that were heterozygous ($PDK4_{wt/del}$) or homozygous ($PDK4_{del/del}$) for the PDK4 mutation displayed significantly reduced oxygen consumption rate, decreased cell viability, mitochondrial stress, and activation of the intrinsic (mitochondrial mediated) apoptotic pathway under conditions of high (unstarved) and low (starved) nutrient availability in vitro. Following treatment with with an adeno-associated virus (AAV)-PDK4, cells were again starved, and re-evalatued. $PDK4_{wt/del}$ and $PDK4_{del/del}$ treated cells showed a significant improvement in their mitochondrial function and increased viability in the starved condition compared to the cells that were not treated with the AAV-PDK4. The restoration of function of cells observed following administration of AAV-PDK4 provides strong support for the translation of this gene therapy approach into the clinical realm for PDK4-affected Dobermans.

C34

Aortoseptal Angle and Systolic Murmur Occurrence in Normal Cats and Cats with Cardiomyopathy

Natalie R. Morgan, Wendy A. Ware, Jessica L. Ward
Iowa State University College of Veterinary Medicine, Ames, IA, USA

Systolic murmurs are common in healthy cats and cats with cardiac disease. The angle formed by the junction of interventricular septum and ascending aorta (aortoseptal angle, AoSA) might contribute to murmur generation. This study aimed to determine whether (1) a systolic murmur is associated with smaller AoSA, (2) AoSA is different in HOCM cats compared to other cardiomyopathic or normal cats, and (3) AoSA is influenced by age or gender.

Echocardiographic records were searched for feline studies containing stored cine loop(s) of the right long-axis left ventricular outflow view, with contemporaneous cardiovascular exam. Exclusion criteria included congenital cardiac malformation, marked anemia, hyperthyroidism, or hypertension. Both systolic and diastolic AoSA measurements were made in triplicate.

Eighty-one cats were included and classified as normal (n = 33), HOCM (n = 18), HCM (n = 14), and other cardiomyopathy (n = 16). Mean systolic AoSA was smaller in cats with murmurs compared to those without ($p < 0.0001$). A significant, although weak, negative correlation existed between AoSA and murmur grade. AoSA in HOCM cats was smaller than AoSA in normal cats, but not different from cats with HCM or other cardiomyopathy. AoSA was smaller in diastole than in systole for all cats combined ($p < 0.0001$), cats with murmurs ($p = 0067$), and HOCM cats ($p = 0.0224$). AoSA was not different between males and females, or among age groups (<4 years, 4-9 years, ≥10 years). Our findings suggest that smaller AoSA could be associated with murmur development in cats.

C35

Investigation of Transfusion-Free Mitral Valve Plasty in Dogs

Akane Yoshikawa[1], Haruhiko Suzuki[2], Masayuki Enokizono[3], Katsuhiro Matsuura[4], Shusaku Yamada[5], Hideki Yotsuida[6], Nobuyuki Kanno[1]
[1]Nihon University, Fujisawa, Kanagawa, Japan, [2]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Nihon University, Sayama, Saitama, Japan, [3]Animal Cardiovascular and Thoracic Surgery Center / Japan Small Animal Medical Center, Tokorozawa, Saitama, Japan, [4]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Tokyo University of Agriculture and Technology, Sayamashi, Saitama, Japan, [5]Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan, [6]National Cerebral and Cardiovascular Center, Suita, Osaka, Japan

Transfusion-free open-heart surgery under cardiopulmonary bypass (CPB) in dogs is difficult because of the mismatch between the CPB priming volume and the patient';s blood volume. Here, we investigated whether transfusion-free mitral valve plasty (MVP) is possible in dogs. Forty-eight dogs (6-14 years) with body weight ranging from 1.85 to 20.9 kg (median 4.0 kg) who underwent MVP under CPB were included. Patients were divided into three groups: non-transfusion group (G1), blood priming group (G2), and postoperative transfusion group (G3). Criteria for blood transfusion included hematocrit (Ht) less than 20% and/or hemoglobin (Hb) less than 7 g/dl during CPB. We performed ultrafiltration in all patients. Among the 48 dogs, 8 (17.0%) received no transfusion, 19 (39.6%) received blood priming, and 21 (44.0%) received postoperative transfusion only. Ht in G1 was significantly higher than that in G2 during CPB (average Ht: G1, 26.2%; G2, 22.0%; $P = 0.034$), and higher than those in both G2 and G3 after CPB (G1, 41.7%; G2, 34.7%; G3, 34.2%; G1 vs G2, $P = 0.037$; G1 vs G3, $P = 0.013$). Predisposing factors for blood priming were body weight < 3.75 kg (OR 0.455; 95% CI 0.261-0.794; $P = 0.006$; sensitivity: 78%, specificity: 82%) and Ht < 37.15% before CPB (OR 0.837; 95% CI 0.706-0.991; $P = 0.039$; sensitivity 68%, specificity 72%). To conclude, it is possible to perform MVP without blood transfusion in dogs, depending on their body weight and Ht before and after CPB.

C37

Changes of Blood Gas Characteristics During Open Heart Surgery

Masayuki Enokizono[1], Haruhiko Suzuki[2], Katsuhiro Matsuura[3], Shusaku Yamada[4], Dai Nagakubo[5], Kenji Teshima[6], Nobuyuki Kanno[7]
[1]Animal Cardiovascular and Thoracic Surgery Center / Japan Small Animal Medical Center, Tokorozawa, Saitama, Japan, [2]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Nihon

University, Sayama, Saitama, Japan, [3]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Tokyo University of Agriculture and Technology, Sayama-shi, Saitama, Japan, [4]Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan, [5]The University of Tokyo/Animal Cardiovascular and Thoracic Surgery Center, Bunko-ku, Tokyo, Japan, [6]Nihon univ., Fujsawa, Kanagawa, Japan, [7]Nihon University, Fujisawa, Kanagawa, Japan

In this study, the purpose was to clarify changes in blood gas during surgery when mitral valve plasty surgery was performed using cardiopulmonary bypass(CPB).

The subjects were 54 dogs with mitral regurgitation.For anesthesia inhalation anesthesia was combined with moderate hypothermia method. Timing of conducting blood gas sample examination was pre: before initiating CPB, partial: after starting CPB, total: after aortic cross-clamping, rebeat: after aortic declamping, post: after completing CPB.

In this case, 74% (40/54) cases showed acidosis, and only one case exhibited alkalosis. BE decreased from Rebeat (-3.1 ± 3.41mmol / L) to Post (-7.3 ± 2.87mmol / L). In addition, Lactate increased from Post (1.5 ± 0.63mmol / L) to Total (1.1 ± 0.39mmol / L) and Post (2.1 ± 1.25mmol / L) increased. Seven cases were observed in PaO2 that showed 300 mmHg or less to post. All seven cases were improved by extubation after performing postoperative posture change and respiratory management. Lastly, Finally, SvO2 was low at pre (68.5 ± 13.6%), but increased at post (76.3 ± 10.1%).

Based on the results of this study, it was shown that after the CPB, it is leaning toward acidosis. It is thought that change was recognized in PaCO2, Lactate, BE by hypothermia method or long operation. It was suggested that surgery was able to maintain blood circulation better than the result of SvO2. Furthermore, regardless of the state of the preoperative patient, PaO2 was finally improved in all cases and it was considered possible to maintain oxygenation appropriately.

C38

Effect of Hemodialysis during Cardiopulmonary Bypass in Dogs with Azotemia Undergoing Mitral Valve Plasty

Katsuhiro Matsuura[1], Shusaku Yamada[2], Masayuki Enokizono[3], Haruhiko Suzuki[4], Mao Hayashi[5], Dai Nagakubo[6], Akane Yoshikawa[7], Kenji Teshima[7], Nobuyuki Kanno[7]

[1]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Tokyo University of Agriculture and Technology, Sayama-shi, Saitama, Japan, [2]Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan, [3]Japan Small Animal Medical Center/Nihon University, Tokorozawa, Saitama, Japan, [4]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Nihon University, Sayama, Saitama, Japan, [5]Shiraishi Animal Hospital, Sayama, Saitama, Japan, [6]The University of Tokyo/Animal Cardiovascular and Thoracic Surgery Center, Bunko-ku, Tokyo, Japan, [7]Nihon University, Fujisawa, Kanagawa, Japan

Azotemia is a situation frequently seen in patients with mitral regurgitation (MR). The decline in renal function can be a prognostic factor at the time of cardiac surgery. In this study, we examined the effect of hemodialysis (HD) during the cardiopulmonary bypass for dogs undergoing mitral valve plasty (MVP).

10 azotemic dogs with MR were separated into non-HD group (n=5, mean BUN 49.9 mg/dl, Cre 1.50 mg/dl) did not perform HD and HD group (n=5, mean BUN 58.1 mg/dl, Cre 1.34 mg/dl) performed HD. BIOCUBE®hemoconcentrator BHC-030 (NIPRO, Osaka, Japan) was set in the circuit as the dialyzer. Sublood-BSG (FUSO Pharmaceutical Industries Ltd., Tokyo, Japan) was used for the dialysate. BUN and Cre values at preoperative, postoperative, day1, day2, day3, after surgery, the day discharged and 1 month after surgery were compared.

Compared with the preoperative day, BUN and Cre were significantly lower in both groups after surgery, on the first day after surgery and at discharge. BUN in the HD group was significantly lower at the first day, the third day, and the one month postoperatively compared to the non-HD group. There was no significant difference in Cre between both groups.

It was suggested that HD showed an effect of lowering the renal value of the perioperative period in dogs with decreased renal function undergoing MVP. In this case group, the decline in renal function was not severe so that the renal value at discharge was stable (HD: mean BUN 24.9 mg/dl, Cre 0.60 mg/dl, non-HD: mean BUN 27.5 mg/dl, Cre 0.76 mg/dl), and no complications due to azotemia were observed. Farther study is necessary in patients with further decreased renal function to verify the usefulness and limitation of HD.

C39

Thyroid Hormone and Cardiopulmonary Bypass in Dogs

Nobuyuki Kanno[1], Karin Ariyama[2], Haruhiko Suzuki[3], Masayuki Enokizono[4], Katsuhiro Matsuura[5], Kenji Teshima[6]

[1]Nihon University, Fujisawa, Kanagawa, Japan, [2]Nihon university, Fujisawa, Kanagawa, Japan, [3]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Nihon University, Sayama, Saitama, Japan, [4]Japan Small Animal Medical Center/Nihon University, Tokorozawa, Saitama, Japan, [5]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Tokyo University of Agriculture and Technology, Sayama-shi, Saitama, Japan, [6]Nihon univ., Fujsawa, Kanagawa, Japan

Thyroid hormones play an important role in regulating cardiac function. Several studies on human pediatric surgery have reported a reduction in thyroid hormones and hormone replacement after heart surgery.The purpose of this study was to define the effects of cardiopulmonary bypass on the concentrations of thyroid hormones in dogs. Twenty-seven dogs with mitral valve regurgitation, aged 10 (8.5-11) years, body weight 3.9 (2.6-6.4) kg, underwent mitral valve plasty (MVP) with cardiopulmonary bypass (CPB) between July 2016 and October 2017. Thyroid-stimulating hormone (TSH), total thyroxine (T4), and free T4 (fT4) were measured using Chemiluminescent Enzyme Immunoassay on postoperative days 1, 2, 3, and 7, and at discharge. Patients showing a marked decrease in thyroid hormone concentration, decreased myocardial contraction, and depression, were prescribed levothyroxine sodium (20 μ/kg, oral every 24 hr).

TSH (median 0.07 ng/ml, range 0.05-0.15), T4 (median 0.3>μg/dl, below measurable limits) and fT4 (median 0.3 pg/dl, below measurable limits) were significantly depressed for up to 24 hours after surgery in all subjects. TSH, T4, and fT4 had returned by the time of discharge (7-14 days) in most subjects. In the levothyroxine treatment group (n=10), T4 was significantly increased, however, fractional shortening and ejection fraction evaluated by echocardiography were not significantly improved after the start of treatment. Anesthesia time, duration of CPB, cross-clamp time, and length of hospital day did not differ

between the treatment and non-treatment groups. There were no adverse effects in the treatment group.

These results indicate that canine patients undergoing MVP under CPB might present with euthyroid sick syndrome. Additional study is necessary to clar

C40

Clinicopathologic, Hemodynamic, and Echocardiographic Effects of Anti-Inflammatory Glucocorticoids in Systemically Healthy Cats

Imal A. Khelik[1], *Darren J. Berger*[2], *Jonathan P. Mochel*[2], *Jean-Sébastien Palerme*[2], *Wendy A. Ware*[2], *Jessica L. Ward*[2]
[1]*Iowa State University, Ames, IA, USA,* [2]*Iowa State University College of Veterinary Medicine, Ames, IA, USA*

Administration of long-acting injectable glucocorticoids has been reported to precipitate congestive heart failure in cats. This prospective clinical trial aimed to investigate mechanisms by which oral intermediate-acting glucocorticoids could predispose cats to progression of heart disease. Systemically healthy cats with allergic dermatitis (n = 10) were given oral prednisolone at anti-inflammatory doses (1 - 2 mg/kg) once daily for 14 days followed by taper and washout. Matched control cats (n = 10) received no treatment. Clinicopathologic, echocardiographic, and hemodynamic variables were measured prior to glucocorticoid administration at baseline (day 0), during prednisolone treatment (days 7 and 14), and post-washout (day 35). Multiple linear regression models were used to assess effects of prednisolone treatment and time on study variables. No significant changes in blood glucose, blood pressure, sodium, potassium, cardiac biomarkers, or echocardiographic measurements occurred with prednisolone treatment at any time point. Expected hematologic and biochemical changes occurred during prednisolone treatment, including mild increases in monocyte count and serum albumin, calcium, cholesterol, and triglycerides ($p < 0.05$). Indices of red blood cell mass decreased from baseline in both treatment and control cats at day 7 and 14 ($p < 0.001$), likely due to repeated blood sampling. Fructosamine increased from baseline in steroid-treated cats on day 7 ($p = 0.021$) and 14 ($p = 0.036$). Other than triglyceride levels, all hematologic and biochemical changes were mild and within reference ranges. Results of this study suggest that anti-inflammatory prednisolone does not result in clinically relevant hemodynamic or diabetogenic effects in systemically healthy cats.

C41

Reversal Effect of Toceranib Versus Sorafenib on Monocrotaline-Induced Pulmonary Arterial Hypertension in Rats

Zi Ping Leong[1], *Yoshiaki Hikasa*[2]
[1]*The United Graduate School of Veterinary Science, Yamaguchi University (Tottori University)/Tottori University, Faculty of Agriculture, Joint Department of Veterinary Medicine, Tottori-shi, Tottori, Japan,* [2]*Tottori University, Faculty of Agriculture, Joint Department of Veterinary Medicine, Tottori-shi, Tottori, Japan*

Pulmonary arterial hypertension (PAH) is characterized by pulmonary arterial muscularization and right ventricular hypertrophy (RVH) with poor treatment outcomes and a guarded prognosis in humans and dogs. Sorafenib has shown to potently reverse cardiopulmonary remodeling and PAH in human patients, but the effect of toceranib, a veterinary tyrosine kinase inhibitor, is unknown.This study compared the anti-remodeling effect of toceranib with sorafenib on monocrotaline-induced pulmonary arterial remodeling and RVH in rats. Since we recently showed that a low-dose imatinib significantly reversed cardiopulmonary remodeling using the same experimental model, here we also investigated the potential use of a low-dose (non-neoplastic) toceranib for treatment of PAH.

Monocrotaline-treated rats were randomized to receive sorafenib (10, 30, or 100 mg/kg), toceranib (1, 3, or 10 mg/kg), or water, orally once daily for two weeks. RVH, pulmonary arterial muscularization, and messenger RNA (mRNA) and protein levels of target receptors tyrosine kinase were evaluated.

Sorafenib significantly and dose-dependently reversed RVH (R^2_{RVH}: 0.35; $P < 0.05$) and B-type natriuretic peptide (BNP) mRNA level (R^2_{BNP}: 0.38). Besides, low, medium, and high doses of sorafenib dose-dependently reduced the proportion of fully muscularized pulmonary arteries (FMPA) by 7.5%, 11.7%, and 31.1% (R^2_{FMPA}: 0.33), respectively. By contrast, the toceranib groups showed a weaker reversal dose-dependency (R^2_{RVH}: 0.32; R^2_{BNP}: 0.27; R^2_{FMPA}: 0.01). A significant reduction in RVH and BNP mRNA level was observed only in rats at the highest dose of toceranib. The FMPA was reduced by 1.4% at the highest dose of toceranib, but further aggravated by 12% at the lowest dose. Pulmonary platelet-derived growth factor receptor beta (PDGFR-β) mRNA expression was significantly downregulated in all treatment groups except for the lowest dose toceranib group. Phosphorylated ERK-1/2 protein level which represents the PDGFR downstream signaling was significantly suppressed in all doses of sorafenib, but solely the highest dose of toceranib. Furthermore, toceranib at lower doses significantly upregulated pulmonary p-62 and microtubule-associated protein 1 light chain 3 beta (MAPLC3) mRNA levels, suggesting an impaired autophagy.

In conclusion, toceranib elicited weaker reversal properties on pulmonary arterial remodeling and RVH, and therefore, a low dose of toceranib in comparison with sorafenib may not be a promising therapeutic agent for cardiopulmonary remodeling and PAH.

C42

The Effects of Obesity on Cardiovascular Parameters in Cats

Fúlvia B. Souza[1], *Danilo V. Golino*[1], *Shayra P. Bonatelli*[1], *Angélica Alfonso*[1], *Maria Jaqueline Mamprim*[1], *Júlio Cesar C. Balieiro*[2], *Alessandra Melchert*[3], *Priscylla T. C. Guimarães-Okamoto*[3], *Maria Lucia Gomes. Lourenço*[1]
[1]*São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Botucatu, Sao Paulo, Brazil,* [2]*Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia., Pirassununga, Sao Paulo, Brazil,* [3]*School of Veterinary Medicine and Animal Science, São Paulo State University - UNESP Botucatu, Botucatu, Sao Paulo, Brazil*

Obesity is the most common nutritional disease in cats and is increasing in prevalence. Excess body fat predisposes the animal to deleterious effects on heart function and systolic blood pressure (SBP) alterations, arrhythmias and radiographic and echocardiographic changes have been described. However, there is not much information regarding the effects of obesity on the cardiovascular system of cats. This study aims to evaluate these effects on cardiovascular system of domestic cats and compare them with cats with normal body

conditions. Thirty-six cats were allocated in two groups (20 obese cats and 20 cats with normal body condition) and submitted to SBP measurement; electrocardiogram (ECG); and chest radiograph to evaluate the cardiac silhouette by vertebral heart size (VHS). The echocardiographic measurements were evaluated, establishing a relationship between the data obtained and Body Weight, Body Condition Score (BCS) and Body Mass Index (BMI). SBP and VHS were statistically higher in obese animals in contrast to normal cats. In obese cats, the mean SBP was 148.5 ± 29.6 and seven animals presented values exceeding 150 mmHg. In normal cats, the SBP was 126.4±19.8 and hypertension was observed in three animals. VHS analyses presented values of 8.1 ± 0.6 and 7.8 ± 0.4 in obese and normal cats, respectively. ECG evaluation revealed a sinus rhythm in 100% of the obese cats. In normal cats, sinus rhythms were present in 19 cats (95 %) and sinus tachycardia in one cat (5 %). Heartrate was 203.5 ± 24.0 and 206.8 ± 23.3 in obese and normal cats, respectively. Obese cats presented higher values than normal cats in most parameters, except for interventricular septum in diastole (IVSd), but there was no statistical difference in most of them. The difference in the left ventricular free wall in diastole (LVFWd) was the only one with statistical significance ($P < 0.05$). The tests applied to verify a possible correlation between the echocardiographic parameters, BMI, BCS, and Body Weight have shown a positive relationship between LVFWd, Body Weight, and BCS; aorta (Ao), Body Weight and BMI; left atrium (LA) and Body Weight; left ventricular internal diameter in diastole (LVIDd) and Body Weight. A quadratic relationship was also observed between left atrial-to-aortic root diameter ratio (LA:Ao) and Body Weight. In addition, the relationship between LVFWd and BMI, although not considered significant, presented P values slightly above 0.05. Obese cats showed higher BPS and VHS than normal cats, which led to an increase in echocardiographic measurements. This study has shown that obesity promoted cardiac function impairment in the studied cats and, therefore, that it is important to monitor these animals since even asymptomatic ones may present changes in cardiac parameters.

C43

Heart Rate and Heart Rate Variability in Pregnant Dairy Cows and in Fetuses

Dario A. C. Quevedo[1], Maria Lucia Gomes Lourenço[1], Carmen Daza. Bolaños[2], Angélica Alfonso[1], Carla Maria Vela. Ulian[3], Simone Biagio. Chiacchio[1]
[1]São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Botucatu, Sao Paulo, Brazil, [2]Department of Animal Health. University of Nariño, FACIPEC, Pasto, Nariño, Colombia, Pasto, Narino, Colombia, [3]Faculdade Pio Décimo - Campus III, Faculdade Pio Décimo - Campus III, Jabotiana, Sergipe, Brazil

The study aims at measuring heart rate variability in Holstein cows, fetuses and neonates through a descriptive evaluation of continuous fetomaternal and neonatal electrocardiogram recordings during the perinatal period. Heart rate (HR) and heart rate variability (HRV) were assessed by fetomaternal electrocardiography (ECG). Fetomaternal measurements were taken six times pre-partum and in neonates six times after calving. Heart rate, time-domain variables and frequency-domain variables were analyzed in 23 Holstein cows and 18 neonates. No significant changes were observed in maternal or fetal RR intervals and HR. In the fetuses, the standard deviation of beat-to-beat interval (SDNN) decreased significantly from 38.08 ± 2.6 ms to 28. 9 ± 2.4 ms ($p < 0.05$), but the root mean square of successive beat-to-beat differences (RMSSD) did not change significantly. Fetal heart rate and RR interval differed statistically from the day before delivery (163 ± 7.5 bpm; 381 ± 24.2 ms) to the day after calving (131 ± 5 bpm; 472 ± 16. 2 ms). Time-domain variables (SDNN and RMSSD) and frequency-domain variables (LF and HF) were significantly different ($p < 0.05$) between the fetal and neonatal stages. Fetomaternal ECG is a reliable technique to detect cardiac signals in bovine fetuses in the last 35 days of gestation. Decreases in the values of SDNN and RMSSD reflect a shift towards sympathetic dominance. After calving, the increase in high frequency and the decrease in low frequency variables indicate activation of the vagal nerve followed by heart and respiratory modulation.

C44

Heart Rate Variability Assessment in Foals Undergoing Hoof Trimming with Use of Equine Maternal Pheromones

Renata Alves, Amanda Sarita Cruz-Aleixo, Maria Lucia Gomes Lourenço, Simone Biagio Chiacchio
São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Botucatu, Sao Paulo, Brazil

The equine maternal pheromone (EMP) has been used as a tool to calm equines facing new or stressful situations, which forces physiological adaptations and alterations related mainly to increases in heart-rate (HR) and behavioral alterations. This study aims at assessing the effects of EMP treatment in colts undergoing hoof trimming for the first time, employing the behavior of heartrate variability (HRV) as the main parameter. We assessed 20 colts with average age of 9 months that underwent hoof trimming for the first time. The animals were divided in two groups and a randomized double blind experimental design was employed. The treatments (EMP and placebo) were administered and the Holter monitor was put in place. After 20 minutes, the procedure was started. The HR (measured through the conventional method) and HRV were measure at two moments: 20 minutes before trimming (M1) and immediately after trimming (M2). The parameter analysis was conducted with a Student's T test with significance level of $p = 0.05$. The results are displayed on Table 1. Statistically significant differences were noted when the HR was measured through the conventional method, with Group B presenting a decrease at M2. However, no statistically significant differences were observed during the Holter test when comparing both groups.

C45

Heart Rate and Heart Rate Variability in Pregnant American Miniature Horse Mares

Juliana A. N. Gama, Maria Lucia Gomes Lourenço, Gabriela A. Campos, Raphael T. Teixeira, Simone Biagio Chiacchio
São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Botucatu, Sao Paulo, Brazil

The measurement of heart rate variability (HRV) was reported as an acceptable method for assessing the level of stress and numerically express the neurohormonal effect exerted on the heart rate. Normal

(C44) Table 1 – Mean (standard deviation) and P values for heartrate measurement through a conventional clinical examination and through Holter examination in equines undergoing hoof trimming with previous treatment with EMP and placebo.

	Group A	Group B	*p*
Conventional Clinic Exam			
HR Before (M1)	60.8 (18.9)	57.6 (10.5)	0.6460
HR After (M2)	71.6 (21.3)	54.4 (10.4)	**0.0353**
Holter Exam			
Minimum HR (bpm)	47.5 (9.1)	40.5 (7.6)	0.2354
Mean HR (bpm)	82.6 (18.2)	73.5 (24.2)	0.9262
Maximum HR (bpm)	230.6 (25.9)	250.0 (15.9)	0.4398
QRS No.	3654.1 (974.2)	2758.5 (1041.3)	0.1788
NNS	1837.7 (919.9)	1802.0 (578.5)	0.8290
Mean NN (ms)	957.9 (241.3)	1205.5 (212.2)	0.1591
SDNN (ms)	331.0 (237.6)	230.0 (148.6)	0.4567
SDANN (ms)	145.2 (98.8)	119.5 (111.0)	0.8417
SDNNIDX (ms)	133.7 (58.7)	156.5 (107.4)	0.4345
NNNs	1725.7 (891.8)	1774.0 (589.1)	0.9481
RMSSD (ms)	78.4 (65.1)	81.0 (182.1)	0.3302
PNN50 (%)	15.61 (13.4)	14.4 (13.3)	0.6892

* Statistical test employed: Student's T test with significance level of $p < 0.05$. Min. max. avg HR: Minimal. Maximum and mean heart rate (bpm); NN: mean of all normal RR intervals in the test; SDNN: standard deviation of all normal RR intervals; SDANN: standard deviation of the mean of the RR intervals every five minutes; SDNNindex: mean of the standard deviations calculated for normal RR intervals every five minutes; pNN50: ratio of differences higher than 50 ms between adjacent normal RR intervals during the 24-hour test; RMSSD: square root of the mean of the squares of the successive differences between adjacent normal RR intervals.

values for physiological parameters in larger breeds are often used as reference values in ponies. However, heart rate increases in smaller animals and, in adult ponies, is higher than in adult warmblood horses. Little is known about the effect of pregnancy signalment on heart rate variability values in the equine species. This study aimed at determining physiological ranges for heart rate (beats per minute) and heart rate variability in equine mares at different months during gestation (10 months). The ECG recordings were made once a month and the data were used for analysis. Heart rate (HR) and HRV were assessed by maternal electrocardiography (ECG). Maternal measurements were taken ten times pre-partum and heart rate variability was analyzed in the time-domain.

Significant changes were observed in the fourth and tenth month of pregnancy for RR interval (1238.53 ± 100.37 ms; 962.60 ± 234.11 ms) and HR (49.42 ± 4.84 bpm; 62.43 ± 11.53 bpm) ($p = 0.018$), corresponding to the smallest and largest value respectively. No significant changes were observed in the standard deviation of beat-to-beat interval (SDNN) ($p = 0.466$) or the root mean square of successive beat-to-beat differences (RMSSD) ($p = 0.760$). Time-domain analysis has demonstrated a significant increase in the mean RR intervals and HR with the progression of pregnancy and has also established that HRV is influenced by HR, as a higher HR was associated with a lower HRV in the mare.

C46

Cardiovascular-Renal Axis Disorder in Cats with Congestive Heart Failure Due to Primary Cardiomyopathy

Mengmeng Liu[1], *Anne French*[1], *Geoffrey Fosgate*[2], *Paul Wotton*[1], *Liza Köster*[1]

[1]*Small Animal Hospital, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK, Glasgow, Scotland, United Kingdom,* [2]*Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa, ONDERSTEPOORT, Gauteng, South Africa*

Renal dysfunction caused by primary cardiovascular disease is considered to be a class of cardiovascular renal disorders (CvRD). In small animal medicine, CvRD in cardiac patients is a novel research area. In this study we aimed to evaluate CvRD in cats with congestive heart failure (CHF) and primary cardiomyopathy (CM) by measuring cardiac and renal functional markers.

Twenty-one client owned cats diagnosed with primary CM (16 CHF and 5 asymptomatic) from a referral Cardiology Service were enrolled. Twenty healthy cats were used as controls. Serum N-terminal-pro brain naturetic peptide (NT-proBNP), symmetric dimethylarginine (SDMA) and creatinine levels were measured in a reference laboratory. Relevant clinical information and survival status of CHF cats were recorded. One-way analysis of variance (ANOVA), Student's T tests, Spearman's rho and Cox proportional hazards models were used for statistical analysis.

NT-proBNP, SDMA and creatinine levels were positively correlated and significantly different among the three groups of cats. CHF cats had higher serum NT-proBNP than asymptomatic CM cats and healthy control cats, and higher SDMA and creatinine than healthy controls ($P < 0.05$). Cats with CHF that died had higher NT-proBNP ($P = 0.001$) and SDMA ($P = 0.007$) but not creatinine ($P = 0.922$) compared to survivors; however, neither of the markers were significant prognostic factors in multivariable survival analysis models.

Study results suggest that CvRD is present in CHF cats with primary cardiomyopathy. Furthermore, SDMA is a novel biomarker in cats with CHF and primary CM that could be used for disease management and prognostication.

C47

Familial Ventricular Arrhythmias in the Rhodesian Ridgeback

Kathryn Meurs[1], *Bruce Keene*[2], *Jess Weidman*[3], *Steve Rosenthal*[4], *Teresa DeFrancesco*[2], *Darcy Adin*[2], *Sandra Tou*[2]
[1]*North Carolina State University, Raleigh, NC, USA,* [2]*NCSU, Raleigh, NC, USA,* [3]*CVCA, Maryland, DC, USA,* [4]*CVCA, Columbia, MD, USA*

Sudden cardiac deaths associated with ventricular arrhythmias have been reported in some families of Rhodesian Ridgeback dogs.

We examined ambulatory electrocardiographic (Holter) recordings from 98 Rhodesian Ridgebacks that were reported to be asymptomatic by their owners. Recordings with at least 20 hours of data were evaluated for the presence of ventricular and supraventricular ectopy. The number of ventricular premature complexes (VPCs) / 24 hours and complexity of ectopy were quantified; dogs that experienced more than 2 VPCs / 24 hours were considered to be abnormal. Complexity of ventricular ectopy was graded as follows: grade 1, isolated, uniform VPCs; grade 2, bigeminy or trigeminy; grade 3, couplets or triplets; grade 4, R on T or nonsustained ventricular tachycardia; there were no dogs with sustained ventricular tachycardia.

Fifty-four of 95 dogs examined (24 male, 30 female) had 2 or fewer VPCs/24 hours, and no supraventricular ectopy; they ranged in age from 3 – 39 months (median 8 months).

Forty-one dogs (16 male, 1 male castrated, 24 female) had 3 or more VPCs/24 hours. They were not statistically different in age (median 12 months, range 3 - 32 months) and experienced a median number of 53 VPCs / 24 hours (range 3 -29,673). Complexity grades ranged from 1 - 4. Two dogs also had frequent supraventricular premature complexes.

This preliminary study identified asymptomatic ventricular ectopy in approximately 44% of Rhodesian Ridgebacks examined at less than 4 years of age. Additionally, supreventricular ectopy was also infrequently observed.

C48

Platelet Function in Dogs with Myxomatous Mitral Valve Disease

Takeshi Mizuno[1], *Masashi Mizuno*[1], *Kayoko Harada*[1], *Hiroshi Takano*[1], *Asako Shinoda*[1], *Arane Takahashi*[1], *Kazuya Mamada*[1], *Ayaka Chen*[2], *Kazuki Takamura*[1], *Masami Uechi*[1]
[1]*JASMINE Veterinary Cardiovascular Medical Center, Yokohama, Kanagawa, Japan,* [2]*JASMINE Vetrinary Cardiovascular Medical Center, Yokohama, Kanagawa, Japan*

As a consequence of the turbulent high-velocity flow and changes in fluid shear stress around the mitral valve, changes in platelet function may be involved in the pathogenesis of myxomatous mitral valve disease (MMVD). The aim of this study is to investigate whether the changes in coagulability vary with the severity of MMVD in dogs.

We retrospectively reviewed charts from dogs with MMVD. The classification of MMVD dogs was based on the ACVIM staging. We compared results from the following test: white blood cell counts (WBC), platelet count (PLT), mean platelet volume (MPV), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fib), C-reactive protein (CRP), antithrombin III (ATIII), fibrin degradation products (FDP), activated clotting time (ACT), platelet function (PF), and clot rate (CR).

A total of 95 dogs were evaluated; 27 dogs with ACVIM Stage B2, 45 dogs with Stage C, and 24 dogs with Stage D. Fibrinogen and CRP were significantly increased as the disease progressed ($P < 0.05$). Dogs in Stage D had significantly higher WBC, PLT, and PF when compared to dogs in Stage B2 ($P < 0.05$). There were no significant differences in MPV, PT, APTT, ATIII, and FDP among the groups.

The platelet function and activity declined in dogs with MMVD. WBC, CRP, and Fib increased, which is consistent with increased inflammation as the MMVD progressed. The inflammatory response may play an important role in increasing platelet function as the mitral valve function deteriorates.

	B2	C	D
WBC	7300 (2700 – 19700)	8000 (4200 – 19200)	10950 (7200 – 29800) *
CRP	0.64 ± 0.34	0.69 ± 0.53 *	1.22 ± 1.12 *
PLT	42.4 (11.2 – 58.9)	44.9 (12.3 – 34.3)	49.1 (18.7 – 73.6) *
MPV	5.2 (3.5 – 8)	4.9 (2.9 – 7.3)	4.45 (3.1 – 7.7)
Fib	245 (123 – 608)	281 (116 – 650) *	373 (187 – 650) *
PF	1.2 (0 – 4.7)	1.9 (0.3 – 4.7)	2.9 (0.7 – 4.9) *
CR	21 (8 – 34)	21 (10 – 40)	24 (15 – 37) *

*; $P < 0.05$ vs Stage B2, ** ; $P < 0.01$ vs Stage B2

C49

N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP) in Feline Arterial Thromboembolism

Michelle Oranges[1], *John Rush*[2], *Lisa Freeman*[2], *Elizabeth Rozanski*[2], *Emily T. Karlin*[3]
[1]*Cummings School of Veterinary Medicine at Tufts University, Waltham, MA, USA,* [2]*Tufts University, North Grafton, MA, USA,* [3]*Tufts University, Westborough, MA, USA*

Arterial thromboembolism (ATE) is a life-threatening condition in cats. However, there is currently no point-of-care test that can identify cats at risk for ATE. NT-proBNP concentrations may be helpful in identification of cats that might develop ATE. The goal of this retrospective study was to compare NT-proBNP concentrations in cats with cardiomyopathy that had or developed ATE within 1 year of NT-proBNP testing to cats that did not develop ATE within 1 year.

Medical records were searched for cats with any form of cardiomyopathy that had NT-proBNP testing. Cats were classified into 3 groups: cats with occult cardiomyopathy (OCM group), cats with cardiomyopathy and congestive heart failure without ATE (CHF group), and cats with cardiomyopathy that had or developed an ATE (ATE group).

Eighty-six cats were enrolled. Cats in both the CHF and ATE groups (P < 0.001) had significantly higher NT-proBNP concentrations than those in the OCM group; however, concentrations were not different between the CHF and ATE groups. An ROC curve was generated using the NT-proBNP concentrations of the ATE and OCM groups (AUC 0.96, 95% confidence interval: 0.914 - 1.000, P < 0.001). Youden';s index (J) estimated the optimal NT-proBNP cutoff point at 490.5 pmol/L (Sensitivity = 96.2%, Specificity = 90.6%). Dividing all cats using this cutoff point, cats with NT-proBNP concentrations > 491 pmol/L had a larger left atrium, thicker left ventricle, and lower fractional shortening.

Prospective studies are needed to evaluate the utility of NT-proBNP > 491 pmol/L in assessing risk of ATE in cats with cardiomyopathy.

C50

Clinical Outcome and Echocardiographic Prediction of Congestive Heart Failure in Dogs with Left Atrial Rupture

Guilherme G. Pereira[1], Mariana Karamm[2], Rodrigo Francisco[3], Isabela E. de Campos[4]
[1]NAYA / Pompeia Veterinary Hospital, Vinhedo, Sao Paulo, Brazil, [2]Pompeia Veterinary Hospital, São Paulo, Sao Paulo, Brazil, [3]NAYA / Pompeia Veterinary Hospital, Sao Paulo, Sao Paulo, Brazil, [4]Pompeia Veterinary Hospital, Sao Paulo, Sao Paulo, Brazil

Left atrial rupture (LAR) is an uncommon complication of myxomatous mitral valve disease, usually leading patients to die suddenly or in a few hours, but some dogs can survive for a long period of time.

Congestive heart failure (CHF) was previously hypothetized to reduce long term survival. Doppler derived echocardiographic parameters influenced by atrial pressure, such as peak early (E) left ventricular filling wave velocity and E to isovolumic relaxation time (E:IVRT) ratio usually provide informations about congestive heart failure. Although echocardiographic prediction of CHF at the time of LAR diagnosis would be helpful for prognosis, reduction in left atrial pressure due to chamber emptying to pericardial space could hinder this predictive analysis.

The study retrospectively reviewed hospital recordings from dogs with diagnosis of LAR based on necropsy and/or echocardiographic findings, which included all of the following criteria: (1) myxomatous mitral valve disease with mitral regurgitant flow aliased on color Doppler study filling over 60% of the left atrium, (2) LA:Ao ratio > 1.5 on two-dimensional right parasternal short-axis view, (3) pericardial effusion and (4) organized echogenic material suggestive of cloth within pericardial space. Dogs that died from non cardiac cause were not included.

Six dogs met the criteria for inclusion, being 4 males and 2 females from different breeds: Toy Poodle (n = 3), Pinscher (n = 2) and Chihuahua (n = 1). Mean age was 12.3 years (range, 10 to 15 years) and mean body weight was 5.0 kg (range, 2.8 to 9.0 kg). Clinical signs included cough (n=6), weakness or colapse (n=5) and tachypnea (n=4). Most dogs (n = 5) had systolic systemic arterial blood pressure lower than 71 mmHg. All dogs were hospitalized after diagnosis and had systemic arterial blood pressure greater than 100 mmHg after therapy with intravenous crystalloid (n=6), dobutamine (n = 5), noradrenaline (n = 2) and/or pericardiocentesis (n = 1).

Thoracic radiographs were performed at the time of LAR diagnosis and 3 dogs had marked interstitial and alveolar perihilar infiltration (CHF group) while the remainder 3 dogs had no pulmonary abnormalities and also no prior history of decompensated heart failure (non-CHF group).

Mean survival time in non-CHF group was 228 days (range 87 – 474 days) which was significantly longer (P = 0.0326) compared to CHF group (2.3 days, range 1 – 4 days). Two dogs from non-CHF group were still alive at the conclusion of the study.

CHF and non-CHF groups were not different when comparing echocardiographic LA:Ao ratio (2.20, range 1.62 – 2.61 vs. 2.21, range 2.12 – 2.35, respectively) and Doppler derived mean peak E wave (0.94 m/s, range 0.70 – 1.17 m/s vs. 1.03 m/s, range 1.00 – 1.10 m/s, respectively) and mean E:IVRT index (1.23, range 0.71 – 2.02 vs. 1.35, range 1.17 – 1.65, respectively). Predictive values of E:IVRT ratio for congestive heart failure (> 2.5) were not found in any dog with radiographic evidence of decompensated congestive heart failure. Cardiac tamponade was found in two dogs, being one from each group.

Informations provided in this retrospective study reinforce the hypothesis that absence of congestive heart failure in dogs with LAR is associated with better long term outcome. Prediction of CHF based on isolated echocardiographic E:IVRT ratio was not accurate in dogs with LAR secondary to myxomatous mitral valve disease and should be better investigated in further studies including larger populations.

C51

Blood Pressure, Serum Fructosamine Concentration, and Insulin Dose Correlations in Diabetic Dogs

Frederico Aécio Soares[1], Mariana Feijó Chagas[2], Tayna Veronezi[3], Lilian Cardoso Heck[2], Barbara Correia[4], Leticia Machado[4], Alan Gomes Poppl[4]
[1]Faculdade de Veterinária / Departamento Medicina Animal / Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, [2]Autonomous, Porto Alegre, Rio Grande do Sul, Brazil, [3]Autonomous, Porto Alegr, Rio Grande do Sul, Brazil, [4]Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Diabetes mellitus (DM) is a common endocrinopathy in dogs and its impact on the cardiovascular system is still unclear in this species. In human medicine, serum fructosamine concentration (SFC) use as a cardiovascular prognostic is growing. The aim of this study was to evaluate correlations among systolic blood pressure (SBP), SFC, and insulin dose in use by diabetic dogs. A cross-sectional study was designed including diabetic dogs under treatment with NPH insulin (mean dose 0.62 U ± 0.24 U/kg, range 0.25 to 1.2 U/kg) for a period of 2 to 60 months (25.6 ± 17.8 months). The patients were assessed by Doppler vascular method to verify SBP in agreement with ACVIM guidelines. SFC were measurement by a colorimetric assay. Eighteen diabetic dogs (10.6 ± 2.9; 10.8 ± 8.1 kg) and twelve healthy control dogs (10.4 ± 3.1 years; 13.0 ± 9.4 kg) paired by breed, sex and age were included. There was a significantly positive correlation between SBP and SFC in diabetic dogs (r = 0.54; P < 0.05) as well as in control dogs (r = 0.57; P < 0.01). Insulin dose and SBP correlation only approach significance (r = 0.41; P = 0.08). Also, there was no correlation between time of diagnosis and SBP (r = 0.17; P = 0.47). Our findings suggests that there is association between SFC and hypertension in dogs. This results emphasize the importance of the SBP monitoring

in diabetic dogs, and suggests that insulin dose may be a more important factor than the time of diagnosis as a risk factor for hypertension in canine diabetic patients.

C52

Elevated Thyroid Stimulating Hormone Levels Following Administration of Amiodarone for Tachyarrhythmias in 10 Dogs

Kevin Render[1], Ioannis Giatis[2], Thaibinh Nguyenba[2], Roger Hostutler[2]
[1]MedVet Medical & Cancer Centers for Pets, Westerville, OH, USA, [2]MedVet Medical & Cancer Center for Pets, Worthington, OH, USA

Amiodarone is predominantly a class III anti-arrhythmic drug that also blocks sodium channels, and calcium channels, and beta2-adrenergic receptors. It is commonly used for the treatment of tachyarrhythmias, most notably ventricular tachyarrhythmias. As a lipophilic, iodinated benzofuran-derivative medication, amiodarone has unique pharmacological and structural properties that not only distinguish it from other anti-arrhythmics, but warrants careful surveillance of certain metabolic processes. Its composition can lead to the inhibition of 5′-deiodinase activity, resulting in a decrease in Triiodothyronine (T3) production. Amiodarone and its main metabolite, desethylamiodarone may also block T3 binding to its respective receptor. Amiodarone induced thyroid dysfunction has been documented in people. We aimed to determine if amiodarone alters thyroid function in dogs treated for significant tachyarrhythmia.

Records from our institution were reviewed to find dogs treated for tachyarrhythmias with amiodarone whose retrievable information was complete in regard to amiodarone dosing and thyroid function testing before and after starting amiodarone. Ten client-owned dogs were identified, and their records were evaluated for data regarding history, signalment, anti-arrhythmic medications, echocardiographic findings, other cardiac medications, electrocardiogram findings, Total T4 (T4), and thyroid stimulating hormone (TSH) levels before and after initiation of amiodarone, amiodarone doses, and length of time between initiation of amiodarone and post T4 and TSH testing.

The median age of evaluated patients was 8 years (range, 7-11). There were 6 males and 4 females with no sexually intact patients. Eight dogs initially received other anti-arrhythmic therapy (2/8 mexiletine monotherapy, 2/8 sotalol monotherapy, 3/8 mexiletine/sotalol dual therapy, 1/8 sotalol/diltiazem dual therapy). Two dogs did not receive anti-arrhythmic medication prior to evaluation. Amiodarone was instituted most commonly in dogs with systolic dysfunction +/- congestive heart failure, intolerance to particular anti-arrhythmic, or tachyarrhythmias refractory to other antiarrhythmics. Median maintenance amiodarone dose was 7.52 mg/kg/d (range, 6.32-10.17) preceded by a median amiodarone loading dose of 15.46 mg/kg/d (range, 14.14-20.34) for 7 days.

The median pre-amiodarone T4 value was 1.2 nmol/L (range, 0.7-1.9), while the median pre-amiodarone TSH value was 0.245 ng/mL (range, 0.06-0.55). Median post-amiodarone T4 was 1.95 nmol/L (range, 0.8-2.8), with median post-amiodarone TSH of 0.565 ng/mL (range, 0.08-1.99). Wilcoxon signed-rank analysis showed that TSH increased ($p = 0.005$) after amiodarone initiation. Median time to post-amiodarone thyroid testing was 37 days (range, 6-239) for T4 and 23.5 days (range, 6-239) for TSH after starting the drug, while median time between pre-amiodarone and post-amiodarone testing was 28.5 days (range, 6-246) for T4 and 24 days (range, 6-246) for TSH. No clinical signs of hypothyroidism were noted at time of recheck.

Amiodarone administration has been shown to alter thyroid function in humans. Our retrospective study aimed to find a relationship between amiodarone administration and thyroid parameters in dogs. In our study population, TSH increased after amiodarone administration, though no signs of hypothyroidism were observed. To our knowledge, thyroid parameter changes have not been previously documented in dogs treated with amiodarone. Additional prospective evaluation with a larger population is needed determine the significance of this finding.

C53

Residual Pulmonary Hypertension After Mitral Valve Plasty in Dogs With Mitral Valve Disease

Haruhiko Suzuki[1], Nobuyuki Kanno[2], Katsuhiro Matsuura[3], Dai Nagakubo[4], Masayuki Enokizono[5], Shusaku Yamada[6]
[1]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Nihon University, Sayama, Saitama, Japan, [2]Nihon University, Fujisawa, Kanagawa, Japan, [3]Shiraishi Animal Hospital/Animal Cardiovascular and Thoracic Surgery Center/Tokyo University of Agriculture and Technology, Sayama-shi, Saitama, Japan, [4]The University of Tokyo/Animal Cardiovascular and Thoracic Surgery Center, Bunko-ku, Tokyo, Japan, [5]Animal Cardiovascular and Thoracic Surgery Center / Japan Small Animal Medical Center, Tokorozawa, Saitama, Japan, [6]Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

The purpose of this study was to assess the effectiveness of mitral valve plasty (MVP) for treating pulmonary hypertension (PH) secondary to mitral valve disease in dogs. Twenty-three dogs (7-14 years of age) with preoperative PH, that underwent MVP under cardiopulmonary bypass between 2014 and 2017, were included. PH was diagnosed if the peak tricuspid valve regurgitation (TR) velocity was >3 m/s at echocardiography. Cardiac examination was performed before the operation and during follow-up (1 month, 3 months). Sildenafil was administered to dogs with residual PH if severe right-side heart overload (flattening of ventricular septum) and/or clinical signs (exercise intolerance, cyanosis) were observed. Median TR was 3.4 m/s (range 3.1–4.4 m/s) at preoperative examination. There were no deaths due to residual PH until 3 months after surgery. PH was improved in 9 (45%) patients at 3 months. TR disappeared in 4 patients and TR velocity was decreased in 5 (median 2.5 m/s; range 2.1–2.9 m/s). Eleven (55%) patients exhibited residual PH. Median TR was 3.3 m/s (range 3.0–3.7 m/s). Sildenafil was administered to 2 (18.2%) patients.

PH was a negative prognostic factor in dogs with mitral valve regurgitation. Our results suggest that MVP is an effective treatment for dogs with PH secondary to mitral valve disease. Further study is needed to clarify long-term prognosis and treatment of patients that exhibit residual PH after surgery.

E01

Equine Urinary N-Acetyl-β-D-Glucosaminidase Assay Validation and Correlation with Other Markers of Kidney Injury

Rosemary Bayless[1], Brittney Byer[1], A Russell Moore[1], Gabriele Landolt[2], Diana Hassel[1], Yvette Nout-Lomas[1]
[1]Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA, [2]Colorado State University College of Veterinary medicine and Biomedical Sciences, Fort Collins, CO, USA

Detection of equine kidney injury in initial stages, when intervention is likely to be most successful, is hindered by limited markers of early renal damage in horses. N-acetyl-β-D-glucosaminidase (NAG), a lysosomal enzyme in renal tubular cells that is released into urine during tubular insult, has shown promise for early identification of acute kidney injury in humans and other species. The aims of this study were to validate an assay for urinary NAG (uNAG) in horses and to correlate uNAG index with other markers of renal tubular dysfunction. An enzymatic assay was validated using coefficients of variation (CV), spike and recovery, and linearity of dilution. Urine and plasma were collected from 7 nonazotemic and 7 azotemic horses. Spearman rank correlation and Wilcoxon rank sum tests were used to compare uNAG index with plasma creatinine, urinary fractional excretion of sodium (FE_{Na}), and urinary gamma-glutamyltransferase (uGGT) index.

Intra- and inter-run CV, percent recovery after spiking, and linearity of dilution were satisfactory. There were significant correlations between uNAG index and plasma creatinine (p-value < 0.05) and between uNAG index and FE_{Na} (p-value < 0.01). Median uNAG indices were significantly higher (p-value < 0.05) in azotemic horses, in horses with increased FE_{Na}, and in horses with increased uGGT index. Urinary NAG can be measured in horses and shows correlation with current biomarkers of renal dysfunction. Additional work is needed to determine the timing of increase in uNAG index relative to onset of kidney injury in horses and to evaluate clinical utility in equine patients.

E02

Urinary Tract Infections: Retrospective Study in an Equine Hospital between 1995 to 2016

Thibaut Lemirre[1], *Daniel Jean*[2]
[1]*Clinical Sciences department, University of Montreal, St-Hyacinthe, PQ, Canada,* [2]*University of Montreal, St-hyacinthe, PQ, Canada*

URINARY TRACT INFECTIONS: RETROSPECTIVE STUDY IN AN EQUINE HOSPITAL BETWEEN 1995 AND 2016. T. Lemirre and D. Jean. Département de Sciences cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Canada.

Urinary tract infections (UTI) are uncommon in horses and data are limited in equine patients. *Escherichia coli* are reported to be the most frequently isolated bacteria but several other bacteria can be identified. The goal of our retrospective study is to describe clinical signs, identify pathogens and quantify microbial sensitivity profiles.

Clinical records from cases presented to an equine hospital between 1995 and 2016 were rewieved. Cases were included if a bacteriuria was identified with a threshold of unit forming colonies in cultures based on a previous study from MacLeay and Kohn (1998).

Twenty-eight cases were identified. Prevalence of UTI in our equine cases was 0.13% between 2009 and 2016. Trakehner and Holsteiner horses were significantly overrepresented compared to the hospital population (p < 0.05) and UTI were more frequent in females (p < 0.05). Urinary tract infections were associated with other diagnostics involving the urinary tract such as bladder emptying problems in 64% of the cases. Eighty-four bacteria were isolated from 54 cultures. *Escherichia coli* and *Enterococcus spp.* were the most common bacteria representing respectively 31% and 29% of isolated bacteria. Trimethoprim-sulfonamide, enrofloxacin and ceftiofur were the most commonly used antibiotics, especially at the beginning of hospitalization, and respectively 22%, 39% and 42% of isolated bacteria were sensible to these drugs. Seventy percent of isolated bacteria were multi-drug resistant. These results have to be placed in a context where two equine patients represented 31% of the isolated bacteria. Evaluation of the response to treatment was limited by the study design.

In conclusion, UTI is uncommon in horses and we identified a breed and sex predisposition. Both gram positive and negative bacteria were identified, and a high prevalence of multi-drug resistant bacteria can represent a therapeutic challenge in horses with urinary tract infections in an hospital setting.

E03

Characterization of Acute Kidney Injury in Hospitalized Horses

Vicky L. Savage[1], *Celia Marr*[2], *Miss Sarah S. Smith*[1]
[1]*Langford Equine Hospital/University of Bristol, Bristol, England, United Kingdom,* [2]*Rossdales Equine Hospital, Newmarket, England, United Kingdom*

The incidence of acute kidney injury in hospitalized humans and dogs ranges from 14.6-22.7% and mild decreases in kidney function have marked effects on mortality, length of hospitalization and cost of treatment. The incidence of acute kidney injury in hospitalized horses is unreported.

The study aim was to apply a validated canine scoring system to assess the incidence of acute kidney injury in hospitalized horses. We hypothesized that the incidence of acute kidney injury in horses is similar to that reported in other species.

Clinical records from hospitalized adult horses, August 2015-October 2017, were reviewed. Horses must have been hospitalized for ≥3 days and had serum creatinine concentration measured twice. Horses that were diagnosed with primary renal pathology or were azotaemic on baseline serum biochemistry were excluded. A veterinary acute kidney injury scoring system was applied based on percentage increase in serum creatinine concentration from baseline: stage 0 (< 150%), stage 1 (150-199% or ≥ 26.5 μmol/L), stage 2 (200-299%) or stage 3 (≥ 300%).

227 horses were included; 17.6% had an acute kidney injury (40/227). 16.7% were classified at stage 1 (38/227) and 0.9% as stage 2 (2/227). No horse had a stage 3 acute kidney injury.

The incidence of acute kidney injury in this population of hospitalized horses is similar to that reported in dogs and humans. Serum creatinine concentrations could be monitored in hospitalized horses to allow identification and early treatment of acute kidney injury. Further work is required to establish the impact of stage 1 acute kidney injury on long term equine health.

E04

Comparison of Two Collection Methods for Cerebrospinal Fluid Analysis from the Standing, Sedate Adult Horse

Hayley B. Chidlow[1], *Steeve Giguere*[2], *Erin McConachie Beasley*[2]
[1]*University of Georgia College of Veterinary Medicine, Athens, GA, USA,* [2]*Department of Large Animal Medicine, University of Georgia College of Veterinary Medicine, Athens, GA, USA*

Cerebrospinal fluid (CSF) analysis is an important component of the evaluation of neurologic horses. Lumbosacral (LS) centesis is routinely

practiced; however, CSF collection from the space between the first and second cervical vertebrae (C1-C2) has also been described. The purpose of this study was to compare collection times, CSF cytology results, and equine protozoal myelitis (EPM) titers between the two collection sites.

Healthy adult horses (n=14) and horses with a complaint of neurologic disease (n=7) were used. Cerebrospinal fluid was collected from both sites in randomized order. Continuous data were analyzed using mixed-effects linear models and count data using mixed-effects negative binomial regression. Statistical significance was set at $P < 0.05$.

Cerebrospinal fluid collected from C1-C2 had a significantly lower mean protein concentration (48 mg/dL C1-C2 versus 53 mg/dL LS; $P = 0.01$), red blood cell count (98 cells/uL C1-C2 vs 540 cells/uL LS; $P = 0.032$) and lower percentage of neutrophils (0.25% C1-C2 vs 4.7% LS; $P = 0.04$). Collection time, total nucleated cell count, percentage of mononuclear cells, EPM titers, and serum:CSF EPM titer ratios were not significantly different between collection sites. No adverse effects were detected for either technique.

Cerebrospinal fluid from the C1-C2 space provides an acceptable alternative to LS CSF collection with decreased likelihood of clinically important blood contamination of samples.

E05

Eastern Equine Encephalitis in Horses - 104 cases (1979 - 2017)

Demia J. de Tonnerre, Robert J. MacKay
University of Florida, Gainesville, FL, USA

The purpose of this study was to describe the epidemiological and clinicopathologic findings of hospitalized adult horses and foals with Eastern Equine Encephalitis.

Medical records of adult horses and foals admitted to a Veterinary Teaching Hospital from 1979 through 2017 were examined for cases presenting with signs of diffuse brain disease, and serological or postmortem diagnosis of Eastern Equine Encephalitis. There were 104 cases that met the inclusion criteria. Data retrieved included season at admission, signalment, physical examination, neurologic evaluation, and clinicopathologic findings at presentation; as well as duration of hospitalization, treatment, and case outcome. Where available, historical information (i.e. vaccination status, treatment prior to referral) was also included.

The median age at presentation was 1.4 years (range: 0.1-13.4 years), with Thoroughbred and Quarter Horse breeds most common. Cases were typically admitted during summer (63 %), and less frequently during spring (17 %), fall (11 %), and winter (9 %). Common findings upon physical examination were abnormal mentation (n = 100/104, 96 %), with dementia seen in 32/94 (34 %) cases. Fever (≥ 101.5°F) prior to presentation was reported in 67/68 (99 %) cases (mean: 104.4°F; range: 98.9-107.0°F), but only evident at admission in 42/92 (46 %) cases (mean: 101.3 F, range: 96.4-107.0 F). Approximately half (51/100, 51%) were recumbent; seizure-like activity was reported in 38/68 (56 %) cases. Cerebrospinal fluid analysis revealed gross discoloration in 51/84 cases (61 %), and a high protein concentration (> 85 mg/dL) in 65/82 (79 %) cases. Total nucleated cell count (> 6/uL) was typically high (80/90, 89 %), with a high neutrophil count (> 5%) in 71/83 (86 %) cases.

Median duration of hospitalization was 1 day (range: 0-13 days), attributed to rapid progression of neurologic disease resulting in recumbency. Mortality rate was high (90 %), with only 10 horses (10 %) surviving to discharge.

Eastern Equine Encephalitis in horses is a severe, rapidly progressive, and typically fatal neurologic condition.

E06

Response of Warmbloods with Type 2 Polysaccharide Storage Myopathy to Diet and Exercise

Zoë J. Williams[1], Andrew G. Huff[2], Megan Bertels[3], Stephanie J. Valberg[3]
[1]Michigan State University, College of Veterinary Medicine, Pinckney, MI, USA, [2]Michigan State University, College of Veterinary Medicine, Large Animal Clinical Sciences, East Lansing, MI, USA, [3]Michigan State University, College of Veterinary Medicine, McPhail Equine Performance Center, East Lansing, MI, USA

The same diet and exercise regime prescribed for type 1 polysaccharide storage myopathy (PSSM1) is recommended for type 2 PSSM (PSSM2); however treatment responses have not been documented for PSSM2. A retrospective case-control questionnaire (2008-2016) was used to capture clinical signs of PSSM2 in Warmblood horses (WB) (n = 42), before and after a recommended low nonstructural carbohydrate/fat supplemented diet and exercise regimen. Twenty-two owners of healthy control WB also provided information. Muscle glycogen concentrations were measured fluorometrically in PSSM2 WB (n = 36) to determine if they were abnormally elevated compared to horses with no myopathy (n = 23). Significantly more PSSM2 WB had performance decline and a reluctance to collect than control WB. With diet and exercise recommendations, 80% of PSSM2 WB owners reported improvement in clinical signs; however, 53% of horses were not advancing as expected in training with reluctance to go forward and collect persisting in approximately one third of horses. Glycogen concentrations in PSSM2 WB were variable and the median was not significantly different from WB with no histopathology. In conclusion, diet and exercise recommendations ideal for PSSM1 may improve but not eliminate clinical signs of PSSM2 in WB. Glycogen concentrations in many PSSM2 WB are not abnormally elevated.

E07

Cyclophosphamide Dose Escalation during Equine Chemotherapy

Daniela Luethy[1], Angela E. Frimberger[2], Barbara A. Byrne[3], Erin S. Groover[4], Rachel Gardner[5], Trisha Lewis[6], Valerie MacDonald[7], Lauren Proctor-Brown[8], Joy E. Tomlinson[9], Ken Rassnick[10], Amy L. Johnson[1]
[1]University of Pennsylvania School of Veterinary Medicine New Bolton Center, Kennett Square, PA, USA, [2]Veterinary Oncology Consultants Pty Ltd, Wauchope, New South Wales, Australia, [3]University of California School of Veterinary Medicine, Davis, CA, USA, [4]Auburn University College of Veterinary Medicine, Auburn, AL, USA, [5]B.W. Furlong & Associates, Oldwick, NJ, USA, [6]Lewis Veterinary Services, Lafox, IL, USA, [7]University of Saskatchewan Western College of Veterinary Medicine, Saskatoon, SK, Canada, [8]Cornell University College of Veterinary Medicine, N/A, NY, USA, [9]Baker Institute for Animal Health, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [10]VETERINARY MEDICAL CENTER OF CNY, East Syracuse, NY, USA

Cyclophosphamide, an alkylating agent, is commonly included in chemotherapeutic protocols for equine neoplasia, including lymphoma. Dosages regularly used in equine chemotherapeutic protocols are

extrapolated from other species, and cyclophosphamide dose optimization has not been reported in horses. Dose intensity refers to the dose of active drug over time; and multiple studies in humans and animals with cancer have shown that increasing chemotherapeutic dose intensity correlates with increased anti-tumor effect. Chemotherapeutic drug dose escalation is a strategy of gradual dose increases with careful monitoring for toxicity, utilized to determine the highest tolerated dose and thereby achieve higher dose intensity and maximum efficacy. The purpose of this report was to describe cyclophosphamide dose escalation during equine chemotherapy. Nine horses were included following medical record search and case recruitment via the ACVIM LAIM and Oncology Diplomate listserves. Seven horses had multicentric lymphoma and two horses had cutaneous lymphoma. T-cell rich large B-cell lymphoma was the most common immunohistochemical classification (n = 6). Initial dose of cyclophosphamide ranged from 150 mg/m^2 to 300 mg/m^2 IV and furosemide was administered concomitantly. Other drugs administered included doxorubicin, L-asparaginase, vincristine, lomustine, cytosine arabinoside, chlorambucil, valacyclovir, and corticosteroids. Cyclophosphamide dose was escalated in 3 horses to maximum doses of 310mg/m^2 IV, 450 mg/m^2 IV, and 800 mg/m^2 IV. Adverse effects attributed to cyclophosphamide administration were noted in one horse. This horse developed VCOG-CTCAE grade 1 lethargy and pelvic limb edema following a cyclophosphamide dose of 800 mg/m^2; these signs resolved without treatment. This horse experienced no adverse effects at previous and subsequent cyclophosphamide doses ≤ 750 mg/m^2 IV. Two pregnant mares received cyclophosphamide at doses of 150 mg/m^2 and 300 mg/m^2 without adverse reactions or apparent detrimental effects on fetal health. These findings suggest that cyclophosphamide dose escalation may be used in treatment of equine lymphoma to achieve higher chemotherapeutic dose intensity while minimizing adverse effects. The therapeutic dose of cyclophosphamide may be higher in horses than previously used and further studies are warranted to determine the optimal dose of cyclophosphamide for horses.

E08

Retrospective Evaluation of Clinical Outcome following Chemotherapy for Lymphoma in 11 Horses

Daniela Luethy[1], Angela E. Frimberger[2], Barbara A. Byrne[3], Erin S. Groover[4], Rachel Gardner[5], Trisha Lewis[6], Valerie MacDonald[7], Lauren Proctor-Brown[8], Joy E. Tomlinson[9], Ken Rassnick[10], Amy L. Johnson[1]
[1]University of Pennsylvania School of Veterinary Medicine New Bolton Center, Kennett Square, PA, USA, [2]Veterinary Oncology Consultants Pty Ltd, Wauchope, New South Wales, Australia, [3]University of California School of Veterinary Medicine, Davis, CA, USA, [4]Auburn University College of Veterinary Medicine, Auburn, AL, USA, [5]B.W. Furlong & Associates, Oldwick, NJ, USA, [6]Lewis Veterinary Services, Lafox, IL, USA, [7]University of Saskatchewan Western College of Veterinary Medicine, Saskatoon, SK, Canada, [8]Cornell University College of Veterinary Medicine, N/A, NY, USA, [9]Baker Institute for Animal Health, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [10]VETERINARY MEDICAL CENTER OF CNY, East Syracuse, NY, USA

Prognosis associated with equine lymphoma is often grave, and treatment is often non-curative. Few studies have evaluated long-term outcome following chemotherapy in horses treated for lymphoma. The purpose of this study was to report long-term outcome of horses with lymphoma treated with chemotherapeutic protocols. Eleven horses were included following medical record search and case recruitment via the ACVIM LAIM and Oncology Diplomate listserves. Eight horses had multicentric lymphoma and three horses had cutaneous lymphoma. T-cell rich large B-cell lymphoma was the most common immunohistochemical classification (n = 6). Three horses were EHV-5 positive on biopsy samples of neoplastic tissue. Chemotherapeutic agents included cyclophosphamide (n = 9), vincristine (n = 9), lomustine (n = 8), L-asparaginase (n = 7), doxorubicin (n = 6), cytosine arabinoside (n = 2), chlorambucil (n = 1), and intra-lesional cisplatin (n = 1). Adjunctive treatments included corticosteroids (n = 9) and valacyclovir (n = 3). Complete remission was achieved in 5 horses (45.5%), partial response was achieved in 3 horses (27.3%), stable disease was achieved in 1 horse, and 2 horses died during treatment. Overall response rate was 73% (8/11). All 3 horses with EHV-5 associated lymphoma achieved complete remission. Two pregnant mares were treated, with one mare surviving to foaling. Overall median survival time was 13 months (range, 1 - 41 months). Median survival time for multicentric lymphoma (n = 8) was 7.5 months (range, 1 - 28 months) and median survival time for cutaneous lymphoma (n = 3) was 21 months (range, 16 - 41 months). Seven of 11 horses exhibited a total of 11 adverse effects directly attributed to chemotherapy. Adverse effects were graded according to the VCOG-CTCAE grading system (grade 1 alopecia, n = 2; grade 1 combined neutropenia and lymphopenia, n = 2; grade 1 lymphopenia, n = 1; grade 1 lethargy, n = 1; grade 2 gastrointestinal signs, n = 1; grade 2 injection site reaction, n = 1; grade 2 hypersensitivity, n = 1; grade 4 hypersensitivity, n = 1; grade 5 hypersensitivity, n = 1). Higher grade adverse effects were most commonly associated with doxorubicin administration (n = 3), including one horse that died 18 hours post-administration. This report is limited by its retrospective nature, particularly the variation in treatment. However, these findings show that chemotherapy can be used successfully for treatment of equine lymphoma. Adverse effects, most commonly mild, occurred in approximately two-thirds of treated cases.

E09

Medical and Surgical Treatment of Primary Hyperparathyroidism in 17 Equids (1999-2016)

Emma B. Gorenberg[1], Amy L. Johnson[2], K. Gary Magdesian[3], François-René Bertin[4], Lais R.R. Costa[5], Mathijs J.P. Theelen[6], Sian Durward-Akhurst[7], Claudia Cruz Villagrán[8], Harry Carslake[9], Nicholas Frank[10], Joy E. Tomlinson[11]
[1]Cornell University School of Veterinary Medicine, Ithaca, NY, USA, [2]University of Pennsylvania School of Veterinary Medicine New Bolton Center, Kennett Square, PA, USA, [3]UC Davis Veterinary Medical Teaching Hospital, Dept. Medicine and Epidemiology, Davis, CA, USA, [4]The University of Queensland, Gatton, Queensland, Australia, [5]University of California Davis School of Veterinary Medicine, Davis, CA, USA, [6]Department of Equine Sciences, Universiteit Utrecht, Utrecht, Utrecht, Netherlands, [7]University of MInnesota School of Veterinary Medicine, Saint Paul, MN, USA, [8]University of Adelaide, Adelaide, South Australia, Australia, [9]University of Liverpool, Wirral, England, United Kingdom, [10]Tufts University, North Grafton, MA, USA, [11]Baker Institute for Animal Health, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Primary hyperparathyroidism is uncommon in large animals. The objective of this study was to describe the diagnostic findings and efficacy of treatment in equids with primary hyperparathyroidism.
Cases were recruited by retrospective review of records at Cornell University and via an ACVIM listserv query. Inclusion criteria were an equid with hypercalcemia and one or more of the following: 1) high

parathyroid hormone (PTH); 2) normal PTH with high calcium and negative PTH-related protein; or 3) histopathologic identification of a parathyroid adenoma.

Sixteen horses and one mule fulfilled the inclusion criteria. The most common presenting complaints included weight loss (12), hypercalcemia (10), anorexia (6), and colic (2). The median ionized calcium at presentation was 2.66 mmol/L (range, 2.14 – 4.95 mmol/L; reference range 1.58 – 1.9 mmol/L), and the median PTH concentration was 23.7 pmol/L (range: 3.8 – 128.63 pmol/L; reference range 0.6 – 11 pmol/L). Suspected abnormal parathyroid tissue was localized in 12/17 equids by ultrasonography (7/13) and/or technetium 99m sestamibi scintigraphy (10/11). Five out of five successfully excised tumors were located at the thoracic inlet, and surgery resulted in complete cure. One peri-thyroid tumor was excised; however, the horse remained hypercalcemic following surgery. Four additional cases treated surgically, five treated medically, and two not treated also remained hypercalcemic.

Parathyroid adenomas in equids can be successfully localized with ultrasonography and/or scintigraphy. Surgical excision appears more likely to be successful if a single abnormal gland is identified at the thoracic inlet.

E10

Pioglitazone in Equids Increases High-Molecular-Weight Adiponectin Concentrations and Decreases Insulin Response After Oral Sugar

Rebecca M. Legere[1], Debra Ruffin-Taylor[2], Kaitlyn Bello[1], Caroline Parker[1], Robert L. Judd[3], Anne A. Wooldridge[1]
[1]Department of Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn, AL, USA, [2]College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [3]Department of Anatomy, Physiology and Pharmacology, Auburn University College of Veterinary Medicine, Auburn, AL, USA

Decreased adiponectin concentrations are linked with the laminitis prone phenotype in equids with insulin dysregulation and equine metabolic syndrome (EMS). The high molecular weight (HMW) form of adiponectin is closely linked with insulin sensitivity, and thiazolidinedione drugs increase total and HMW adiponectin concentrations and improve insulin sensitivity in humans. The thiazolidinedione drug pioglitazone is orally absorbed in the horse and is safe and affordable for long-term management. The hypothesis was that pioglitazone would decrease insulin response to oral sugar and increase HMW adiponectin concentrations in horses and ponies.

Two cohorts of healthy equids, 7 horses (mean Henneke body condition score [BCS] 6.8 of 9) and 8 ponies (mean BCS 6.1 of 9), were treated with pioglitazone (2 mg/kg per os q24 h) for 28 days. Serum HMW adiponectin concentrations were measured by ELISA at 0, 14, and 28 days after treatment. Oral sugar tests (OST) were performed at days 0 and 28. A two-way repeated measures ANOVA with Sidak';s multiple comparisons test was used for data analysis. Cohorts compared were the ponies, horses, and insulin dysregulated (ID) equids (defined as horses or ponies having insulin concentrations > 65 μU/ml during the OST at ≥ 60 minutes).

Insulin concentrations were significantly lower after pioglitazone at the 90 and 120 minute time points of the OST in ponies [$P = 0.0035$] and ID equids [$P = 0.0028$], but not the horses [$P = 0.4970$]. HMW adiponectin was significantly higher after treatment in horses [$P < 0.01$, baseline 2.5 ± 1.0 μg/ml; endpoint 4.9 ± 2.9 μg/ml], ponies [$P < 0.05$, baseline 1.6 ± 2.6 μg/ml; endpoint 3.3 ± 4.4 μg/ml], and ID equids [P=0.0272, baseline 1.0 ± 8.0 μg/ml; endpoint 2.3 ± 1.8 μg/ml]. Lower insulin concentrations during the OST and increased HMW adiponectin concentrations indicate positive effects of pioglitazone for treatment of metabolic derangements in equids with EMS.

E11

Investigation of Novel Biomarkers for Early Detection of Equine Metabolic Syndrome

Valentina M. Ragno[1], Fabienne D. Uehlinger[1], Katherine A. Robinson[1], Gordon A. Zello[2], Julia B. Montgomery[1]
[1]Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, Saskatoon, SK, Canada, [2]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada

Methylglyoxal (MG), D-lactate and several inflammatory markers are increased in human diabetes and metabolic syndrome. The objective of this prospective cross-sectional study was to determine if concentrations of MG, D-lactate, L-lactate and the inflammatory markers, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and macrophage chemoattractant protein-1 (MCP-1), differ between horses with and without insulin dysregulation, as determined by a combined glucose-insulin tolerance test (CGIT).

Client-owned horses were recruited. Horses with abnormal physical examination or complete blood cell count, incomplete data, or with signs of pituitary pars intermedia dysfunction were excluded from further testing. The CGIT was performed after overnight grain fasting. Serum samples were stored at -80°C and the biomarkers were tested via previously validated ELISA or colorimetric assays. Age, body condition score (BCS), cresty neck score (CNS), baseline glucose, baseline insulin and triglycerides were also recorded. Characteristics of horses categorized as having insulin dysregulation and of those that did not were compared.

Of the 32 horses that met inclusion criteria, 12 horses (37.5%) were determined as having insulin dysregulation. Age, BCS, baseline glucose, triglycerides, MG, D-lactate, L-lactate, TNF-α, IL-6 and MCP-1 did not differ significantly between the two groups of horses. In univariate analysis, baseline insulin was significantly associated with insulin dysregulation ($P < 0.05$), but not in multivariate logistic regression. Of note, horses with CNS ≥ 3 had 11 times higher odds of having insulin dysregulation (OR 11.3, 95% C.I. 2.04-63.08, $P < 0.05$). In this study, inflammatory markers, MG and D-lactate were not good predictors of insulin dysregulation.

E12

Enteroinsular Axis Response to Fasting and Dextrose in Healthy Neonatal Foals

Lindsey Rings, Laura K. Dunbar, Teresa A. Burns, Stephanie Vijan, Ramiro Toribio
The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

The enteroinsular axis (EIA) comprises intestinal factors (incretins) that promote insulin release and suppress glucagon secretion. Glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1

(GLP-1) are the main incretins. EIA alterations could contribute to energy dysregulation in critically ill foals.

The EIA has been evaluated in horses, however, information is lacking in healthy and sick newborn foals. Our study sought to evaluate GLP-1 (total and active), GIP, and insulin response to fasting and dextrose (oral and intravenous) administration in healthy newborn foals.

Oral and intravenous glucose tolerance tests were performed in 17 healthy Standardbred foals < 72 hours old. Following 1 hour of fasting, a bolus of dextrose (300 or 500 mg/kg) was administered orally or intravenously. Blood incretin and insulin concentrations were measured at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 minutes by immunoassay.

GIP concentrations decreased steadily following dextrose (300 and 500 mg/kg) regardless of route of administration by 68-75% of baseline values ($P < 0.05$). GLP-1 total and active concentrations showed a steady decrease of 52-70% of baseline values ($P < 0.05$). Mild but not significant GLP-1 elevations were noted at 5-15 minutes.

In foals allowed to nurse, incretin concentrations increased above baseline within 15 minutes of nursing. Minimal incretin response with oral dextrose but rapid incretin release after nursing indicates that 500 mg/kg was insufficient for a strong EIA stimulation, that higher dextrose dosing is required, or that factors in milk may be important to activate the EIA in newborn foals.

E13

Evaluation of an Oral Sugar Test to Diagnose Insulin Dysregulation in Horses

Sanna Lindåse, Katarina EA. Nostell, Johan Bröjer
Swedish University of Agricultural Sciences, Uppsala, Uppsala Lan, Sweden

An oral sugar test (OST) has been developed as a clinical diagnostic technique to identify horses with insulin dysregulation (ID). Diagnosis is currently based on arbitrary cut-off values. The aim of the study was to establish appropriate cut-off insulin values for ID at different time points during an OST.

Forty-nine horses of different breeds with a wide range of insulin sensitivity were subjected to a modified OST (0.2 mL/kg Dansukker glykossirap) and a euglycemic hyperinsulinemic clamp (EHC) 24 hours apart. Using a glucose disposal rate (M-value) of < 2.5 mg/kg/min as a definition for insulin resistance by the EHC, the insulin cut-off values for the different time points during the OST were calculated by receiver operating characteristic (ROC) curve analysis. Spearman correlations were used to study correlations between the M-value and OST insulin concentrations at 60, 90 and 120 minutes.

Oral sugar test derived 60, 90 and 120 minutes insulin concentrations of > 44.4, 50.9, 38.8 μIU/mL respectively, were indicative of ID. Using EHC as the gold standard, optimal sensitivity and specificity for diagnosing ID were 88 and 96%, respectively, for time point 60 minutes; 84 and 92%, respectively, for time point 90 minutes and 84 and 88%, respectively, for time point 120 minutes. Oral sugar test derived insulin concentrations (60, 90 and 120 minutes) were inversely related ($rho \geq -0.81$, $p < 0.001$) to the M-value.

Results indicate that time-specific OST insulin cut-off concentrations should be used, when diagnosis of ID is based on single blood sampling.

E14

Prevention of Laminitis in Ponies Using Velagliflozin, a Novel Treatment for Insulin Dysregulation

Alexandra Meier[1], Dania Reiche[2], Melody de Laat[3], Christopher Pollitt[4], Donald Walsh[5], Martin Sillence[3]
[1]Queensland University of Technology, Corlette, New South Wales, Australia, [2]Boehringer Ingelheim Vetmedica, Ingelheim am Rhein, Rheinland-Pfalz, Germany, [3]Queensland University of Technology, Brisbane, Queensland, Australia, [4]The University of Queensland, Gatton, Queensland, Australia, [5]Animal Health Foundation/Homestead Veterinary Hospital, Pacific, MO, USA

The aim of this study was to determine if hyperinsulinaemia could be reduced and laminitis prevented in insulin-dysregulated ponies, by using the sodium-glucose co-transport 2 (SGLT-2) inhibitor velagliflozin. Forty-nine ponies with varying degrees of insulin dysregulation, based on an oral glucose test (1 g dextrose/kg BW), received either velagliflozin (0.3 mg/kg, *p.o.*, *s.i.d.*, *n = 12*), or a placebo (n = 37), throughout the study. A maintenance diet of lucerne hay was fed for 3 weeks, followed by a high-NSC challenge diet (12 g NSC/kg BW/day) for up to 18 days. On the second day of the diet challenge blood glucose and serum insulin were measured over 4 h after feeding. Results are expressed as geometric mean (95% CI). The maximum concentration of glucose was lower ($P = 0.022$) for the velagliflozin group at 9.4 mM (8.0 -11.0) versus 11.9 mM (10.5 -13.4) in controls; and likewise, the maximum concentration of insulin was lower for treated ponies at 149 mIU/mL (97 - 228) versus 269 mIU/mL (206 - 351) in controls ($P = 0.017$). Fourteen of the 37 controls (38%) developed Obel grade 1 or 2 laminitis, whereas none of the velagliflozin-treated ponies did ($P = 0.011$). As there were no clinical signs of adverse effects, velagliflozin could be a valuable tool for the prevention of insulin-associated laminitis and the treatment of insulin dysregulation in susceptible animals.

E15

The Impact of Different Glucose Dosages in Oral Glucose Test for Assessment of Insulin Dysregulation

Tobias Warnken[1], Svenja Schumacher[1], Pia Schmidt[1], Korinna Huber[2], Karsten Feige[1]
[1]Clinic for Horses, University of Veterinary Medicine Hannover, Foundation, Hannover, Niedersachsen, Germany, [2]Institute of Animal Science, Faculty of Agricultural Sciences, University of Hohenheim, Stuttgart, Baden-Wurttemberg, Germany

Oral glucose tests (OGT) are currently recommended for diagnosis of insulin dysregulation (ID). As horses suffering from ID are prone to laminitis especially when exposed to high amounts of sugar it would be desirable to reduce the amount of diagnostic glucose. Furthermore, a reduced glucose amount enables various routes of application.

Aim of this study was to determine whether a dosage of 0.25g or 0.5g glucose per kg bodyweight (BW) instead of 1g/kg BW would be useful for clinical settings and sufficient to distinguish between insulin sensitive and insulin dysregulated horses.

Eighteen Icelandic horses of different sex, age, bodyweight and uncertain metabolic status were tested each by application of 0.25g/kg BW (LOGT), 0.5g/kg BW (MOGT) and 1g/kg BW (OGT) glucose dissolved in 2L water and administered via naso-gastric-tube. Blood samples were collected for five hours and were analyzed for insulin and glucose.

Blood glucose concentration was significantly lower in LOGT compared to OGT ($P < 0.0004$) from 60 minutes and significantly lower in MOGT compared to OGT from 90 minutes ($P < 0.0001$) after application. Insulin concentration after 30 minutes was significantly lower in LOGT compared to MOGT ($P < 0.05$) and OGT ($P < 0.01$). No statistically different insulin concentrations were detected between MOGT and OGT until 135 minutes ($P < 0.001$). Moreover, insulin dynamics in MOGT and OGT allowed satisfying differentiation in insulin sensitive and insulin dysregulated horses.

Summing up, a reduction of glucose to 0.5g/kg BW in OGT can be recommended without loss of diagnostic value.

E16

Comparison of Insulin and Glucose Response in Horses Using Two Different Formulations of Corn Syrup

Kira Moser[1], Heidi Banse[2]
[1]University of Calgary, Calgary, AB, Canada, [2]Louisiana State University, Baton Rouge, LA, USA

Diagnosis of insulin dysregulation in adult horses is frequently performed using Karo Light corn syrup. However, Karo Light syrup is not readily available worldwide. Composition of available corn syrups may vary, leading to different insulin and glucose results. In this study, we evaluated agreement between two formulations of corn syrup, Karo Light and Crown Lily White. This study consisted of two experiments. In experiment 1, 14 horses were allowed access to hay or pasture prior to the OST. In experiment 2, 10 horses were fasted overnight prior to the OST. For both studies, corn syrup formulation order was randomized and oral sugar tests were performed one week apart. Blood was drawn for measurement of blood glucose and insulin concentrations at T0 (prior to administration of corn syrup) and 30, 60, 75, 90, and 120 minutes after administration of corn syrup. Blood glucose was measured using a handheld glucometer and serum insulin was measured using a radioimmunoassay. Data was analyzed for normality. Insulin concentrations were log transformed for normality. Changes in glucose and insulin concentration at each time point, area under the curve (AUC), maximum concentration (C_{max}), and time at C_{max} (T_{max}) were compared between formulations using a two way analysis of variance with repeated measures. Bland Altman analysis was used to determine agreement in insulin concentrations between formulations. There were no significant differences between Karo and Crown syrup formulations at any individual time points for insulin or glucose concentrations in either experiment ($P > 0.2$) There were no significant differences between the area under the curve, T_{max}, or C_{max} for insulin or glucose concentrations with Karo compared to Crown syrup ($P > 0.1$). Bland-Altman analysis of insulin concentrations indicated a mean bias (Karo-Crown) of 4 μIU/mL (95% limits of agreement, −11.7 to 19.6 μIU/mL; experiment 1) or a mean bias of 1.6 μIU/ml (95% limits of agreement, −11 to 14.2 μIU/ml; experiment 2) for insulin concentrations at 75 minutes. This study suggests that horses have similar glucose and insulin responses to these two formulations of corn syrup.

E17

Combination of TRH Stimulation Test and 2-Step Insulin Sensitivity Test to Diagnose PPID and EMS

Remona Horn, François-René Bertin
The University of Queensland, Gatton, Queensland, Australia

Equine metabolic syndrome (EMS) and pituitary *pars intermedia* dysfunction (PPID) are the most common hormonal disorders in horses and can coexist in the same patient. The aim of this study is to combine two diagnostic tools to diagnose PPID and EMS at once within 30 minutes. It was hypothesised that measured values from the 2-step insulin response test and the thyrotropin-releasing hormone (TRH) stimulation test performed in combination would not differ from the values obtained when tests are performed independently.

Twenty-one horses were tested for EMS and PPID using a 2-step insulin response test and a TRH stimulation test respectively and classified as EMS, PPID, EMS and PPID or controls. For combined testing, insulin and TRH were injected simultaneously. Results were compared among protocols by paired *t* tests or Wilcoxon signed rank test and Bland-Altman analysis.

Based on independent testing, 8 horses were considered as controls, 4 as EMS only, 3 as PPID only and 6 as EMS and PPID. Independent or combined testing conditions did not significantly affect ACTH concentrations before or after TRH injection nor it changed the percentage of reduction in blood glucose after insulin injection when compared within groups or overall ($p > 0.05$). In one control horse, combined testing resulted in a larger increase in ACTH after TRH injection consistent with a diagnosis of PPID.

Combination of the TRH stimulation test and the 2-step insulin sensitivity test appears as an attractive and rapid tool to diagnose EMS and PPID at the same time in horses.

E18

Effect of Using Corn Syrup with Fructose on Equine Oral Sugar Test Results

Emma D. Stapley[1], Molly McCue[2], Jane M. Manfredi[1]
[1]Michigan State University, East Lansing, MI, USA, [2]University of Minnesota, St. Paul, MN, USA

Early identification of horses affected with insulin dysregulation (ID) allows veterinarians to recommend preventative measures to reduce the risk of laminitis. An oral sugar test (OST) using Karo® Light corn syrup (KLCS) has been validated as a screening test for ID (positive if insulin is ≥45 μIU/mL at 60 or 90 minutes). Veterinarians and owners often use whatever type of corn syrup is convenient, despite brand differences in sugar content. In dogs and humans, fructose increases hepatic glucose metabolism, lowering insulin and glucose responses to OSTs. A similar effect in horses would increase the number of false negatives from OST screening. OSTs using KLCS (with glucose and maltose) and OSTs using Fox';s® corn syrup (FCS, with high fructose corn syrup) were performed twice each on seven Arabian horses previously diagnosed with ID (via OST and a frequently

sampled intravenous glucose tolerance test). Differences in area under the curve (AUC) and peak concentrations for insulin and glucose were assessed using a one-way ANOVA (significant at $P < 0.05$). Repeatability was assessed using Bland-Altman Plots. Significant differences were not noted between KLCS and FCS for either AUC or peak concentrations. However, when insulin results were compared to the ID positive insulin cut off, tests with fructose correctly identified horses with ID only 8 of 14 times; OSTs without fructose identified ID in 12 of the 14 tests. Based on this, fructose does not have a substantial impact on glucose metabolism in horses, but may interfere with insulin responses during an OST.

E19

Serum and Cerebrospinal Fluid Alpha-Tocopherol Concentration in Adult Horses Supplemented with Subcutaneous Alpha-Tocopherol

Callum G. Donnelly[1], *Ms. Erin Burns*[1], *Carrie Finno*[2]
[1]*University of California, Davis, Davis, CA, USA*, [2]*Univeristy of California, Davis/Department of Population Health and Reproduction, Davis, CA, USA*

Vitamin E is essential for neuromuscular function. Oral supplementation with natural ("RRR") α-tocopherol has been the mainstay of therapy in horses with hypovitaminosis E. However, there is a subset of non-responsive horses. The objectives of this pilot trial were to evaluate the safety and efficacy of an injectable RRR-α-tocopherol preparation delivered subcutaneously. We hypothesized that RRR-α-tocopherol injection would increase serum and cerebrospinal fluid (CSF) α-tocopherol concentrations in healthy adult horses. Six mixed breed horses (3 mares and 3 geldings) and two untreated horses (1 mare and 1 gelding) were enrolled. In Phase I, horses were randomly assigned to receive RRR-α-tocopherol (5000 IU/450kg of 600 IU/mL) by subcutaneous (n=3) or oral (n=3) administration. Moderate tissue reaction following injection necessitated adjustment of the preparation through reduction of the RRR-α-tocopherol concentration to 500 IU/mL. Following an 8-week washout period, horses received the reciprocal treatment in Phase II with the new preparation at an equivalent dose. Alpha-tocopherol concentrations of serum and CSF collected over a 7d period were determined by HPLC. There was no difference in baseline serum (P=0.07) or CSF (P=0.20) concentrations and no residual effect noted, indicating appropriate washout. Serum ($P < 0.0001$) and CSF (P=0.0.007) α-tocopherol concentrations increased significantly post-injection only when the 500 IU/mL product was administered, with serum concentrations peaking at 24h post-injection. This injectable formulation may therefore be useful in cases refractory to oral supplementation. However, caution is warranted due to the marked local tissue reaction observed in all horses.

E20

Serum Phosphorus, Magnesium, and Potassium Concentrations in Small Equids with Dyslipidemias

Laura K. Dunbar, *Teresa A. Burns*, *Ramiro Toribio*
The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

Hypophosphatemia, hypokalemia, and hypomagnesemia are associated with various diseases in equids. Despite the interactions between these analytes and energy metabolism, evaluation of their concentrations in critically ill equids with lipid disorders is not frequently performed; therefore, the incidence of these abnormalities in these patients remains unclear. Medical records from 54 hospitalized small equids [ponies (multiple breeds), American Miniature Horses, and donkeys] greater than 2 years of age with serum triglyceride concentrations greater than 200 mg/dL admitted between 2002-2017 were reviewed, and relationships between biochemical analytes were assessed (Chi-square analysis).

Most patients (59%) survived to discharge, while 41% were euthanized or died. Patients were stratified into quartiles based on triglyceride concentrations (1^{st} quartile 207-343 mg/dL, 2^{nd} quartile 386-638 mg/dL, 3^{rd} quartile 668-989 mg/dL, and 4^{th} quartile 1014-3281 mg/dL). Equids with triglycerides in the 1^{st} and 2^{nd} quartiles were more likely to survive than those with triglyceride concentrations in the 3^{rd} and 4^{th} quartiles (OR = 1.66; $p < 0.05$). Hypophosphatemia ($P < 2.5$ mg/dL), hypokalemia ($K < 3.0$ mEq/L), and hypomagnesemia (total $Mg < 1.29$ mg/dL) were observed in 25%, 16.1%, and 10.7% of all cases, respectively. Only potassium concentration was significantly correlated with triglyceride concentrations ($\rho = -0.318$; $p < 0.05$). Equids with triglyceride concentrations in the 3^{rd} and 4^{th} quartiles were more likely to be hypokalemic (OR = 3.92; $p < 0.05$). Hypophosphatemia was not associated with survival, and most (72%) equids with hypertriglyceridemia and hypophosphatemia survived. Evaluation of phosphorus, potassium, and magnesium is recommended in equids with dyslipidemias.

E21

Lipidome of Thoroughbred Horses Before and After Supra-Maximal Exercise Using an Untargeted Lipidomics Approach

Lucas J. Nolazco Sassot, *Nairanjana Dasgupta*, *Jillian Morrison*, *Warwick Bayly*, *Macarena G. Sanz*
Washington State University, Pullman, WA, USA

Lipidomics enables global screening of the effect of a stimulus like exercise on lipid metabolism. Changes in the lipidome of Arabian horses during endurance racing and its influence on performance have been studied, but information is lacking for Thoroughbred racehorses. Our objective was to assess the effect of supra-maximal exercise on the lipidome of racehorses.

Four Thoroughbred geldings of similar body condition and age were used. Horses were housed in the same barn and fed the same diet. Each horse underwent treadmill exercise to fatigue at 115% of its $\dot{V}O_2$max (10.6±0.6m/s; 10% slope). Venous plasma samples were obtained before and immediately, 15 and 30mins post-exercise, and evaluated using an untargeted lipidomics approach at West Coast Metabolomics Center. Data was analyzed using principal components analysis and 1-way RM ANOVA (significance was set at $p < 0.05$).

965 plasma lipids were detected. Of these, 184 were "known" as their spectra and retention index are linked to library entries. After exercise, plasma levels of eight lipids changed significantly, likely reflecting the occurrence of lipolysis. Briefly, an increase of unsaturated fatty acids (11,14-eicosadienoic, 9-11-octadecadienoic and α-linoleic acids) and a decrease of saturated fatty acids (Icosanoic, heptadecanoic, lauric and myristic acids) and phospholipids (Sphingomyelin) were observed.

This pilot study provides valuable information regarding changes in the lipidome of Thoroughbreds associated with supramaximal exercise and will serve as a reference to guide future studies of the racehorse metabolome.

E22

Quantification of Left-Atrial Stunning in Horses After Cardioversion of Atrial Fibrillation Using Two-Dimensional Speckle Tracking

Christina Eberhardt, Katharyn J. Mitchell, Colin C. Schwarzwald
Zurich University, Zurich, Zurich, Switzerland

In horses, persistent depression of left atrial (LA) mechanical function is a prognostic indicator of recurrence of atrial fibrillation (AF) after cardioversion. The objective of this prospective study was to characterize LA mechanical function in Warmblood horses after successful transvenous electrical cardioversion (TVEC) of AF using standard two-dimensional echocardiography (2DE) and 2D speckle tracking (2DST) analyses. 2DST was recently described to be a feasible method to quantify LA function in healthy horses. We aimed to provide proof of concept for use of 2DST to detect compromised LA booster pump function in horses.

Warmblood horses with atrial fibrillation, successful TVEC and continued normal sinus rhythm for at least one month thereafter were included in this study. Echocardiography was performed 24h, 72h and 1mt after TVEC. Maximum LA area (LAA_{max}) and LA diameter (LAD_{max}) as well as active and passive LA fractional area change (FAC) were measured using 2DE on a right-parasternal 4-chamber view focusing on the left atrium. LA dimensions were measured at end-systole and allometrically scaled to a body weight of 500 kg. Global longitudinal peak strain (S_L) and strain rate (SR_L) during active booster pump function were measured on the same recordings using 2DST analyses. Measurements were judged in relation to previously established reference intervals. Friedman one-way ANOVA with Dunn';s multiple comparison test was performed to detect differences between timepoints. Linear regression analysis was performed to describe associations of S_L and SR_L with conventional 2DE measurements of LA size and function, considering for repeated measurements within horses. $P < 0.05$ was considered significant.

Thirteen Warmblood horses fulfilled the inclusion criteria. Measurement of all indices at all 3 timepoints was feasible in 6/13 horses, whereas severe LA enlargement prevented measurement in 7 horses. LA contractile dysfunction 24h after TVEC was detected in 5/6 horses based on active LA FAC and global S_L. Active LA FAC did not change over time ($p = 0.14$) and remained below reference limits in 5/6 horses. Global S_L significantly increased from 24h to 1mt after TVEC ($p = 0.028$); at that time, S_L of all 6 horses was within (but at the lower range) of the reference interval. SR_L was below the reference interval in 2/6 horses 24h after TVEC and within reference limits in all 6 horses 1mt after TVEC ($p = 0.30$). Strain and strain rate were significantly associated with active FAC ($p2_{adj}=0.76$ and $p2_{adj}=0.77$), passive FAC ($p=0.016$, $R^2_{adj}=0.55$ and $p=0.016$, $R^2_{adj}=0.55$), LAA_{max} ($p2_{adj}=0.85$ and $p2_{adj}=0.88$), and LAD_{max} ($p2_{adj}=0.83$ and $p2_{adj}=0.85$), with lower S_L and SR_L in larger atria.

This study shows that 2DST is useful to detect compromised LA contractile function in horses after conversion of AF to NSR using TVEC. Strain and strain rate are negatively related to LA enlargement. The clinical value of 2DST to predict the risk of recurrence of atrial fibrillation will have to be established in future studies.

E23

Acute, Subacute and Chronic Assessment of Horses Exposed to Lethal and Sublethal Doses of Monensin

Clémentine Gy[1], Marie-Claude Belanger[2], Marion Allano[2], Guy Beauchamp[2], Mathilde Leclere[2], Jean-Pierre Lavoie[2]
[1]Université de Montréal, St-hyacinthe, PQ, Canada, [2]Université de Montréal, St-Hyacinthe, PQ, Canada

Monensin is highly toxic to horses and inadvertent ingestion can result in cardiac dysfunction and death. The objectives of this prospective study were to determine the cardiovascular and athletic outcome of horses exposed to monensin.

Physical examination, exercise stress testing, ECG (pre- and during exercise) and echocardiography (pre- and post-exercise) were performed in 76 horses exposed to monensin-contaminated feed. Four horses were examined within 2 weeks of exposure (acute period). Twenty-nine horses were examined between 15 and 45 days post-exposure (subacute period) and 70 horses were examined after 4 to 10 months of rest (chronic period). Follow-up information was obtained by telephone interviews approximately 16 months after exposure for 56 horses.

Three of the 4 horses (75 %) presented during the acute period died or were euthanized; all horses had clinical signs and cardiac anomalies at rest. Nineteen of the 29 horses (66 %) examined during the subacute period had cardiac anomalies (upon physical examination (11), cardiac examination at rest (16) and with exercise (5)). Four to 10 months after exposure, 31 of the 70 horses (44 %) had cardiac abnormalities (upon physical examination (6), cardiac examination at rest (22) and with exercise (13)). Sixteen months after exposure, 34 (53 %) of the 64 horses with known outcome had returned to their intended use, including 10 horses with cardiac anomalies in the previous months.

Clinical outcome of horses exposed to sublethal doses of monensin is highly variable and commonly result in long-term cardiac dysfunction leading to exercise intolerance and even death.

E24

Comparison of Non-invasive, Invasive Central and Invasive Peripheral Blood Pressure in the Standing Horse

Natalia Rodriguez[1], Joanne Hardy[2], Mauricio Lepiz[2], Noah Cohen[2], Cristobal Navas De Solis[2]
[1]Department of Large Animal Clinical Studies, Texas A&M University, College Station, TX, USA., Lexington, KY, USA, [2]Department of Large Animal Clinical Studies, Texas A&M University, College Station, TX, USA., College Station, TX, USA

Accuracy and precision of devices that measure non-invasive blood pressure (NIBP) in horses are variable and there are no devices validated against central invasive blood pressure in the standing horse. The objective of this study was to compare invasive pressures obtained from the carotid artery (IBPc), facial artery (IBP) and NIBP.

An arterial catheter was placed in the transverse facial artery routinely and in the carotid artery under sonographic guidance. NIBP

(coccygeal) was measured using a commercial oscillometric device (Mindray Passport 12) and corrected for the vertical distance between the base of the tail and the heart base. Measurements were obtained under baseline, high (dobutamine), and low (acepromazine) blood pressures. Mean bias, SD of the bias, and Pearson';s product correlation (R) were calculated from data obtained from 11 horses.

Mean bias/SD of the bias/R between IBPc and IBP were -3 mmHg/12 mmHg / 0.92 for systolic, 2.8 mmHg/ 6.1 mmHg /0.96 for mean, and 5.5 mmHg/ 7.3 mmHg/ 0.91 for diastolic pressures. Mean bias/SD of the bias/R between IBPc, and NIBP were -1.3 mmHg/ 12.1 mmHg/ 0.91 for systolic, 6.4 mmHg/ 11.4 mmHg/ 0.86 for mean, and 10.1 mmHg/ 15.7 mmHg/ 0.72 for diastolic pressures. Mean bias/SD of the bias/R between IBP and NIBP were 0.1 mmHg/ 12.8 mmHg/ 0.91 for systolic, 3.2 mmHg/ 11.1 mmHg/ 0.88 for mean, and 4.7 mmHg/ 15.7 mmHg/ 0.72 for diastolic pressures.

Measuring IBPc without a surgical approach was feasible. IBP is an acceptable surrogate for IBPc but these are not interchangeable under all circumstances. The NIBP device tested had acceptable accuracy and precision for systolic pressure following ACVIM suggested criteria and approximated the criteria for mean blood pressure. Diastolic pressure measurements were less accurate and precise.

E25

A Comparison of the Stability of Equine Blood D-Lactate in Sodium Fluoride and Serum Tubes

Valentina M. Ragno[1], Nicole S. Sereda[2], Ekaterina E. Charles-Dudko[2], Deborah L. Michel[3], Gordon A. Zello[3], Fabienne D. Uehlinger[1], Julia B. Montgomery[1]
[1]Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, Saskatoon, SK, Canada, [2]Western College of Veterinary Medicine, Saskatoon, SK, Canada, [3]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada

Continuation of glycolysis in erythrocytes after blood collection may cause an artifactual in vitro increase of methylglyoxal, and consequently of its metabolite D-lactate. Sodium fluoride (NaF) inhibits enolase in the glycolysis pathway. Our hypotheses were that equine blood collected in NaF tubes would result in both valid and lower D-lactate values when compared to serum tubes, and that D-lactate concentrations would be stable up to 6 months at -80°C.

Blood was collected in both serum and NaF tubes from 23 horses and kept on ice until centrifugation for separation of serum or plasma. Samples were frozen at -80°C until analysis (after 1, 2 and 6 months) with a previously validated commercial assay (D-Lactate Colorimetric Assay Kit, BioVision, Inc., Milpitas, CA, USA).

D-lactate values measured from blood collected in serum tubes had a mean (± SD) concentration of 0.26mmol/L (0.09), 0.27mmol/L (0.09) and 0.17mmol/L (0.07) after 1, 2 and 6 months, respectively. From the NaF tubes, the mean D-lactate concentrations were 0.05mmol/L (0.01), 0.06mmol/L (0.01) and 0.03mmol/L (0.01) after 1, 2 and 6 months, respectively. D-lactate was significantly different ($P < 0.0001$) between serum and NaF samples, and when comparing 6 months to 1 and 2 months within the NaF tube type ($P < 0.0001$).

Use of NaF tubes is recommended for equine blood collection for determination of D-lactate concentration, as use of serum tubes results in inflated and inaccurate values. Furthermore, longer-term storage (>2 months at -80°C) appears to result in sample degradation and should be interpreted with caution.

E26

Activated Platelets and Platelet-Leukocyte-Aggregates in Equine Systemic Inflammatory Response Syndrome

Kim Theuerkauf, Carmen Schroeck, Carsten Staszyk, Andreas Moritz, Katja A. Roscher
Justus-Liebig-University, Giessen, Hessen, Germany

Activated platelets contribute in humans to sepsis complications and to multiple organ failure. The aim of the prospective study was to determine, if platelets are activated in clinical cases of equine systemic inflammatory response syndrome (SIRS).

Adult horses and ponies fulfilling at least two criteria of an adapted SIRS-score were included. A standard human protocol measuring activated platelets and platelet-leukocyte-aggregates (PLA) with fluorescence flow cytometry in platelet-leukocyte-rich-plasma (PLRP) was established in horses. Activation of platelets was determined by increased presentation of CD62P and CD154 on platelets. Activation and PLA were measured before and after in vitro activation of platelets with collagen. Ten healthy adult horses and ponies served as controls. Statistical analysis included proof of normal distribution followed by two-way ANOVA and post-hoc Bonferroni tests.

The 19 included horses and ponies with SIRS had significantly more activated platelets and PLA in native PLRP than controls: CD62P 11.73 ± 3.74 % in SIRS and 1.74 ± 0.36% in controls ($P = 0.0004$); CD154 2.10 ± 0.91 % and 0.40 ± 0.08% respectively ($P = 0.119$); PLA 6.23 ± 1.18 % and 2.46 ± 0.32% respectively ($P = 0.031$). Six horses survived. There was a trend for more activation and PLA in non-surviving horses. Furthermore a trend for reduced in vitro activation with collagen was detected in the non-survivors.

This is the first study demonstrating increased platelet activation and platelet-leukocyte-aggregates with fluorescence flow cytometry in clinical cases of equine SIRS. Likewise platelet activation could be a prognostic factor in these patients. Antiplatelet therapy (e.g. clopidogrel) could be an additional therapeutic option in clinical cases of SIRS and other inflammatory diseases to prevent complications and improve outcome.

E27

First Detection and Frequent Occurrence of Equine Hepacivirus in Horses on the African Continent

Marcha Badenhorst[1], Birthe Tegtmeyer[2], Daniel Todt[3], Alan Guthrie[4], Karsten Feige[5], Eike Steinmann[6], Jessika M.V. Cavalleri[7]
[1]Faculty of Veterinary Science, University of Pretoria / University of Veterinary Medicine, Vienna, Vienna, Wien, Austria, [2]Institute for Experimental Virology, Twincore Centre for Experimental and Clinical Infection Research, Medical School Hannover (MHH)-Helmholtz Centre for Infection Research (HZI), Hannover, Germany., Hannover, Niedersachsen, Germany, [3]Institute for Experimental Virology, Twincore Centre for Experimental and Clinical Infection Research, Medical School Hannover (MHH)-Helmholtz Centre for Infection Research (HZI), Hannover, Germany, Hannover, Niedersachsen, Germany, [4]Faculty of Veterinary Science, University of Pretoria, Pretoria, Gauteng, South Africa, [5]Clinic for Horses, University of Veterinary Medicine Hannover, Foundation, Hannover, Niedersachsen, Germany, [6]Institute for Experimental Virology, Twincore Centre for Experimental and Clinical Infection Research, Medical School Hannover (MHH)-Helmholtz Centre for Infection Research (HZI),

Hannover, Niedersachsen, Germany, [7]University of Veterinary Medicine, Vienna / University of Veterinary Medicine, Hannover Foundation / Faculty of Veterinary Science, University of Pretoria, Vienna, Wien, Austria

Since initial discovery of equine hepacivirus (EqHV) in 2011, the virus has been detected in horse populations from more than 12 countries, across five continents. EqHV seroprevalence is reported to be as high as 61.8% and EqHV ribonucleic acid (RNA) prevalence to range between 0.9% and 34.1%. Molecular and serological indications of EqHV infection have never been reported in equids on the African continent. Therefore, investigation of EqHV prevalence in South African horses and subsequent viral genetic characterisation would contribute to understanding global epidemiology of this emerging virus.

In a cross-sectional study, serum samples from 454 Thoroughbred foals (aged 58-183 d) were analysed for anti-EqHV non-structural (NS)3-specific antibodies (Abs) with the luciferase immunoprecipitation system (LIPS) and for EqHV RNA by quantitative real-time polymerase chain reaction (qRT-PCR). Farms of origin (n=26) were situated in the Western Cape Province, South Africa. Descriptive analysis was performed to study associations between EqHV infection status, age and gender. Identified EqHV isolates were sequenced, with subsequent phylogenetic analysis of genomic portions located in the NS3-gene.

Abs were detected in 83.7% (380/454) of samples - the highest seroprevalence reported yet in an equine population. The RNA prevalence of 7.9% (36/454) was within the previously reported range. Increasing foal-age was associated with decreasing prevalence of Abs and increasing prevalence of EqHV viraemia. South African EqHV strains didn';t cluster separately to published sequences of EqHV strains.

In conclusion, EqHV is circulating in the South African Thoroughbred population and appears to be more prevalent than in other horse populations worldwide.

E28

Validation of an In-Clinic Enzyme-Linked Immunosorbent Assay for Diagnosis of Leptospirosis in Horses

Emma B. Gorenberg[1], Hussni O. Mohammed[2], Thomas J. Divers[1]
[1]Cornell University School of Veterinary Medicine, Ithaca, NY, USA, [2]Department of Population Medicine and Diagnostic Sciences, Cornell University School of Veterinary Medicine, Ithaca, NY, USA

Infection with serovars of *Leptospira interrogans* and *Leptospira kirschneri* is an important cause of abortion, acute renal failure, recurrent uveitis, and rarely neonatal disease in horses. An in-clinic rapid ELISA is marketed for dogs. This study evaluated the performance of the LipL32-based ELISA (IDEXX SNAP Lepto) compared with the microscopic agglutination test (MAT) using serum from experimentally and naturally infected horses.

Serum samples were obtained from a previous study of horses experimentally infected with *Leptospira interrogans* serovar Pomona (n = 97). Serum from naturally infected horses was randomly selected from samples submitted to the Animal Health Diagnostic Center at Cornell University that were identified as positive on the MAT in any serovar (n = 31). A random selection of MAT negative samples was also included for testing (n = 20). All samples (n = 148) were tested with the IDEXX SNAP Lepto. The performance of the test kits was compared to the MAT. The accuracy was measured in terms of the relative sensitivity and specificity with a cut-off point for positivity in the MAT of ≥ 100. The relationship between the MAT titer and the probability of positivity in the IDEXX SNAP Lepto was assessed using logistic regression analysis.

Results indicated that the test kits have high specificity (100%). Sensitivity was poor at titers ≤400 (0 – 36%) and moderate at titers 800-1600 (50%), but was excellent at titers ≥ 3200 (90-100%).

The IDEXX SNAP Lepto provides a rapid in-clinic test for evidence of antibody response to *Leptospira* species in horses, with excellent specificity and high sensitivity with increasing titers. Leptospirosis associated abortion and acute renal disease often result in MAT titers of ≥ 6,400. This test kit may allow for prompt stall-side or field determination of Leptospirosis status in these cases.

E29

Prevalence of Equine Leptospiral Shedding using Urine Polymerase Chain Reaction and Serum Microscopic Agglutination Testing

Amanda C. Trimble, Chris A. Blevins, Laurie A. Beard, Ram K. Raghavan, Ashley R. Deforno, Elizabeth G. Davis
Kansas State University, Manhattan, KS, USA

Leptospirosis is a worldwide veterinary and public health concern, and emerging infectious disease of horses. The spirochete can be directly transmitted by contaminated urine, placental fluids, semen, infected tissues, reservoir hosts, or flood waters. Seroprevalence and infecting serovar vary with geography, yet diagnosis using the gold standard microscopic agglutination test (MAT) merely confirms high exposure rate. Subclinical infection can complicate diagnosis. The aims of this study were to use quantitative PCR on urine from apparently healthy horses to determine period prevalence of Leptospiral shedding and to correlate these findings with MAT results to establish associations with client based survey data regarding horse management and environment.

Serum and free-catch urine were collected from 204 healthy horses between May 2016 - December 2017. Serum was used to determine GGT, creatinine concentrations, and six serovar MAT (Canicola, Hardjobovis, Icterhemorrhagiae, Pomona, Grippotyphysa, Bratislava). Urine samples were submitted for PCR testing of Leptospiral DNA. Client consent and survey data were collected for all subjects. Potential risk factors included drinking water source, exposure to livestock and dogs, geographical location, season, and precipitation.

Two horses were positive on urine PCR for leptospirosis (shedding prevalence 1%), yet only 1 had a high MAT titer (> 1:800). Both horses were negative on urine PCR one month later without treatment. Seventy-seven percent of horses (157/204) were seroreactive (MAT > 1:100) for at least one serovar, and Bratislava was detected more frequently than others (47.5%; (97/204).

Apparently healthy horses infrequently shed Leptospira spp. in urine, yet seroreactivity in clinically normal horses is high (77%), confirming high exposure rates to Leptospira spp. in the Central Midwest. Further studies should target serovar specific PCR tests and incorporate PCR testing in horses clinically affected with leptospirosis.

E30

Nasal Bacterial Microbiota of Adult Horses Shedding Equine Herpes Virus 1

Diego Gomez-Nieto[1], Luis Arroyo[2], Brandon Lillie[3], J. Scott Weese[4]
[1]University of Florida, Gainesville, FL, USA, [2]Ontario Veterinary College, Guelph, ON, Canada, [3]University of Guelph, Guelph, ON, Canada, [4]Dept of Pathobiology Ontario Veterinary College University of Guelph, Guelph, ON, Canada

Bacterial and viral microbiotas often inhabit and share the same microenvironments, however, interest in their potential contribution in promoting health or disease has only recently gained attention. The objective of this study was to characterize the nasal bacterial microbiota of healthy horses (control) and horses shedding equine herpes virus-1 (EHV-1) using next generation sequencing technology.

The nasal bacterial microbiota of 10 EHV-1 and 10 control horses from a single farm experiencing an outbreak of EHV-1 was characterized using the Illumina MiSeq platform targeting the V4 region of the 16S rRNA gene. All EHV-1 horses had fever, limb edema and were positive on PCR of nasal swabs, within one week after the first case was confirmed EHV-1 positive. Control horses were animals from the same farm that showed no clinical signs during the outbreak and were negative for EHV-1 on PCR.

Nasal bacterial microbiota of healthy and EHV-1 was significantly different in community membership (Jaccard index) and structure (Yu and Clayton index) (Parsimony and AMOVA; $P < 0.05$). Horses shedding EHV-1 had lower bacterial richness [Chao-1: median and (range) = 241 (139 – 348) vs. 366 (288 – 420)]; $P = 0.0017$), evenness (Shannoneven: 0.47 (0.1 – 0.6) vs. 0.6 (0.45 – 0.72)]; $P < 0.008$) and diversity (Inverse-Simpson: 4 (1.1 – 15) vs. 11 (3.5 – 31)]; $P = 0.026$) than control horses. Healthy horses had higher relative abundance of Firmicutes (median: 36%) and Bacteroidetes (1%) than EHV-1 horses (20% and 0.3%, respectively) ($P < 0.05$). Based on LefSe analysis, enriched phylotypes from control horses were predominantly from the phylum Firmicutes, whereas most phylotypes that were enriched EHV-1 infected horses were from Proteobacteria and Actinobacteria The genera *Brachybacterium*, *Dietzia*, *Arthrobacter*, *Psychrobacter* and *Moraxella* were significantly associated with shedding EHV-1, whereas 19 genera including *Lactobacillus*, and unclassified genera of the family Clostridiales and Lachnospiraceae were enriched in control horses.

This study provides the basis for generation of hypotheses and investigations on the roles that bacterial-viral interactions play in health and diseases of adult horses.

E31

Very High Streptococcus equi Subspecies equi M Protein Titers (≥1:12,800) with and without Complications Post-Outbreak

Katherine M. Delph[1], Laurie A. Beard[1], Amanda C. Trimble[1], Maureen E. Sutter[2], John F. Timoney[3], Jennifer K. Morrow[4]
[1]Kansas State University, Manhattan, KS, USA, [2]Red Oak Animal Hospital, Bucyrus, KS, USA, [3]Maxwell H. Gluck Equine Research Center University of Kentucky, Lexington, KY, USA, [4]Equine Diagnostic Solutions LLC, Lexington, KY, USA

Streptococcus equi subspecies *equi* M protein (SeM) titers are useful for confirming complications of strangles infection (titer ≥1:12,800) or for evaluating immunologic risk of complications prior to vaccination (titer ≥1:1600). There are generally-accepted interpretations of SeM titers; however, specific references for data interpretation are lacking. The purpose of this study was to compare SeM titers with clinical disease displayed by horses post-outbreak to determine if the generally-accepted titer interpretation was consistent with clinical signs in these horses. Following an outbreak of *S. equi*, serum was collected to measure SeM titers on all horses at 8 weeks post-outbreak and on select horses at 12 weeks and 28 weeks post-outbreak. Horses were categorized as having no disease, uncomplicated strangles, persistent guttural pouch infection (>40 days post-infection), and/or complicated cases (metastatic abscess formation or purpura hemorrhagica, for example) based on clinical findings. Twenty-eight out of forty-eight (58.3%) developed clinical signs of *S. equi* infection. Of the 28 affected horses, 11 (39.3%) had uncomplicated strangles, 12 (42.8%) had persistent guttural pouch infection, and 8 (28.6%) were complicated cases, 3 of which were dually-categorized as persistent guttural pouch infection and complicated cases. Sixteen out of 28 (57.1%) horses had titers ≥1:12,800 at 8 weeks post-outbreak. Only 6/16 horses with SeM titers ≥1:12,800 had evidence of strangles complications at that or later time points. SeM titers declined in all horses tested at 28 weeks (n=36) to levels ≤1:6,400. This outbreak demonstrates that SeM titers can be significantly elevated post-outbreak (≥1:12,800) and the horse may not have strangles infection complications. It is still prudent to monitor a horse with a very high titer to ensure that complications are absent.

E32

Medical Treatment of Dorsal Displacement of the Soft Palate in Sport Horses

Fe ter Woort[1], Emmanuelle van Erck[2]
[1]Equine Sports Medicine Practice, Tervuren, Vlaams-Brabant, Belgium, [2]Equine Sports Medicine Practice, Waterloo, Brabant Wallon, Belgium

Dorsal Displacement of the Soft Palate (DDSP) has been well described in race horses[1,2]. Even though a link between DDSP and lower airway inflammation has been suggested in race horses[3], the treatment of choice remains surgical. DDSP has also been described in sport horses[4] although no efforts have been made to correlate this with lower airway inflammation.

A retrospective study was performed over a period of 3 years: 270 overground endoscopies were performed on sport horses presented to a private referral practice with a complaint of cough, respiratory noise or poor performance. Of these, 27 horses had DDSP. The DDSP was associated with coughing in 22/27 horses, noise in 4/27 horses and was silent in 1 horse. A tracheal wash was performed in all cases and an additional bronchoalveolar lavage was performed in 24 cases. Tracheal inflammation was present in 25/27 horses and lower airway inflammation was present in 24/24 horses. The bacterial culture of the tracheal wash was positive in 14/27 horses and the fungal culture was positive in 16/27 horses. Medical treatment was attempted in 25 horses. This involved topical anti-inflammatory treatment with furacine and DMSO and/or inhaled corticosteroids, bronchodilators and antimicrobial therapy where appropriate. Follow-up was available for all 25 cases. This included a recheck overground endoscopy in 3 horses, recheck of the respiratory samples in 8 horses

and phone contact of 22 horses. Treatment was successful in 22/25 cases. These horses stopped coughing or making a noise and were performing up to expectation. Treatment failures occurred in 2 very chronic cases and 1 case where the DDSP occurred after tie back surgery.

This study shows that in this sport horse population, DDSP was associated with lower airway inflammation in a large proportion of cases and can be managed medically.

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E33

Pharmacokinetics of Maropitant Citrate in Horses

Emily H. Berryhill[1], *K. Gary Magdesian*[2], *Heather Knych*[3], *Judy Edman*[2]
[1]*UC Davis Veterinary Medical Teaching Hospital, Davis, CA, USA,* [2]*UC Davis Veterinary Medical Teaching Hospital, Dept. Medicine and Epidemiology, Davis, CA, USA,* [3]*K.L. Maddy Equine Analytical Chemistry Laboratory, Davis, CA, USA*

The neurokinin-1 (NK) receptor antagonist maropitant citrate (Cerenia™, Zoetis Services) is commonly used in cats and dogs to mitigate nausea and vomiting. While horses do not vomit, nausea is poorly understood and likely under recognized in this species. In dogs and cats maropitant has significant visceral analgesic properties, as well. Investigation into the use of NK-1 receptor antagonists in horses has not been reported. The purpose of this study was to determine the pharmacokinetic profile of maropitant citrate in seven adult horses after a single intravenous (IV; 1 mg/kg) and intragastric (IG; 2 mg/kg) dose. A randomized, crossover design was performed and blood samples were collected serially after maropitant administration. Plasma maropitant concentrations were measured using LC-MS. Pharmacokinetic parameters were determined using noncompartmental analysis. The mean (± SD) plasma maropitant concentration 3 min after IV administration was 0.80 ± 0.14 μg/mL, elimination half-life was 10.37 ± 2.07 h and volume of distribution was 6.54 ± 1.84 L/kg. The mean (± SD) maximum concentration following IG administration was 0.08 ± 0.04 μg/mL and elimination half-life was 9.64 ± 1.27 h. The oral bioavailability was variable (13.31 ± 5.30%). Maropitant concentrations achieved after IG administration were comparable to those in canines and felines, however concentrations after IV administration were lower and higher than dogs and cats, respectively. Elimination half-life was longer than that in dogs and shorter than in cats. This study describes the pharmacokinetic profile of single dose maropitant in horses and can serve as the basis for further pharmacologic investigations.

E34

Effect of Soluble Epoxide Hydrolase Inhibitor, t-TUCB, on Recovery of Ischemic Injured Porcine Jejunum

Alexandra R. Carlson[1], *Kristen Messenger*[2], *Liliana Hernandez*[2], *Emily Griffith*[2], *Alonso Guedes*[3], *Sung Hee Hwang*[4], *Bruce Hammock*[4], *Anthony Blikslager*[2]
[1]*North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA,* [2]*North Carolina State University, Raleigh, NC, USA,* [3]*University of Minnesota, Saint Paul, MN, USA,* [4]*University of California Davis, Davis, CA, USA*

Ischemic intestinal injury disrupts mucosal barrier function in diseases such as strangulating obstruction in horses. Prostaglandins (PGs) play an important role in mucosal recovery, and soluble epoxide hydrolase (sEH) inhibitors may modulate the production of PGs. This study aimed to determine whether the sEH inhibitor, *trans*-4-{4-[3-(4-Trifluoromethoxy-phenyl)-ureido]-cyclohexyloxy}-benzoic acid (*t*-TUCB), modulates recovery of mucosal barrier function following ischemic injury in porcine jejunum. Ten juvenile pigs were anesthetized and complete ischemic injury was induced in the jejunum for 45 minutes. Non-ischemic and ischemic-injured mucosal tissues were collected and mounted on Ussing chambers and bathed in oxygenated Ringer';s solution. Ischemic-injured tissues were untreated controls or treated with *t*-TUCB (1μM) after 30-minutes of equilibration. Transepithelial electrical resistance (TER) data were calculated every 15 minutes over a 240-minute recovery period. Serosal fluid samples collected at 60 and 240-minute time points were assessed for PGE_2 concentration. There was a significant increase in PGE_2 levels in ischemic-injured tissues treated with *t*-TUCB as compared to untreated ischemic-injured tissues ($p = 0.0151$). The untreated and *t*-TUCB-treated ischemic-injured tissues had significantly reduced TER compared to the non-ischemic tissues ($p < 0.0001$ and $p = 0.0001$). However, there were no statistical differences between ischemic-injured tissues treated with or without *t*-TUCB. We conclude that *t*-TUCB is a novel modulator of PG levels, resulting in increased PGE_2 in tissues injured by ischemia without significantly impairing recovery of barrier function. Therefore, *t*-TUCB may have a future use to treat animals with diseases such as strangulating obstruction.

E35

Pharmacokinetics of 4-Methylaminoantipyrine, the Active Metabolite of Dipyrone, in the Horse

Jen Davis[1], *Emily A. Sundman*[2], *Matt Edmunds*[3], *Melinda Poole*[2]
[1]*VA-MD College of Veterinary Medicine, Blacksburg, VA, USA,* [2]*Kindred Biosciences Inc., Burlingame, CA, USA,* [3]*Johnson Research, LLC, Parma, ID, USA*

The purpose of this study was to evaluate the pharmacokinetics of the active metabolite of dipyrone, 4-methylaminoantipyrine (4-MAA), in adult horses following intravenous administration of four different dosing regimens.

Dipyrone was administered intravenously (IV) to 20 healthy adult horses for nine days in a parallel design laboratory study. Horses were randomly assigned to receive 30 mg/kg q12h (n=6), 30 mg/kg q8h (n=4), 60 mg/kg q8h (n=6) or 90 mg/kg q12h (n=6). Blood was

collected at predetermined times after administration and plasma 4-MAA concentrations were measured using a high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) method validated for use in horses.

4-MAA was rapidly detected in the plasma, reaching maximum measured concentrations (C_{max}) at the first time point for all doses studied. Following the first dose, the C_{max} increased linearly over the range of 30-90mg/kg (R^2 = 0.884). Following multiple doses, significant accumulation occurred and the area under curve (AUC) increased disproportionate to dose for 60 and 90 mg/kg. Significant differences including increases in minimum concentration (5.89µg/mL vs 2.47 µg/mL), average concentration (15.61 µg/mL vs 8.93 µg/mL), and half-life (11.37 h vs 4.48 h) were noted when dipyrone was administered 30 mg/kg q8h compared to q12h (P < 0.001). No significant differences were noted in clearance or AUC_{tau}.

Higher doses or shorter dosing intervals result in nonlinear pharmacokinetics for 4-MAA following multiple doses. This may be a result of drug accumulation at the dosing regimens utilized in this study, or may represent alterations in metabolism.

E36

Susceptibility of Horses and Sheep to Hypoglycin A Intoxication: In/Ex Vivo Bioavailability and Cellular Effects

Sonia Gonzalez-Medina[1], *William Bevin*[2], *Rafael Alzola*[2], *Carolyne Hyde*[3], *Richard Piercy*[4]
[1]*Royal Veterinary college, London, England, United Kingdom*, [2]*Oakham Veterinary Hospital, Oakham, England, United Kingdom*, [3]*London Bio-analysis center, London, England, United Kingdom*, [4]*Laboratory of Comparative Neuromuscular diseases, London, England, United Kingdom*

Atypical myopathy / seasonal pasture myopathy is a toxic rhabdomyolysis of horses linked to the ingestion of hypoglycin A (HGA) contained in seeds and seedlings of some *Acer* tree species. Although the toxic effect of HGA';s metabolite, methylenecyclopropylacetic acid (MCPA), has been studied in several species, it remains unclear why animals (such as ruminants) that graze similar pastures to horses, appear unaffected. The objective of this study was to investigate the hypothesis that apparent low susceptibility in sheep is due to either low bioavailability of HGA or to HGA degradation in ruminal fluid, or to relative increased MCPA sensitivity of horse muscle. HGA concentration was measured by a validated liquid chromatography-mass spectrometry method in serum samples from clinically healthy ewes (n=10) and horses (n=11) grazing on sycamore seedling-contaminated pastures. Additionally, sycamore seed homogenates were incubated with equine gastric fluid (n=6) and sheep ruminal fluid (n=5) samples for 1 hour and extracts assayed for HGA concentration. Finally, primary cells cultured from horse (n=4) and sheep (n=4) muscle samples were exposed to a range of MCPA concentrations for 24h and cytotoxic effects compared by MTT assay. Data were analysed using Mann-Whitney U or ANOVA tests. Similar concentrations of HGA were detected in serum of clinically-unaffected sheep [range 5.2-125ng/ml] and horses [6.5-81.4 ng/ml] grazing contaminated pastures. There was no statistically significant difference in HGA concentration following sycamore seed homogenate incubation with either gastric or rumen fluid. Compared to sheep cells, horse cells had significantly higher *in vitro* compromise at MCPA concentrations between 4-6mM (p < 0.0001). This study reveals that both sheep and horses absorb HGA following sycamore seedling ingestion and no evidence for HGA degradation after exposure to rumen fluid. Primary horse muscle-derived cells were more sensitive than sheep cells to the active HGA metabolite at low concentrations, suggesting greater muscle metabolic inhibition by MCPA in horses than in sheep. Though further study is necessary, this work potentially explains the apparent disparity in susceptibility to HGA intoxication between these species.

E37

Effect of Different Antimicrobial Treatment Regimes on Occurrence of Fecal Extended- Spectrum β-Lactamase-Producing Enterobacteriaceae

Angelika Schoster[1], *Julia van Spijk*[2], *Arshnee Moodley*[3], *Peter Damborg*[4], *Constanze Kirchgässner*[5], *Sonja Hartnack*[6], *Sarah Schmitt*[7]
[1]*University of Zurich, Zurich, Zurich, Switzerland*, [2]*University of Zurich Clinic for Equine INternal Medicine, Zurich, Zurich, Switzerland*, [3]*University of Copenhangen Departement of Veterinary and Animal Science, Frederiksberg, Hovedstaden, Denmark*, [4]*University of Copenhagen Departement of veterinary and Animal Science, Frederiksberg, Hovedstaden, Denmark*, [5]*University of Zurich, Institute of Veterinary Bacteriology, Zurich, Zurich, Switzerland*, [6]*University of Zurich, Section of Epidemiology, Zurich, Zurich, Switzerland*, [7]*University of Zurich, Institute for Veterinary Bacteriology, Zurich, Zurich, Switzerland*

Cephalosporins administration induces high shedding rates of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in humans. The objective was to prospectively evaluate the effect of different antimicrobial treatments on fecal shedding of ESBL-producing Enterobacteriaceae in horses.

Horses presented to the University of Zurich between 2014-2015 were randomly allocated to receive penicillin and gentamicin (P/G, n = 43) or cefquinome (CEF, n = 43) - if antibiotic treatment was needed - or no antimicrobial treatment (NOAMD, n = 33). Fecal samples were taken on admission (Day 0), 3 days after admission (Day 3) and 28 days after discharge (Day 28). Samples were semi-quantitatively cultured on ESBL-agar. Differences between groups were analysed using the Chi-square test, odds ratios and two-way repeated measures ANOVA.

Prevalence on Day 0 was not different between groups (p = 0.47). Horses treated with CEF and P/G were more likely to shed ESBL-producing Enterobacteriaceae compared to NOAMD on Day 3 (OR: 0.4, p = 0.07 and OR: 6.8, p = 0.04, respectively) and Day 28 (OR: 0.2, p = 0.02 and OR: 6.3, p = 0.003, respectively). ESBL counts increased from Day 0 to Day 3 in all groups (CEF p = 0.001, P/G p < 0.001, NOAMD p = 0.02). ESBL bacterial count was significantly higher in the P/G group compared to CEF and NOAMD group (p = 0.001 and p < 0.001, respectively) and was also significantly higher in the CEF group compared to NOAMD group (p = 0.001).

Hospitalization and antimicrobial treatment increases colonization with ESBL-producing Enterobacteriaceae. Administration of cefquinome did not induce higher shedding rates compared to administration of penicillin/gentamicin as a standard antibiosis. This highlights the need for reducing antimicrobial use overall.

E38

Inflammatory Effect of Mitochondrial Fragments in Equine Whole Blood

Jennifer R. Bauquier, Simon R. Bailey
University of Melbourne, Werribee, Victoria, Australia

Mitochondrial products, especially mitochondrial DNA, are pro-inflammatory in human studies, presumably due to their bacterial evolutionary origins. Therefore their release from tissue necrosis may be an important contributor to the inflammatory response in the horse. The aim of this study was to determine the inflammatory response to mitochondrial products in an *in vitro* equine whole blood assay.

Mitochondria were isolated from liver tissue of a systemically healthy horse by cell lysis and centrifugation. Mitochondria were then fragmented by freeze-thawing and serially diluted from 1:1 to 1:512 in PBS, and added to aliquots of whole blood from 6 healthy horses, mixed 1:1 with RPMI medium, with one aliquot serving as a negative control. Aliquots were incubated rotating at 37°C for 21 hours. The inflammatory response was determined by measuring TNF-α in the supernatant using a murine L929 fibroblast bioassay.

Mitochondrial fragments caused an inflammatory effect in equine whole blood, producing a concentration-dependent increase in TNF-α release into the plasma/supernatant, which fitted to a sigmoidal concentration-response curve. There was a 7.3-fold increase in TNF-α production at the lowest dilutions of mitochondrial fragments (557.5 ± 470.5 pg/mL at 1:2 dilution compared with 76.1 ± 33.9 pg/mL in whole blood incubated with no fragments).

The release of mitochondrial fragments from localized tissue damage, inflammation or necrosis might contribute to the clinical severity of SIRS, and possibly offer a mechanism for the development of SIRS in horses in the absence of obvious gastrointestinal disease or infection. Further work is warranted to investigate the significance of inflammation caused by mitochondrial fragments in horses, including novel anti-inflammatory therapies.

E39

Electrocardiographic Parameters Pregnant Donkeys (Equus Asinus) of the Pega Breed

Raíssa Karolliny S. Cruz [1], Gabriela A. Campos[1], Angélica Alfonso[1], Mariana Herman[1], Miriam H. Tsunemi[2], Maria Lucia Gomes. Lourenço[1], Simone Biagio Chiacchio[1]
[1]São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Botucatu, Sao Paulo, Brazil, [2]São Paulo State University (Unesp), Institute of Biosciences, Botucatu, Botucatu, Sao Paulo, Brazil

The electrocardiogram is an indispensable tool in the diagnosis of arrhythmias and electric conduction disorders in the equine heart. However, several electrocardiographic variables may be influenced by factors such as species, age, gender, breed and morphofunctional constitution, highlighting the importance of knowing normal characteristics for distinct species, breeds and development stages. This study aims at assessing the electrocardiographic development in the frontal plane and apical-to-basal derivations during the last days of pregnancy (35, 28, 21, 14, 7 and 1 day before birth) in 10 donkeys of the Pega breed. Therefore, the electrocardiogram was conducted in a computer-interpreted electrocardiography device in the frontal plane and apical-to-basal derivations for 5 minutes without sedatives, tranquilizers or anesthetics. The results are shown in Table 1. We did not observe physiological or pathological cardiac arrhythmias or electric conduction disorders in the animals assessed. The heartrates observed in frontal plane and apical-to-basal derivations presented minimal changes between the studied moments during pregnancy and the predominant cardiac rhythm observed in 100% (60/60) of the females was sinus tachycardia, with the heartrate increasing as the birth got closer (58±8.47 bpm in the 35th day before birth and 61,3±11 bpm at birth). Bifid and biphasic P waves were predominant at all moments. We observed significant differences in the duration of PR intervals (ms) in the frontal plane and apical-to-basal derivations, and in the QT interval (ms) in both derivations; as well as a significant increase in R wave amplitude. The electrocardiographic parameters of pregnant donkeys diverge in terms of duration, amplitude and morphology in

Table 1. Mean electrocardiographic variables and standard deviations in the frontal plane and apical-to-basal derivations of 10 pregnant donkeys of the Pega breed during the last days of pregnancy

	MOMENT													
	35 days before birth		28 days before birth		21 days before birth		14 days before birth		7 days before birth		Birth		P Value	
VARIABLES	Frontal Plane	Apical-to-Basal	Frontal Plane	Apical-to-Basal	Frontal Plane	Apical-to-Basal	Frontal Plane	Apical-to-Basal	Frontal Plane	Apical-to-Basal	Frontal Plane	Apical-to-Basal	Frontal Plane	Apical-to-Basal
FC (bpm)	60.2±17.2	58±8.47	59.6±9.7	58±8.5	56.7±5.7	55.2±8.5	59.3±6.8	58.7±6.2	58±7.6	58.2±6.7	56.7±4.6	61.3±11	**0.000***	**0.000***
P (ms)	102.4±20.8	98.6±10.2	96±17.2	99±10.1	94.6±15.3	98.5±9.8	93.7±18	102.2±16.3	93±16.5	95.4±18.1	99±25.4	98.3±8.1	0.772	0.783
P (mV)	0.17±0.02	0.17±0.03	0.15±0.02	0.17±0.04	0.15±0.02	0.17±0.03	0.15±0.02	0.16±0.04	0.14±0.04	0.16±0.05	0.15±0.04	0.17±0.02	0.080	0.982
PR (ms)	245.4±42	251.2±29.7	231.8±37	254.1±34.3	229.6±28.3	234.8±32.2	233.8±30.1	249±31.5	227.3±35	241±34.7	231.2±33.7	249.7±32	**0.017***	**0.001***
R (mV)	0.74±0.29	0.0±0.01	0.65±0.32	0.01±0.02	0.63±0.33	0.0±0.01	0.52±0.29	0.02±0.03	0.63±0.33	0.04±0.0	0.55±0.29	0.04±0.05	0.377	**0.007**
QRS (ms)	105.5±12.1	114.8±13.1	109.8±10.1	115.6±12	111±10	115.6±11.8	104.6±16.6	114.1±13	106.5±41	113.3±13.5	100.6±15.3	108.4±8.7	0.086	0.429
QT (ms)	454.4±75.4	463.1±36	469±43	467.7±30	475.1±24.5	477.4±20.4	460±33.7	464±21.2	464±37.5	461±23.7	453.5±21.7	431.3±36.4	**0.015***	**0.000***
T (ms)	150.3±33.7	145.2±15.1	151±33.2	141±15.4	153.4±12.7	157±24.6	144.2±24.3	145.7±15.1	145±27.6	141.7±20.1	147.6±22.2	128±17.6	0.062	**0.012***
T (mV) (+)	0.11±0.07	0.12±0.07	0.07±0.05	0.10±0.08	0.07±0.06	0.09±0.07	0.07±0.05	0.12±0.11	0.07±0.05	0.10±0.07	0.06±0.06	0.11±0.14	0.269	0.994
T (mV) (-)	-0.29±0.12	-0.31±0.14	-0.27±0.11	-0.29±0.11	-0.27±0.09	-0.34±0.16	-0.30±0.09	-0.29±0.10	-0.23±0.24	-0.28±0.05	-0.33±0.15	-0.29±0.14	0.119	0.875

***Statistical significance (P<0.05)**

Bpm = beats per minute, ms = millisecond, mV = millivolt, p = p values obtained through the One Way Repeated Measures test.

contrast with other donkey and equine breeds and these findings highlight the importance of obtaining specific values for distinct species, breeds and pregnancy stage so a safer prenatal care can be achieved.

E40

Expression of Micropeptides that Regulate Intracellular Calcium Concentrations in Horse Skeletal and Cardiac Muscles

Melissa AP Schott[1], Sudeep Perumbakkam[2], Zoë J. Williams[3], Keri L. Gardner[2], Joseph M. Autry[4], David D. Thompson[4], Stephanie J Valberg[2]
[1]Michigan State University, Bath, MI, USA, [2]Michigan State University, East Lansing, MI, USA, [3]Michigan State University, College of Veterinary Medicine, Pinckney, MI, USA, [4]University of Minnesota, Minneapolis, MN, USA

Key genes involved in the transport of calcium (Ca^{2+}) across the sarcoplasmic reticulum (SR) encode proteins such as the ryanodine receptor (RYR), which releases Ca^{2+} from the SR, and SR calcium ATPase (SERCA), which pumps Ca^{2+} back into the SR. Newly discovered regulatory micropeptides such as MRLN, PLN and SLN inhibit SERCA, thereby controlling intracellular Ca^{2+} and contractility. The transcript expression or protein abundance of these micropeptides in horses is unknown. The purpose of this study is to determine the relative expression of equine Ca^{2+} regulatory genes in left ventricle, right atrium, and gluteal muscle. RNA was extracted from tissues collected from 7 horses and reverse transcribed into cDNA. Transcript abundance was quantified using qRT-PCR and data normalized to *GAPDH* (within sample). Data was compared using an ANOVA, with gluteal used as a baseline, and results were corrected for multiple testing. Gluteal muscle, compared to right atrium and left ventricle, had the highest expression of *RYR1*, *SERCA1*, and *SLN*, with *MRLN* expressed but at low levels. Whereas *SLN* was found to be the most abundantly expressed SERCA inhibitor in gluteal muscle, *PLN* had higher expression in the right atrium and left ventricle. The atrium and ventricle expressed higher levels of *RYR2* and *SERCA2* compared to gluteal muscle. Differential expression of these newly discovered micropeptides suggests varying roles in their regulation of SERCA in different tissues. This new expression data complements findings in other species and opens exploration of the role of SERCA inhibitors in equine skeletal and cardiac diseases.

E41

Pharmacokinetics and Safety of Repeated Oral Dosing of Acetaminophen in Adult Horses

Melissa Mercer[1], Jen Davis[2], Bridgett McIntosh[3], Katie Wilson[1], David Hodgson[1], Harold McKenzie[1]
[1]Virginia Maryland College of Veterinary Medicine, Blacksburg, VA, USA, [2]VA-MD College of Veterinary Medicine, Blacksburg, VA, USA, [3]Virginia Polytechnic Institute and State University, Middleburg, VA, USA

The safety and pharmacokinetics of acetaminophen (APAP, INN: paracetamol) were determined using 8 healthy adult Thoroughbred geldings. Commercially available APAP (20 mg/kg; 500 mg tablets) was administered orally as a single dose followed by multiple doses every 12 hours for 14 days. Blood samples were collected for determination of plasma APAP concentrations using HPLC-UV. Serum biochemistries and gastroscopy were performed prior to initiation of dosing and following the final dose.

Following a single dose, mean maximum concentration (C_{max}) was 16.61 ug/mL at 1.35 hours (T_{max}). APAP remained above presumed therapeutic concentrations (10 ug/mL) for two hours post-administration and was undetected by 12 hours. Elimination half-life ($T_{½}$) was 2.78h. No significant accumulation was noted following multiple doses. Average C_{max} of APAP following repeated oral dosing was 15.85 ug/mL, with a T_{max} of 0.99 hours and $T_{1/2}$ of 4 hours. SDH was significantly decreased (pre: 13.84 U/L, post: 10.52 U/L, p = 0.013) and total bilirubin was significantly increased (pre: 1.99 mg/dl, post: 3.47 mg/dl, p = 0.004) following the last dose. No statistically significant changes were noted in gastroscopy scores (pre: 9.67 mean, post: 10.08 mean, p = 0.75). Dose simulations suggested higher doses and/or shorter dosing intervals may be indicated.

This study demonstrated the safety of acetaminophen with repeated oral dosing over 14 days. This study also confirms that the 20 mg/kg dose used in previous clinical reports reaches proposed therapeutic concentrations after oral administration to fed horses, however a shorter 6-8 hour dosage interval may be required for improved efficacy.

E42

Pharmacodynamics of Doxycycline in a Streptococcus equi Subsp. zooepidemicus Infection Model in Horses

Ronan J.J Chapuis[1], Fabienne D. Uehlinger[2], Melissa Meachem[3], Patricia M. Dowling[4]
[1]Department of Large Animal Clinical Sciences, Saskatoon, SK, Canada, [2]Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, Saskatoon, SK, Canada, [3]Department of Veterinary Pathology, Saskatoon, SK, Canada, [4]Department of Veterinary Biomedical Sciences, Saskatoon, SK, Canada

This study describes the pharmacodynamics of oral doxycycline in a *Streptococcus equi* subsp. *zooepidemicus* infected tissue chamber model in adult horses.

Six healthy adult light breed horses were included in the study with a median age of 8 years [range: 5-16 years]. Tissue chambers (TC) were implanted subcutaneously on the neck and were allowed a median healing time of 39.5 days [range: 27-73 days] prior to *S. zooepidemicus* inoculation. On day 0, 2 mL of $4.45x10^6$ colony-forming units (CFU) were inoculated in the TC. Doxycycline hyclate (10 mg/kg, PO, q 12 h) mixed with poloxamer gel and xanthum gum was administered on days 1 to 5. TC fluid was sampled before and after inoculation and on days 2, 4, 7, 21 and 28 for total solids measurement, quantitative culture, total nucleated cell and differential cell counts. Rectal temperatures were measured daily from days 0 to 8. Matched pairs sign rank tests were used to compare the distribution of cytologic and CFU results.

Before inoculation (day 0), TC fluid was sterile and cytology showed mild inflammation (total solids: median 50.5 g/L [range: 45-60 g/L], neutrophils: median 42.5% [range: 24-61%], macrophages: median 27.5% [range 20-38.5%], lymphocytes: median 25.5% [range 17-37.5%]). All but two horses developed a transient hyperthermia [median 39.7°C, range: 38.8-40]. All horses developed an abscess at the TC site. Between days 0 to 2, a significant increase in the total nucleated cell counts ($P < 0.04$), total solids ($P < 0.03$) and CFU concentration ($P < 0.03$)

occurred in the TC. The cytological profiles also changed significantly characterized by an increased proportion of neutrophils and decreased proportion of macrophages and lymphocytes (P < 0.03).

These findings were consistent with a septic reaction in the TC after inoculation and a failure of doxycycline treatment. In conclusion, the oral dose and formulation of doxycycline hyclate used in this study failed to cure *S. zooepidemicus* infection in the TC model.

E43

Pharmacokinetics and Pharmacodynamics of Subcutaneous Dexmedetomidine in Anaesthetized Horses

Sarah Bigby[1], *Andrew Woodward*[1], *Heather Knych*[2], *Jennifer Carter*[1]
[1]*The University of Melbourne, Werribee, Victoria, Australia,* [2]*K.L. Maddy Equine Analytical Chemistry Laboratory, Davis, CA, USA*

The pharmacokinetics of dexmedetomidine were described in 12 horses undergoing anaesthesia for arthroscopy procedures. Dexmedetomidine was administered by intravenous bolus (6 horses) or subcutaneous injection (6 horses), and blood samples collected for 90 minutes post-dosing. Clinical observations included the systolic, mean arterial, and diastolic blood pressures, and heart rate. Concentrations of dexmedetomidine in plasma samples were determined using a validated LCMS method. The dexmedetomidine concentration data were analysed using Monolix (Lixoft, France) fitting a population pharmacokinetic-pharmacodynamic model consisted of a two-compartment PK model with first-order absorption. Elimination rate constant for dexmedetomidine was median 0.0102 min $^{-1}$, with median central compartment apparent volume of distribution 0.644 L. Median subcutaneous bioavailability was 30.9 % with median absorption rate constant 0.225 min $^{-1}$. Following optimization of the pharmacokinetics, these parameters were locked and the pharmacodynamic variables assessed in a sequential modelling strategy. Parameters of a Michaelis-Menten model were not identifiable, so this model was rejected. Model goodness-of-fit statistics supported the inclusion of a simpler power model for the effect of dexmedetomidine on all blood pressure parameters, describing a concentration-dependent rise in blood pressure. No effect of dexmedetomidine on heart rate was detected. This study identified that dexmedetomidine was rapidly but incompletely absorbed after subcutaneous administration. Higher plasma concentrations after intravenous administration were associated with increased blood pressure, compared with subcutaneous administration. These results suggest a need for dosage adjustment based on route of administration for dexmedetomidine.

E44

Retrospective Evaluation of Plasma Phosphate and Calcium Concentrations as Biomarkers in Horses with Gastrointestinal Disease

Lucia Mentink[1], *Simon R. Bailey*[2], *Jennifer R. Bauquier*[2]
[1]*University of Melbourne, Wollongong, New South Wales, Australia,* [2]*University of Melbourne, Werribee, Victoria, Australia*

Plasma phosphate concentration (P) and plasma calcium concentration (Ca) change in human sepsis and experimentally-induced systemic inflammatory response syndrome in horses. These easily measured parameters might be useful biomarkers for equine colic. This study aimed to determine the usefulness of P and Ca as prognostic and diagnostic biomarkers in horses with colic.

Records of horses presenting for colic (including colitis) were reviewed. Controls were healthy teaching horses. P and Ca, and Ca:P were compared between horses with colic and controls. Additional comparisons were made between horses with strangulating, non-strangulating and inflammatory colic lesions, and survivors and non-survivors. Correlations with haematocrit, white cell count (WCC) and fibrinogen were examined.

Records from 101 colic cases and 24 controls were included. Colic cases had significantly higher P (1.2-fold increase, P=0.037) and significantly lower Ca (1.06-fold decrease, P=0.03) and Ca:P (1.15-fold decrease, P=0.005) than controls. There was no difference in P, Ca or Ca:P between colic lesion groups, but Ca was significantly lower for strangulating and inflammatory lesions compared to controls (P < 0.05), and Ca:P was significantly lower for inflammatory lesions compared to controls (P < 0.05). Weak but significant correlations were found between P and haematocrit, WCC and fibrinogen, but not Ca and these parameters. Ca:P was significantly correlated with fibrinogen and haematocrit but not WCC. Ca and P were not significantly correlated. There was no association between P, Ca or Ca:P and survival.

Neither P, Ca or Ca:P were useful alone as biomarkers, but can contribute to the overall clinical picture.

E45

Gastric Outflow Obstruction in Four Foals Associated with an Outbreak of Rotaviral Enteritis

Laura K. Dunbar[1], *Teresa A. Burns*[1], *Lindsey Rings*[1], *Margaret Gabour*[2], *Eric Schroeder*[1], *Ramiro Toribio*[1]
[1]*The Ohio State University College of Veterinary Medicine, Columbus, OH, USA,* [2]*Sea Coast Equine, Columbus, OH, USA*

Nine halter-bred American Quarter Horse foals (age range 1 day to 3 months) from a single farm in central Ohio were presented to the OSU CVM for evaluation of diarrhea, lethargy, and inappetance. One foal died within 12 hours of admission, while two foals were euthanized within 2 hours of admission (poor response to resuscitation in one and suspected gastrointestinal rupture in another). Rotavirus PCR performed on feces was positive in 4 foals. One foal was positive for *Clostridium difficile* on fecal culture, while the rest were negative for *Clostridium perfringens/difficile*, *Salmonella*, and Coronavirus. Four foals were discharged from the hospital, but re-presented within 2-3 weeks for persistent inappetance, lethargy, and failure to thrive, and were subsequently diagnosed with pyloric outflow obstruction based on gastroscopy, persistent nasogastric reflux, and barium gastrography. One foal re-presented for caudal esophageal stricture, and was euthanized after poor response to treatment. Three foals were euthanized due to pyloric outflow obstruction, while 1 was discharged. Necropsies performed on 3 foals revealed severe gastric distention and pyloric/duodenal stenosis.

While gastroduodenal ulceration (EGUS) has been suspected in association with rotavirus, the incidence of multiple cases of pyloric outflow obstruction in association with rotaviral enteritis has not been documented as well in horses as it has in human neonates. EGUS in foals is

common (prevalence ranging from 25-50%), and frequently clinically inapparent. Rarely, these lesions may produce stricture and gastric outflow obstruction. This report describes gastric outflow obstruction diagnosed in multiple foals in association with an outbreak of rotavirus.

E46

Association of Progestogens with Inflammation and Immunity in Critically Ill Foals

Stephanie Vijan[1], Katarzyna Dembek[2], Steven Reed[3], Nathan Slovis[4], Ramiro Toribio[5]
[1]The Ohio State University College of Veterinary Medicine, Westerville, OH, USA, [2]Iowa State University College of Veterinary Medicine, Ames, IA, USA, [3]Rood and Riddle Equine Hospital, Lexington, KY, USA, [4]Hagyard Equine Medical Institute, Lexington, KY, USA, [5]The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

Septicemia is the leading cause of mortality in newborn foals. Progesterone is mainly known for its role in pregnancy; however, it is also a precursor to adrenocortical and neuroactive steroids and likely plays important functions in equine neonates. Human studies have demonstrated that progesterone modulates immunity and predisposes to inflammatory conditions. However, the mechanisms by which progesterone influences outcomes in sick foals remain unclear. The goal of our study was to measure blood concentrations of inflammatory cytokines and serum amyloid A (SAA) in healthy and hospitalized foals, and to determine their association with progesterone, severity of disease, and mortality. We hypothesized that hospitalized foals will have higher progesterone, 17 α -hydroxyprogesterone and cortisol concentrations that will be associated with the inflammatory response, disease severity and mortality. Foals (n = 62) were divided into three groups based on severity of disease (septic [n = 31], sick non-septic [SNS; n = 21], and healthy [n = 10]), and hospitalized foals (SNS and septic) were divided into two groups based on survival (survivors and non-survivors). Blood samples were collected on admission. Hormones were measured by radioimmunoassay, and cytokines by ELISA. Progesterone, 17 α -hydroxyprogesterone, cortisol, and interleukin 1-β concentrations were significantly higher in septic and SNS compared to healthy foals ($P < 0.05$). Leukocyte and neutrophil count as well as IgG concentrations were lower, while SAA concentrations were significantly higher in hospitalized compared to healthy foals ($P < 0.05$). Interleukin 4 concentrations were significantly higher in non-survivors than survivors. IgG and interleukin 6 concentrations were positively correlated. Progesterone was significantly lower in non-survivors and negatively correlated with SAA ($P < 0.05$). These results suggest that progesterone, in addition to cortisol, may be involved in the adrenocortical response to stress from critical illness and could influence the inflammatory response in sick foals. This is the first study to associate progestogens with markers of inflammation in hospitalized foals.

E47

Proof of Concept Study of Subcutaneously Administered Anti-TNF Antibody in Neonatal Foals with Sepsis

Emily A. Sundman[1], Tianhua Hu[1], Peter Morresey[2], Carole K. Clark[3], Laszlo Hunyadi[4], Melinda Poole[1]
[1]Kindred Biosciences Inc., Burlingame, CA, USA, [2]Roode and Riddle Equine Hospital, Lexington, KY, USA, [3]Peterson & Smith Equine Hospital, Ocala, FL, USA, [4]Equine Sports Medicine and Surgery, Weatherford, TX, USA

Anti-TNF antibodies (TNF blockers) have been used in human sepsis and in animal models of sepsis, but have not been FDA approved for use in horses in the US. The objective of the pilot study was to evaluate a single dose of novel anti-TNF antibody in neonatal foals with sepsis.

The study was a single-dose, multi-center, clinical trial. Foals aged 0-96 hours with sepsis as defined by a sepsis score ≥ 11 or sick (non-septic) defined as rectal temperature 102.5°F *and* blood glucose 180 mg/dL were enrolled. Foals were administered anti-TNF antibody (2-4 mg/kg SQ) or placebo (matched volume saline SQ) once, along with concurrent treatment for the underlying disease(s). Foals were assessed daily for up to eight days by the study veterinarians.

Nineteen septic foals with a variety of diagnoses were enrolled in the study; thirteen foals were treated with anti-TNF antibody and six foals were treated with placebo. Kaplan-Meier survival analysis showed a statistically significant difference in survival to study termination in anti-TNF antibody treated foals in comparison to placebo ($p = 0.0293$). Post-treatment abnormal clinical signs and clinical pathology changes were related to the underlying diseases. No injection site reactions were noted. Foals treated with anti-TNF antibody showed improvement in survival at study termination in comparison to scores at baseline. Anti-TNF antibody was well tolerated when administered to septic neonatal foals at 2-4 mg/kg.

E48

Comparison Between Euglycemic-Hyperinsulinemic Clamp Measurements and Proxies for Insulin Sensitivity and B-Cell Response in Horses

Johan Bröjer, Sanna Lindåse, Katarina EA. Nostell
Swedish University of Agricultural Sciences, Uppsala, Uppsala Lan, Sweden

Proxies for prediction of insulin sensitivity and β-cell response calculated from basal plasma glucose and insulin concentrations have been described for use in horses. There is a non-linear inverse relationship between β-cell response and insulin sensitivity (IS) in the horse. The euglycemic-hyperinsulinemic clamp (EHC) is a gold-standard method for measuring IS but the complexity of the technique limits its use to research.

Insulin sensitivity was quantified using the EHC in 56 horses of different breeds with a wide range of IS including clinical patients diagnosed with insulin dysregulation. Quantitative measures of IS were expressed as M (mean glucose disposal rate) and M/I values. Basal plasma glucose and insulin concentrations to calculate proxies were determined from blood samples collected prior to the EHC. Proxies to estimate IS (quantitative insulin sensitivity check [QUICKI] and reciprocal of the square root of insulin [RISQI]) as well as proxies for β-cell response (basal insulin concentration [BIC], modified insulin-to-glucose ratio [MIRG], homeostasis model assessment for β-cell function [HOMA-β%]) were calculated. Spearman correlations were used to evaluate the correlation between proxies and IS assessed by the EHC.

There was a strong positive linear correlation (*rho* = 0.82 – 0.83, P < 0.001) between proxies for IS (RISQI, QUICKI) and M and M/I-values. Proxies for β-cell function (BIC, MIRG, HOMA-β %) were inversely related (*rho* = -0.80 – -0.84, P < 0.001) to quantitative measurements of IS (M, M/I).

The use of proxies for estimates of IS or β-cell response offers an attractive alternative for large population studies where a relatively simple and inexpensive assessment is necessary.

E49

Effects of Freezing on Measurement of Plasma Adrenocorticotropic Hormone Concentrations in Horses

Steve T. Grubbs[1], John Haffner[1], Dwana L. Neal[2]
[1]Middle Tennessee State University, Murfreesboro, TN, USA, [2]Boehringer Ingelheim, St. Josephh, MO, USA

Measurement of adrenocorticotropic hormone (ACTH) has become the most common diagnostic method utilized for the diagnosis of pituitary pars intermedia dysfunction (PPID) in horses. Adrenocorticotropic hormone has been reportedly understood to be fragile in whole blood samples and most affected by heat and time spent on cells. Consequently, the recommendations have been to refrigerate whole blood samples prior to centrifugation and separate plasma within 8 hours of collection. If plasma samples cannot be shipped the day of collection to the respective laboratory, plasma should be frozen until shipment. It is imperative for the veterinarians to understand if freezing plasma has any negative effects on the stability of resting ACTH concentration. The objective of the study was to determine the stability of ACTH in plasma after freezing for different lengths of time prior to determination of resting ACTH concentration. Plasma samples were obtained from 12 horses and resting ACTH levels were measured at Day 0 (baseline) and over time (variable by storage method). Plasma samples were stored in either -80° C, -20°C or samples placed between ice packs and stored at -20°C. Plasma samples were stored at -80°C for 3, 7, 30, 60 and 90 days, or stored at -20°C for 3, 7, 30 and 60 days, or stored between ice packs at -20°C for 3 and 7 days prior to determination of resting ACTH concentration. Plasma samples were shipped to the Animal Health Diagnostic Center, Cornell University, Ithaca, NY for measurement of resting ACTH concentration. Within each storage method, ACTH levels over time were compared to baseline (non-frozen Day 0 plasma) using a paired t-test (i.e. each horse serving as its own control and p < 0.05 was considered statistically significant). Data were summarized with mean, standard error (SE) and percent change from baseline (%CFB). Mean ACTH level on Day 0 for plasma stored at -80°C was 392.2 pg/mL that declined 6.9% to 365.0 pg/mL by Day 90. Through Day 60, the %CFB never varied more than 2%. On Day 90, the %CFB was -6.9% although not reaching statistical significance (p = 0.1042), a trend toward significant degradation was observed. Mean ACTH level on Day 0 for plasma stored at -20°C was 392.2 pg/mL that declined 5.3% to 371.4 pg/mL by Day 60. The %CFB was not statistically significant (p = 0.0590) at Day 60 although a decreasing trend was observed. Mean ACTH level at Day 0 for plasma stored between ice packs at -20°C was 392.2 pg/mL that declined 1.1% to 387.9 pg/mL by Day 7. No significant degradation (p = 0.4860) of ACTH was observed by Day 7. None of the storage methods resulted in sample degradation that differed significantly from baseline. Trends toward degradation were observed on Day 90 in -80°C stored samples and on Day 60 at -20°C stored samples. No significant degradation was observed in ACTH from plasma samples stored between ice packs at -20°C for 7 days. Freezing plasma for short periods of time at -20°C or at -80° C for at least 90 days resulted in no statistically significant degradation of plasma ACTH compared to non-frozen plasma baseline samples. Further studies should be conducted to further evaluate the effects of freezing on plasma ACTH concentrations in larger populations of horses.

E50

Blood Pressure and Cortisol in Horses with Diet Induced Obesity and Effect of Pasture

Katarina EA Nostell, Sanna Lindåse, Johan Bröjer
Swedish University of Agricultural Sciences, Uppsala, Uppsala Lan, Sweden

The aim of the study was to evaluate the effects of weight gain, achieved by providing healthy horses a low-carbohydrate and fat rich diet followed by pasture, on insulin sensitivity, blood pressure and serum cortisol concentrations.

Weight gain was induced in 9 Standardbred mares fed a fat supplemented forage diet at 2.5 times the daily requirements for 22 weeks. Horses were then turned out to pasture for 4 weeks. Insulin sensitivity was measured before and after weight gain and after 4 weeks of pasture using the euglycemic hyperinsulinemic clamp method. Body weight, body condition (BCS), blood pressure and serum cortisol was monitored throughout the study.

All horses became obese during the weight gain period (BCS ≥ 7.0). Mean arterial blood pressure increased gradually during the weight gain period and was significantly higher than initial values at the end of the weight gain period (78 ± 3 mmHg vs 92 ± 3 mmHg). The mean arterial blood pressure remained at a higher level on pasture (93 ± 3 mmHg). Insulin sensitivity ($M:I_{60}$) did not change during weight gain (4.3 ± 0.5 vs 4.8 ± 0.5 ([mg/kg/min x 10^3]/ [mU/L]) but improved after 4 weeks on pasture (7.3 ± 0.5 ([mg/kg/min x 10^3]/ [mU/L]). Serum cortisol concentrations increased during the weight gain period (80 ± 9 nmol/L vs 112 ± 9 nmol/L) and remained elevated during the pasture period.

In conclusion, obesity was associated with an increase in blood pressure that was not associated with change in insulin sensitivity.

E51

Metacarpal Bone Thickness and Density in Aged Horses with and without Pituitary Pars Intermedia Dysfunction

Harold C. Schott[1], Nathan C. Nelson[2]
[1]Michigan State University, East Lansing, MI, USA, [2]North Carolina State University, Raleigh, NC, USA

Pathologic fractures have occasionally been reported in aged horses with pituitary pars intermedia dysfunction (PPID), suggesting that

PPID may be associated with loss of bone thickness and / or density. The purpose of this study was to compare cortical bone thickness and density of the third metacarpal bone in horses with PPID ($PPID^+$ with pars intermedia [PI] histology scores of 4-5 / 5; n = 9), aged horses without PPID ($PPID^-$, PI histology score ≤ 3; n = 6), and young horses (YOUNG [< 8 years]; n = 5). Following euthanasia the right third metacarpal bone was removed and CT scans were performed. Images were used to measure thickness (mm) and density (Hounsfield Units [HU] of a region of interest) of the dorsal, palmar, medial, and lateral cortices at the midpoint of the diaphysis. Data were analyzed by ANOVA and ANOVA on ranks. Significant ($p < 0.05$) differences were found in palmar cortex thickness between $PPID^+$ and YOUNG (YOUNG greater) and in lateral cortex thickness between $PPID^-$ and both $PPID^+$ and YOUNG ($PPID^-$ greater than both $PPID^+$ and YOUNG). For bone density the only significant ($p < 0.05$) difference detected was in palmar HU between $PPID^+$ and YOUNG (YOUNG greater). Although minor differences were found in cortical bone thickness and HU between groups, there was not a consistent effect of $PPID^+$, as compared to $PPID^-$ and YOUNG. Further, it was difficult to separate effects of age and PPID status. In conclusion, substantial changes in third metacarpal cortical thickness or density were not found in this cohort of $PPID^+$ horses.

E52

Hyperlipemia in Hospitalized Equids: Treatment and Prognosis

Laura K. Dunbar, Teresa A. Burns, Ramiro Toribio
The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

Hyperlipemia is a common problem in hospitalized equids. Medical records of 84 hospitalized equids (> 2 years of age; median age 10.5 years, range 2 to 30 years) with triglyceride concentrations > 500 mg/dL admitted between 2002 and 2017 were reviewed. Breeds included donkeys (8), 24 American Miniature Horses (24), pony breeds (12), Quarter Horses (12), Friesians (6), American Paint Horses (3), Arabians (3), Belgians (3), Warmbloods (3), Tennessee Walking horses (3), and Morgans (3). Sixty-one percent were female, while 31% were geldings, and 7% stallions. Fifty-eight percent survived to discharge, while 41% died or were euthanized. Degree of hyperlipemia was not associated with survival. The most common primary diagnosis was enterocolitis (36%); 17% were diagnosed with a form of colic (medical or surgical), while other diagnoses included primary hyperlipemia, botulism, pneumonia, dystocia, guttural pouch empyema, and lymphoma. Thirty-three percent were treated via voluntary alimentary intake, 28% were administered intravenous dextrose (IVD), 15% were administered enteral nutrition via nasogastric tube (EN-NGT), and 11% were administered both IVD and EN-NGT. Thirteen percent were treated with partial parenteral nutrition, often in combination with nasogastric feeding and intravenous dextrose. Fourteen percent were treated with insulin. Average duration of hospitalization was 5.7 days. Hyperlipemia in hospitalized equids is a common comorbid disorder and does not necessarily require expensive or intensive treatment. Prognosis for survival was fair and not associated with the degree of hypertriglyceridemia.

F01

Risk Factors Associated with Mycobacterium avium Subsp. paratuberculosis Herd Status in Québec Dairy Herds

Maria Puerto-Parada[1], Juan Carlos Arango-Sabogal[2], Julie Paré[3], Élizabeth Doré[2], Geneviève Côté[4], Vincent Wellemans[5], Sébastien Buczinski[6], Jean-Philippe Roy[7], Olivia Labrecque[8], Gilles Fecteau[2]
[1]Université de Montréal - Faculté de médecine vétérinaire, Saint-hyacinthe, PQ, Canada, [2]Université de Montréal - Faculté de médecine vétérinaire, St-Hyacinthe, PQ, Canada, [3]Agence canadienne d'inspection des aliments/Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, PQ, Canada, [4]Ministère de l'Agriculture, des Pêcheries et de l'Alimentation, Québec, PQ, Canada, [5]Université de Montréal - Faculté de médecine vétérinaire, Saint-Hyacinthe, PQ, Canada, [6]Université de Montréal, Saint-Hyacinthe, PQ, Canada, [7]Université de Montréal - Faculté de médecine vétérinaire, Saint Hyacinthe, PQ, Canada, [8]Laboratoire d'épidémiosurveillance animale du Québec/ Université de Montréal - Faculté de médecine vétérinaire, Saint-Hyacinthe, PQ, Canada

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the etiological agents of paratuberculosis, a chronic and contagious enteric disease of ruminants. Economic losses and the potential role of MAP in Crohn';s disease in humans, justify the study of paratuberculosis. Management practices that limit exposure of susceptible animals to MAP are more effective at reducing disease prevalence than testing and culling infected cows. The objective of this retrospective case-control study was to study the association between management practices and MAP status in dairy herds in Québec. Twenty-six case herds (MAP isolated from at least 1 environmental sample) and 91 control herds (no clinical cases of paratuberculosis and negative on 2 consecutive yearly environmental samplings) were selected among herds enrolled in the Québec Voluntary Paratuberculosis Control Program. Exposure was measured using a risk assessment questionnaire, completed at enrolment. Culture of MAP was achieved using the BACTEC 960 detection system. Multivariable logistic regression was used to evaluate the association between risk factors and MAP herd status. Herd size was significantly associated with a positive MAP herd status (OR = 1.17; 95 % CI: 1.02 - 1.33). Farms buying more than 4 % of the cows in their herds per year in the last 5 years have significantly greater odds of being MAP positive compared to closed herds (OR = 5.44; 95 % CI: 1.23 - 23.98). These risk factors are consistent with the literature and should be prioritized in control programs.

F02

Risk Factors Associated with Bovine Leukemia Virus Infection in Dairy Herds in Atlantic Canada

Emily John, Greg Keefe, Marguerite Cameron, Henrik Stryhn, J McClure
Atlantic Veterinary College, Charlottetown, PI, Canada

Over the past 30 years the prevalence of bovine leukemia virus (BLV) infection has increased in North America, including Atlantic Canada, at both the herd and individual cow levels. This has occurred despite increased awareness of the disease and its deleterious effects and in the face of management practices aimed at reducing disease transmission.
Our objective was to identify risk factors associated with the within-herd prevalence of BLV-infected cows. We hypothesized that, as well as previously established risk factors, management practices associated with calf rearing and fly control would affect within-herd BLV prevalence.

A risk assessment and management program questionnaire (RAMP-Q) was developed and distributed to all bovine veterinarians in Atlantic Canada. All dairy farms shipping milk and who had bulk tank milk (BTM) samples collected in January and April 2017 (n=605) were eligible to participate in the RAMP-Q. BTM samples were tested with ELISA for levels of anti-BLV antibodies to estimate within-herd prevalence. RAMP-Q results were combined with demographic information collected from each farm and with the mean BTM ELISA results. Multivariable linear regression was performed to investigate the association between RAMP-Q risk factors and within-herd prevalence of BLV infection.

One hundred RAMP-Qs were returned, with participants from each province in Atlantic Canada (New Brunswick, 22; Newfoundland, 3; Nova Scotia, 49; Prince Edward Island, 26). Factors significantly associated with increasing level of within-herd BLV prevalence included history of clinical BLV within the herd (23.7% increase; 95% CI 6.8-39.9%) and history of purchasing BLV-suspect cattle (19.0% increase; 95% CI 3.4-37.0%). No significant difference in within-herd prevalence was associated with needle and injection (P-value 0.69), fly control (P-value 0.56), or rectal sleeve practices (P-value 0.48), or the use of bulls for natural breeding (P-value 0.81).

Important factors associated with increasing within-herd BLV prevalence include a history of prior diagnosis of clinical BLV and history of purchasing cows of unknown BLV status. In this study, there was no significant association of any previously established risk factors with increasing within-herd prevalence of BLV infection.

F03

The Nemabiome: A New Tool for the Small Ruminant Clinician

Michel Levy[1], Camila de Queiroz[2], Elisabeth Redman[3], Fabienne D. Uehlinger[4], John Gilleard[3]
[1]University of Calgary, Calgary, AB, Canada, [2]University of Calgary, department of Comparative Biology & Experimental Medicine, Calgary, AB, Canada, [3]University of Calgary department of Comparative Biology and Experimental Medicine, Calgary, AB, Canada, [4]Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, Saskatoon, SK, Canada

Gastro intestinal nematodes (GIN) have a significant impact on small ruminant production. Co-infection with multiple species of parasites are common and different parasites have different pathogenesis so investigation of the different populations of GIN is important to plan control strategies, understand interactions between parasites and the host as well as surveillance of regional diversity and determination of the specific populations involved in anthelmintic resistance. Several methods of identification are available, such as fecal culture and morphologic examination of the larval stage of the parasite or PCR, however these methods are time consuming, have a low throughput and/or require specific expertise.

We originally developed deep amplicon sequencing of the nematode rDNA ITS-2 (the Nemabiome) to quantify the species composition of cattle GIN and we have recently adapted and validated its use in small ruminants. Field applications of the Nemabiome have included epidemiological studies of the nematode species affecting sheep flocks in Western Canada (fig.1) the identification of the parasite populations resistant to anthelmintics as a complement to the Fecal Egg Count Reduction Test (fig.2). The information gathered using deep amplicon

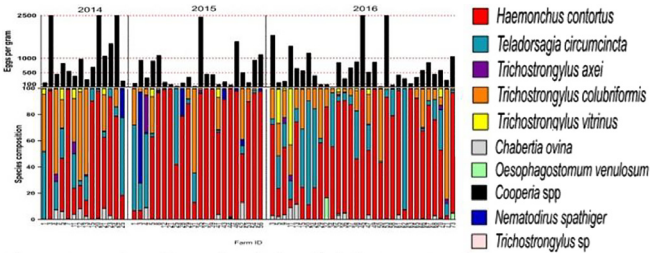


FIGURE 1 Nematode species prevalence in sheep flocks in Western Canada 2014-2016

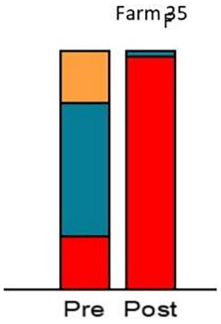


FIGURE 2 Example of Nematode composition pre and post-treatment with fenbendazole

sequencing provides important qualitative and quantitative information about the epidemiology and resistance status of the different species of gastrointestinal parasites, which is essential to make informed decisions about treatment and control. We are currently developing the nemabiome approach to screen directly for anthelmintic drug resistance mutations in GIN populations.

F04

The Nasopharyngeal Microbiota of Pre-Weaned Dairy Calves with and without Ultrasonographic Lung Lesions

Sarah M. Raabis, Theresa Ollivett, Garret Suen, Allison Quick
University of Wisconsin-Madison, Madison, WI, USA

The purpose of this prospective case-control study was to describe bacterial communities in the nasopharynx (NP) of preweaned dairy calves with and without ultrasonographic lung lesions. Additional objectives included evaluating the effects of previous antibiotic therapy and age on the NP microbiota composition. A total of 257 Holstein heifer calves were enrolled into a separate study investigating the genomics of resistance to bovine respiratory disease (BRD) over a 4-week follow up period. Calves were examined twice using thoracic ultrasound and clinical respiratory score (early exam: 4 weeks old; late exam: 7 weeks old). From this population, case and age-matched controls were selected to undergo deep NP swabbing for the current study. Cases were defined by the presence of significant ultrasonographic lung lesions (≥ 1 lobe completely consolidated). Controls were defined by the lack of these lesions.

The NP swabs were taken at the time of exam and calf information was collected from farm management software. Swabs were placed in phosphate buffer solution, and stored at -80°C. Following DNA extraction, the V4 region of the 16S rRNA gene was amplified using PCR. Libraries were sequenced using the MiSeq platform. The

generated sequences were processed through Mothur 1.36.1 and outputted taxonomy and diversity data were analyzed in RStudio. Diversity data was analyzed using t-tests and general linear models. Comparisons of relative abundance (RA) of genera between groups were performed using Kruskal Wallis tests. Multiple linear regression was used to investigate the impact of time point of exam and antibiotic treatment, in the month prior to examination, on RA of genera.

In total, 50 swabs (1:1 case to control) were collected. Two swabs were lost during transport, therefore, 48 swabs (cases = 23, controls = 25) obtained from 44 calves, were used for analysis. Thirty-five (73%) swabs were collected during the late exam. Overall, the most common genera were *Acinetobacter* sp, *Escherichia* sp, *Mycoplasma* sp, *Pasteurella* sp and *Psychrobacter* sp. Alpha diversity was not different between cases and controls (P = 0.78), nor between early and late time points (P = 0.37). The RA of *Mycoplasma* sp. was higher in controls (P = 0.03) and the RA of *Pasteurella* sp. tended to be higher in controls (P = 0.08). The RA of both *Mycoplasma* sp. and *Pasteurella* sp. was not affected by exam time point or antibiotic treatment (P > 0.75 and P > 0.12, respectively).

To the authors'; knowledge, this is the first study to evaluate the community dynamics of the NP microbiota from calves with and without ultrasonographic lung lesions. We did not expect to identify a higher RA of *Mycoplasma* sp. in the control calves, as previous studies have shown a higher RA of *Mycoplasma* sp. in calves with BRD compared to healthy calves. Future studies are needed to better understand this discrepancy, including metagenomics studies to infer speciation and determine pathogenicity of the identified *Mycoplasma* genus. It would also be important to know if the duration of ultrasound lesions affected the RA of pathogenic bacteria. This study helps to demonstrate the complexities of identifying NP microbiota changes associated with disease status. This may impact future attempts to utilize NP microbiota characteristics in diagnosis and treatment of BRD.

F05

Evaluation of a Rapid Immunoassay for Detection of Cryptosporidium parvum in Bovine Calf Fecal Samples

Carlie Stilwell1, Sierra Guynn[2], David Lindsay[2], Brandy A. Burgess[1]
[1]University of Georgia, Athens, GA, USA, [2]Virginia Polytechnic and State University, Blacksburg, VA, USA

Cryptosporidium parvum is not only an occupational exposure risk for veterinary personnel, but poses a risk to other patients. In general, C. parvum oocysts are environmentally hardy and are resistant to standard disinfection. As such, rapid detection and early implementation of preventive measures are key in risk reduction. The objective of this study was to evaluate a commercially available rapid test for detection of C. parvum in comparison to an immunofluorescent antibody test (IFA) when used to test bovine fecal samples.

A diagnostic test evaluation was performed. Fecal samples were collected per rectum from calves less than 6 months of age (n=119). The study population was stratified on disease status - healthy or scouring. Two-grams of each sample were fixed and tested via a commercially available rapid test marketed for use in human healthcare, and IFA, the recognized gold standard testing method. Isolates were submitted for genetic typing.

Overall, there was moderate agreement among the tests evaluated (kappa statistic = 0.52 [95%CI 0.30, 0.75]) with the prevalence of test-positive samples being higher when using the rapid test (16.8%; 20/119) as compared to IFA (9.2%; 11/119). Among samples with health status recorded (n=113), cryptosporidium was identified more commonly among scouring calves (14.3% rapid test; 21.4% IFA) than healthy calves (9.1% rapid test; 17.2% IFA), irrespective of the testing method used.

This evaluation demonstrates that this rapid test may be a useful tool in managing this risk in the hospital setting, and shows the relatively high prevalence of cryptosporidium among apparently healthy calves.

F06

Abomasitis in Calves: Retrospective Study of 20 Cases (2006-2016)

Eloi Guarnieri[1], Gilles Fecteau[2], Julie Berman[1], Desrochers André[2], Marie Babkine[2], Sylvain Nichols[2], David Francoz[3]
[1]Université de Montréal - Faculté de Médecine Vétérinaire, Saint-hyacinthe, PQ, Canada, [2]Université de Montréal - Faculté de médecine vétérinaire, St-Hyacinthe, PQ, Canada, [3]Université de Montréal, Saint-Hyacinthe, PQ, Canada

Abomasitis is a pathological condition reported in calves. The current description is mainly limited to post-mortem findings. The objectives of this retrospective study are to describe clinical presentation and outcomes in calves with a primary ante-mortem diagnosis of abomasitis.

Medical archives from the Centre Hospitalier Universitaire Vétérinaire of the Université de Montréal from 2006 to 2016 were searched using terms referring to abomasum. After exclusion of calves with clinical co-morbidity factors and/or older than three months old, twenty calves with clinical diagnosis of abomasitis were included. The median age was 2.5 days old (range: 0-30 days). Most calves were Holstein (19/20). The median clinical duration prior to admission was 24 hours (range: 0-4 days). Abomasal tympany (20/20), positive succussion (11/12), abnormal mucous membrane color (13/15), dehydration (12/14), depression (14/17), recumbency (13/16), tachycardia (14/19; range 80-240 beats/min) and tachypnea (12/19; range 32-128 breaths/min) were the most frequent clinical signs noted. Hematological and biochemical profiles revealed hyperlactatemia (13/13; range: 3.3-16.3 mmol/L), hypochloremia (12/16; range 77.8-106 mmol/L), left shift (13/19), neutrophils'; toxic changes (10/15), hypoglycemia (11/17; range: 0.3-10.9 mmol/L), hyperkaliemia (9/18; range: 3.6-8.3 mmol/L), acidemia (6/13; range: 6.8-7.5) and neutropenia (5/15; range: 1.6-22.7 cellsx10^9/L). Based on univariate statistical analysis, fatal outcome was significantly ($p < 0.05$) related to dehydration, recumbency, hypothermia, metabolic acidosis, hypoproteinemia, leucopenia and neutropenia. Fatality rate was 60% (12/20). Necropsy was performed on 11 calves and confirmed the clinical diagnosis.

Abomasitis has a guarded prognosis in calves. Clinical and clinicopathologic findings were shown to be associated with a fatal outcome.

F07

Investigation of the Fecal Virome of Neonatal Dairy Calves with Diarrhea

Diego Gomez-Nieto[1], Laura Avellaneda-Franco[2], Alejandro Reyes[2], Luis Arroyo[3], J. Scott Weese[4]
[1]University of Florida, Gainesville, FL, USA, [2]Department of Biological Sciences, Universidad de los Andes, Bogotá, Colombia., Bogota, Cundinamarca, Colombia, [3]Ontario Veterinary College, Guelph, ON, Canada, [4]Dept of Pathobiology Ontario Veterinary College University of Guelph, Guelph, ON, Canada

The concept of the existence of a "gut virome" is very recent although the presence of viruses as pathogens in calf intestine has been documented for more than 40 years. In contrast to the bacterial microbiota, little is known regarding the development, establishment and factors that modify the gut virome during the neonatal period. The objective of this study was to characterize the fecal virome of healthy and diarrheic neonatal dairy calves.

Twenty diarrheic calves (DC) (ages of 1 to 30 days) and 20 age-matched healthy control calves (HC) were enrolled. Viral nucleic extraction was performed using a commercial Kit. Synthesis of viral cDNA was completed using a primer containing a fixed 18bp plus a random nonamer. Twenty samples from HC were mixed in equimolar quantities in 4 sets as well as 20 DC samples. The 8 total pooled samples were sequenced using MiSeq-lllumina-Platform. Raw sequences quality was assessed using FastQC. Then, low-quality reads were removed with Trimmomatic. CD-HIT-EST was used to cluster reads with >90% sequence identity. The five more representative sequences of each cluster were used to perform a *de novo* assembly using SPADES. LefSe was used to obtain molecular markers, which can be used in diagnosis.

Caudovirales viruses, mainly viruses belonged to Myoviridae family, were found in all pools. A total of 223 contigs that could be associated with neonatal calf diarrhea were identified but these had no available annotation with any database. Sequences belonging the Retroviridae family were found predominantly associated to all the DC. *Escherichia* phage was found in 3 of the 4 pools that composed the DC group. HC shared Enterobacteria *phage phiX174 sensu lato*, and the DC shared *Escherichia coli* O157 typing phage 3.

Our results demonstrate the limited knowledge of bovine-associated viral sequences and the limitations of existing annotation datasets. Detection of sequences associated with viruses could support the hypothesis that gastrointestinal diseases in neonatal calves are a consequence of complex interactions between several microorganisms in the gut.

F08

Use of a Digital Brix Refractometer to Estimate Serum Immunoglobulin in Goat Kids

Rachel E. Oman[1], Robert Streeter[2], Jared Taylor[2], Lindi Gilliam[2], Lionel Dawson[2]
[1]University of Missouri, Columbia, MO, USA, [2]Oklahoma State University, Stillwater, OK, USA

The objective of this study was to evaluate the utility of a digital Brix refractometer for estimating serum immunoglobulin concentration in neonatal goats.

Blood was collected from 30 Alpine goat kids at 0-12 hours of age prior to receiving any colostrum, at 24-48 hours of age after receiving colostrum, and at 7-14 days of age. Serum was harvested and the protein measured using a digital Brix refractometer, an optical refractometer, and the Beckman colorimetric method at a commercial laboratory. Linear regression and correlation coefficient analysis was performed for all two-way results: Brix x optical, Brix x laboratory colorimetric, and optical x laboratory colorimetric.

There was significant relationship between the three serum protein analysis methods with a high degree of positive correlation between the digital Brix and optical refractometer ($P < 0.0001$; $r = 0.87$), and a moderate positive correlation between the laboratory colorimetric measurements and the digital Brix ($P < 0.0001$; $r = 0.50$) and optical refractometer ($P < 0.0001$; $r = 0.62$). Regression analysis showed a digital Brix percentage of 8.4% could be used to predict a serum protein of 5.4 g/dl and thus adequate passive transfer of maternal immunoglobulin.

The digital Brix refractometer demonstrated utility for point-of-care estimation of serum immunoglobulin to assess passive transfer status in goat kids.

F09

Prognostic Indicators in Downer Cows Presented to a Hospital: Retrospective Study (1472 cases)

Marie-Eve Bilodeau[1], David Francoz[2], Desrochers André[1], Sylvain Nichols[1], Marie Babkine[1], Juan Carlos Arango-Sabogal[1], Gilles Fecteau[1]
[1]Université de Montréal - Faculté de médecine vétérinaire, St-Hyacinthe, PQ, Canada, [2]Université de Montréal, Saint-Hyacinthe, PQ, Canada

Survival rate of downer cows treated on farm or in a hospital setting is estimated between 11.3% and 50%. Identifying prognostic indicators could be useful in reducing unnecessary treatments on animals with a poor prognosis.

The primary objective of this study was to predict prognosis associated with downer cows treated at a referral hospital. A secondary objective was to identify prognostic factors associated with the outcome.

This retrospective study included all recumbent dairy cattle older than 15 months that were presented between 1994 and 2016 (n=1472 cases). Information regarding history (age, lactation status, duration of recumbency), hospitalization (outcome, duration, number of days of flotation therapy, diagnosis) and blood analysis results (CBC, biochemistry) were obtained. A multivariable logistic regression model was built to explore the association between hematology and biochemistry parameters and survival.

Overall survival rate was 50.1%. Cows that survived were significantly younger and were recumbent for a shorter period of time. They had lower fibrinogen values and higher neutrophils counts at arrival than cows that did not survive. Finally, cows that survived had significantly lower values of AST, CK, BUN, creatinine, and phosphorus and higher total CO_2 value at arrival than cows that did not survive. An increase in fibrinogen, AST, and creatinine values were significantly associated with lower odds of survival.

We concluded that the overall survival rate of downer cows treated in-hospital with flotation therapy is approximatively 50%. Further,

some historical elements, CBC and biochemistry values can be used to select cases with a better chance of survival.

F10

Pharmacokinetics of Tulathromycin in the Fetal and Maternal Compartments of Sheep

Evelyn MacKay
Texas A&M University, College Station, TX, USA

Macrolides are important antimicrobials that may be useful agents for the control of infectious ovine abortion, which has economic, animal health, and human health impacts. Pregnant ewes were given a single dose of 2.5mg/kg tulathromycin subcutaneously and drug concentrations were measured in fetal plasma, maternal plasma, and amniotic fluid. Catheters were surgically placed in the fetal vasculature and the amnion at 115 (+ / - 2) days gestation for sampling at 4, 8, 12, 24, 36, 48, 72, 144, and 288 hours after administration. Maternal plasma samples were collected at time points of 0, 2, 4, 6, 8, 12, 24, 36, 48, 72, 144, and 288 hours. Serum concentrations and pharmacokinetic values in the ewe were similar to those previously reported in non-pregnant ewes. Tulathromycin was present in fetal plasma and amniotic fluid, indicating therapeutic potential for use against organisms in these compartments, though concentrations were lower than those found in maternal plasma. Notably, the pharmacokinetics in the fetus were quite different than those found in the ewe, with plasma concentrations reaching a plateau at 4 hours and remaining at this concentration for the remainder of the sampling period (288 hours), raising questions about how this drug is handled and metabolized in the fetus.

F11

Sodium Iodide as a Preventative Strategy against Respiratory Disease in Pre-Weaned Dairy Calves

Lisa Gamsjaeger[1], Gary McArthur[2], Meera Heller[1], Brian Vander Ley[3]
[1]UC Davis, Davis, CA, USA, [2]Swinging Udders Veterinary Clinic, Elk Grove, CA, USA, [3]UNIVERSITY OF NEBRASKA-LINCOLN, Clay Center, NE, USA

Bovine Respiratory Disease (BRD) remains a major economic problem in the dairy industry and the aim of preventing disease as well as reducing antimicrobial use warrants evaluation of potential alternatives. The LPO/H_2O_2/I^- system is able to inactivate common bacterial and viral BRD pathogens *in vitro*. Administration of oral sodium iodide (NaI) significantly increases the I^- concentration of respiratory fluids in pre-weaned dairy calves, suggesting potential as a preventative strategy against respiratory disease. The objective of this study was to evaluate the effect of orally administered NaI on BRD treatment frequency and weaning weights of pre-weaned dairy calves.

427 female pre-weaned dairy calves, aged 18 (±2) days and housed in individual hutches on a ranch in central California, were used for this study.

The calves were divided into treatment (20mg/kg NaI orally on day 0 and day 4) and control groups (no treatment). A subset of calves of both groups (70 treatment calves and 70 control calves) were given respiratory and ultrasound scores on day 0 (baseline) and day 7. Medical treatments were recorded for the entire study period and all calves were weighed at weaning.

Ultrasound scores, change in ultrasound scores from baseline and respiratory scores of treatment calves on day 7 were significantly higher (P=0.001, P=0.0127 and P=0.0243, respectively) than those of control calves. In addition to having higher respiratory and ultrasound scores, treatment calves had higher odds of being treated for respiratory disease (OR 2.04, P=0.0003) than controls. Despite these apparent increases in respiratory disease in the treatment group, average daily gain and weaning weights did not differ between groups.

The findings of this study do not support the use of oral NaI as a preventative strategy against respiratory disease in pre-weaned dairy calves.

F12

Accuracy of Systematic Thoracic Ultrasound for Diagnosis of Active Pneumonia in Pre-Weaned Calves

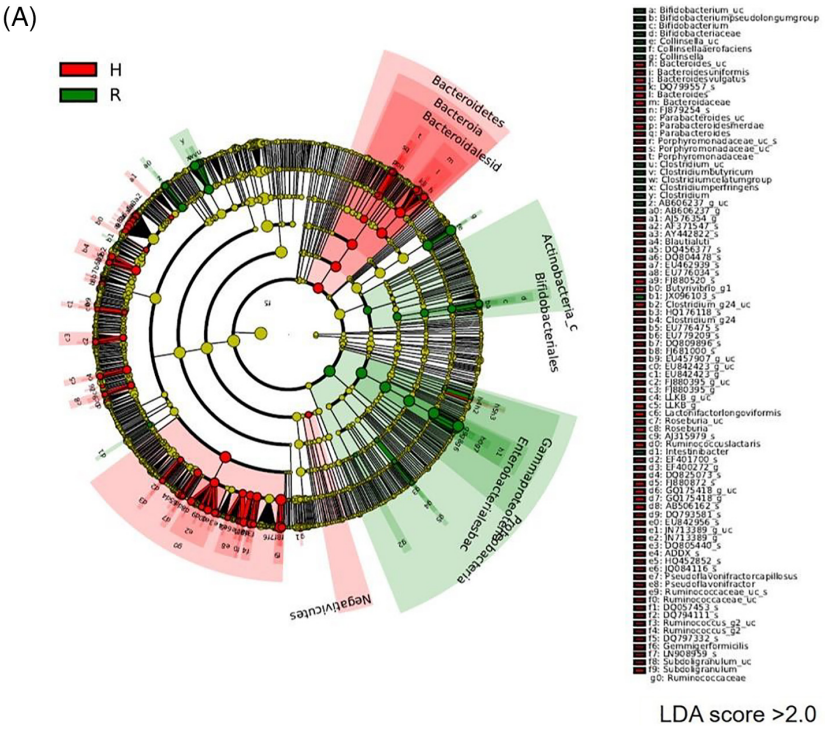
Julie Berman[1], David Francoz[2], Simon Dufour[2], Sébastien Buczinski[2]
[1]Université de Montréal - Faculté de Médecine Vétérinaire, Saint-hyacinthe, PQ, Canada, [2]Université de Montréal, Saint-Hyacinthe, PQ, Canada

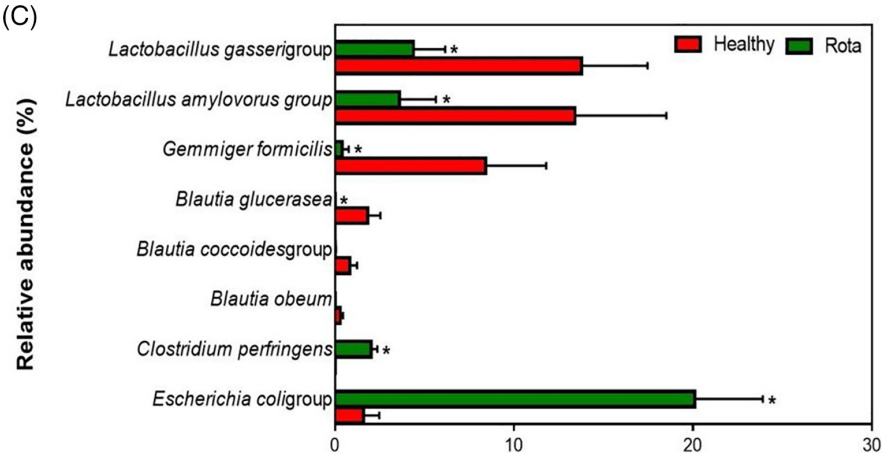
Among the different presentations of bovine respiratory disease, active pneumonia (**ActP**) (defined as an infection of the lower airway with signs of inflammation) is the most important to diagnose to institute appropriate treatments. Thoracic ultrasonography (**TUS**) seems promising but data are scant regarding its accuracy for diagnosis of ActP. The objective of this study was to estimate the accuracy of TUS (positive if maximal depth of consolidation (**DEPTH**) ≥3cm) compared to reference tests (Wisconsin Clinical Respiratory Scoring Chart (**CRSC**, positive if ≥5) and serum haptoglobin concentration (**Hap**, positive if ≥15mg/dL)), for the diagnosis of ActP in pre-weaned calves, using a latent-class model methodology (**LCM**). One population of veal calves (n=209) and one of dairy calves (n=301) were enrolled. TUS, CRSC and Hap (imperfect tests) were performed at the same day, in each calf. Bilateral TUS was performed by screening the mid to ventral portion of the lung caudal of the heart (caudal sites) as well as the right parenchyma cranial to the heart (cranial site). The DEPTH on TUS was recorded and noted separately for caudal and cranial sites. The accuracy of TUS was estimated by LCM for three tests conducted in two populations. The ActP prevalence in both populations was low (0.05). Adding the cranial site had little effect on TUS accuracy. Using DEPTH ≥3cm for caudal sites only, posterior sensitivity and specificity median estimates of 0.88(95%BCI: 0.52-1.0) and 0.95(95%BCI: 0.92-0.98), respectively, were observed. In conclusion, we suggest using a DEPTH ≥3cm caudal to the heart to detect ActP.

F13

Gut Microbiota in Diarrheic Calf with Bovine Rotavirus infection in Korea

Jayoung Jang[1], Suhee Kim[2], Sukhan Jung[3], Jinhee Kang[3], Hayoung Yang[4], Dong-In Jung[5], Do-Hyeon Yu[5], Hakjong Choi[6], Jinho Park[3]
[1]World institute of Kimchi, Gwangju, Cholla-namdo, Republic of Korea, [2]National Institute of Animal Science, Rural Development Administration, Wanju, Cholla-bukto, Republic of Korea, [3]College of Veterinary Medicine, Chonbuk National University, Iksan, Cholla-bukto, Republic of Korea, [4]College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju, Kyongsang-namdo, Republic of Korea, [5]College of Veterinary Medicine, Gyeongsnag National University, Jinju





52828, South Korea, Jinju-si, Kyongsang-namdo, Republic of Korea, [6]World institute of kimchi, Gwangju, Cholla-namdo, Republic of Korea

Neonatal diarrhea accounts for more than 50% of total deaths in dairy calves. Few population-based studies of calve have investigated how the microbiota is impacted during diarrhea. In this study, to characterize the fecal microbiota of the microbial communities and identify possible relationships of the microbiota profiles with health status. Five rotaviral diarrheic calves between the ages of 1 and 30 days and 10 age-matched healthy control calves were enrolled. Viral infection was confirmed by RT-PCR, and feces from five calves without *E. coli* K99+, Salmonella, Cryptosporidium, Coronavirus infection were selected. The Illumina MiSeq sequencer was used for high-throughput sequencing of the V3-V4 region of the16S rRNA gene. Significant differences in community membership and structure were identified between healthy and rotaviral diarrheic claves. Based on linear discriminant analysis effect size (LEfSe), the representative genera from *Lactobacillus*, *Subdoligranulum*, *Blautia*, *Bacteroides*, *Escherichia* and *Clostridium* were closely affiliated to rotaviral diarrhea in calves. Results presented here provide new information regarding the intestinal microbiota of rotaviral diarrhea in calves and its association with health status. Fecal microbial diversity was also associated with disease status such as diarrhea. Results suggesting a possible beneficial effect of *Lactobacillus spp.* on health and growth are promising.

F14

Regression of Squamous Cell Carcinoma in a Mature Bucking Bull and Longhorn Cow Using Immunocidin©

Jennifer L. Halleran, Robert Streeter
Oklahoma State University, Stillwater, OK, USA

Ocular squamous cell carcinoma (OSCC) is the most common malignant neoplasia of beef cattle causing significant economic losses to

cattle producers. Genetic selection for ocular pigmentation can reduce the prevalence in certain breeds. Surgical excision with clean margins can be curative. Non-resectable lesions are very difficult to manage and treatment modalities for such cases (radiation therapy and photodynamic therapy) are limited to tertiary referral hospitals. Recently, an immunomodulating drug has been approved for mixed mammary carcinoma in dogs and cats and sarcoids for horses via intralesional injection. The product (Immunocidin©) is a mycobacterial cell wall extract with immunomodulating effects. It works as a non-specific immunotherapy activating natural killer cells, monocytes and macrophages. This report describes the use of Immunocidin© in two mature bovines with non-resectable OSCC. These animals were considered companion animals and the clients agreed they would not enter the food chain. A 3-year-old bucking bull was examined for a rapidly growing mass of the lower lid and medial canthus of two-weeks duration. An extirpation was performed under local blocks and sedation. Reoccurrence was noted at suture removal and the mass was debulked with cryotherapy applied. Aggressive reoccurrence occurred within two weeks. A total of four intralesional injections of Immunocidin© were administered at about two week intervals. After 9 months of therapy, the bull has regression of OSCC. The bull continues to do well with no reoccurrence at this time, 13 months after the first appointment. A 12-year-old Longhorn cow was examined for an extensive ocular and periocular mass of several months duration. An extirpation and regional debulking was performed under general anesthesia; intralesional injection of Immunocidin© was performed at initial surgery and twice more at 3 week intervals. Initial results were promising, however the owner reported that the Longhorn';s health regressed; she was having difficulty chewing, hearing, developed a head tilt and could not see from her remaining eye. There was no external growth from the surgical site. She was found dead in pasture one morning, three months after the initial surgery. This is a limited case series with the hopes of utilizing Immunocidin© in more OSCC cases to further our understanding of treatment with this agent. This may be an appropriate alternative treatment for economically or emotionally valuable animals that will not be consumed.

F16

Efficacy of Mycobacterium Cell-Wall Fraction on Influx of Polymorphonuclear Cells in Uterus of Dairy Cows

Aleksandar Milovanovic[1], *Gabriel Bo*[2], *Pablo Chesta*[2], *Tomislav Barna*[3], *Jelena Apic*[3], *Marina Stosic*[4], *Bojana Prunic*[4], *Aleksandar Masic*[5]
[1]*NovaVive Inc/Ontario veterinary College, Unevirsity of Guelph, Belleville, ON, Canada*, [2]*Instituto de Reproducción Animal Córdoba (IRAC), Cordoba, Cordoba, Argentina*, [3]*Scientific Veterinary Institute Novi Sad, Serbia, Novi Sad, Vojvodina, Serbia*, [4]*Scientific Veterinary Institute Novi Sad, Novi Sad, Vojvodina, Serbia*, [5]*NovaVive Inc, Belleville, ON, Canada*

The main objective of the present study was to evaluate the efficacy of three different doses of Mycobacterium Cell Wall Fraction (MCWF; Amplimune™) following intrauterine (IU) infusion as chemoatractant in dairy cows without signs of clinical endometritis. In addition, the functional activity of polymorphonuclear leukocytes (PMNL) attracted by the MCWF was measured byReactive Oxidative Species (ROS) tests following uterus lavage.We hypothesised that influx of functional PMNLs in uterus can be achieved by different MCWF concentrations in dose-dependent manner following IU administration. It is anticipated that increased presence of active PMNLs would have beneficial effect on the reproductive performance in dairy cows by reducing the incidence of clinical and subclinical endometritis at the end of histological puerpuerium thus improving conception rate.
Study was conducted on commercial dairy operation with 1200 milking Holstein cows. Experimental study protocol was reviewed and approved by institutional Animal Care Committee prior to the study start. Forty lactating cows in average 45.8 days postpartum were selected for the study purposes. Inclusion criteria included history of easy calving, no retained placenta, absence of signs of clinical endometritis and average body condition score of 3 (1 to 5 scale). Prior to MCWF treatment, baseline status of uterine lining was determined by cytology examination. Stained slides were evaluated under microscope to determine the percentage of PMNL's per 300 cells. Following cytology assessment, cows were randomly assigned in four experimental groups with 10 cows per group. On day 0, cows in control group were treated IU with 0.33g of oyster-glycogen (proven PMNL attractant), dissolved in 60 ml 0.9%NaCl while animals in MCWF groups received IU dose of either 2.5ml, 5ml or 10ml MCWF diluted in 0.9% NaCl to a total volume of 60ml. Twenty-four hours later, uterine cytology sample from same spot (*corpus uteri*) was taken and subjected to new cytomorphology analysis and PMNL counts. In addition, uterine lavage samples were obtained immediately after second cytology sampling. PMNL activity was analyzed by Flow-Automated-Cell Sorting (FACS) using Phagoburst® commercial kit within 4 hours post collection. The strength of the respiratory burst (Oxidative burst index-OBI) of individual cells was calculated by multiplying percentage of oxidative burst positive PMNL and their mean fluorescence intensity (MFIs)) using following formula OBI = [(% oxidative burst positive cells) × (MFI)] / 100). All data were subjected to statistical analysis using one-way ANOVA followed by Kruska-Wallis multiple comparison test.
None of the cows had elevated PMNL counts prior to the treatment as determined by cytology. At 24h post treatment PMNL counts in all groups were elevated, however, and statistically significant differences were noted only in groups receiving 5 and 10 ml of MCWF ($p < 0.0012$ and $p < 0.0048$ respectively). Even though there were no statistically significant differences observed in control and 2.5ml MCWF groups there was a trend in PMN increase with 54% and 14% increase respectively ($p < 0.052$ and $p < 0.16$) compared to day 0. FACS analysis on ROS activity revealed similar ROS % for all experimental groups (39.7, 37.6, 40.6 and 38.9% for control, 2.5,5 and 10ml MCWF groups, respectively). Calculated OBI demonstrated that cows receiving 5 and 10ml of MCWF had the highest reaction (OBI=779.7 and 812.02 respectively) compared to control (OBI=578.8) and 2.5ml MCWF (OBI=221.9). Data on reproductive performance showed that 9/10 cows in 2.5 and 5ml MCWF groups and 7/10 cows in 10ml MCWF and control group were pregnant at 200 days post insemination. In total 3 cases of subclinical and 1 case of clinical endometritis were observed in all MCWF treated cows (4/40) while cows in control group were diagnosed with 2 subclinical and 2 clinical cases (4/10) within 60 days post treatment. Overall, MCWF has a potential to attract significant numbers of PMNs in uterus following IU administration. MCWF attracted PMNs have a stronger oxidative burst activity and could lead to accelerated clearance in case of the postpartum infection. Dose of 5ml MCWF appears sufficient for IU administration.

Additional larger studies are underway to demonstrate efficacy of 5ml MCWF dose in prevention and treatment of subclinical and clinical endometritis in dairy cows.

F17

Physiologically-Based Population analysis for Determination of Milk Discard Times for Intra-Mammary Drugs

Andrew Woodward, Ted Whittem
The University of Melbourne, Werribee, Victoria, Australia

The pharmacokinetics of intra-mammary drugs are complex and novel physiologically-based pharmacokinetic models for the mammary gland provide a useful description of processes contributing to the time course of drugs in milk. A major application for milk drug pharmacokinetics is the determination of milk discard times. This study utilized a previously-unpublished dataset describing milk pirlimycin concentrations from 20 cows with clinical mastitis, treated with the label-recommended dose regimen, with pirlimycin milk concentrations determined in triplicate with a microbiological method. The milk pirlimycin concentrations and volumes of milk production from each milking were analyzed with a minimal physiologically-based pharmacokinetic model. The model included a compartment for each quarter and predicted milk pirlimycin concentrations at the quarter level and the whole gland. Log-normal probability distributions for the pharmacokinetic parameters were estimated using the stochastic-approximation expectation-maximization method in Monolix. A hypothetical population of cows was generated using a Monte Carlo simulation, and the milk discard time determined from the simulated population. The result of this method was compared to the safe-concentration-by-linear-regression, safe-concentration-per-milking, and time-until-safe-concentration methods recommended by regulatory agencies. Comparable milk discard times were generated from all methods, which suggested a longer milk discard time is necessary for this cohort of cows with mastitis. The prediction of the population method was sensitive to the range of milk production volume selected for the simulation. This novel method utilized all available data, including data below the assay quantification limit. The study demonstrated the viability of a population physiologically-based model for estimation of milk discard times.

F18

Effect of Oral Iodide Supplementation on Iodine Concentration in Respiratory Fluids of Pre-weaned Dairy Calves

Lisa Gamsjaeger[1], Meera Heller[1], Heather Knych[2]
[1]UC Davis, Davis, CA, USA, [2]K.L. Maddy Equine Analytical Chemistry Laboratory, Davis, CA, USA

Augmenting innate mucosal defense mechanisms with iodine is effective at killing bovine bacterial and viral respiratory pathogens *in vitro* and could be a valuable preventative strategy against respiratory disease in dairy calves. The objective of this study was to determine the kinetics of iodine in nasal secretions following oral administration of 20mg/kg inorganic iodine (NaI), in order to assess the feasibility of using NaI to prevent bovine respiratory disease *in vivo*.

7 female pre-weaned Holstein calves aged 3-5 weeks and housed in Davis, CA, were used for this prospective clinical study. Calves consumed 20mg/kg of NaI, which was added to their milk once, after baseline samples (blood and nasal secretions) were collected (T=0). Consecutive samples were then obtained at 1,3,6,12,48 and 72 hours post-administration. Samples were submitted to a diagnostic lab for analysis of iodine concentration via mass spectrometry.

Iodine concentrations were significantly elevated over baseline levels in nasal secretions ($P = 0.0017$) and serum ($P < 0.0001$) at all time points sampled. Both serum and nasal fluid iodine concentrations peaked at 6 hours, with a serum mean concentration of 17,188ng/ml and a nasal fluid mean concentration of 56,383ng/ml. No adverse effects were noticed in any of the calves.

This study shows that oral administration of NaI increases iodine concentrations in airway fluids of pre-weaned dairy calves, to above effective concentrations *in vitro*, and could augment upper respiratory defense mechanisms. Further studies are needed to determine if sodium iodide is effective as a preventive strategy against respiratory disease in dairy calves.

F19

Pharyngeal Trauma and Perforation in Dairy Cattle: 27 Cases

Diego Gomez-Nieto[1], Desrochers André[2], David Francoz[3], Sylvain Nichols[2], Marie Babkine[2], Gilles Fecteau[2]
[1]University of Florida, Guelph, ON, Canada, [2]Université de Montréal - Faculté de médecine vétérinaire, St-Hyacinthe, PQ, Canada, [3]Université de Montréal, Saint-Hyacinthe, PQ, Canada

The objective of this retrospective study was to describe the signalment, history, clinicopathological, endoscopic, ultrasonographic, radiographic and post-mortem findings as well as treatments and outcomes of adult cattle diagnosed with pharyngeal laceration/trauma.Medical records of cattle > 1 month of age admitted to a Veterinary Teaching Hospital from January 1995 to January 2017 with pharyngeal perforation/trauma identified by oral or endoscopic examination were reviewed.

Twenty-seven cases met the inclusion criteria. In all cases but one, the primary complaint was dysphagia and swelling of the pharyngeal area. Twelve (45%) cows had a history of being administered a bolus of monensin and 5 (20%) of calcium; 3 a magnet (11%); 1 (4%) iodine; 1 aspirin; and 1 (4%) vitamins; 3 (16%) unknown bolus. Reported clinical signs included subcutaneous emphysema in 23 (85%) cows, swelling of the throatlatch in 17 (63%), pain on palpation in 17 (63%), nasal discharge in 14 (52%), foul odour from the nose and mouth in 12 (44%), ptyalism in 12 (44%), dyspnea in 15 (56%) and an upper respiratory noise in 12 (44%). Oral examination findings were reported in 22 (81%) cows. A foreign body (FB) (monensin bolus) lodged in the pharynx was observed in 5 (20%) cows while pharyngeal laceration without FB was reported in 12 (44%) cows. Endoscopic examination was performed in 21 (78%) cows. A FB lodged in the pharynx was observed in 2 cows and in 12 cows the perforation was identified in the dorsal aspect of the pharynx. Radiographs of the neck and throatlatch were performed in 21 (78%) cows and a FB was detected in 8 cows (rumensin bolus, n=6; magnet, n=2). The presence of gas, either subcutaneously or between fascial planes, emphysema of the pharyngeal region (n=20), soft tissue swelling (n=17) and pneumomediastinum (n=16) were commonly reported. Treatment was attempted

in 24 cows. Manual retrieval of the monensin bolus lodged in the pharynx was possible in 5 cows in which the FB was observed during oral exam. Surgical removal of FB was carried out in 4 cows. In 2 cows a monensin bolus was retrieved and a magnet in the other 2 cows. A combination of antimicrobial agents and analgesic anti-inflammatory drugs were administered to all 24 treated cows. Seventeen (63%) cows were discharged from the hospital whereas 10 (37%) were euthanized. Eight (46%) out of 17 surviving cows had pharyngeal trauma associated with monensin, 1 (6%) with an iodine bolus, 3 (18%) with a magnet, 1 (6%) with a vitamin bolus, and 4 (24%) with an unknown bolus. All 5 cows that suffered pharyngeal trauma associated with administration of a calcium bolus were euthanized due to the severity of the clinical signs.

Pharyngeal trauma is a rare condition in adult cattle which can cause serious morbidity and mortality if not diagnosed and treated early. Successful treatment appears to be related with the nature of the penetrating FB.

N01

Nanofiber Lined Catheter Treatment of Intracranial Gliomas in Dogs

Susan Arnold[1], *Simon Platt*[2], *Georgina Stewart*[2], *Marc Kent*[2], *Renee Barber*[2]
[1]*University of Georgia, Athens, GA, USA,* [2]*University of Georgia Veterinary Teaching Hospital, Athens, GA, USA*

Primary intraparenchymal brain tumors, including astrocytomas, glioblastomas, and oligodendrogliomas, collectively comprise approximately 35% of all primary brain tumors in dogs. The prognosis for dogs with any of these tumor types is generally guarded to poor. The median survival time regardless of treatment type is approximately 230 days. Due to numerous challenges associated with diagnosing, grading, treating, and reporting survival outcomes, there is currently no gold standard therapy for these tumors in dogs. Additionally, the similarities between canine and human primary intraparenchymal brain tumors lend translational opportunity to the development of novel treatment delivery systems for canine brain tumors. Glial tumors invade and migrate along white matter tracts and blood vessels, which is a characteristic that can be exploited. Catheters lined with nanofiber particles have been developed to structurally mimic these conduits. The conformation guides tumor cells away from the tumor site to an extracranial reservoir. In this study we describe the techniques associated with stereotactically-guided implantation of nanofiber-lined catheters in dogs with naturally occurring and histopathologically confirmed astrocytomas and oligodendrogliomas. The purpose of this study was to evaluate the safety and feasibility of stereotactically-guided insertion of nanofiber catheters and to confirm tumor cell migration along internal catheter walls. Nanofiber catheter placement technique was evaluated in four client-owned dogs with MRI-confirmed prosencephalic neoplasms that had imaging characteristics consistent with gliomas. A frameless surgical navigation system (StealthStation Surgical Navigation System; Medtronic) was used to guide the catheters (FiberCath Tumor Extraction Device; NeuroLab) through a small, standard rostrotentorial craniectomy window into the tumor center. Intra-tumor catheter placement was confirmed with post-operative MRI evaluation. One month post-operatively, the reservoirs were extracted and replaced. The extracted reservoirs were histologically examined for evidence of intraluminal tumor cells. The surgical outcomes of all dogs were good and microscopic evaluation of the extracted catheters confirmed intraluminal tumor cells. Although this study is small and cannot prove treatment efficacy, these findings support our hypotheses that stereotactic guidance of nanofiber catheters is safe, feasible, and encourages tumor cell migration out of the cranial cavity. This is the first stage in a large clinical trial that ultimately aims to evaluate the safety and efficacy of nanofiber catheters in the treatment of canine brain tumors.

N02

Repetitive Transcranial Magnetic Stimulation for the Treatment of Drug-resistant Canine Idiopathic Epilepsy.

Marios Charalambous[1], *Luc Van Ham*[2], *Bart J. G. Broeckx*[3], *Sofie F. M. Bhatti*[2]
[1]*Faculty of Veterinary Medicine - Ghent University, Merelbeke, Oost-Vlaanderen, Belgium,* [2]*Ghent University, Faculty of Veterinary Medicine, Small Animal Department, Merelbeke, Belgium, Merelbeke, Oost-Vlaanderen, Belgium,* [3]*Ghent University, Faculty of Veterinary Medicine, Department of Nutrition, Genetics and Ethology, Merelbeke, Belgium, Merelbeke, Oost-Vlaanderen, Belgium*

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive, safe and painless procedure that is applied in humans to modify the activity of specific neural circuits of the brain. Via electromagnetic induction, a magnetic field generator produces small electric currents on cortical neurons in order to affect neuronal function. The antiepileptic effect of rTMS has been demonstrated in humans with drug-resistant epilepsy. Therefore, a single-blinded randomized placebo-controlled clinical trial was designed to assess the efficacy and safety of rTMS in dogs with drug-resistant idiopathic epilepsy.

After random assignment into the treatment or placebo group, dogs received active or sham stimulation, respectively. All dogs in both groups were sedated during the whole procedure using the same anesthetic protocol. Both groups received repetitive active or sham stimulation for 90 minutes per day for five consecutive days in total. The efficacy of the procedure was evaluated by comparing monthly seizure frequency (MSF) and monthly seizure day frequency (MSDF) during a retrospective 3-month period with a prospective 3-month follow-up period. Three months after sham stimulation, the dogs in the placebo group also received treatment. Adverse effects and complications were reported.

Until this stage, five dogs were included, four in the treatment and one in the placebo group. In the treatment group, both median MSF and MSDF decreased ($P = 0.1$ and $P = 0.059$, respectively) showing a statistical trend of potential interest. In the placebo group, the limited sample size ($n = 1$) does not permit statistics at the current stage. However, this dog did show no reduction in seizure frequency following sham stimulation, while the seizure frequency reduced by 94% when the dog received active stimulation afterwards. Overall, the procedure seems safe as no significant or serious adverse effects or complications were reported.

In conclusion, these preliminary results suggest that rTMS is a safe procedure that might decrease median MSF and MSDF and result in an improvement in seizure management in dogs with drug-resistant

epilepsy. However, a larger number of dogs is required for both groups before definitive conclusions can be made.

N03

Pharmacokinetics of Approved and Compounded Extended Release Levetiracetam After Single Oral Dose Administration in Cats

Erik R. Johnson[1], Amanda R. Taylor[1], Dawn M. Boothe[1], Heather L. Gray-Edwards[2], Randolph L. Winter[1], Doug R. Martin[3]
[1]Auburn University College of Veterinary Medicine, Auburn, AL, USA, [2]Scott-Ritchey Research Center, Auburn, AL, USA, [3]Auburn University College of Veterinary Medicine/Scott-Ritchey Research Center, Auburn, AL, USA

The half-life of levetiracetam (LEV) in cats approximates 3 hours, prompting the need for an extended release LEV (ER-LEV) formulation. The purpose of this study was to describe and compare pharmacokinetics of ER-LEV in both plasma and CSF in cats after a single dose of approved and compounded ER-LEV.

Using a non-randomized cross-over design, nine clinically healthy cats received a single dose of 500 mg of approved ER-LEV PO. Thirteen blood and 1-2 CSF samples were collected over 24 hours. After a one-week washout period, the study was repeated with a single oral dose of 500 mg compounded ER-LEV. LEV was quantitated by immunoassay. Plasma and pooled CSF LEV concentrations versus time data were subjected to noncompartmental pharmacokinetic analysis and data were compared between formulations using a paired t-test.

Regardless of formulation, CSF LEV closely mimicked plasma concentrations. Significant differences ($P < 0.05$) between formulations were limited to plasma Cmax (ug/mL) (approved 126 +/- 33; compounded 169 +/- 51) and tmax (approved 5.1 +/- 1.6; compounded 3.1 +/- 1.5). With a half-life of 12.6 +/- 1.6 hours and 12.7 +/- 2.0 for approved and compounded, respectively, both formulations maintained concentrations above the human therapeutic reference interval for at least 12 hours.

This study demonstrates that both approved and compounded ER-LEV maintains human therapeutic LEV concentrations in healthy cats 12 hours after oral administration, supporting 12-hour dosing intervals. This study also demonstrates that plasma LEV monitoring can be used as an accurate representation of CSF LEV concentrations in cats.

N04

Spatial Resolution of 3D Printed Stereolithographic Models of the Canine Thoracolumbar Vertebral Column

Amanda Jurkoshek[1], Fred Wininger[2], John Snyder[3]
[1]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia MO/Department of Neurology and Neurosurgery, Veterinary Specialty Services, Manchester MO, Columbia, MO, USA, [2]Department of Neurology and Neurosurgery, Veterinary Specialty Services, Manchester MO/Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia MO, Manchester, MO, USA, [3]Department of Statistics, University of Missouri, Columbia MO, Columbia, MO, USA

Anatomic models of the spine are essential for surgical planning, implant evaluation and biomechanical studies. Cadaver spines are traditionally used despite acquisition and preparation challenges. Additive (3D) models are easily created, replicated, and customized to specific patients. The purpose of this study was to compare 3D printed spinal models to ex-vivo spines and computed tomographic (CT) images with the eventual goal of replacing cadaver spines. We hypothesized that the spatial accuracy of 3D printed stereolithographic models of the canine thoracolumbar vertebral column would not differ from cadaveric bones.

Thoracolumbar vertebral columns were harvested from 3 adult canine cadavers. CT scans were acquired and rendered as 3-D surface models. Stereolithograph models were generated and printed by a stereolithography printer with clear methacrylate photoactivated resin. Cadaveric specimens were prepared by heated soft tissue maceration. Uniform measurements were acquired from T12 through L4 from the cadaver spines, CT images, and models. Measured parameters included vertebral height, vertebral foramen height, vertebral foramen width, pedicle widths, vertebral body length, width of cranial articular processes, and width of transverse processes. Repeated measures were acquired in triplicate by a single investigator. Average values were compared using a linear mixed model with random effect and nested individual vertebrae. There were no significant differences in the 8 individual measurements from the 18 vertebrae between the cadaver, CT scan, and 3D model cohorts ($p = 0.3223$). Stereolithographic models of the canine thoracolumbar spine are spatially accurate and may be used in place of cadaveric spines for future veterinary studies.

N05

Clinical Characteristics of Steroid Responsive Meningitis-Arteritis in a Population of Dogs in North America

Jeanie Lau, Ms. Julie A. Nettifee, Karen R. Munana
North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA

Steroid responsive meningitis-arteritis (SRMA) is a common inflammatory neurological disorder of dogs. This retrospective study evaluated a population of North American dogs with SRMA to explore whether breed differences exist in clinical disease course and treatment response, and examine caregivers'; perception of the effect of SRMA on quality of life (QoL).

Medical records from 61 dogs presenting to NC State Veterinary Hospital between 2003-2017 (n=32) or identified in an AKC Canine Health Foundation survey (n=29) with a diagnosis of SRMA based on results of cerebrospinal fluid (CSF) analysis were reviewed. Caregivers were asked to complete an online survey to evaluate the impact of SRMA on the dogs'; and caregivers'; QoL.

Breeds represented most often included the Golden Retriever (n=12), Bernese Mountain Dog (n=10), Wirehaired Pointing Griffon (n=9), Boxer (n=9), and Beagle (n=6). No breed difference in overall severity of clinical signs or CSF findings was identified. A higher CSF nucleated cell count (NCC) was positively correlated with the frequency of disease relapse ($P = 0.0032$), but no association was found between prednisone dose or duration and disease relapse. Caregivers'; perception of their dogs'; QoL during treatment was associated with the severity of prednisone adverse effects ($P = 0.0331$).

These results suggest that Golden Retrievers and Wirehaired Pointing Griffons be considered among the breeds predisposed to SRMA. Treatment with higher prednisone doses is correlated with more severe adverse effects and worse QoL, but does not appear to

improve clinical outcome. The relationship between CSF NCC and disease relapse warrants further investigation.

N06

Serum Levetiracetam Concentrations Following Chronic Administration of Three Times Daily Transdermal Levetiracetam in Six Cats

Casey J. Smith[1], Heidi Barnes Heller[2], Nicole Reif[1], Matthew Van Hesteren[1], Jennifer Reinhart Dungar[3]
[1]University of Madison-Wisconsin, Madison, WI, USA, [2]University of Wisconsin- Madison, Madison, WI, USA, [3]Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802., Urbana, IL, USA

The purpose of this prospective clinical trial was to 1) determine whether transdermal levetiracetam in Lipoderm vehicle (LL) results in serum concentrations ≥ 5 mcg/ml (minimum human therapeutic interval) following 6 days of every 8 hour administration in healthy cats and 2) identify any adverse drug reactions during the administration period. Six staff or student owned healthy cats weighing ≤ 5 kg were enrolled. Cats were determined to be healthy based on physical examination, neurologic examination, packed cell volume and total protein evaluation, and serum biochemistry panel. LL was applied to the inner pinna at a dosage of 60 mg/kg (400 mg/ml) at home for 6 days. On day 7, cats were hospitalized for serum levetiracetam sampling through peripheral venipuncture at times 0 (prior to dose administration), 30, 60, 120, 180, 240 and 300 minutes post administration. Median (range) timed serum concentrations were 16.6 (8.6 - 39.6), 16.1 (6.8 - 34.4), 15.4 (10.1 - 36.7), 17.4 (9.2 - 32.7), 15.1 (8.3 - 25.9), and 14.8 (11.9 - 28.4) mcg/ml, respectively. Adverse events were limited to sedation (cat 3: day 3). This study demonstrated that thrice daily transdermal LL resulted in median serum concentrations ≥ 5 mcg/ml throughout the sampling period and adverse events were minimal. Additional studies are necessary to evaluate efficacy of transdermal LL in epileptic cats.

N07

Clinical Safety and Magnetic Resonance Imaging Characteristics of Brain Implanted Nanofiber-Lined Catheters in Healthy Dogs

Georgina V. Stewart[1], Simon Platt[1], Harold Solomon[2], Tamas Nagy[3], Barun Brahma[4], Ravi Bellamkonda[5]
[1]University of Georgia Veterinary Teaching Hospital, Athens, GA, USA, [2]Georgia Tech, Atlanta, GA, USA, [3]University of Georgia, Athens, GA, USA, [4]Emory University, Atlanta, GA, USA, [5]Duke University, Durham, NC, USA

Brain tumors, specifically gliomas, have a poor prognosis in both human and veterinary medicine. New treatments are needed to improve survival times. Polycaprolactone (PCL) based nanofiber lined catheters represent a novel biological device recently developed and used for the treatment of brain tumors in dogs. The nanofiber-lined structure of the catheter has been shown to allow tumor growth into its lumen toward an extracranial reservoir, outside of the brain. The purpose of the study was (i) to determine the clinical safety and magnetic resonance imaging (MRI) characteristics associated with use of the nanofiber lined catheters implanted into the brain; (ii) to determine the degree of cellular invasion into the catheter reservoirs; and (iii) to determine cerebral pathology associated with catheter placement, all in healthy dogs.

Four healthy adult female spayed beagle dogs were anesthetized and each underwent freehand brain implantation of 4 different PCL based nanofiber lined catheters via 2 small craniectomy defects on each side of the skull. The 4 catheters included a standard unlined catheter plus 3 PCL-based catheters of varying shapes. Catheter A: standard silicone unlined blunt nose ventricular catheter (control). Catheter B: modified silicone blunt nose ventricular catheter with PCL nanofiber insert. Catheter C: open nose polyurethane catheter with PCL nanofiber insert. Catheter D: blunt nose polyurethane catheter with PCL nanofiber insert.

Neurological examinations were performed prior to the study and daily after surgery. A standard brain MRI with intravenous gadolinium contrast agent was performed immediately, 7 days and 30 days following surgery, after which the dogs were humanely euthanized for histopathological analysis of the brains.

All dogs had transient neurological signs present for approximately 2 weeks after surgery. Mild to moderate T2 weighted hyperintensity was present on MRI, likely representing edema secondary to inflammation. The degree of change noted was similar between catheter types. These changes decreased over the study period, and was markedly reduced at the 30 day MRI. On histopathology, all brain samples were found to have variable amounts of astrogliosis, malacia and gitter cells, representing inflammation. Some catheter lumens contained neuropil with different degrees of degeneration and malacia. Changes were found in both grey and white matter and were most severe in the peri-catheter region. There was a variable degree of hemorrhage associated with all types of catheter. Changes were more severe in catheters A and B.

Each of the catheters instigate mild to moderate inflammation, based on MRI and histopathology, associated with transient neurological signs, but can be considered safe following this study. This study helps understand what to expect when these catheters are implanted into intracranial gliomas in dogs.

N08

Microsurgery and intratumoral Concentration and Safety of Metronomic Chlorambucil for Spontaneous Canine Glioma

R. Timothy Bentley[1], Stephanie Thomovsky[2], Margaret A. Miller[3], Deborah W. Knapp[1], Aaron Cohen-Gadol[4]
[1]Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA, [2]Veterinary Clinical Sciences, Purdue University, Lafayette, IN, USA, [3]Comparative Pathobioogy, Purdue University, West Lafayette, IN, USA, [4]Indiana University School of Medicine, Indianapolis, IN, USA

Investigate the distribution and safety of metronomic (daily low-dose) chlorambucil in naturally occurring canine glioma.

Eight client-owned (pet) dogs with newly diagnosed spontaneous glioma were prospectively enrolled. Chlorambucil was administered preoperatively at 4 mg/m^2 q 24hr for ≥ 3 days, and continued postoperatively until death or dose-limiting adverse events. The chlorambucil concentration of the surgical glioma specimen, cerebrospinal fluid and serum were analyzed. Dogs additionally received lomustine postoperatively. Dogs were monitored for seizures, myoclonus, cytopenias, and tumor recurrence.

Seven oligodendrogliomas and 1 astrocytoma (6 high-grade, 2 low-grade) underwent complete microsurgical resection. Median surgical glioma specimen chlorambucil concentration was 0.52 ng / g (range, 0 – 2.62 ng / g), or 37% (range, 0–178%) of the serum concentration. Median cerebrospinal fluid concentration was 0.1 ng / mL (range, 0–0.3 ng / mL). Chlorambucil was not associated with an increase in seizure activity. Six dogs displayed prolonged seizure-free intervals. There was no myoclonus. Three dogs developed asymptomatic thrombocytopenia after 8 to 12 months of chlorambucil. Median progression-free survival was 253 days (range, 63 – 860 days). Median overall survival was 257 days (range, 64–860 days).

The presence of intratumoral chlorambucil indicated an altered blood brain barrier that varied from case to case. Despite sporadic previous reports of neurotoxicity, prolonged seizure-free intervals supported a high safety margin at this dose in this species. Metronomic chlorambucil was well tolerated.

N09

Development of an Intraventricular Hemorrhage Model of Obstructive Hydrocephalus in Pigs

R. Timothy Bentley[1], *Qi Yang*[2], *Stephanie Thomovsky*[3], *L. Tiffany Lyle*[4], *Hyowon Lee*[2]
[1]*Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA*, [2]*Biomedical Engineering, Purdue University, West Lafayette, IN, USA*, [3]*Veterinary Clinical Sciences, Purdue University, Lafayette, IN, USA*, [4]*Comparative Pathobiology, Purdue University, West Lafayette, IN, USA*

Ventriculoperitoneal shunt obstruction is a major complication in canine and human hydrocephalus, however obstruction typically occurs in the chronic setting. In order to develop a self-clearing ventricular catheter, a hydrocephalus model prone to early shunt obstruction would be beneficial. Autologous blood was injected into the right lateral ventricle of 32.3 ± 5.2 kg, male, 6 month old pigs during terminal (n=3) and survival (n=14) procedures. Median intraventricular intracranial pressures were 9 mmHg (baseline), 66 mmHg (peak) and 20 mmHg (6 minutes post-blood injection). 14.4 ml of blood caused profound hypertensive hydrocephalus in the terminal procedures but the first survival pig could not be recovered from anesthesia. Using 10 ml of blood, hypertensive hydrocephalus occurred in 12 of 13 pigs. In one pig, the subarachnoid space was inadvertently injected and hydrocephalus did not occur. Biweekly computed tomography for 6 weeks after survival procedures revealed blood injection alone failed to produce persistent hematoma (n=2) whereas injection of 10 ml of blood mixed with 140 units of thrombin produced persistent obstruction due to hematoma (n=10). Six pigs were additionally treated with ventriculoperitoneal shunting. Shunt obstruction by coagulated blood in 4 pigs (75%) caused profound ventricular dilation evident on computed tomography and post-mortem examinations. Acute neurological deterioration occurred 12 hours to 4 days post-operatively, and was fatal in 3 of the 4 pigs. A model of obstructive hydrocephalus prone to early shunt obstruction can be generated in 27 - 37 kg pigs with lateral ventricle injection of 10 ml of autologous blood and thrombin.

N10

Pharmacokinetics of Fenbendazole in Canine CSF and Plasma: A Pilot Study

Amanda C. Brenna[1], *Amanda R. Taylor*[1], *Randolph L. Winter*[1], *Crisanta Cruz-Espindola*[2], *Dawn M. Boothe*[1]
[1]*Auburn University College of Veterinary Medicine, Auburn, AL, USA*, [2]*Department of Anatomy, Physiology & Pharmacology, Auburn, AL, USA*

Certain benzimidazoles, fenbendazole and mebendazole, have demonstrated *in vitro* efficacy against glioma cells due to inhibition of tubulin polymerization and disruption of microtubule formation. Fenbendazole produces these effects at a mean inhibitory concentration (IC_{50}) of 150 ng/ml, which might be an initial target for therapeutic CSF concentrations. Our study aimed to describe the time course of fenbendazole in plasma and CSF after oral administration to dogs. Doses were designed to target a CSF concentration approaching 150 ng/ml. Fenbendazole was detected in canine plasma and CSF using high performance liquid chromatography; the limit of detection was 10 ng/ml and 5 ng/ml, respectively. Fenbendazole administered to two dogs at 50 mg/kg was not detectable. Dogs were subsequently treated with 100 mg/kg (n = 4) and then 200 mg/kg (n = 2 due to adverse effect), resulting in an average peak plasma concentration (ng/ml) of 131.5 and 133.4, respectively. CSF concentrations were detectable in less than 25 % of the samples. The highest CSF concentration (at 200 mg/kg) was 21.19 ng/ml at 360 min. However, dogs given 200 mg/kg had acute onset of hematochezia, suggesting doses this high may not be clinically appropriate. This study demonstrates that plasma fenbendazole concentrations increased with dose, but CSF concentrations were well below the target of 150 ng/ml even at 200 mg/kg. Relevance of this target concentration to efficacy for treatment of gliomas remains to be determined. Future studies might focus on a more potent benzimidazole such as mebendazole, for which the in vitro IC_{50} is lower.

N11

Risk Factors for Progressive Myelomalacia in Dogs with Complete Sensorimotor Loss Following Intervertebral disc Extrusion

Aude MH. Castel[1], *Natasha J. Olby*[2], *Christopher L. Mariani*[2], *Karen R. Munana*[3], *Peter J. Early*[2]
[1]*The University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA*, [2]*NC State University College of Veterinary Medicine, Raleigh, NC, USA*, [3]*North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA*

Progressive myelomalacia (PMM) is a dire complication associated with acute spinal cord injury (from intervertebral disc extrusion (IVDE). Understanding of the pathophysiology of this disease is limited. The objective of this retrospective study was to identify risk factors for the development of PMM by comparing client-owned paraplegic dogs without pain perception following IVDE that did and did not develop the disease.

Dogs with acute IVDE causing paraplegia with loss of pain perception were identified from the Canine Spinal Cord Injury Program database and by search of medical records. Dogs were categorized as PMM yes or no based on clinical progression and/or histopathology. Signalment,

onset and duration of signs (categorized), steroids (yes or no), non-steroidal anti-inflammatory drugs (yes or no), spinal cord appearance during surgery (normal or abnormal), location (lumbar intumescence or thoracolumbar) and amount of disc material were retrieved and their association with PMM was examined in all dogs and dogs treated surgically using logistic regression.

Two hundred and eight dogs met the inclusion criteria, 56 with histopathologically confirmed or presumptive PMM (44 surgically decompressed) and 152 without PMM (all surgically decompressed). Disc extrusions at the lumbar intumescence (p = 0,0147), longer time from loss of ambulation to surgery (p = 0,0171), and no steroids prior to presentation (p = 0,0119) were significantly associated with development of PMM. We conclude that dogs with IVDE at the intumescence are at increased risk of PMM. Timing of surgery and steroid use may warrant further investigations.

N12

Accuracy of a Novel Magnetic Resonance Imaging-Based Patient-Individual Stereotactic Brain Biopsy Device in the Dog

Thomas Flegel[1], Dirk Winkler[2], Marcel Müller[3], Robert Möbius[4], Peter Böttcher[5], Ingmar Kiefer[6], Ronny Grunert[3], Sarah Gutmann[6]
[1]Department of Small Animal Medicine, University of Leipzig, Leipzig, Sachsen, Germany, [2]UNiversity of Leipzig, Leipzig, Sachsen, Germany, [3]Fraunhofer IWU, Dresden, Sachsen, Germany, [4]University of Leipzig, ZESBO, Leipzig, Sachsen, Germany, [5]Free University of Berlin, Small Animal Clinic, Berlin, Sachsen, Germany, [6]University of Leipzig, Department of Small Animal Medicine, Leipzig, Sachsen, Germany

The aim of the study was to determine the precision of the novel MRI-based patient-individual stereotactic brain biopsy device in dogs.

Twenty-two canine cadavers with 2 target points each were used to determine accuracy. First, specific bone anchors and MRI-markers were secured to canine cadaver heads. Afterwards CT and MRI examinations of the heads were performed. Two target points and corresponding trajectories were defined on each MRI: left caudate nucleus and right piriform lobe. Based on MR-images, patient-individual frames including rigid needle placement ports to reach defined target points were constructed and printed with a 3D-printer. The needle was to enter the brain in a gyrus and not to penetrate the ventricles. The frames were secured to the bone anchors. Minimal-invasive access to the brain was created using a tool guide. The biopsy needle was placed through the needle placement port up to the predetermined depth. Afterwards CT examinations of the heads with biopsy needles placed in each target point were performed. Needle placement error was determined after fusion of MRI and CT examinations. Error was defined as deviation in mm between needle tip and anticipated target points.

The total median needle placement error for all 42 target points was 0.84 mm (range: 0.09-2.76; outlier: 4.11). The median needle placement error for the caudate nucleus only was 0.67 mm (range: 0.09-1.25) and for the piriform lobe 0.85 mm (range: 0.14-2.76). Therefore, the MRI-based patient-individual stereotactic brain biopsy device reaches higher accuracy than most other described brain biopsy systems.

N13

Can Neuronavigation Aid in Pituitary Removal in Horses?

Sarah M. Kappel, Rebecca A. Packer, Lawrence Santistevan, Amy Downey, Colleen Duncan, Jeremiah T. Easley
Colorado State University, Fort Collins, CO, USA

The purpose of this study was to surgically extract pituitary gland tissue from horses through a transcranial approach as a possible treatment option for pituitary pars intermedia dysfunction (PPID). PPID, also known as Cushing';s disease, is one the most common neuroendocrine diseases that affects aged horses. There is currently no surgical treatment for PPID and the treatment method of choice is with pergolide or cyproheptadine. The treatment of choice in humans and dogs for Cushing';s disease is to surgically remove the pituitary gland. We hypothesized that intracranial neurosurgery with the assistance of the neuronavigation would allow accurate extraction of the pituitary gland tissue from horses. This study consisted of 13 total cadaver horse heads chosen at random varying between the ages of 2-30 years old. Magnetic resonance imaging (MRI) (N = 6) or computed tomography (CT) (N=7) was performed on each head followed by image registration with the neuronavigation and biopsy. Histopathological analysis was performed on all samples. Pituitary gland tissue was found in 6 of the 13 samples (46%) submitted. The higher contrast resolution of MRI compared to CT provided improved accuracy for pinpointing and acquiring pituitary tissue. An automated tissue resection device system showed increased success for pituitary tissue harvest of 80% compared to a traditional blunt brain biopsy needle, which had a 46% success. The pituitary gland has a thick, fibrous capsule making the use of the blunt biopsy needle an inappropriate instrument for performing pituitary tissue removal. Future studies will be aimed at utilizing cadavers and live horses with confirmed PPID to further ensure the safety and efficacy of this neuronavigation system.

N14

Morphometric Analysis of Brachycephalic Features Identified by Machine Learning Technique in Dogs with/without Syringomyelia

Susan P. Knowler[1], Michaela Spiteri[2], Angus K McFadyen[3], Felicity Stringer[4], Kevin Wells[2], Clare Rusbridge[5]
[1]University of Surrey, Guildford, Scotland, United Kingdom, [2]University of Surrey, Guildford, England, United Kingdom, [3]akm-stats, Glasgow, Scotland, United Kingdom, [4]Fitzpatrick Referrals Veterinary Specialist Hospital, Godalming, England, United Kingdom, [5]University of Surrey/Fitzpatrick Referrals Veterinary Specialist Hospital, Guildford, England, United Kingdom

The aim of the study was a follow up morphometric analysis of features associated with Cavalier King Charles spaniel (CKCS) with syringomyelia (SM) secondary to Chairi-like malformation (CM) that had first been identified by an innovative machine learning technique which removes human bias.

Recent studies have suggested that CM is a more complex disorder than originally thought and affected by features associated with brachycephaly such as olfactory bulb rotation and rostral flattening of the forebrain. T2W sagittal DICOM anonymized images of 66 CKCS,

over 4 years of age were obtained, comprising i) 26 dogs without SM with no or < 2mm wide central canal dilation ii) 40 dogs with SM with syrinx width 4mm or over. Morphometric analysis of the shape and position of the soft palate relative to the skull base and the nature of rostral skull flattening was obtained.

In SM dogs, the distance between the rostral end of the palate and i) sella turcica (P = 0.049) ii) foramen magnum basioccipital (P = 0.030) were significantly reduced, as was the maximum distance through the palate centroid (P = 0.015). However, the reduced distance between the brain and frontal bone was highly significant (P < 0.001). The reduction in distance between the olfactory bulb and the sella turcica may be of interest (P = 0.054). Discriminate analysis/logistic regression revealed that rostral skull flattening dominated any model but when removed, ratio of maximum distance through the palate centroid to cerebral height to cerebrum height was highly significant (P < 0.001).

The characteristics of CKCS with SM include osseous insufficiency in the skull and changes in the shape of the soft palate which were implicated by machine learning technology.

N15

Morphometric Analysis of Spinal Cord Termination in Cavalier King Charles spaniels

Courtney R. Sparks[1], Ian Robertson[2], Natasha J. Olby[3]
[1]NCSU College of Veterinary Medicine, Raleigh, NC, USA, [2]North Carolina State University, Raleigh, NC, USA, [3]NC State University College of Veterinary Medicine, Raleigh, NC, USA

Cavalier King Charles spaniels (CKCS) suffer from Chiari-like malformation (CM), a skull malformation that causes crowding of the caudal fossa, and syringomyelia (SM). Affected dogs show signs of pain and frequently show lumbosacral pain. Tethered cauda equina has been reported in people with CM but currently there is no morphometric data on the caudal aspect of the vertebral column and spinal cord in CKCS. The purpose of this study was to compare the location of the conus medullaris in CKCS with other size-matched breeds. We hypothesized that the spinal cord terminates more caudally in CKCS.

A retrospective study was conducted on 90 dogs with thoracolumbar magnetic resonance imaging (MRI). Dog breeds were grouped as CKCS (n=48), and brachycephalic (n=21) and non-brachycephalic (n=20) size-matched controls. MRI identifiers were removed to blind the observer. Termination of the spinal cord was determined from T2-weighted sagittal and axial images as the 6^{th} (L6), or 7^{th} lumbar vertebra (L7), or sacrum. Breed was revealed after Chi-squared analyses were performed.

Among 48 CKCS, the spinal cord terminated at L6 in 3, L7 in 23, and sacrum in 22 dogs compared with 8 at L6, 27 at L7 and 5 at sacrum in 41 controls. Spinal cord termination was significantly more caudal in CKCS as compared to brachycephalic *(P = 0.015)* and non-brachycephalic breeds *(P = 0.005)*. There was no difference between size-matched controls *(P = 0.157)*. There is a need to investigate whether tethering of the cauda equina contributes to signs of pain in CKCS.

N16

A Comparison of Erythrocyte Membrane Fatty Acids Between Shiba and Non-Asian Dogs

Go Togawa[1], Miyoko Saito[2], Akiko Harauma[1], Toshio Tanaka[1], Kenji Mori[3], Yukihiro Fujita[1], Akikazu Ishihara[1], Nanami Iizuka[1], Toru Moriguchi[1]
[1]Azabu University, Sagamihara, Kanagawa, Japan, [2]School of Veterinary Medicine, Azabu University, Sagamihara, Kanagawa, Japan, [3]Mori Animal Hospital, Oumihachimann, Shiga, Japan

Canine cognitive dysfunction (CCD) is a common neurobehavioral syndrome in aged dogs, especially in Japanese dog breeds such as Shiba. Polyunsaturated fatty acids (PUFA), including arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are constituents of biological membranes and nervous tissue. EPA and DHA can be synthesized from alpha-linolenic acid (ALA), but the conversion rate is lower in both humans and dogs, making them prone to deficiency. In humans, it has been reported that high erythrocyte long chain omega-3 fatty acid (FA) levels appear to be associated with better cognitive function later in life and lower risk of cognitive decline. On the other hand, the activity of desaturase (delta-5 desaturase and delta-6 desaturase), which metabolizes EPA and DHA from precursors, has been reported to be lower in Japanese than in Westerners, possibly due to dietary culture as Japanese have historically ingested EPA and DHA directly from seafood. We hypothesized that Japanese dogs share historically similar diets with Japanese and may have a lower desaturase activity compared with Western dogs. The aim of this pilot study was to investigate the composition of FAs in the erythrocyte membrane and plasma, and to evaluate desaturase activity in Japanese dogs and non-Asian dogs.

Prospective study. Twenty-five Shiba dogs without CCD and 57 non-Asian dogs without CCD were used. The FAs (omega-3 PUFA, omega-6 PUFA, monounsaturated PUFA, and saturated PUFA) composition of the erythrocyte membrane and plasma were measured from blood samples using gas chromatography. The difference between Shiba dogs and non-Asian dogs was evaluated using the Wilcoxson rank-sum test.

Shiba dogs had a significantly lower composition (%) of EPA (median: 1.09, range: 0-2.43), DHA (median: 1.15, range: 0-4.12) and total omega-3 FA (median: 3.22, range: 0.48-7.10) in erythrocyte membranes compared with non-Asian dogs (median: 1.20, range: 0.40-2.12; median: 1.51, range: 0-2.56; median: 4.03, range: 1.31-7.08) (p = 0.0055; 0.0379; 0.0419).

These findings might suggest that Shiba dogs have lower desaturase activity than non-Asian dogs. The high incidence of CCD in Shiba dogs may be associated with the low composition of omega-3 FAs in erythrocyte membrane. Studies on FAs using dogs with a unified diet are required to clarify the metabolic difference between Japanese dogs and non-Asian dogs, and to elucidate the association between FAs and CCD.

N17

Genetic Association Analyses of Familial Spontaneous Epileptic Cats

Yoshihiko Yu[1], Daisuke Hasegawa[2], Reuben M. Buckley[3], Aki Fujiwara-Igarashi[2], Yuji Hamamoto[2], Shunta Mizoguchi[2], Takayuki Kuwabara[2], Toshinori Omi[2], Michio Fujita[2], Leslie A. Lyons[3]

[1]Nippon Veterinary and Life Science University/College of Veterinary Medicine, University of Missouri, Columbia, Musashinoshi, Tokyo, Japan, [2]Nippon Veterinary and Life Science University, Musashinoshi, Tokyo, Japan, [3]College of Veterinary Medicine, University of Missouri, Columbia, Columbia, MO, USA

Familial spontaneous epileptic cats (FSEC) is the only strain of cats with a suspected genetic cause of epilepsy. The FSEC colony consists of large multigenerational pedigrees with multiple cases. The mode of inheritance is undetermined since the cats have different clinical presentations. The aim of this study was to identify loci that influence susceptibility to epilepsy using genetic association analyses.

Eighty-one cats, including affected and unaffected, from the FSEC colony and the kindred colony were genotyped using the illumina Infinium iSelect Feline 63K DNA array. Single nucleotide polymorphisms (SNPs) were remapped to feline genome assembly v9.0.

Considering 78 cats, 14 trios, including nine with unaffected parents and one affected offspring and five trios with affected individuals were available for transmission disequilibrium tests (TDT). TDT indicated the strongest association of disease with SNPs on cat chromosome B3. SNPs were located between cat chromosome positions ~120.2 to 121.6 Mb, however, genome-wide significance was not obtained. SNPs on cat chromosomes A3, B2, and D4 also indicated association. Among the SNPs with the strongest association, a gene encoding a zinc finger protein was present. Furthermore, several other potential candidate genes harboring the suggested region were detected.

The analysis of the pedigree and the disease presentation in the FSECs, which includes spontaneous seizures, induced seizures or, abnormal electroencephalogram, suggest this form of epilepsy may have a co-dominant mode of inheritance. Although TDT did not reach significance, other methods such as linkage analysis and whole genome sequencing may provide additional support.

N18

Radiographic Indices for the Diagnosis of Atlantoaxial Instability in Dogs

Kelly Cummings[1], Federico Grosso[2], George Moore[3], Mark Rochat[4], Stephanie Thomovsky[5], R. Timothy Bentley[6]
[1]Purdue University Veterinary Teaching Hospital, Lafayette, IN, USA, [2]Univeristy of Florida Veterinary Teaching Hospital, Gainesville, FL, USA, [3]Purdue University College of Veterinary Medicine, West Lafayette, IN, USA, [4]Purdue University Veterinary Teaching Hospital, West Lafayette, IN, USA, [5]Veterinary Clinical Sciences, Purdue University, Lafayette, IN, USA, [6]Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA

Toy breed dogs are overrepresented for atlantoaxial (AA) instability. Radiography is valuable, however published toy breed-specific radiographic measurements are lacking and diagnosis remains largely subjective. The purpose of this study was to establish objective radiographic criteria for the diagnosis of AA instability in toy breed dogs. Neutral lateral and ventrodorsal radiographs of 102 toy breed dogs (92 control, 10 affected) were retrospectively reviewed. The median C1-C2 overlap (the distance of overlap between the C2 spinous process and the dorsal arch of C1) was +4.65 mm in control dogs and −5.00 mm in AA cases. A C1-C2 overlap ≤ +1.55 mm was the most sensitive (100%) and specific (94.5%) radiographic measurement in the diagnosis of AA instability. We performed three relative measurements: the atlantodental interval to dorsal atlantodental interval ratio (ADI/DADI ratio), the relative dens length and the C1-C2 angle. These three relative measurements had good specificity (94.5%, 86.9%, 98.9% respectively), lower sensitivity (80.0%, 66.7%, 60.0%) and were not influenced by body weight ($p > 0.05$). Absolute measurements (e.g. absolute dens length, atlantoaxial distance) were significantly correlated with body weight ($p < 0.05$) and their utility in the diagnosis of AA instability could not be established. Decreased C1-C2 overlap strongly supports AA instability. The ADI/DADI ratio, relative dens length and C1-C2 angle may provide further support but may be normal in individual cases.

N19

Non-Invasive Vagal Nerve Stimulation for Refractory Epilepsy in Dogs

Georgina V. Stewart[1], Simon Platt[1], Jill Hicks[2], Marc Kent[1], Renee Barber[1]
[1]University of Georgia Veterinary Teaching Hospital, Athens, GA, USA, [2]Animal Neurology and MRI Center, Commerce, MI, USA

Vagal nerve stimulation is a validated treatment for some forms of refractory epilepsy and status epilepticus in human medicine, as well as migraine headaches. Both invasive and non-invasive forms have been used, including transcutaneous stimulation of the nerve. Afferent vagal nerve fibers project to a large number of cortical and subcortical locations in the brain where they exert an inhibitory effect, leading to a widespread decrease in excitability. These changes can be induced with as little as 2 minutes of treatment every 8 hours. This property is exploited by vagal stimulation for seizure management.

The purpose of this study is to evaluate the efficacy of non-invasive vagal nerve stimulation as an adjunctive treatment for refractory idiopathic epilepsy in dogs. The device used was a hand held GammaCore VET transcutaneous stimulator. Client owned dogs were recruited at multiple locations in the United States. Dogs had to have a 6 month history of seizures, with a minimum frequency of 4 seizures per month for the 8 weeks prior to the study, while maintained on either phenobarbital and/or potassium bromide for at least 2 months. Dogs were randomly assigned to group 1 or 2. Group 1 cases received 16 weeks of continuous treatment while, group 2 cases received 8 weeks of no treatment followed by 8 weeks of treatment with the device. Dogs received 90 seconds of treatment every 8 hours, where the device is held against an area of shaved skin, with coupling gel, in the cervical region in the approximate region of the nerve.

Thirteen cases have been recruited from multiple institutions. All recruited dogs were between the ages of 2-7 years of age, with normal neurological examinations. All dogs were receiving either phenobarbital, potassium bromide or both. Recruited cases had bloodwork performed prior to the study which showed serum concentrations of antiepileptic medications within accepted ranges. Seizure frequency varied between dogs, with some experiencing frequent solitary seizures, and others experiencing cluster seizure episodes. Most dogs experienced both generalized tonic-clonic and focal seizures. The mean pre trial seizure frequency for both groups was calculated – group 1: 1 seizure every 9 days; group 2: 1 seizure every 7 days. The

overall mean post trial seizure frequency for both groups was decreased. Group 1: 1 seizure every 16 days; group 2: 1 seizure every 9 days. Side effects were limited, with 1 dog experiencing an increase in seizure frequency and 1 dog displaying negative aversive behavior when the device was applied.

In conclusion, this treatment modality represents a possible additional treatment for refractory epileptic dogs that is non-invasive and has limited side effects.

N20

Long-Term Computed Tomography Follow-Up in Great Danes with or Without Signs of Cervical Spondylomyelopathy

Daniella Vansteenkiste[1], *Ronaldo C. da Costa*[1], *Paula Martin-Vaquero*[2], *Marilia Bonelli*[1], *Luciana B. da Costa*[1]
[1]*The Ohio State University, College of Veterinary Medicine, Columbus, OH, USA*, [2]*Madrid, Columbus, OH, USA*

Cervical spondylomyelopathy (CSM) has a high prevalence in Great Danes. In order to understand the progression of osseous changes, we aimed to perform a long-term clinical and computed tomographic (CT) study of Great Dane dogs with and without CSM.

Great Danes previously diagnosed with CSM and clinically normal Great Danes previously enrolled in a prospective study had clinical and CT follow-up studies at least 18 months after diagnosis. Twelve dogs were investigated, six CSM-affected and six normal. Median time between CT studies was 28 months (CSM dogs) and 25 months (normal dogs).

Morphologic and morphometric assessments were performed in both CT studies. Morphologic assessment evaluated vertebral canal stenosis, which was classified as ventral, dorsal, lateral or a combination. The regularity, degree of proliferation of the articular processes and presence or absence of foraminal stenosis was also recorded. Morphometric assessment included the vertebral canal area at three separate locations per disc space and foraminal heights.

On follow-up CT, three CSM-affected dogs developed new sites of stenosis (three sites total), whereas two clinically normal dogs developed new lesions (five sites total). Disc spaces most commonly affected were C4-C5, C5-C6 and C6-C7. New sites of foraminal stenosis were noted in two of the CSM-affected and four of the clinically normal group. Morphometric evaluation showed no statistically significant differences between the initial and follow-up CT studies in the CSM-affected or normal groups.

In this study we observed development of compressive lesions occurring in both groups, with the majority of dogs not developing new lesions.

N21

Nerve Growth Factor Gene Therapy Increases the Plasticity in Pyridoxine Induced Neuropathic Dogs

Hyunkee Cho[1], *Sumin Yun*[2], *Yoonhwan Kim*[2], *Jung Hoon Choi*[2], *In Koo Hwang*[3] *Jinyoung Chung*[1]
[1]*Kangwon National University, Chuncheon-si, Kangwon-do, Republic of Korea*, [2]*Kangwon National University, Chuncheon-si, Kangwon-do, Republic of Korea*, [3]*Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea*

Nerve growth factor (NGF) is known as a major factor for neuronal plasticity. Therefore we can expect the neuronal plasticity with NGF gene therapy in neuropathic dogs.

For this experiment, twelve dogs were divided into 3 groups; control (n = 4), non-gene therapy group (n = 4), and gene therapy group (n = 4). NGF gene transfection was performed through intrathecal injection in gene therapy group. At 24 hours later, pyridoxine (150 mg/kg s.i.d. 7 days) was injected subcutaneously in non-gene therapy group and gene therapy group to induce neuropathy.

To evaluate the impact of NGF gene therapy on pyridoxine-induced dorsal root ganglia (DRG) damage, βIII - tubulin, Doublecortin (DCX), and neuron-glial antigen 2 (NG2) - Chondroitin sulfate proteoglycans (CSPGs) immunohistochemical staining were performed. The number of βIII - tubulin immunoreactive neurons decreased significantly in non-gene therapy group compared to that in the control group. NGF gene therapy ameliorated the decrease in βIII - tubulin immunoreactive cells in dorsal root ganglia (DRG) from pyridoxine-induced neuronal death. Similarly, the number of DCX immunoreactive cells decreased significantly in non-gene therapy group compared to that in the control group. In addition, DCX immunoreactivity in non-gene therapy group was concentrated at the center of DRG neurons. DCX immunoreactivity in gene therapy group showed similar distribution pattern of DCX - immunoreactive neurons with the control group. In control group, NG2 - CSPG immunoreactivity was found in the sciatic nerve. In the non - gene therapy group, NG2 - CSPG immunoreactivity was similarly detected in the sciatic nerve compared to that in the control group. NGF gene therapy significantly increased the expression of NG2 - CSPG in the sciatic nerve compared to that in the non - gene therapy group. NGF gene therapy could be powerful therapeutic agents for pyridoxine-induced damage in DRG by recovering the DCX expression in the DRG and NG2 - CSPG in the sciatic nerve.

N22

Magnetic Resonance Imaging Characterization of Combined Osseous and Disc-associated Cervical Spondylomyelopathy in Dogs

Ronaldo C. da Costa, *Marilia Bonelli*
The Ohio State University, College of Veterinary Medicine, Columbus, OH, USA

Traditionally, cervical spondylomyelopathy (CSM) has been classified into disc- or osseous-associated forms. However, there are dogs that suffer from both forms, and this has not been thoroughly evaluated. Our goal was to report the MRI findings associated with single or multi-level osseous, disc and ligamentous compressions in dogs diagnosed with CSM.

We retrospectively reviewed 180 magnetic resonance imaging (MRI) cases previously diagnosed with CSM. Inclusion criteria were diagnosis of CSM using MRI and presence of compression in two or more directions. Location of compressive lesions, most severe compressive lesion, direction of the compression (dorsal, ventral, lateral, and combined), and presence of spinal cord hyperintensity were recorded.

Forty-five dogs (25%) met the inclusion criteria: 32 were large-breed (21 Dobermans) and 13, giant-breed (7 Great Danes). The majority was older than 6 years of age (36/45), with 29 (64%) being males. Forty two (93%) dogs had dorsal and ventral spinal cord compression in the same level; of these, 27 (64%) also had spinal cord compression at one or more other sites. Seven dogs (15%) had circumferential spinal cord compression. The most severe compressive lesion was at

C6-7 or C5-6. Spinal cord hyperintensity on T2-W images was present in 35 (77%) dogs.

Our findings indicate that a sizeable portion of CSM-affected dogs suffer from combined compressions. It is important to routinely and carefully evaluate all cervical levels to make sure these are not overlooked, as the high proportion of spinal cord hyperintensity may suggest they represent a more severe form of the disease.

N23

Expression of PDGFR-α/-ß, VEGFR-2, c-Abl, and c-Kit in Canine Granulomatous Meningoencephalitis and Necrotizing Meningoencephalitis

Joong-Hyun Song[1], Do-Hyeon Yu[2], Hee-Chun Lee[2], Dong-In Jung[2]
[1]Gyeongsang National University Animal Medical Center, Jinju City, Kyongsang-namdo, Republic of Korea, [2]College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju-si, Kyongsang-namdo, Republic of Korea

Granulomatous meningoencephalitis (GME) and necrotizing meningoencephalitis (NME) is the common idiopathic inflammatory diseases of the central nervous system (CNS) of dogs. It has long been assumed to be autoimmune-mediated disorders. Systemic medical therapy with a combination of a glucocorticoid and an immunosuppressive agent is the current mainstay of treatment. Due to lack of understanding of the exact treatment mechanism, however, most of which are dependent only on conventional immunosuppressants, which can have serious adverse side effect. It is well recognized that tyrosine kinases (TKs) play an important role in the pathogenesis of malignant tumors. With the active research of targeted therapy using tyrosine kinase inhibitors (TKIs) in oncologic area, recently, the role of TKs and TKIs in autoimmune disorder has been investigated in numerous animal models and human medicine.

The objective of this study was to evaluate the expression of multiple TKs in MUE tissues and determine the possible use of multitargeted TKIs as treatments for canine MUE.

Formalin-fixed, paraffin-embedded sections from 5 GME and 3 NME (total 8 dogs) samples were used in this study. Staining for PDGFR-α, PDGFR-ß, VEGFR-2, c-Abl, and c-Kit was carried out using standard immunohistochemical procedures developed for use in formalin fixed tissues. Immunohistochemical evaluation assessed staining positivity of each TKs and staining intensity and distribution of TK positive cells. Overall staining intensity was graded as no immunostaining, weak immunostaining, moderate and intense immunostaining at ×40 magnification. Distribution of positively staining cells (% cells affected) was evaluated in five separate fields at ×200 magnification and was graded as follows: 0%, 1-9%, 10-50%, 51-100%.

All samples stained positive for PDGFR-ß (8/8 [100%]) with the majority of samples exhibiting relatively intense staining and broad distribution. A large number of the samples also had weak to moderate staining for PDGFR-α (4/8 [50%]) and c-Kit (4/8 [50%]) with various distribution. c-Abl was identified in 3/8 (37.5%) with relatively moderate staining intensity and distribution. Only 1/8 sample showed a weak and restricted VEGFR-2 (12.5%). There was no statistically significant association between GME and NE samples in terms of TK expression ($P > 0.05$ for all).

Known targets of conventional multitargeted TKIs are expressed in canine GME and NME of this study. These TKs could be involved in key aspects of the pathogenesis of autoimmune encephalitis and especially PDGFR-ß may have important role in biologic activity of the disease. Based on this proof, the targeted therapy for the over-expressed TKs may have potential as new treatment modality of GME and NME.

N24

Interobserver Agreement of Mechanical Sensory Thresholds in Normal Dogs

Austin Kerns[1], Nina Kieves[1], Laurie Cook[2], Sarah Moore[1]
[1]Ohio State University, Columbus, OH, USA, [2]Ohio State University, columbus, OH, USA

Electronic von Frey anesthesiometry (VFA) has been previously reported by our laboratory and others as a useful method of mechanical quantitative sensory testing (QST) for evaluating neuropathic pain in dogs. Intraobserver agreement has been previously shown to be good to excellent; however, interobserver agreement has not been previously reported and is vital to the use of this technique in multicenter veterinary clinical trials in neuropathic pain. The goal of this study was to evaluate the interobserver agreement of sensory thresholds obtained using electronic VFA in a group of normal small breed dogs.

Twenty healthy dogs (< 20kg) were recruited from the general practice population at the Ohio State University Veterinary Medical Center. Three novice evaluators used an electronic von Frey device (IITC Systems) to measure mechanical sensory threshold (ST) after a training session conducted by an expert evaluator. Each dog was evaluated by all three investigators on the same day with both evaluator and limb test order randomized and testing sessions separated by 5 minutes.

Mean ST values were averaged for all four limbs to produce a single value per dog for comparison between evaluators. Agreement between evaluators was determined using the intra-class correlation coefficient (ICC; two-way model for consistency, single measures). ICC across all three evaluators was 0.48, indicating moderate agreement. Moderate interobserver agreement is likely not sufficient to support the use of this technique in multi-center clinical trials, and our results underscore the importance of using a single evaluator for this QST technique in canine neuropathic pain studies.

N25

Trace Elemental Profiles of Serum and Cerebrospinal Fluid in Dogs with Idiopathic Epilepsy

Kazuyoshi Sasaoka[1], Hiroshi Ohta[1], Hazuki Mizukawa[2], Shouta Nakayama[2], Noboru Sasaki[1], Keitaro Morishita[1], Yoshinori Ikenaka[2], Mayumi Ishizuka[2], Mitsuyoshi Takiguchi[1]
[1]Department of Veterinary Clinical Sciences, Hokkaido University, Sapporo, Hokkaido, Japan, [2]Department of Environmental Veterinary Sciences, Hokkaido University, Sapporo, Hokkaido, Japan

Idiopathic epilepsy (IE) is a chronic brain disease which causes epileptic seizure. In human, serum and CSF trace elemental profiles were changed in IE patients. Previous studies suggested that supplementation of trace elements can improve refractory IE in human. Currently, there has been no reports about trace elemental profiles in dogs with IE. Therefore, the purpose of this study was to determine trace elemental profiles in IE dogs.

Dogs with IE were recruited between June 2016 and May 2017 at the Hokkaido University Veterinary Teaching Hospital. Thirty-nine

healthy client-owned dogs and 5 laboratory beagles were used as serum controls and CSF controls, respectively. Serum and CSF trace element concentrations (copper, selenium, rubidium, molybdenum, tin, median [range]) were measured by inductively coupled plasma-mass spectrometry. Trace element concentrations of IE dogs were compared with those of controls. IE dogs were classified as responders or non-responders by the response to anti-epileptic drugs 6 months later the starting treatment. Trace element concentrations of responders were compared with those of non-responders.

Ten dogs with IE were enrolled in this study. Dogs with IE had significantly lower serum tin concentration (IE, 0.48 [0.38–0.77] μg/dL; control, 0.59 [0.42–1.04] μg/dL), higher CSF copper concentration (IE, 3.61 [2.88–4.68] μg/dL; control, 2.28 [2.07–2.76] μg/dL) and higher CSF selenium concentration (IE, 13.6 [4.33–21.9] μg/dL; control, 0.98 [0.38–0.77] μg/dL). There were no significant differences in trace element concentrations between responders and non-responders. However, a dog with severe refractory seizure showed lowest serum tin concentration (0.38 μg/dL) and high CSF copper concentration (4.36 μg/dL).

These results indicated that serum and CSF trace elemental profiles in IE dogs were different from those in healthy dogs. Further studies should be conducted to clarify the correlation between responsiveness to anti-epileptic drugs and trace elemental profiles.

N26

Magnetization Transfer and Diffusion Tensor Imaging in Dogs with Spinal Cord Injury

Richard Levon Shinn[1], *Krystina Stadler*[2], *John Rossmeisl*[2]
[1]*Virginia Maryland College of Veterinary Medicine, Christiansburg, VA, USA*, [2]*Virginia Tech, Blacksburg, VA, USA*

MRI findings have been evaluated with variable success in predicting prognosis in dogs with spinal cord injury (SCI). In humans with SCI, magnetization transfer (MT) and diffusion tensor imaging (DTI) provide quantitative values that correlate with neurologic dysfunction. In dogs that fail to recover, we hypothesized that MT ratio (MTR) would be significantly higher and fractional anisotropy (FA), axial diffusivity (AD) and mean diffusivity (MD) would be significantly lower.

This prospective study consisted of 39 dogs with IVDH confirmed with MRI and surgery. Imaging was performed using a 1.5T MRI. MT and DTI images were obtained following conventional sequences. MTR, FA, AD, and MD were calculated using open source software. Dogs were followed for 12 weeks to assess clinical outcome via Modified Frankel Scoring. For statistical analysis, recovered and non-recovered groups were compared using Wilcoxon signed rank test ($p < 0.05$). Median with range of MTR, FA, AD, and MD and p-valve between groups is detailed in the table below.

No statistical difference between MTR and FA was noted between groups. AD and MD were significantly lower in dogs that did not recover. Findings support that quantitative imaging surrogates of axonal integrity may be important prognostic indicators in dogs with SCI.

	Recovered (N=35)	Not Recovered (N=4)	P value
MTR	49.82 (40.86 - 53.46)	51.22 (49.17 - 53.25)	0.21
FA	0.63 (0.46 - 0.76)	0.59 (0.48 - 0.71)	1.0
AD (mcm^2/msec)	1.92 (1.28 - 2.68)	1.24 (0.67 - 1.34)	0.002*
MD (mcm^2/msec)	1.07 (0.71 - 1.49)	0.73 (0.45 - 0.95)	0.01*

O01

Acid Suppressants Modulate in Vitro Mast Cell Structure, Degranulation, and Viability

Emily N. Gould[1], *Elizabeth M. Lennon*[1], *Thomas E. Cecere*[3], *M. Katherine Tolbert*[2]
[1]*University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA*, [2]*University of Tennessee, Knoxville, TN, USA*, [3]*Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA*

Mast cell tumors (MCTs) are the most common cutaneous neoplasm of dogs and third most common intestinal tumor in cats, with a 20% incidence worldwide. Mast cell granules contain pro-inflammatory mediators and vasoactive amines, and potential sequela of mast cell degranulation include life-threatening anaphylaxis and gastrointestinal (GI) ulceration. Patients with MCTs are treated with acid suppressants, including histamine-2 receptor antagonists (H2RAs) and proton pump inhibitors (PPIs), to prevent GI ulceration. Acid suppressants have pH independent effects, including alteration of leukocyte cytokine production, modulation of cell number and function, and changes in mast cell degranulation ability. Mast cell granules require an acidic pH and contain a vacuolar-type ATPase to achieve acidity. This vacuolar ATPase is related to the H^+ / K^+ ATPase in gastric parietal cells, thus, PPIs may impact MCT function and survival. The effect of acid suppressants, most notably PPIs, on mast cells has not been fully explored.

Using a previously validated *in vitro* rat mast cell line, RBL-2H3, and mouse bone marrow derived mast cells (BMMCs), our central objectives were to evaluate the effect of H_2RA (famotidine) and PPI (esomeprazole) treatment on mast cell degranulation, granule morphology, and cell death. Mast cell degranulation and cell death were assessed using beta-hexosaminidase and flow cytometric assays, respectively. Changes to granule morphology were evaluated via light and ultrastructural microscopy.

Degranulation percentage of RBL-2H3 cells was significantly increased when treated with esomeprazole ($P < 0.05$; One-way ANOVA with Holm-Sidak post hoc) but not with famotidine or vehicle treatments. Structural changes were observed with both acid suppressants but were more pronounced with esomeprazole.

Acid suppressant treatment altered mast cell degranulation, viability, and structural morphology *in vitro*, with PPIs having more pronounced effects. Ongoing work will assess the effects of acid suppressant therapy on mast cell tumors in dogs and cats.

O02

Plasma Cytokeratin-18 Levels as Non-invasive Biomarker of Early Gastrointestinal Toxicity in Dogs Receiving Toceranib

Rachel L. Kovac[1], *Joelle Fenger*[1], *Cheryl London*[2], *Emma Warry*[1]
[1]*The Ohio State University, Columbus, OH, USA*, [2]*Tufts University Cummings School of Veterinary Medicine/The Ohio State University, North Grafton, MA, USA*

Toceranib phosphate is a tyrosine kinase inhibitor (TKI) frequently used in veterinary medicine for treatment of a number of tumor types.

Despite studies demonstrating that lower doses of toceranib (2.4 - 2.9 mg/kg every other day) provides drug exposure sufficient for target inhibition while reducing the frequency of drug-related adverse events, gastrointestinal (GI) toxicity continues to be the most commonly encountered side effect in dogs receiving toceranib at this dose. The degree of toxicity is variable among individual dogs and currently there are no clinical or molecular markers to identify those dogs that would benefit from the use of concomitant medications to prevent GI toxicities. This is critical as the development of GI toxicity significantly impacts patient morbidity and quality of life, decreases treatment intensity, increases the cost of treatment, and leads to discontinuation of therapy.

Cytokeratin 18 (CK18) is a member of the intermediate filament family of cytoskeletal proteins and is highly expressed in epithelial cells, notably gastrointestinal mucosal epithelium. Epithelial cells undergoing necrosis and/or apoptosis release full-length or caspase-cleaved CK18 fragments that are detectable in bodily fluids such as serum and plasma. In humans, circulating levels of CK18 have been shown to have potential clinical utility as non-invasive prognostic or predictive biomarkers in a number of solid and hematopoietic tumor types. Data from human lymphoma studies indicate that serum CK18 levels increase following cytotoxic chemotherapy and that high CK18 expression correlates with patients experiencing epithelial toxicity, suggesting that circulating CK18 may have potential utility as predictive biomarker for early gastrointestinal toxicity. Given the lack of clinically relevant biomarkers for the early detection of GI toxicity in veterinary patients receiving anti-cancer drugs, the objective of our study was to determine plasma CK18 levels in dogs with cutaneous and subcutaneous mast cell tumors (MCTs) receiving single-agent toceranib therapy as a reference for understanding the relevance of any changes for the detection of early GI toxicity.

Blood was collected from 20 healthy dogs > 1 year of age with no medical history of gastrointestinal disease or recent gastrointestinal signs including vomiting and diarrhea, to establish a reference range for plasma CK18 levels in healthy control animals. Twenty five client-owned dogs with a histopathological diagnosis of cutaneous or subcutaneous MCTs and no evidence of gross and/or metastatic disease at the time of evaluation were enrolled. Patients were treated with toceranib (2.75 mg/kg EOD) and received Omeprazole (1 mg/kg SID). No dogs received concurrent prednisone and/or other concomitant medications. Plasma was collected from treatment dogs at day 0, day 7, day 14, day 21, and day 28.Clients were instructed to record any gastrointestinal or other clinical signs over the 28 day period. Drug-related adverse events (AEs) were defined and graded according to the published VCOG-CTCAE criteria. Plasma CK18 measurements were obtained for control dogs and treated dogs at all study timepoints using a commercially available canine enzyme-linked immunosorbent assay (ELISA) kit to detect CK18 (NeoBiolab). Determination of plasma VEGF concentrations in treated dogs was performed at all timepoints using a canine VEGF Quantikine ELISA kit (R & D Systems) as a surrogate biomarker of pathway inhibition by toceranib. Preliminary data indicate that baseline plasma CK18 levels are increased in dogs with a diagnosis of cutaneous or subcutaneous MCT compared to healthy control animals. Interestingly, while baseline CK18 levels were elevated in treated dogs, we observed a decline in CK18 levels over the period of the study with plasma CK18 concentrations appearing to plateau over days 14 to 21. No changes in plasma CK18 were correlated with toceranib-related adverse effects and/or grade. However, of the dogs experiencing gastrointestinal toxicity, most were mild. 14 dogs experienced a grade I gastrointestinal toxicity. 7 experienced a grade II toxicity, while only 1 experienced a grade III toxicity. No patients experienced a grade IV or greater gastrointestinal toxicity during the study. Despite the small number of subjects assessed in the current study, these data suggest that plasma CK18 is not a suitable biomarker for the early detection of toceranib-related gastrointestinal toxicity in dogs. However, further investigations to fully assess the predictive value of circulating CK18 detecting early GI toxicity in a larger cohort of patients is required.

O03

Comprehensive Analysis of Gene Mutations in Canine Histiocytic Sarcoma by Whole Exome Sequencing

Hajime Asada[1], *Takao Uchikai*[2], *Hirotaka Tomiyasu*[1], *Genki Ishihara*[2], *Yuko Goto-Koshino*[1], *Koichi Ohno*[3], *Hajime Tsujimoto*[3]
[1]*The University of Tokyo, Bunkyo-ku, Tokyo, Japan,* [2]*Anicom Specialty Medical Institute Inc., Shinjuku-ku, Tokyo, Japan,* [3]*The University of Tokyo, Tokyo, Tokyo, Japan*

Gene mutations in various canine tumors have been explored. In canine histiocytic sarcoma (HS), the mutations of *TP53* and *PTPN11* genes have been reported to be frequently observed. However, there has been no report on the comprehensive analysis of gene mutations in canine HS. The objective of this study was to conduct the comprehensive analysis of mutations of protein coding genes in dogs with HS by whole exome sequencing.

Genomic DNA was extracted from the tumor tissues of three dogs that were histologically diagnosed with HS. For the analysis of germline DNA sequences, genomic DNA was also extracted from normal tissues or peripheral blood of these dogs. Whole exome sequencing was performed using Illumina NextSeq 500. The alignment of processed reads to the canine reference genome (CanFam 3.1) was carried out using Genome Analysis Toolkit. VarScan was used for calling of the somatic variants in each dog and SnpEff was used for the annotations of the variants. The extracted somatic variants were filtered by the read depth and the putative impact for the gene functions. The variants in the genes known to be associated with the pathogenesis of human tumors were validated using Sanger sequencing.

Whole exome sequencing generated the mean read depth of 630×, and 99.2 % of the unique reads could be mapped to the canine reference genome on average. After the analysis of the data, 11 variants in 11 genes, 17 variants in 16 genes, and 14 variants in 13 genes were extracted as the somatic mutations in Dogs 1, 2, and 3, respectively. Of these variants, the mutations of *TP53* (Dog 1), *PDGFRB* and *N4BP2* (Dog 2), and *SH3KBP1* (Dog 3) were confirmed by Sanger sequencing.

This study revealed somatic mutations of *TP53*, *PDGFRB*, *N4BP2*, and *SH3KBP1* genes in canine HS. We previously reported the high incidence of *TP53* gene mutations in canine HS. N4BP2 was reported to interact with BCL3, a target of PI3K/Akt and MAPK/ERK pathways, and SH3KBP1 was shown to induce down-regulation of protein tyrosine kinase that activates PI3K/Akt and MAPK/ERK pathways (Fig. 1). Although further studies are needed to know the frequencies of the

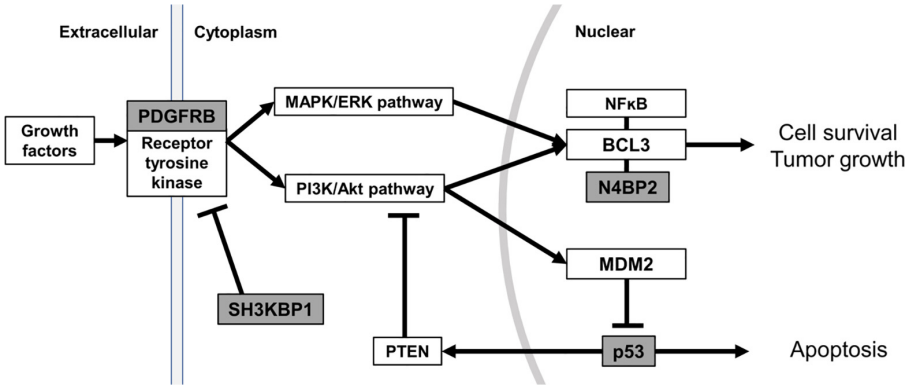


FIGURE 1 Schematic diagram of MAPK/ERK, PI3K/Akt, and p53 pathway. Gray boxes represent the genes where mutations were identified in the present study

gene mutations, findings obtained in the present study will lead to the introduction of molecular targeted therapy directed to PI3K/Akt, MAPK/ERK, and p53 pathways in canine HS.

O04

Predicting the Response of Canine B-Cell Lymphoma to CHOP Chemotherapy with Ex Vivo Biodynamic Imaging

Michael O. Childress[1], *Honggu Choi*[2], *Zhe Li*[2], *Zhen Hua*[2], *Ms. Sarah A. Lahrman*[2], *José A. Ramos-Vara*[2], *Christopher M. Fulkerson*[2], *Deborah W. Knapp*[3], *John J. Turek*[2], *David D. Nolte*[2]
[1]*Purdue College of Veterinary Medicine, West Lafayette, IN, USA,* [2]*N/A, West Lafayette, IN, USA,* [3]*Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA*

Combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) is the standard treatment for dogs with diffuse large B-cell lymphoma (DLBCL). While CHOP affords durable cancer remission to most dogs with DLBCL, some dogs benefit minimally from this therapy. Current methods of prognostic assessment do not adequately discriminate those dogs that will and will not derive significant benefit from CHOP. However, biodynamic imaging (BDI) a novel imaging technology that measures changes in intracellular motion induced within living tissues by drug treatment, has shown promise as a means to predict a tumor's sensitivity to chemotherapy. We previously showed that BDI, performed *ex vivo* on tumor biopsy tissues from dogs with lymphoma, accurately predicted *in vivo* response to doxorubicin. The purpose of this study was to expand the use of BDI to predicting clinical outcome of CHOP chemotherapy in dogs with DLBCL.

Twenty-two dogs with WHO substage a DBLCL were enrolled in a prospective clinical trial. At the time of study enrollment, all dogs underwent standardized staging tests and surgical lymph node biopsy to provide tissue for histopathologic and immunohistochemical confirmation of DLBCL. All dogs subsequently received treatment with a previously described 25-week CHOP chemotherapy protocol. The primary clinical outcome of interest was progression-free survival (PFS), defined as the time in days between initiation of CHOP chemotherapy and detection of measurable disease progression, or death due to any cause, whichever came first.

At the time of enrollment, a portion of each dog's lymph node biopsy (approximately 100 mm^3) was reserved for *ex vivo* BDI. Within 1 hour of biopsy collection, the tissue was processed into several small fragments of approximately 1 mm^3 size. All fragments were immobilized in 96 well plates and treated ex vivo with 4-hydroxycyclophosphamide (5 μM), doxorubicin (10 μM), vincristine (0.06 μM), and prednisolone (0.6 μM), as well as the drugs in combination. Tissue samples treated with 0.1% DMSO served as negative controls. Changes in intracellular motion following ex vivo drug treatment were recorded from all tissue samples from each dog for approximately 24 hours following biopsy collection. A machine learning classifier was developed to analyze the intracellular motion-based tumor responses measured by BDI and to associate these responses with poor or favorable clinical responses based upon PFS. The classifier was trained in a "one-left-out" approach, first on data sets from 18/19 dogs, then adding in data from the remaining dog for cross-validation. This process was repeated for each enrolled dog.

Clinical outcome following CHOP chemotherapy was evaluable in 19/22 dogs. The best objective response to chemotherapy was complete remission in 17 dogs and partial remission in 2 dogs. Progression-free survival times ranged from 43 to > 769 days. When PFS times for the study population were segregated into low-PFS (≤ 98 days, n=6) or high-PFS (≥ 232 days, n = 13) groups, the machine learning classifier correctly predicted assignment of dogs to the appropriate group in 18/19 cases. The classifier consistently assigned one dog with low PFS (97 days) incorrectly into the high-PFS group. These results suggest a possible important application of BDI as a method for predicting the responsiveness of cancers to chemotherapy drugs.

O05

The Association between Symmetric Dimethylarginine Concentrations and Neoplasia in Dogs and Cats

Michael Coyne, Corie Drake, Donald J. McCrann
IDEXX Laboratories, Inc., Westbrook, ME, USA

The purpose of this study was to examine the association between SDMA concentrations in dogs and cats with different types of neoplasia. The IDEXX Laboratories pathology database was used to identify cases of lymphoma, hemangiosarcoma, mammary carcinoma, mammary adenocarcinoma, and lipoma in dogs, and lymphoma and visceral mast cell tumor in cats. Potential cases needed a serum SDMA and creatinine measured within 3-months prior to diagnosis. Control animals were randomly selected from the clinical laboratory database

and age- and breed-matched (in dogs) and age-matched (in cats) to cases. SDMA and creatinine concentrations were compared between cases and controls by cancer type. SDMA concentrations were significantly higher in dogs and cats with lymphoma ($P < 0.0001$) and significantly lower in dogs with lipoma, mammary adenocarcinoma and mammary carcinoma ($P \leq 0.003$) as compared to controls. Creatinine concentrations were significantly lower in dogs with mammary adenocarcinoma and mammary carcinoma and in cats with lymphoma ($p \leq 0.0008$) when compared to controls. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine the association between SDMA and tumor type (Table 1). The results indicate that in both cats and dogs, cases of lymphoma are associated with increased SDMA concentrations with a large portion of these cases having concurrent creatinine concentrations within the reference interval. Although the etiology of increased SDMA concentrations in these cases was not determined, SDMA may be an early marker of renal involvement and reduced GFR in recently diagnosed cases of lymphoma.

Table 1.

Cancer Type	N	OR (95% CI)	P
Canine Lymphoma	307	10.00 (5.98 -16.72)	P < 0.001
Feline Lymphoma	224	3.04 (1.95-4.73)	P < 0.001
Feline Visceral Mast Cell Tumor	55	1.63 (0.67-3.92)	P = 0.275
Canine Hemangiosarcoma	230	1.11 (0.66 -1.87)	P = 0.691
Canine Mammary Carcinoma	388	0.49 (0.28 -0.84)	P = 0.009
Canine Mammary Adenocarcinoma	388	0.41 (0.231- 0.71)	P = 0.001
Canine Lipoma	212	0.39 (0.18 -0.85)	P = 0.013

O06

Treatment with Enterococcus faecium NCIMB 10415 Does Not Affect the Outcome of Chemotherapy-Induced Diarrhea

Linda A. Matthewman[1], Ana Lara-Garcia[1], Dirk Werling[2]
[1]Royal Veterinary College, Hatfield, England, United Kingdom, [2]Pathobiology and Population Sciences Royal Veterinary College, Hatfield, England, United Kingdom

Chemotherapy-induced diarrhoea (CID) is an adverse event associated with administration of cytotoxic anticancer drugs in dogs. In many cases, chemotherapy reduces the quality of life (QoL) of the patient and can prejudice prognosis. Concurrent treatment with probiotics may support the functions of the intestinal microbiota and reduce the prevalence and severity of CID.

To assess the impact of probiotic treatment on the prevalence and severity of CID, a randomised, double-blinded, placebo-controlled, crossover trial was performed in dogs with cancer scheduled to receive doxorubicin. Dogs received treatment for 14 days, starting on the day of doxorubicin administration, with either a probiotic formulation containing *Enterococcus faecium* NCIMB 10415 (2×10^9 CFU) or a placebo. A CID assessment form was developed for daily completion by the owner for five days, starting four days after treatment with doxorubicin, and a score was calculated to describe the severity of the event. In addition, a quality of life (QoL) assessment form was developed to assess the QoL of the dog during each treatment period. Faecal samples were collected before administration of doxorubicin and during and after treatment with the probiotic or the placebo. Faecal DNA was extracted and a quantitative polymerase chain reaction was developed to detect and quantify the presence of the specific probiotic strain.

Fifty dogs were recruited, 32 took both the probiotic formulation and the placebo, 11 took either product only, and seven dogs died of their cancer or were removed from the trial, leaving 43 cases for evaluation. Faecal consistency was described as abnormal in 80% of dogs in the probiotic group and 83% in the placebo group. Faecal frequency was increased in 51% of dogs in the probiotic group and 44% in the placebo group. There was no difference in the diarrhoea event scores whilst the dogs took either trial product ($p > 0.05$). In addition, there was no difference in QoL score between each trial period, but there was a significant correlation between QoL and diarrhoea score; QoL was considered to be worse when the diarrhoea was more severe ($p < 0.0001$). Also, there was a significant correlation between QoL and tumour type; dogs with lymphoma were described as having a significantly worse QoL compared to dogs with sarcomas or carcinomas ($p < 0.05$). The probiotic bacteria could be detected in the faeces of most dogs ($n = 37$) whilst they took the probiotic formulation but only in four dogs, seven days after treatment stopped, independent of the tumour-type, suggesting only transient colonisation of the gastrointestinal tract during the period of probiotic therapy.

Our data clearly indicate that CID reduces QoL. Quality of life as well as prevalence or severity of CID were not improved by treatment with *E. faecium* NCIMB 10415 for 14 days.

O07

Felis catus Gammaherpesvirus 1 Associated with Shorter Survival but not Risk of Lymphoma in Cats

Alicia J. McLuckie[1], Vanessa R. Barrs[1], Scott Lindsay[2], Mahdis Aghazadeh[1], Cheryl Sangster[3], Julia A. Beatty[1]
[1]The University of Sydney, Sydney, New South Wales, Australia, [2]The University of Adelaide, Roseworthy, South Australia, Australia, [3]The University of Sydney/The University of Glasgow, Sydney, New South Wales, Australia

The pathogenic capacity of *Felis catus* gammaherpesvirus 1 (FcaGHV1), a common infection of domestic cats, is unknown. To explore a possible role for FcaGHV1 in feline lymphoma, a retrospective, cross-sectional, disease-association study was carried out.

DNA from FFPE biopsy and/or whole blood from 122 lymphoma cases, excluding small cell tumors, diagnosed by histopathology ($n = 93$) or cytology ($n = 29$), was tested for viral DNA. Control DNAs were derived from paired, autologous uninvolved FFPE tissue ($n = 33$) and FFPE lymph node ($n = 31$) or whole blood ($n = 86$) from non-lymphoma cases. Results of viral testing, signalment, epidemiologic data and treatment received were recorded.

Neither a molecular diagnosis of FcaGHV1 nor whole-blood virus load was related to outcome in lymphoma cases compared with controls matched for age and sex. Molecular analysis of lymphoma-derived DNAs paired with autologous uninvolved tissue did not suggest restriction of FcaGHV1 DNA to tumour tissue. FcaGHV1 DNAemia was associated with significantly shorter survival in lymphoma cases ($P = 0.0019$), an observation that could not be adequately explained by differences in treatment received. In addition, a history of fighting with other cats and roaming were identified as novel epidemiological risk factors for FcaGHV1 detection, lending further support to inter-cat aggression as a potential route of virus transmission.

Future studies addressing virus localisation and expression in neoplastic cells are indicated to assist in ruling out a lymphomagenic role for FcaGHV1. Prospective investigation of FcaGHV1 DNAemia as a negative prognostic marker in feline lymphoma is warranted.

O08

YM155 Inhibits the Growth of Canine Squamous Cell Carcinoma Cells with High Expression of Survivin

Ryo Miyamoto[1], *Hiroyuki Tani*[2], *Sena Kurita*[3], *Miss Tomoyo Ikeda*[4], *Moe Ishizaka*[4], *Miss Hono Saima*[4], *Kyoichi Tamura*[5], *Makoto Bonkobara*[5]
[1]*Nippon veterinary and life science university, Musashino, Tokyo, Japan,* [2]*Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan,* [3]*Nippon Veterinary and Life Science University, musashino, Tokyo, Japan,* [4]*Nippon veterinary and life science university, Musashino-shi, Tokyo, Japan,* [5]*Nippon Veterinary and Life Science University, Musashino-shi, Tokyo, Japan*

Survivin is highly expressed in various malignancies and functions as an inhibitor of apoptosis. YM155, an imidazolium-based small molecule, has potent inhibitory activity to survivin; therefore, it is considered a potential new class of anti-tumor agents. In the current study, we screened for various canine tumor cell lines that have susceptibility to YM155. On the basis of the screening, we focused on squamous cell carcinoma (SCC) and growth inhibitory mechanisms of YM155 in SCC cells were investigated.

Among ten cell lines (eight tumor types) screened, only SCC cell line showed substantial susceptibility to YM155. Based on this finding, seven canine SCC cell lines were examined for susceptibility to the cytotoxic effects of YM155. YM155 clearly inhibited the growth of two cell lines HAPPY and SQ4. These cell lines, but not other cell lines, highly expressed survivin. In HAPPY cells, YM155 inhibited expression of survivin at the both mRNA and protein level. In contrast, YM155 down-regulated survivin expression only at the protein level in SQ4 cells. Moreover, YM155 suppressed cell growth mostly by induction of apoptosis in HAPPY cells but not in SQ4 cells.

Our results suggested that canine SCCs with high cellular expression of survivin are susceptible to survivin inhibitor YM155. There are different mechanisms in YM155-induced suppression of survivin expression, namely at the transcriptional and post-transcriptional levels. Moreover, YM155 was found to induce growth inhibition of SCC cells by induction of not only apoptotic cell death, but also non-apoptotic cell death.

O09

Nanoparticle and Laser Thermal Ablation in Canine Low Grade Mast Cell Tumors

Lisa Parshley[1], *Abbey Bradley*[1], *LIsa Miller*[2], *Luis De Taboada*[3], *Jon Schwartz*[4], *Scott Gustafson*[1], *Emily Mouat*[1]
[1]*Olympia Veterinary Specialists, Olympia, WA, USA,* [2]*LiteCure, Companion Therapy, Newark, DE, USA,* [3]*LiteCure LLC, Carlsbad, CA, USA,* [4]*Nanospectra Biosciences, Houston, TX, USA*

Use of nanoparticles as a possible cancer therapy has intriguing potential. Studies are currently in progress that explore their use in immunotherapy, targeted therapy delivery, and thermal ablation. In this pilot study we employ a nanoparticle consisting of silicone and gold. Due to the unique nature of the nanoparticle design they vibrate when exposed to the appropriate wavelength of light generating controlled and targeted heat. Our hypothesis is that heat generated with laser light induced nanoparticle vibration will cause cancer cell death, thereby provide definitive therapy in low grade mast cell tumors.

Eighteen with low grade mast cell tumors, < 1.5 cm, were randomized into either treatment or control groups. All dogs received Benadryl, 2 mg/kg/12 hours, and Pepcid AC, 0.5mg/kg/24 hours. On day one dogs received a 2 hour IV infusion of nanoparticles. On day two, the treatment group dogs had light therapy applied to the tumor using a diode laser - 810 nm, 12W/cm^2. Dogs were evaluated once a week for four weeks and then monthly. Data collected included signalment, tumor size pre-infusion and pre-laser therapy application, response to therapy, duration of response, and any toxicities.

All laser treated dogs responded and were in clinical remission by one month from laser therapy. Three dogs had recurrence at 3 and 4 months after therapy. No serious or long term toxicities were noted in any of the dogs.

Conclusion - Nanoparticle laser ablation therapy was effective in treating low grade mast cell tumors with a low risk of toxicities.

O10

Amiloride Sensitizes Canine Osteosarcoma Cells to Doxorubicin Chemotherapy

Andrew C. Poon, Anita K. Luu, Anthony J. Mutsaers
Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Canine osteosarcoma (OSA) is the most common primary bone tumour in dogs. Chemotherapy delays metastasis, yet most dogs succumb within one year of diagnosis. Recent studies show that certain chemotherapeutics are sequestered within acidic endosomes, leading to chemoresistance. Furthermore, the high metabolic demand of cancer cells results in a preferential glycolytic shift that leads to tumor acidosis.

Amiloride, a potassium-sparing diuretic, is among a class of proton pump inhibitors prescribed by veterinary cardiologists for refractory heart failure. In this study, we hypothesized that amiloride sensitizes canine OSA cells to carboplatin and doxorubicin by reducing their sequestration within acidic endosomes.

Assessments of cell viability and apoptosis were performed in three OSA cell lines after single agent or combination treatment, and the synergism of each combination was evaluated with combination index (CI) calculations. Metabolic profiling of extracellular acidification rates (ECAR) and oxygen consumption rates (OCR) was performed, and immunoblotting was used to evaluate apoptotic protein expression.

Amiloride sensitized canine OSA cells to doxorubicin (CI < 1.0), but not carboplatin (CI ≥ 1.0) in combination therapy. Consistent with these findings, combination treatment with doxorubicin significantly increased late apoptosis ($P < 0.0001$), but decreased necrosis ($P < 0.001$) in OSA cells compared to treatment with doxorubicin alone. Cells treated with amiloride upregulated proteins involved in p53-mediated apoptosis and downregulated Akt-specific survival. Energetic stress tests further demonstrated metabolic switching to a less glycolytic phenotype with significant decreases in ECAR ($P = 0.021$).

The well-known safety profile of amiloride and its likelihood of synergism with doxorubicin may justify drug repurposing for clinical trial evaluation in canine OSA.

O11

Antitumor Effect of Lapatinib in Canine Transitional Cell Carcinoma Cell Lines

Kosei Sakai, Shingo Maeda, Masaya Tsuboi, Tomohiro Yonezawa, Naoaki Matsuki
Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Tokyo, Japan

Transitional cell carcinoma (TCC) accounts for > 90% of canine malignant tumors occurring in urinary bladder, and the prognosis is poor. Our previous study, using RNA sequencing, showed that the oncogene human epidermal growth factor 2 (HER2) was the significantly activated gene pathway related to carcinogenesis in canine TCC. However, information on HER2 in canine TCC is lacking. The aims of this study were to examine the antitumor effect of lapatinib, a tyrosine kinase inhibitor of HER2, on canine TCC cell lines *in vitro* and *in vivo* and to assess HER2 protein expression in dogs with TCC.

Five canine TCC cell lines (TCCUB, Love, Sora, LTCC, and CTCC) were used. Cells were incubated with lapatinib. Expression and phosphorylation of downstream molecules in the HER2 signaling pathway were then analyzed by western blotting. Cell proliferation, cell cycle, and apoptosis were examined by methylthiazolyl tetrazolium assay, flow cytometry, and TdT-mediated dUTP nick end labeling (TUNEL) method, respectively. For the *in vivo* experiments, the canine TCC cells were subcutaneously injected into nude mice. Lapatinib or vehicle were administered daily via intraperitoneal administration for 14 days. At the endpoint, the mice were euthanized, and the tumors were collected. Histologically, necrotic areas in the tumor tissues were evaluated. Finally, immunohistochemistry of HER2 was performed using urinary bladder tissues of 23 dogs with TCC and 8 healthy dogs. Intensive staining localized to cell membrane was defined as HER2 protein overexpression.

HER2 protein expression was observed in all of the canine TCC cell lines. Lapatinib inhibited phosphorylation of HER2 and the downstream molecules and cell proliferation in a dose-dependent manner. Cell cycle analyses showed that lapatinib significantly increased the sub-G_1 and G_0/G_1 phase fractions and significantly decreased the S and G_2/M phase fractions in the cells. TUNEL staining showed few apoptotic cells after treatment with lapatinib. Six to fourteen days after treatment, tumor volumes of the canine TCC-engrafted mice were significantly smaller in the lapatinib group compared to the vehicle control group. Lapatinib treatment significantly increased the necrotic areas in the tumor tissues. Immunohistochemistry of HER2 showed that HER2 protein overexpression was observed in 14/23 (61%) dogs with TCC but not in the healthy dogs.

This study demonstrated that the antitumor effects of lapatinib on the canine TCC cell lines by inhibiting HER2 signaling and inducing cell cycle arrest. Importantly, lapatinib also exerted the antitumor effects on the canine TCC-engrafted mice. In addition, HER2 protein was overexpressed in more than half of the dogs with TCC. These results suggest that lapatinib has therapeutic potential for dogs with TCC.

O12

Adjuvant Electrochemotherapy with Bleomycin and Cisplatin Combination for the Canine Soft Tissue Sarcoma: 30 Cases

Enrico P. Spugnini
Biopulse S.r.l., Roma, Lazio, Italy

Electrochemotherapy (ECT) couples the administration of anticancer drugs with the delivery of electric pulses that increase the drug uptake through the cell membranes, thus resulting in an improved efficacy. This study has been undertaken to evaluate the tolerability and efficacy of the combination of systemic bleomycin and local cisplatin as ECT agents for incompletely excised canine soft tissue sarcoma (STS). Thirty dogs with incompletely excised STSs were enrolled. The dogs received intravenous 20 mg/m^2 bleomycin and the tumor bed and margins were infiltrated with cisplatin at the dose of 0.5 mg/cm^2, and then exposed to trains of permeabilizing pulses under sedation. More precisely, five minutes after the injection of the chemotherapy agents, sequences of 8 biphasic pulses lasting 50+50 microseconds each, were delivered in bursts of 1300 V/cm using caliper electrodes. A second session was performed two weeks later. The treatment was well tolerated and side effects were minimal. Twenty-six dogs had no evidence of recurrence at different times (remission range 6-36 months), four had recurrence and one of the four recurring dogs, died of lung metastases. ECT using combination of bleomycin and cisplatin appears to be effective in the treatment of incompletely resected STSs in dogs and could be a useful addition to the current options in consideration of its low cost, limited toxicity, and ease of administration.

O13

Treatment of Histiocytic Sarcoma with Nimustine in Dogs: 9 Cases

Hiroyuki Tani[1], Sena Kurita[2], Ryo Miyamoto[1], Shizuka Iijima[1], Kyoichi Tamura[3], Makoto Bonkobara[3]
[1]Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan,
[2]Nippon Veterinary and Life Science University, musashino, Tokyo, Japan,
[3]Nippon Veterinary and Life Science University, Musashino-shi, Tokyo, Japan

In the treatment of canine histiocytic sarcoma (CHS), a nitrosourea agent lomustine (CCNU) is often used. CCNU is a potent myelosuppressive agent and can cause cumulative dose-related irreversible hepatotoxicity, indicating a need of accurate dose adjustment. However, CCNU is an oral capsule drug, and thus, a fine dose adjustment is difficult. Nimustine (ACNU), the same nitrosourea agent, is an intravenous injection drug and its safety in dogs has been demonstrated in a phase I study. ACNU is therefore an attractive agent for the treatment of CHS. In this study, we thus examined the therapeutic potential of ACNU in the cases of CHS.

Nine CHS cases (with gross disease, n = 4; in the adjuvant setting, n = 5) were treated with ACNU (20-30 mg/m^2) for 1-527 days. The therapeutic effects were evaluated by overall survival (OS) and progression-free interval (PFI). Tumor responses to ACNU were evaluated by c-RESIST v.1.0 criteria and/or by improvement of tumor-associated symptoms.

Median OS and median PFI were 256 days (range, 6 to >1286 days) and 131 days (range, 1 to >1286 days), respectively. Tumor responses of two cases with measurable disease were CR or SD. Of the five

cases with tumor-associated symptoms, three were improved the symptoms.

In this study, median OS/PFI appeared similar to those reported in CHS treated with CCNU. Moreover, tumor regression and/or improvement of tumor-associated symptoms were observed in three dogs. ACNU could be potential therapeutic agent for CHS. Our results warrant further investigation of ACNU in larger clinical studies.

O14

Generation of Myeloid Derived Suppressor Cells by Tumor Exosomes (VCS Award Winner)

Jennifer Lenz, Nhungoc Ti Luong, Julie Olson
University of Minnesota, Minneapolis, MN, USA

Introduction Accumulation of myeloid-derived suppressor cells (MDSCs) diminishes antitumor immune responses necessary to control tumor growth and progression. MDSCs are discriminated from other monocytes in that they possess strong immunosuppressive activities rather than immunostimulatory properties. Exosomes are small membrane-bound vesicles that provide a method of communication between cells through their ability to transfer proteins and miRNA. We hypothesize that tumor cells secrete exosomes that are taken up by monocytes and promote MDSC differentiation through defective myeloid cell maturation.

Methods Exosomes were isolated from tumor cell lines (osteosarcoma, glioma, colon carcinoma, melanoma, fibrosarcoma) and from primary cultures of each parental cell of origin. Following addition of exosomes to monocytes over 3 consecutive days, cells were collected for flow cytometry, transcriptional analysis, and T cell proliferation assays.

Results Increased expression of anti-inflammatory mediators (IL-10, iNOS, Arg1) and decreased expression of pro-inflammatory cytokines (IL-12, TNFa) was observed in the monocytes after the addition of exosomes from all the tumor lines supporting the conversion to a suppressor phenotype. Flow cytometry and T cell proliferation assays confirmed a MDSC phenotype. Exosomes isolated from the primary control cultures did not promote suppressive functions by the monocytes.

Conclusion Our data supports the hypothesis that tumor-derived exosomes promote MDSC differentiation. A better mechanistic understanding of this interaction is critical for the discovery of novel therapeutic targets in cancer treatment, with the ultimate goal of preventing the conversion of monocytes to a suppressor phenotype.

O15

Comparison of Various Imaging Modalities for Setup Verification Prior to Delivery of Stereotactic Radiation Therapy for Head and Neck Cancers in Veterinary Patients (VCS Award Winner)

Leanne Magestro, Michael Nolan, Tracy Gieger
North Carolina State University, Raleigh, NC, USA

INTRODUCTION The “gold standard” for patient positioning verification before linac-based stereotactic radiation therapy (SRT) is cone-beam computed tomography (CBCT), which is not available in all clinics; planar imaging is sometimes used instead. The purpose of this study was to quantify errors inherent to orthogonal megavoltage (MV) portal and kilovoltage (kV/kV) imaging versus CBCT when used to verify setup of bony targets. Our hypothesis was that use of planar imaging would necessitate inclusion of a setup margin in the planning target volume (PTV) expansion.

MATERIALS AND METHODS All patients receiving head and neck SRT were considered for inclusion in this prospective clinical study. Planar (MV ports and kV/kV images) and rotational (CBCT) images were acquired before treatment, and manually co-registered with the reference image set. Differences in patient position (vertical, lateral, longitudinal, pitch, yaw, and roll) when matched based on MV ports, kV/kV images, and CBCT were compared.

RESULTS Eighty-two independent data sets were evaluated. Mean differences in lateral, longitudinal and vertical directions from MV ports to kV/kV images were 1.0, 0.4 and 0.8 mm, respectively, with a mean 3D vector of 1.7 mm^3. Mean angular difference in pitch and yaw were 0.3 and 0.4°. Mean differences from MV ports to CBCT images were 1.2, 0.7, and 1.3 mm, respectively, with a mean vector of 2.2 mm^3. Mean angular differences were 0.7 and 0.5°. The mean roll detected on CBCT was 0.6°; this could not be quantified with planar imaging.

CONCLUSIONS The mean shift discrepancies described herein should be accounted for in the setup margin for linac-based SRT; failure to do so could result in early treatment failure due to geographic miss. Size of the PTV expansion can be minimized through use of CBCT and correction of visible positioning errors using a robotic couchtop, or meticulous manual positioning.

O16

Efficacy of a Doxorubicin and Cytarabine Chemotherapy Protocol in 6 Dogs with Acute Myeloid Leukemia

Arata Matsuyama[1], Danielle Richardson[1], Jerome Calvalido[2], Anthony J. Mutsaers[1], Dorothee Bienzle[1]
[1]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Tufts Veterinary Emergency Treatment & Specialties, Walpole, MA, USA

The aim of this study was to assess the toxicity and efficacy of a doxorubicin/ cytarabine (DC) combination protocol in dogs with acute myeloid leukemia (AML). A cohort of 6 dogs with persistent cytopenia, and bone marrow cytology and/or core biopsy diagnosis of AML were treated with same day doxorubicin at 30 mg/m^2 or 1 mg/kg IV over 20 minutes, followed by cytarabine at 300 mg/m^2 with constant rate IV infusion over 4 hours, every 14 days. Prednisone (2 mg/kg PO q24h) was also administered. Hematologic results, adverse effects and outcomes were assessed every 1-2 weeks during DC therapy.

Six dogs were treated with the DC protocol over a period of two years. Median age was 9.4 years (range 1-10) with median weight of 17.4 kg (range 3.7-32.2 kg). Dogs presented with anemia (n=6) and leukopenia (n=2) or thrombocytopenia (n=2). Two dogs had circulating blast cells. Median number of treatment cycles was 6 (range 1-9). Dose reduction was required in 2 dogs due to grade 4 neutropenia or grade 3 gastrointestinal adverse effects (GIAE). One dog developed dilated cardiomyopathy (DCM) after a cumulative doxorubicin dose of 180 mg/m^2. Two dogs are still alive 305 and 799 days after diagnosis; one dog died due to DCM at 380 days while cytopenia was in

remission, and three dogs died from progressive AML 13, 29 and 217 days after diagnosis, respectively. Improvement in cytopenia was noted in all but the two dogs that died within 30 days.

The DC protocol was overall well tolerated. Four of 6 dogs survived longer than 6 months. Further studies with larger sample size are warranted.

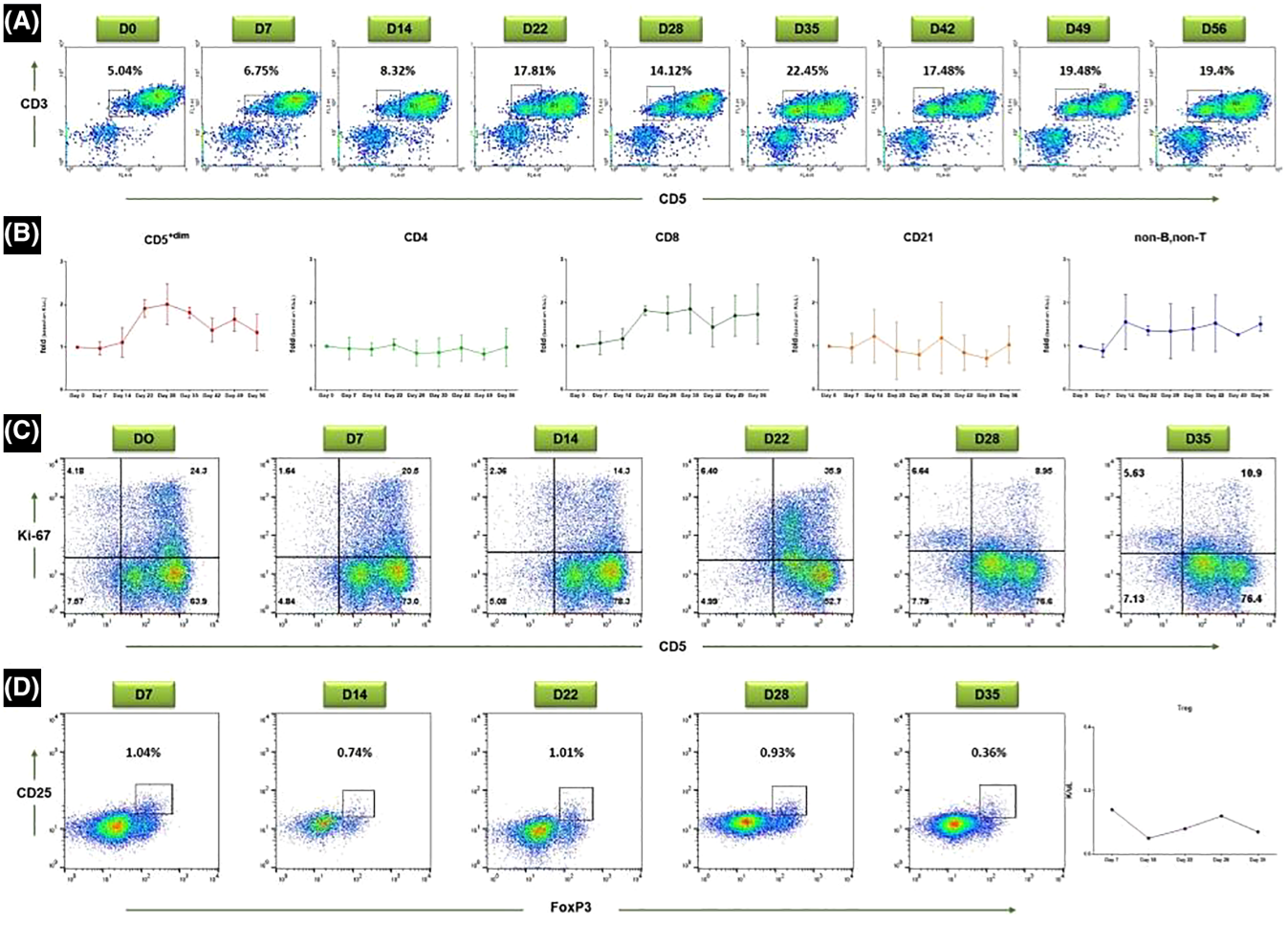
O17

Clinical and Immunological Anti-Cancer Effects of Canine IL-15 with Metronomic Cyclophosphamide in Dogs with Cancer

Soomin Ahn[1], Soohyeon Lee[2], Miseon Lim[3], Dongjun Shin[4], Sangki Kim[4], Dong-In Jung[5], Do-Hyeon Yu[5]

[1]College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju, Kyongsang-namdo, Republic of Korea, [2]Department of Integrated Life Science and Technology, Kongju National University, Yesan-gun, Chungnam, Republic of Korea, yesan, Ch'ung-ch'ong-namdo, Republic of Korea, [3]JBRC BIC 201, Sandangil 12-55, Hwasun, Jeollanam-do, Republic of Korea, Vaxcell-Bio Institute, Hwasun, Cholla-namdo, Republic of Korea, [4]Department of Laboratory and Companion Animal Science, College of Industrial Science, Kongju National University, Yesan-gun, Chungnam, Republic of Korea, Yesan, Ch'ung-ch'ong-namdo, Republic of Korea, [5]College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju-si, Kyongsang-namdo, Republic of Korea

Interleukin-15 (IL-15) is a pleiotropic cytokine that plays a pivotal role in both innate and adaptive immunity. IL-15 is also a promising cytokine for treating cancer. Despite a growing interest in the use of IL-15 as an immunotherapeutic agent, it has not been reported the immunological and clinical effects of IL-15 on cancers in dogs. In this study, we generated a recombinant canine IL-15 (rcIL-15), and determined its biological effects on immune effector cells, and confirmed its safety *in vivo*. After then, clinical and immunomodulatory effects of rcIL-15 in combination with metronomic cyclophosphamide were evaluated in canine patients with various tumor types. To determine the safety and immunologic effects, rcIL-15 was injected intravenously in doses of 20 μg/kg daily for 8 days and monitored for 32 days. Treatment outcomes in dogs with cancers were evaluated in a prospective clinical trial. Low dose of cyclophosphamide (15 mg/m^2, PO, SID) was continuously administrated for 7 weeks. From day 14 after administration of cyclophosphamide, rcIL-15 (20 μg/kg daily) was injected intravenously for 8 days. Although several dogs experienced temporal and mild pyrexia, any other significant adverse events were not detected during and after injection of rcIL-15. Administration of rcIL-15 in combination with metronomic cyclophosphamide remarkably increased the number of NK cells and its anti-cancer activities, and significantly decreased the number of regulatory T cells in the peripheral blood of all patients, which were associated with improved clinical outcomes. Our results suggest rcIL-15 is safe and has tremendous potential for antitumor therapy for dogs.



O18

Ecological-Level Analysis of Canine and Feline Primary Lung Tumors and Environmental Radon Levels

Brittany L. Fowler [1], Chad M. Johannes[2], Annette M. O'Connor[2], Deanna Collins[2], Jonathan L. Lustgarten[3], Kristen M. Weishaar[4], Kelly E. Sullivan[5], Kelly R. Hume[5], Jennifer Mahoney[6], Brittany A. Vale[7], Alicia Schubert[8], Valerie E. Ball[8], Katie M. Cooley-Lock[9], Katie Curran[10], Laura Nafe[11], Allison Gedney[12], Megan T. Weatherford[13], Dana N. LeVine[2]
[1]Iowa State University, College of Veterinary Medicine, DES MOINES, IA, USA, [2]Iowa State University, Ames, IA, USA, [3]Compassion First Pets Red Bank Veterinary Hospital, Tinton Falls, NJ, USA, [4]Colorado State University, Fort Collins, CO, USA, [5]Cornell University, Ithaca, NY, USA, [6]University of Pennsylvania, Philadelphia, PA, USA, [7]Purdue University, West Lafayette, IN, USA, [8]North Carolina State University, Raleigh, NC, USA, [9]Mississippi State University, Mississippi State, MS, USA, [10]Oregon State University, Corvalis, OR, USA, [11]Oklahoma State University, Stillwater, OK, USA, [12]University Pennsylvania, Philadelphia, PA, USA, [13]University of Georgia, Athens, GA, USA

Epidemiologic studies suggest that residential radon exposure may increase the risk of primary lung cancer in people, however, these studies are limited by subject mobility outside of the home. This limitation may be overcome by evaluating the association in dogs and cats. We hypothesized that the rate of primary pulmonary neoplasia would be increased in dogs and cats residing in counties with higher environmental radon levels.

Systematic retrospective review of medical records at ten veterinary schools identified client-owned dogs and cats diagnosed with primary lung tumors between 2010 and 2015. Patient radon exposure was determined by correlating the patient's zip code with published county EPA radon levels. County pulmonary neoplasia rates were determined using average annual county cat/dog populations; rates were compared between radon zones using ANOVA with subsequent pairwise comparisons.

Record review identified 690 dogs and 205 cats with primary pulmonary neoplasia from veterinary schools with high (n = 4), mid-level (n = 2) or low (n = 4) radon levels. Average county level rates of canine pulmonary neoplasms were higher in high environmental radon areas (> 4 pCi/L) compared to counties with mid-(2-4 pCi/L) ($p < 0.0006$) or low (< 2 pCi/L) radon ($p < 0.0037$). There was no significant difference in the pulmonary neoplasia rate by radon level in cats ($p = 0.1011$).

Higher environmental radon levels may play a role in development of primary pulmonary neoplasia in dogs. Further studies investigating the residential radon level along with other environmental factors are warranted to fully define the potential effects of radon on development of primary pulmonary neoplasia in dogs and cats.

O19

Immunohistochemical Expression of Lung Resistance-Related Protein (LRP) in Dogs with Cutaneous Lymphoma

Ana Luíza Nairismagi Alves[1], Valter Medeiros. Winkel[2], Bruno Cogliati[2], Renata Afonso. Sobral[3], Juliana Satie Hatarashi[4], Alexandre Rocha Novaes[2], Silvia Regina Ricci Lucas[5]
[1]School of Veterinary Medicine and Animal Science - University of São Paulo, São Paulo, Sao Paulo, Brazil, [2]School of Veterinary Medicine - University of São Paulo, São Paulo, Sao Paulo, Brazil, [3]Specialist Animal Hospital - Oncology, São Paulo, Sao Paulo, Brazil, [4]Achool of Veterinary Medicine - University of São Paulo, São Paulo, Sao Paulo, Brazil, [5]School of Veterinary Medicine - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil

Canine cutaneous lymphomas are characterized by a rapid progression and poor prognosis. Cutaneous lymphomas have a low response to the treatment, and dogs with non-epithelitropic lymphoma (NEL) has a lower survival time when compared to dogs with epitheliotropic form (EL). The low rate of response to systemic chemotherapy can be associated to a multidrug resistance (MDR). Cell mechanisms of MDR may include proteins involved in nucleo-cytoplasmic transport, such as lung resistance-related protein (LRP), the major vault protein, that was found to be overexpressed in many chemoresistant primary tumors, including lymphomas, and it was associated to poor response to the treatment. The aim of this study was to investigate LRP expression in dogs with cutaneous lymphoma. Immunohistochemistry was performed in 20 samples (11 NEL and 9 EL), using the polyclonal antibodies rabbies anti-CD-20 (Spring Bioscience), anti-CD3 (DAKO) and mouse anti-LRP (clone 1014; Santa Cruz Biotech). LRP immunoreactivity was analyzed by percentage of labeled cells and it was considered positive when more than 10% of cells was labeled. The predominant immunophenotype was CD3+CD20- (75%), 15% of the cases were CD3-CD20+ and 10% were CD3+CD20+. All samples were positive for LRP and the median of positive cells was 80% for NEL and 60% for EL. The median of overall survival time was 4 months and all animals had partial response to the treatment. In conclusion, despite the high expression of LRP in canine cutaneous lymphomas, there was no difference between NEL and EL forms and overall survival time.

O20

Retrospective Evaluation of Palladia® Use in the Treatment of inoperable, Metastatic, Or Recurrent Canine Pheochromocytomas

Meg Musser[1], Kathryn Taikowski[2], Chad M. Johannes[1], Philip Bergman[3]
[1]Iowa State University, Ames, IA, USA, [2]Ohio State University, Columbus, OH, USA, [3]VCA, Bedford Hills, NY, USA

Effective treatment options for inoperable, metastatic, or recurrent canine pheochromocytomas are lacking. In humans, specific germline mutations drive the development of pheochromocytomas; targeting these abnormalities with small molecule inhibitors are an effective treatment strategy. Similar mutations may exist in the dog. Thus, treatment with similar small molecule inhibitors, such as toceranib phosphate (Palladia), may provide a survival advantage.

The purpose of this study was to assess the role of Palladia in the treatment of inoperable, metastatic, or recurrent canine pheochromocytomas. Retrospectively, medical records of dogs that had a diagnosis of a pheochromocytoma and treatment with Palladia were reviewed for information regarding response to Palladia, observed side effects, and overall outcome. Five dogs were identified that fit the inclusion criteria. All five experienced clinical benefit (1 partial response, 4 stable disease) based on periodic staging evaluations. Progression-free interval for the dog with the partial response was 61 weeks. Progression-free interval for the two dogs with stable measurable primary disease were 36 weeks and 28 weeks. Progression-free interval in the two dogs with stable metastatic disease were at least 11 weeks and 18 weeks.

Based on this limited series of cases, the results suggest that Palladia has biological activity in dogs with measurable primary and metastatic pheochromocytomas. Prospective clinical studies are warranted to

more fully define the role of Palladia in treatment of canine pheochromocytomas.

O21

Effect of Glucose Transporter Inhibition on Cell Growth in Canine Melanoma

Shinichi Namba[1], Rei Nakano[2], Taku Kitanaka[2], Nanako Kitanaka[3], Tomohiro Nakayama[4], Hiroshi Sugiya[2]
[1]Laboratory of Veterinary Biochemistry, College of Bioresource Sciences, Nihon University/Marble Veterinary Medical Center, Fujisawa, Kanagawa, Japan, [2]Laboratory of Veterinary Biochemistry, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa, Japan, [3]Laboratory of Veterinary Biochemistry, College of Bioresource Sciences, Nikon University, Fujisawa, Kanagawa, Japan, [4]Laboratory of Veterinary Radiology, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa, Japan

Melanoma is the most common malignant tumor in oral cavity of dogs. It is incurable once infiltrated into bone and lung metastasis, and the traditional therapeutic approach is less effective. Tumor cells rely on glycolysis, rather than mitochondrial oxidative phosphorylation, for energy production even under oxygen-rich conditions, which is termed as "Warburg effect". Since Warburg effect is characteristic for tumor cells, glucose metabolism is considered to be a therapeutic target in this regard. In this study, we investigated the contribution of glucose transporters on the cell growth of canine melanoma.

Canine melanoma cells (MCM-N1) were cultured in Dulbecco';s Modified Eagle';s medium containing 1 g/L glucose and 10% fetal bovine serum. Cell growth was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide reduction assay. The cells proliferated for three days in a time-dependent manner. When treated with WZB-117, a pan-glucose transporter inhibitor, the cell growth was clearly attenuated. We observed, by RT-PCR, that mRNAs of glucose transporter subtypes GLUT1 and 3 were dominantly expressed in canine melanoma. The protein expression of GLUT1 and 3 was confirmed by Western blotting. We, then, investigated the contribution of GLUT subtypes on the cell growth by subtype-specific knockdown of GLUT1 and 3. When the cells were transfected with GLUT1 and 3 siRNAs, the cell growth was inhibited. We also observed that the expression of proliferation marker Ki67 was inhibited in GLUT3 knockdown cells, whereas the inhibition was less in GLUT1 knockdown cells, hence suggesting that GLUT3 dominantly contributes to cell growth in canine melanoma.

In conclusion, it is likely that glucose transporter is a promising therapeutic target for canine melanoma.

O22

Intratumoral Expression of Serotonergic System Genes htr1b, htr2a, htr2b, tph1, and slc6a4 in Canine Osteosarcoma

Austin K. Viall[1], Rachel Phillips[2], Shannon McLeland[3], Jesse Hostetter[1], Shay Bracha[4], Meg Musser[1], Chad Johannes[1]
[1]Iowa State University, Ames, IA, USA, [2]Iowa State university, Deprtment of Pathology, Ames, IA, USA, [3]The Ohio State University/International Veterinary Renal Pathology Service, Columbus, OH, USA, [4]Oregon State University, Corvallis, OR, USA

The serotonergic signaling system is a major regulator of osteogenesis and bone homeostasis. The role of this system in canine osteosarcoma biology is largely unexplored. Serotonin receptors 1B and 2A are expressed in canine osteosarcoma cell lines. Incubation of tumor cells with specific receptor agonists and antagonists decreases cell viability and induces apoptosis. In vitro evidence suggests serotonin system elements are present in canine osteosarcoma and may represent novel therapeutic targets.

To investigate the presence of serotonergic components in vivo, we examined the expression of five serotonin system associated genes in archived, formalin-fixed appendicular osteosarcoma samples. Three genes encoding serotonin receptors (*htr1b*, *htr2a*, and *htr2b*) were evaluated, along with genes for the serotonin transporter (*slc6a4*) and serotonin synthesis (*tph1*). Expression was assessed using in situ RNA-Scope® RNA hybridization; evaluation of *ucb* was used to screen for adequate residual RNA. Presence and magnitude of expression were correlated with the tumor grade, vascular invasion, necrosis, matrix, cellular density, and pleomorphism. We expected to find expression of all genes amongst the tumor samples and that expression magnitude would correlate with histologic tumor features.

Of the 20 appendicular osteosarcoma tumor samples examined, 12 contained sufficient residual RNA for analysis. *Htr1b* expression was detected in 63% of the analyzed tumor samples, *htr2a* in 42%, *htr2b* in 42%, *slc6a4* in 75%, and *tph1* in 42%. However, average expression was < 1 transcript / cell for all genes. No correlations were identified between gene expression or magnitude relative to any histologic tumor features.

In conclusion, we identified low-level expression of certain serotonergic system genes in canine osteosarcoma tumor samples. Assessment of expression of the associated protein products is warranted to help determine if these elements may potentially have a role in osteosarcoma biology or represent viable therapeutic targets.

O23

Unravelling the Chaotic Genomic Landscape of Canine Osteosarcoma with Current Sequencing Technologies and Bioinformatic Approaches (VCS Award Winner)

Shirley Chu[1], Zachary Skidmore[2], Jason Kunisaki[2], Jason Walker[2], Malachi Griffith[2], Obi Griffith[2], Jeffrey Bryan[1]
[1]University of Missouri, Columbia, MO, USA, [2]McDonnell Genome Institute, Washington University, St. Louis, MO, USA

Introduction Understanding the genomic landscape of canine osteosarcoma (OSA) is critical for translational modeling. The objectives of this study were to develop the first high- resolution map of small-scale (single nucleotide variants (SNVs) and insertions/deletions), large-scale (copy-number variants (CNVs), gene fusions, and structural variants (SVs)) somatic alterations and clonal architecture in OSA.

Methods Whole genome (WGS), exome and RNA sequencing were performed on matched primary appendicular OSA and metastatic pulmonary tumors with matching normal samples in 2 dogs. HiSeqX (2x150bp reads) and HiSeq2500 (2x125bp reads) platforms were used. The McDonnell Genome Institute's cancer informatics pipelines were adapted for the canine genome.

Results Mean WGS and exome coverage was 28.5x and 140X respectively. The mean non- synonymous mutation rate was low at 0.46/Mb. The patients shared only one commonly mutated gene, both harboring TP53 missense mutations in exon 6. Mutations common to the

matched primary and metastatic tumor included ARHGEF18, ADGRV1, HHIPL1, MYO1A, BRPF1, BEST2, SLCO2B1, ROBO1, PHLDB2, FSIP2 and UNC80. Loss of heterozygosity (LOH) was extensive, on chr5 (TP53), chr12, chr16, chr20, chr26 (PTEN), chr37 and chr38. CDKN2A/B was deleted in both primary tumor samples.

Conclusion The genomic landscape of canine and human pediatric OSA are similar. TP53 suffered two-hits, point mutation and LOH, resulting in complete loss of the wild-type allele, a common early event and key driver of canine OSA. Application of established methods for human tumor genome analysis to canine samples revealed widespread genomic instability as seen in human tumors.

O24

Incidence and Risk Factors Associated with the Development of Symptomatic Cardiotoxicity in Dogs Receiving Doxorubicin (VCS Award Winner)

Briana Hallman[1], *Steven Suter*[1], *Paul Hess*[1], *Marlene Hauck*[1], *Laurel Williams*[2]
[1]*North Carolina State University, Raleigh, NC, USA,* [2]*Veterinary Specialty Hospital of the Carolinas, Cary, NC, USA*

Introduction: Doxorubicin is an anthracycline anti-tumor antibiotic that causes cumulative cardiotoxicity in dogs. This study aimed to characterize the overall incidence and risk factors of doxorubicin-induced clinical cardiotoxicity.

Methods: Medical records for dogs that received three or more doses of doxorubicin 2006-2015 were reviewed. Dogs were considered to have clinical cardiotoxicity if they lacked pre-existing heart disease and developed syncope, collapse, exercise intolerance, congestive heart failure, or sudden death during or after doxorubicin treatment.

Results: Of 444 cases in the study population, 20 (4.5%) developed clinical cardiotoxicity. The median time from treatment initiation to clinical sign development was 194 days. Dogs that developed clinical cardiotoxicity had a higher cumulative dose of doxorubicin and a higher body weight. Dogs that received a 1-hour infusion rather than a 10-15 minute infusion were also more likely to develop clinical cardiotoxicity. Boxer dogs had a higher incidence of cardiotoxicity. No association was found with dexrazoxane administration, dosing interval, or other chemotherapy drugs. A total of 225 dogs had an echocardiogram and ECG prior to the first doxorubicin treatment and at least one subsequent evaluation. Dogs with clinical cardiotoxicity had a significant decrease in fractional shortening from their baseline (-31.4%) compared to the fractional shortening change in unaffected dogs (+2.1%). Development of arrhythmias was associated with cardiotoxicity.

Conclusion: Overall incidence of the development of clinical cardiotoxicity was 4.4%. Risk factors identified include higher cumulative dose, higher body weight, Boxer dogs, longer infusion time, decreased fractional shortening, and development of arrhythmias.

EN01

Effect of Sample Type on Measurement of Cortisol and T4 by Immulite in Dogs

Dana A. Schechter, Ellen N. Behrend, Hollie Lee, Robert J. Kemppainen
Auburn University College of Veterinary Medicine, Auburn, AL, USA

Serum is the recommended sample to use for measurement of cortisol and T4 using Immulite technology. However, cortisol is more stable in EDTA plasma than serum. The use of EDTA plasma can significantly affect results obtained by Immulite, and addition of magnesium (Mg) can potentially overcome the effects of EDTA (Kemppainenet al. *J Vet Diagnost Invest*, in press). Our objective was to compare the effect of EDTA with and without addition of Mg on measurement of cortisol and T4 by Immulite in canine samples.

Paired canine EDTA-plasma and serum samples leftover after use for indicated diagnostic testing were obtained. The EDTA concentration in each plasma sample was unknown and likely varied depending on the degree of underfilling of the tube. Cortisol and T4 were measured in the EDTA-plasma and serum samples with and without Mg added using a previously validated, solid-phase, automated, chemiluminescent enzyme immunoassay (Immulite 1000). For statistical analysis, we used a one-way repeated measures ANOVA to compare the measured concentrations. A post-hoc test using Dunnett's method was performed with the concentrations in serum without added Mg as the control group. To detect differences in concentrations between plasma with and without Mg, data were analyzed using a paired t-test. Significance was set at the $P < 0.05$ level.

Blood samples from 15 dogs were included. Cortisol and T4 values were significantly different between plasma and serum samples (both without Mg). For cortisol and T4, concentrations measured in EDTA plasma were 34% and 45% higher than in serum samples, respectively ($P < 0.001$ for both). The addition of Mg to plasma significantly decreased the measured cortisol and T4 concentrations ($P < 0.001$ and $P = 0.016$, respectively). After addition of Mg, the cortisol concentrations measured in EDTA plasma were no longer significantly different from those measured in serum; however, for T4, the concentrations measured in EDTA plasma remained significantly different from serum.

Use of EDTA plasma significantly increases the measured concentration of cortisol and T4 obtained by Immulite. Addition of Mg to plasma samples can overcome the effects of EDTA when measuring cortisol, but not T4. Thus, EDTA plasma can be used to maximize cortisol stability if Mg is added to the sample before assay via Immulite.

EN02

Isolated Renal Glycosuria Does Not Lead to Polyuria in a Feline Model

Arnon Gal, Ms. Stacey E. Burton, Karin Weidgraaf, Richard Burchell
School of Veterinary Sciences, Massey University, Palmerston North, Manawatu-Wanganui, New Zealand

Osmotic diuresis is considered the cause of polyuria in diabetes mellitus. However, people with a mutation in the renal sodium-glucose cotransporter 2, and diabetic patients treated with drugs that block this transporter (SGLT2i) have profound glycosuria without polyuria. The purpose of this study was to determine if isolated renal glycosuria will induce polyuria in cats.

The study was approved by the University Animal Ethics Committee. The study consisted of four, 5-day study periods separated by 7-day washout periods, in which eight healthy male cats were randomly allocated to control or treatment with 10mg of dapagliflozin (SGLT2i)

once daily based on a previous pilot study. In each of the four 5-day study periods, we recorded daily food and water intake and urine output, and a urine aliquot was collected. Blood was obtained at the beginning and end of each study period. Data were analyzed with a linear mixed-effect model that included treatment and day-of-treatment as fixed effects, and cat as a random effect. Statistical significance was set at 0.05.

Dapagliflozin induced a 566-fold increase in urine glucose loss (41.0 ± 12.6 mmol/d vs. 0.07 ± 0.15 mmol/d). There were no differences in daily urinary excretion of sodium, potassium, and chloride between groups. Also, there were no differences in water intake (259 vs. 282 mL), body weight (4320 vs. 4321 g), and urine production (231 vs. 190 mL).

These results call in to question the generally accepted hypothesis that glycosuria-induced osmotic diuresis is the primary cause of polyuria in diabetes mellitus.

EN03

Transcription Factors Involved in the Development and Reprogramming of the Endocrine Pancreas in Diabetic Cats

Lune Geurts[1], Elena Salesov[2], Katarina Macha[3], Thomas Lutz[2], Claudia Reusch[2], Eric Zini[4]
[1]University of Utrecht, Utrecht, Utrecht, Netherlands, [2]-, Zurich, Zurich, Switzerland, [3]University of Zurich, Switzerland, Zurich, Zurich, Switzerland, [4]University of Zurich, Switzerland/University of Padua, Italy/Istituto Veterinario di Novara, Italy., Zurich, Zurich, Switzerland

In humans and rodents the development of the endocrine pancreas is under the control of tightly regulated, cross-interacting transcription factors. Two of them, paired box gene-4 (PAX4) and aristaless-related homeobox gene (ARX), play a central role in the allocation of endocrine progenitors towards β-cell and α-cell specification, respectively. Of note, in adulthood in both species, the expression of PAX4 has been shown to contribute to reprogramming of exocrine cells and α-cells into insulin-producing β-cells; induction of ARX expression in β-cells leads them to reprogramming into α-cells. We recently discovered that diabetic cats have an increased number of cells in the exocrine pancreas that stain positive for a marker of proliferation (proliferating cell nuclear antigen; PCNA), in particular nearby islets. Because proliferation increases reprogramming efficiency in rodents, we hypothesized that these cells may anticipate reprogramming in cats. Aim of the study was to test if diabetic cats have an increased number of cells in the endocrine and exocrine pancreas expressing developmental markers of β- and α-cells, suggesting reprogramming.

In 9 diabetic and 9 well-matched control cats, the pancreas was collected within 1 hour from death, formalin-fixed and paraffin-embedded for immunohistochemistry. Tissue sections were stained for insulin, glucagon, PAX4 and ARX. The number of cells positive for each marker and double-positive for any of their combination were counted in 5 images at 400x magnification in the exocrine pancreas and islets. Results were compared between groups with non-parametric tests.

As expected, compared to controls diabetic cats had less insulin-positive cells in the islets (median: 14.8, range: 2.4-51 vs. median: 64, range: 33.4-71.4; p=0.001) and scattered in the exocrine pancreas (median: 5, range: 1.8-14.6 vs. median: 10, range: 4.4-19; p=0.038); the number of glucagon-positive cells did not differ. Diabetic cats had more insulin/glucagon double-positive cells in the islets than controls (median: 0.2, range: 0-11 vs. median: 0, range: 0-0.4; p=0.024). In the islets, diabetic cats had more PAX4-positive cells (median: 9.6, range: 0-29.6 vs. median: 2.2, range: 0.2-6.4; p=0.038) and more PAX4/insulin double-positive cells (median: 4.4, range: 0-12 vs. median: 0.2, range: 0-1; p=0.027). The percentage of PAX4/insulin double-positive relative to the number of insulin-positive cells was 50-fold higher in diabetic cats (p=0.019); 7 of 9 cats had >5% of cells double-positive while none of the controls (78 vs. 0%, p=0.002). Diabetic cats had more ARX/glucagon double-positive cells in the exocrine pancreas than controls (median: 0, range: 0-0.8 vs. median: 0, range: 0-0; p=0.029). None of the markers or their combination differed between groups in the exocrine pancreas.

In the islets of diabetic cats, the decreased number of insulin-positive cells along with the increased number of PAX4-positive and PAX4/insulin double-positive cells suggest that some β-cells may change to an earlier stage of differentiation or that new β-cells are formed. The increased number of islet cells double-positive for insulin and glucagon in diabetic cats may indicate that α-cells are transforming into β-cells or vice versa, however the absolute number of these cells was very low. Collectively, these data suggest that histological evidence for reprogramming of cells is present in diabetic cats and that this event occurs in the islets and not in the exocrine pancreas. The reason behind the increased number of ARX/glucagon double-positive cells in the exocrine pancreas of diabetic cats is unclear.

EN04

Comparison of Pharmacodynamics Between Insulin Degludec and Insulin Glargine 300 U/mL in Healthy Cats

Chen Gilor[1], William Culp[2], Ms. Sonia Ghandi[3], Juliana do Carmo Emidio e Silva[4], Ms. Amarpreet Ladhar[5], Sean Hulsebosch[3]
[1]UC Davis, Davis, CA, USA, [2]School of Veterinary Medicine, UC Davis, Davis, CA, USA, [3]University of California, Davis, Davis, CA, USA, [4]FCAV/Universidade Estadual Paulista (UNESP), Jaboticabal, Sao Paulo, Brazil, [5]University of California, San Francisco, Benicia, CA, USA

Insulin glargine 300 U/ml (Toujeo®) and insulin degludec (Tresiba®) are synthetic insulin analogs that are used in people as basal insulin. Toujeo® is more predictable and longer-acting compared to glargine 100 U/ml (Lantus®) in people. The duration of action of Tresiba® is over 40 hours which allows a flexible daily schedule of administration. We hypothesized that Tresiba® would have longer duration of action compared to Toujeo® in healthy cats. Six healthy purpose-bred cats, each received 0.4 U/kg SQ injections of Tresiba® and Toujeo® on two different days, > 1 week apart. Blood glucose (BG) was measured every 5 min and glucose was administered intravenously at a variable rate with the goal of maintaining BG = 85 mg/dL ("isoglycemic clamp"). Glucose infusion rate was used as a measure of exogenous insulin action. The Shapiro-Wilk test was used to assess normality and normally distributed parameters were compared using paired t-tests.

Onset of action (T_{OA}) was similar (79 ± 27 min for Toujeo®, 60 ± 21 min for Tresiba®, P = 0.3) but the end of action (T_{EA}) and duration of action ($T_{DUR} = T_{EA}$-T_{OA}) were longer for Toujeo® vs. Tresiba® (T_{DUR} = 828 ± 130 min vs. 620 ± 148 min, respectively, P = 0.04;

T_{EA} = 907 ± 135 min vs. 679 ± 127 min, respectively, P = 0.03). There were no other significant differences between the two formulations.
Based on these preliminary data, Toujeo® is longer-acting and therefore better suited than Tresiba® as a once-daily insulin formulation in cats.

EN05

Thyroid Status in Dogs with Renal Proteinuria

Jean-Sébastien Palerme[1], *Laura R. Van Vertloo*[2]
[1]*Iowa State University College of Veterinary Medicine, Ames, IA, USA,* [2]*Iowa State University, Ames, IA, USA*

Renal proteinuria has been associated with both subclinical and clinical hypothyroidism in people; however, little is known about the effect of renal proteinuria on the thyroid status of companion animals. The purpose of this study was to determine if thyroid status, assessed by total T4 (TT4), free T4 (fT4) and thyroid-stimulating hormone (TSH), differed between ill dogs that were proteinuric versus non-proteinuric.
Ill proteinuric dogs, defined by a urine protein/creatinine ratio (UPC) > 1 with negative urine culture, and ill non-proteinuric dogs were prospectively enrolled in this cross-sectional study. All dogs underwent physical examination, complete blood count, serum chemistry, urinalysis, and thyroid panel; urine from proteinuric dogs was submitted for culture and UPC. Descriptive statistics and student';s t-tests were used to determine differences between groups, with significance of $p < 0.05$.
Twenty-two ill proteinuric dogs and 8 ill non-proteinuric dogs were enrolled in the study. Serum total protein concentrations were higher in non-proteinuric dogs (p =0.015), though albumin levels did not differ between groups (p = 0.06). Median UPC for proteinuric dogs was 5.1 (range 1.9 – 16.5). Interestingly, proteinuric dogs had a significantly higher mean serum cholesterol than non-proteinuric dogs (p = 0.04). No significant difference in mean TT4 (p = 0.403) or fT4 (p = 0.396) was found between proteinuric and non-proteinuric dogs; however, mean TSH was significantly higher in proteinuric dogs (p = 0.002).
These results suggest that renal proteinuria influences thyroid homeostasis and should be considered when interpreting thyroid status of dogs.

EN06

Diagnosis of Hypothyroidism following Thyroid Stimulating Hormone Stimulation Testing of Radio-Iodine-Treated Hyperthyroid Cats

Jennifer Wakeling[1], *Teresa Hall*[2], *Timothy L. Williams*[3]
[1]*Douglas College, British Columbia, Canada, Coquitlam, BC, Canada,* [2]*North West Nuclear Medicine, Vancouver, BC, Canada,* [3]*Department of Veterinary Medicine, University of Cambridge, Cambridge, England, United Kingdom*

Hyperthyroid cats are commonly treated with radio-iodine (I131) which may result in iatrogenic hypothyroidism. Although the thyroid stimulating hormone (TSH) stimulation test has been used to assess thyroid reserve in several studies of hypothyroid dogs, limited data are available for cats. This study tests the hypothesis that I131 treated cats diagnosed with iatrogenic hypothyroidism using the TSH stimulation test, will have low total-thyroxine (tT4), low free-thyroxine (fT4) and high TSH.
This was a prospective study of 118 client-owned I131 treated cats (> 12 weeks post-treatment). Total thyroxine, fT4, and TSH were measured, and a TSH stimulation test was performed. Previously published criteria from canine hypothyroidism studies and TSH stimulation test responses of 32 mature adult control cats were used to define group cut-off criteria. I131 treated cats were divided into hypothyroid (post-stimulation tT4 ≤ 20nmol/l), euthyroid (post stimulation tT4 ≥ 30nmol/l OR post stimulation tT4 20.0-29.9nmol and pre: post tT4 ratio > 1.5) or intermediate (post stimulation tT4 20.1-29.9nmol/l and tT4 ratio < 1.5) groups.
Twenty-five I131-treated cats were diagnosed with hypothyroidism of which 22/25 had high TSH (≥ 0.3ng/ml) and 23/25 had low fT4 (< 9pmol/l). Only 6/25 hypothyroid cats had low tT4 (< 10nmol/l) based on the laboratory reference interval (RI); therefore, tT4 below RI is an insensitive test for feline hypothyroidism. Twenty-two cats had intermediate thyroid function. In the euthyroid group 10/71 cats had high TSH, 5/71 had low fT4 and one cat had low tT4. Only 1 euthyroid cat with low fT4 also had high TSH. Measurement of both fT4 and TSH is recommended for diagnosis of iatrogenic feline hypothyroidism.

EN07

Effect of Illness on Response of Euthyroid cats to Thyroid Stimulating Hormone Stimulation Test

Jennifer Wakeling[1], *Teresa Hall*[2], *Timothy L. Williams*[3]
[1]*Douglas College, British Columbia, Canada, Coquitlam, BC, Canada,* [2]*North West Nuclear Medicine, Vancouver, BC, Canada,* [3]*Department of Veterinary Medicine, University of Cambridge, Cambridge, England, United Kingdom*

The thyroid stimulating hormone (TSH) stimulation test has been used to assess thyroid reserve in studies of hypothyroid dogs, however limited data are available for cats. The purpose of this study was to assess the effect of mild to moderate concurrent illness on the response of euthyroid cats to the TSH stimulation test.
This was a prospective study of 35 client-owned and shelter-housed mature adult cats. A full examination including systolic blood pressure, routine hematology, biochemistry and urinalysis, measurement of urine protein-creatinine ratio, total thyroxine (tT4), free thyroxine (fT4) and TSH, and a TSH stimulation test were performed. Cats thought to be 7 years or older were recruited, however the age of shelter cats could not always be verified. Hospitalized cats and cats receiving thyroid-suppressive medications were excluded. Cats were divided into 3 groups based on their physical examination and laboratory results: group 0 (no concurrent illness detected), group 1 (mild concurrent illness e.g. moderate dental disease or stable IRIS stage II chronic kidney disease) and group 2 (moderate concurrent illness e.g. untreated moderate to severe hypertension, anemia, hypoalbuminemia, proteinuria and/or hypokalemia). Results are reported as median (range). Post-TSH tT4 concentrations (post-tT4) and the ratio of tT4 concentrations before and after TSH administration (tT4 ratio) were compared between the 3 groups using the Kruskal Wallis H-test. Twelve cats were owned and 23 cats were shelter-housed. Two shelter-housed cats were diagnosed with hyperthyroidism and

excluded from further evaluation. In the remaining 33 cats, baseline tT4 was 25.9nmol/l (17.2-39.3), post-tT4 was 64.4nmol/l (29.4-102) and tT4 ratio was 2.41 (1.56-3.95). There was no significant difference ($p > 0.05$) in post-tT4 or tT4 ratio between the 3 groups (group 0, n=8; group 1, n=16; group 2, n=9), therefore no effect of mild-moderate illness on the TSH stimulation test results in this study.

EN08

Oxidative Stress of Erythrocytes in Cats with Diabetes Mellitus

Eric Zini[1], *Elena Salesov*[2], *Gabriele Gerardi*[3], *Laura Da Dalt*[3], *Thomas Lutz*[2], *Claudia Reusch*[2], *Gianfranco Gabai*[3]
[1]*University of Zurich, Switzerland/University of Padua, Italy/Istituto Veterinario di Novara, Italy., Zurich, Zurich, Switzerland,* [2]*-, Zurich, Zurich, Switzerland,* [3]*-, Legnaro, Veneto, Italy*

In diabetic humans, erythrocytes have lower antioxidant capacity and increased protein and lipid oxidation byproducts as a consequence of hyperglycemia and hyperlipidemia. Similarly, erythrocytes from diabetic cats have increased Heinz bodies formation which may suggest increased oxidative stress, but this topic has not yet been thoroughly investigated. Furthermore, because remission of diabetes in cats has been shown to be associated with less marked hyperglycemia and normal serum cholesterol, it is possible that antioxidant capacity of cats achieving remission is higher than in those without remission. Thus, we assessed if erythrocyte oxidative stress is increased and antioxidant capacity decreased in diabetic cats, if antioxidant capacity differs between cases with and without remission, and if oxidative status is ameliorated by treatment.

Healthy cats and cats with newly diagnosed diabetes not affected by obvious concurrent diseases were enrolled. Diabetic cats received insulin glargine and a low-carbohydrate diet and were followed-up for 3-4 months. Remission was defined as euglycemia without insulin for >4 weeks. Blood was collected at first admission, at 1-2 and 3-4 months. Erythrocytes were used to quantify carbonyls (protein oxidation byproducts) and thiols (group of antioxidants) in membranes, advanced oxidized protein products (AOPP, protein oxidation byproducts), thiobarbituric acid reactive substances (TBAR, lipid peroxidation byproducts) and thiols in cytoplasm. Nonparametric tests were used for comparisons.

Eleven healthy cats and 27 diabetic cats were included; 15 diabetic cats achieved remission. Compared to controls, diabetic cats at diagnosis had higher median membrane carbonyls [4.6 nmol/mg (range: 0.1-37.7) vs. 0.7 nmol/mg (range: 0.1-4.7), $p<0.001$] and lower cytoplasmic thiols [0.4 µmol/mg (range: 0.2-0.6) vs. 0.6 µmol/mg (range: 0.4-0.8), $p<0.01$]. Cytoplasmic TBAR was lower in diabetic cats [1.9 nmol/mg (range: 0.5-2.4) vs. 2.4 nmol/mg (range: 1.4-3.5), $p<0.01$]. At 3-4 months, membrane carbonyls remained higher ($p<0.01$), cytoplasmic thiols and TBAR lower ($p<0.05$ and $p<0.01$, respectively). Differences between cats with and without remission were not documented, except for cytplasmic AOPP being lower in those with remission at 1-2 months ($p<0.05$).

In conclusion, diabetic cats have erythrocytes with increased protein oxidation byproducts and reduced antioxidant capacity, suggesting increased oxidative stress. Because oxidative status was not ameliorated by treatment, it is possible that oxidative stress is persistent or needs longer treatment duration to decrease. The reason behind reduced TBAR in diabetic cats remains elusive. Lastly, oxidative status is not different between cats with and without remission.

EN09

Molecular Prognostic Markers in Canine Cortisol-Secreting Adrenocortical Tumours (ESVE Award Winner)

Karin Sanders[1], *Erik Teske*[1], *Koen Cirkel*[1], *S. A. (Bas) van Nimwegen*[1], *M. C. M. Uijens*[1], *Hans S. Kooistra*[2], *Jan A. Mol*[1], *Jan Willem Hesselink*[3], *Sara Galac*[3]
[1]*Utrecht University, Utrecht, Utrecht, Netherlands,* [2]*Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht University, Utrecht, Netherlands,* [3]*Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Utrecht, Netherlands*

Assessment of malignancy in canine cortisol-secreting adrenocortical tumors (ATs) remains challenging. No previous studies have linked molecular markers to survival times in dogs after adrenalectomy, making it difficult to give a reliable prognosis. The aim of this study was to identify molecular prognostic markers in a large cohort of canine ATs. This could not only enhance insight in individual prognosis, but could also provide potential future treatment targets.

Fifty-nine dogs with hypercortisolism due an AT that underwent adrenalectomy between 2002 and 2015 at the authors'; institution and of which follow-up information was available, were included in this study. Three classes of potential prognostic factors were reviewed: firstly clinical data, including body weight, age at time of surgery, gender, neuter status and tumor size; secondly immunohistochemical Ki67 labeling index, and thirdly mRNA expression of factors associated with proliferation of ATs, including SF-1, PTTG1, PBX1, VAV2, RRM2, TOP2A, Ki67, CCND1, MC2R and BCL2. Univariate analysis was performed with the Cox proportional hazards model for continuous variables and the Log Rank test for bivariate variables. Multivariate analysis was performed using multiple linear regression with forward selection.

Median survival time was 63.6 ± 9.4 months. In the univariate analysis, significant prognostic factors were tumor volume in cm^3 ($P=0.015$, hazard ratio (HR)=1.004), maximal diameter of tumor in cm ($P=0.047$, HR=1.284), Ki67 labeling index (P6) as independent predictors of poor survival.

In conclusion, most important predictors of poor survival are Ki67 labeling index and SF-1 expression. These results show the importance of including Ki67 staining in histopathological assessment of canine ATs. Moreover, since pharmacological manipulation of SF-1 is possible, the considerable impact of SF-1 expression on prognosis indicates great potential of SF-1 as a treatment target in canine ATs in the near future.

EN10

Use of Lispro Insulin for Treatment of Diabetic Ketoacidosis in Cats: A Pilot Study - Sponsored By Dechra Veterinary Products

Jodie D. Anderson[1], *Danielle A. Rondeau*[2], *Rebecka Hess*[3]
[1]*Department of Clinical Sciences & Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, Philadelphia, PA, USA,* [2]*Not applicable, Not applicable, VT, USA,* [3]*Department of Clinical Sciences & Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA., Philadelphia, PA, USA*

The objectives of this study were to evaluate the safety of lispro insulin for treatment of cats with diabetic ketoacidosis (DKA) and to

compare the times required to resolve hyperglycemia, ketosis, and acidosis, in DKA cats treated with lispro or regular insulin.

Hospital population cats with DKA were enrolled into a randomized, prospective, blinded, clinical trial. Cats with blood glucose (BG) > 300mg/dL, glucosuria, blood pH < 7.35 but > 7.0, and a blood beta-hydroxybutyrate (BOHB) concentration > 2.0mmol/L were enrolled. Cats were randomly assigned to receive an IV continuous rate infusion of lispro or regular insulin administered at an initial dose of 0.09U/kg/hr and adjusted as previously described (doi: 10.1111/vec.12298).

Six cats were enrolled into each treatment group. No adverse events were observed in association with IV lispro administration. The median time to resolution of hyperglycemia (BG < 250mg/dL) was significantly shorter in cats treated with lispro (7h [range 2-10h]) compared to cats treated with regular insulin (12.5h [8-20h]; P = 0.02). There were no significant differences in the median times to resolution of ketosis (BOHB ≥ 2.0mmol/L, 60h [18-80h], 68h [18-92h]) or acidosis (pH ≥ 7.35, 32h [10-40h], 30 [18-62h]) in lispro versus regular insulin treated cats. There was also no difference in the median time to hospital discharge in lispro versus regular insulin treated cats (64h [13-132h] and 94h [51-112h], respectively). Two cats from each study group were euthanized due to comorbidities.

It is concluded that lispro insulin is safe, and as effective as regular insulin for resolution of metabolic derangements in DKA cats.

EN11

Effects of EDTA on Chemiluminescent Immunoassay Measurement of ACTH, Cortisol, and Thyroid Hormones in Dogs - Sponsored By Dechra Veterinary Products

Aubrey L. Hirsch, Mark A. Mitchell, Jon M. Fletcher
Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, Baton Rouge, LA, USA

Previous studies in dogs found improved cortisol stability when measured in ethylenediaminetetraacetic acid (EDTA) plasma and interchangeability of serum and plasma when cortisol, total thyroxine (TT4), and free thyroxine (FT4) were measured by radioimmunoassay. Interestingly, a study using the IMMULITE® 2000 chemiluminescent immunoassay found that the increased EDTA concentration in sample tubes ≤ 50% filled affected the alkaline phosphatase secondary enzyme leading to significantly different human parathyroid hormone concentrations. This study evaluated the effect of EDTA on the measurement of adrenocorticotropic hormone (ACTH), cortisol, TT4, FT4, and thyroid stimulating hormone (TSH) in healthy dogs using the Siemens IMMULITE® 1000.

Whole blood from forty dogs was aliquoted into three Monoject™ sample tubes; plain, completely filled EDTA tube, and 50% filled EDTA tube. Handling and storage conditions were identical and all samples were analyzed on the same day. ACTH, cortisol, TT4, FT4, and TSH were measured in each sample.

Bland-Altman plots and Passing-Bablok regression were used to assess agreement and risks for error, respectively. Proportional errors were found between serum and plasma samples for ACTH, cortisol, TT4, FT4, and TSH; systematic errors were also found for FT4. Results confirm significant differences between these sample types and that they are not directly interchangeable. Incompletely filled EDTA tubes are associated with significantly higher cortisol concentrations and significantly lower ACTH concentrations when compared to completely filled EDTA tubes.

When measured by chemiluminescent immunoassay, serum should be used for cortisol, TT4, FT4, and TSH, while plasma from completely filled EDTA tubes should be used for ACTH.

EN12

Investigating the Effect of Thyroid Stimulating Hormone Administration on Radioactive Iodine Uptake in Hyperthyroid Cats - Sponsored By Dechra Veterinary Products

Amy E. Oberstadt[1], Nathan C. Nelson[2], Andrew K. Claude[3], Kent R. Refsal[4], Brian K. Petroff[4], Daniel K. Langlois[3]
[1]Michigan State University College of Veterinary Medicine, Brighton, MI, USA, [2]North Carolina State University, Raleigh, NC, USA, [3]Michigan State University College of Veterinary Medicine, East Lansing, MI, USA, [4]Michigan State University College of Veterinary Medicine, Lansing, MI, USA

Radioactive iodine therapy is considered the treatment of choice for feline hyperthyroidism. In humans with thyroid neoplasia or toxic nodular goiter, administration of recombinant human thyroid stimulating hormone (rh-TSH) can increase thyroidal iodine uptake, thereby allowing lower doses of radioactive iodine to be used for treatment. Similar strategies could be valuable for treating feline hyperthyroidism. The primary objective of this study was to investigate the effects of rh-TSH administration on iodine uptake in hyperthyroid cats. In randomized crossover design, 10 client-owned cats with hyperthyroidism were treated with placebo, 50 μg rh-TSH (low-dose), and 100 μg rh-TSH (high-dose) with treatments separated by 8-10 days. Following each treatment, thyroid scintigraphy was performed by administering 300 μCi ^{123}I and assessing radionuclide uptake at 8 and 24 hour time points. Serum thyroid hormone profiles also were measured at each visit. Thyroidal iodine uptakes (mean ± SD at 8 and 24 hours) were not different among placebo (25.2 ± 13.4, 30.0 ± 12.8), low-dose (24.1 ± 12.5, 29.4 ± 13.7), and high-dose (24.2 ± 16.3, 30.8 ± 15.3) treatments (P = 0.81). Independent of rh-TSH administration, cats with severe increases in serum thyroid hormone concentrations had greater radioactive iodine uptake at 8 hours (P = 0.002), but not at 24 hours(P = 0.08), as compared to cats with mild increases. Administration of rh-TSH as performed in this study would not be expected to lower the radioactive iodine dose needed for treatment. Investigations of alternate strategies to increase thyroidal uptake of radioactive iodine are warranted.

EN13

Comparison of Urine Cortisol:Creatinine Ratio and Basal Cortisol for the Diagnosis of Canine Hypoadrenocorticism - Sponsored By Dechra Veterinary Products

Allison J. Rowland[1], Adam J. Birkenheuer[1], Lisa Mamo[1], Katharine F. Lunn[2]
[1]North Carolina State University, Raleigh, NC, USA, [2]NC State University, Apex, NC, USA

Hypoadrenocorticism in dogs is confirmed by performing an ACTH stimulation test. Although rare, hypoadrenocorticism is a differential diagnosis for many clinical and clinicopathological findings in canine patients, therefore a simple inexpensive way to rule it out is desirable. A prospective study was performed to test the hypothesis that the urine cortisol:creatinine ratio (UCCR) is more specific than basal

cortisol for the diagnosis of hypoadrenocorticism. Client-owned dogs with clinical signs or clinicopathological abnormalities for which hypoadrenocorticism was a reasonable differential diagnosis were enrolled. Cases were excluded if they had received prior therapy with glucocorticoids, mitotane, or trilostane. Urine for UCCR was obtained in all dogs prior to performing an ACTH stimulation test.

A total of 135 dogs were enrolled. 5 dogs were diagnosed with hypoadrenocorticism (pre- and post-ACTH serum cortisol < 2.0 mcg/dL) and 130 dogs were diagnosed as non-hypoadrenal (NH). UCCR ranged from 0 to 1 (RR:8-24) in the hypoadrenocorticism dogs, and from 4 to > 613 in the NH group. Basal cortisol ranged from ≤ 1 to 14.7 in the NH group. For the diagnosis of hypoadrenocorticism, sensitivity and specificity of basal cortisol ≤ 1 or ≤ 2 were 100% and 93.1%, and 100% and 65.4% respectively. Using a cut-off of ≤ 3, UCCR was 100% sensitive and 100% specific for diagnosis of hypoadrenocorticism. UCCR is significantly more specific than basal cortisol ≤ 2 (p=0.0001) and basal cortisol ≤ 1 (p=0.0034). These results suggest that UCCR allows hypoadrenocorticism to be ruled out in more patients, compared to basal cortisol.

EN14

Accuracy Over Time of Bayer Diastix® Strips for Detection of Glucosuria in Cat Urine - Sponsored By Dechra Veterinary Products

Thao T. Vo[1], Ellen N. Behrend[1], Hollie Lee[1], Presleigh Wiley[2]
[1]Auburn University College of Veterinary Medicine, Auburn, AL, USA, [2]Auburn University College of Veteirnary Medicine, Auburn, AL, USA

Monitoring control of diabetes mellitus can be challenging, especially in cats. Diastix® reagent strips can be used to assess the presence and degree of glucosuria, which may aid in assessing diabetic control. The purpose of this prospective study was to evaluate the accuracy of Bayer Diastix® for detection of glucosuria in cat litter over 8 hours.

Glucose was added to previously frozen feline urine samples to achieve 90 urine samples with approximate glucose concentrations of 50, 125, 375, 750, and 1500 mg/dL. For each sample, the pad that detects glucose was cut off from 3 Diastix® reagent strips, added to clay litter-filled Petri dishes and soaked with 3 drops of one of the urine samples. An estimate of the urine glucose concentration was determined by a trained, blinded observer by comparing the color change of the pieces to the color chart in the Diastix® package insert immediately, as per manufacturer instructions, and at 30, 120, 240, 360, and 480 minutes after exposure to urine. A chi square test was used to compare the distribution of categorizations (accurate, overestimate and underestimate) between time points. Significance was set at the $p < 0.05$ level.

Reference ranges that correlate with the colors on the Diastix® color chart (Diastix® 0 mg/dL [0 mg/dL], 50 mg/dL [0-100 mg/dL], 125 mg/dL [100-200 mg/dL], 375 mg/dL [250-500 mg/dL], 750 mg/dL [500-1000 mg/dL], or 1500 mg/dL [1000-2000 mg/dL]) were devised because of lack of published concentration ranges for the Diastix®. According to our ranges, the glucose concentration was accurately measured immediately after soaking in only 34 of 90 samples (38%). Of the inaccurate measurements, 54 of 56 samples (96%) were underestimated. At 30 minutes, the accuracy improved to 88% (79 of 90 samples), and 8 of the 11 inaccurate measurements (73%) were underestimated. At 2 hours and beyond, the % of accurate measurements was 71-76%. Of the inaccurate measurements, the majority were underestimates (64% -77%). The categorizations changed significantly over time ($p < 0.001$), specifically between the readings at 0 and 30 minutes.

Thus, if using the Diastix® in clay-based cat litter, the most accurate readings are obtained after 30 minutes. After the 2-hour time point, the color change remains stable although with an approximate 65% accuracy. The product tends to underestimate glucose concentrations in cat urine.

EN15

Dexmedetomidine's Effect on Glucose Homeostasis in Healthy Cats - Sponsored By Dechra Veterinary Products

Juliette Bouillon[1], Alexandra Focken[1], Tanya Duke-Novakovski[2], Elisabeth Snead[1], Kevin Cosford[1]
[1]Department of Small Animal Clinical Sciences, Saskatoon, SK, Canada, [2]Department Small Animal Clinical Sciences, Saskatoon, SK, Canada

Alpha-2 agonist administration has been documented to increase blood glucose concentration in a number of species. The objective of this study was to describe the effect of dexmedetomidine on glucose regulation in healthy cats.

A randomized crossover study using 8 healthy cats with a 14-day washout period was used to assess the effect of dexmedetomidine(10 μg/kg IV) and saline on glucose, lactate, cortisol, insulin, glucagon, and non-esterified fatty acid (NEFA) concentrations at baseline (t=0), and then 20, 60, 120, and 180 min post administration. Nonparametric distribution was established with the Shapiro-Wilk test. Comparisons between groups were performed with a Wilcoxon matched pair signed rank test. Comparisons within groups were performed with a Friedman test using Dunn's multiple comparisons for post-hoc analyses.

No significant differences were identified between groups, within the saline group, and within the dexmedetomidine group for insulin, cortisol and lactate concentrations. Significant differences ($P < 0.05$) were observed within the dexmedetomidine group: increased blood glucose concentration at 60 min (11.55, [5.9-16.6], mmol/L) and 120 min (12, [6.1-13.8], mmol/L) compared to t=0 (6.05, [4.8-13.3], mmol/L); decreased serum glucagon concentration at 120 min (3.8, [2.7-8.8], pg/mL) and 180 min (4.7, [2.1-8.2], pg/mL) compared to t=0 (11.85 [8.3-17.2], pg/mL); and decreased serum NEFA concentrations at 60 min (0.28, [0.04-1.36], mmol/L) and 120 min (0.41, [0.035-1.36], mmol/L) compared to t=0 (0.94, [0.68-1.48], mmol/L).

In conclusion, the transient effects of dexmedetomidine on glucose homeostasis are unlikely to affect clinical practice.

EN16

Changes in Serum total Thyroxin Concentrations in Hyperthyroid Cats Do Not Affect Serum Glucose Concentrations - Sponsored By Dechra Veterinary Products

Mackenna Dent, Richard Burchell, Stacey E. Burton, Karin Weidgraaf, Arnon Gal
School of Veterinary Sciences, Massey University, Palmerston North, Manawatu-Wanganui, New Zealand

This prospective study investigated the veracity of a recent retrospective study, where we found a significant negative correlation between serum total thyroxine (TT4) and glucose concentrations in cats. To that end, we used a naturally-occurring hyperthyroid cat model to

determine the effect that a range of serum TT4 concentrations would have on serum glucose concentrations.

The study was approved by the University Animal Ethics Committee. An apriori power analysis indicated that six cats per group would suffice to detect a difference of 15 ± 15 mg/dL on repeated measures of serum glucose on the same cat with a power of 0.8. Ten hyperthyroid cats were randomly allocated in a crossover design to receive treatment with a long-acting carbimazole or to have treatment withheld. The experimental design consisted of four, 14-day study periods that were separated by 21-day washout periods. We collected serum on days 1, 3, 7, 10, and 14 of each of the four study periods and measured serum glucose and TT4. Data were analyzed with a linear mixed-effect model that included TT4 as a fixed effect, and cat as a random effect. Statistical significance was set at 0.05.

Serum glucose concentration (median 94 mg/dL, interquartile range 90-99, range 59-142) was not affected by changes in serum TT4 (median 4.2 μg/dL, interquartile range 2.8-6.4, range 0.8-20.7; $P = 0.63$)

In contrast to previous findings, our study suggests that serum TT4 in hyperthyroid cats may not substantially affect serum glucose concentrations.

EN17

Perioperative Characteristics, Histological Diagnosis and Outcome in Cats Undergoing Surgical Treatment of Primary Hyperparathyroidism - Sponsored By Dechra Veterinary Products

Ameet Singh[1], Michelle Giuffrida[1], Jared Baum[2], Chris Thomson[3], Betty Kramek[3], Julius Liptak[4], Phil Hyndman[5], Brendon Ringwood[6], Dan Smeak[7], Kathleen Ham[8], John Berg[9], Kelley Thieman-Mankin[10], Sarah Boston[11]

[1]University of California, Davis, Davis, CA, USA, [2]Ontario Veterinary College, Guelph, ON, Canada, [3]University of Minnesota, St Paul, MN, USA, [4]Alta Vista Animal Hospital, Ottawa, ON, Canada, [5]Veterinary Emergency and Referral Centre, Toronto, ON, Canada, [6]Veterinary Emergency Clinic and Referral Centre, Toronto, ON, Canada, [7]Colorado State University, Fort Collins, CO, USA, [8]The Ohio State University, Columbus, OH, USA, [9]Tufts University, North Grafton, MA, USA, [10]Texas A&M University, College Station, TX, USA, [11]University of Florida, GAinesville, FL, USA

The objective of this study was to determine peri-operative characteristics and outcome in cats undergoing surgical treatment for PHPT.

This was a multi-institutional, retrospective study with medical record data collection and via telephone follow-up. Cats undergoing surgical treatment and histopathological evaluation of resected tissue were included. Cats were divided into pre-operative ionized calcium (iCa) groups corresponding to the 33rd, 67th, and 100th percentiles of the study population's pre-operative iCa results.

Thirty-two cats were included in the study. Mean cat age was 13.3 ± 2.4 years and mean cat body weight was 4.9 ± 1.3 kg. iCa was above reference range in all cats (median 1.8 mmol/L (IQR 1.5,1.9)). All cats underwent cervical exploratory surgery and abnormal tissue was identified and removed in all cats. Histopathologic diagnosis was parathyroid adenoma in 20/32 (62.5%) cats, parathyroid endocrine carcinoma in 7/32 (21.9%) cats, parathyroid hyperplasia in 3/32 (9.4%) cats, and parathyroid cystadenoma in 2 (6.3%) cats. At discharge, 6/32 (18.8%) cats had hypercalcemia, 5/32 (15.6%) had hypocalcemia, and 21/32 (65.6%) cats had iCa within reference range. Overall median survival time was 1109 days (95% CI 856 - 1332). Survival time was not significantly associated with pre-operative iCa group ($p = 0.139$), hypocalcemia at discharge ($p = 0.326$), hypercalcemia at discharge ($p = 0.955$), or diagnosis of carcinoma ($p = 0.930$).

In this cohort of cats, parathyroid adenoma was the most common cause of PHPT and surgical treatment results in favorable survival times.

EN18

Serum Amino Acid Concentrations in Dogs with Naturally Occurring Pituitary-Dependent Hyperadrenocorticism - Sponsored By Dechra Veterinary Products

Ji-Soo Kim[1], Hyun-Shik Yoon[1], Ji-Houn Kang[1], Chan-Joo Sung[1], Jinhee Lee[1], Hakhyun Kim[1], Minji Lee[2], Byeong-Teck Kang[1], Kyu-Bong Kim[3], Hyang-Yeon Kim[3], Mhan-Pyo Yang[1]

[1]Veterinary Medical Center, College of Veterinary Medicine, Chungbuk National University, Cheongju, Ch'ungch'ong-bukto, Republic of Korea, [2]Veterinary Medical Center, College of Veterinary Medicine, Chungbuk National University, cheongju, Ch'ungch'ong-bukto, Republic of Korea, [3]College of Pharmacy, Dankook University., Cheonan, Ch'ungch'ong-namdo, Republic of Korea

Amino acid metabolism in dogs might be influenced by prolonged endogenous and exogenous hypercortisolemia. Dogs suffering from chronic hypercortisolemia display a variety of clinical signs. Cushing's syndrome has been shown to result in the disruption of amino acid (AA) metabolism in humans, but there is a lack of information on the changes in AA profiles in dogs with hyperadrenocorticism (HAC). Therefore, the objective of the present study was to determine the changes in circulating AA concentrations in dogs with HAC.

Twenty-eight dogs newly diagnosed with pituitary-dependent hyperadrenocorticism (PDH) were enrolled in this case-controlled study, and 6 healthy beagles were also included as controls. Serum concentrations of 21 AA (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, and valine) were analyzed using a 600 MHz ^{1}H nuclear magnetic resonance spectrometer.

Orthogonal partial least square-discriminant analysis (OPLS-DA) plots showed a separation between PDH and healthy dogs. Dogs with PDH showed significant increases in median serum concentrations of arginine ($P = 0.0039$) and aspartate ($P = 0.0123$) but significant decreases in glutamine ($P = 0.0127$) and serine ($P = 0.0287$), compared to healthy dogs.

Our study showed that circulating concentrations of some AA were affected in dogs with PDH, indicating that chronic hypercortisolemia may precipitate a disturbance in AA metabolism. Further studies are necessary to clarify the association between AA profiles and consequences of hypercortisolemia in dogs.

EN20

Untargeted Metabolomic Analysis in Non-Fasted Diabetic Dogs - Sponsored By Dechra Veterinary Products

Allison L. O'Kell[1], Timothy Garrett[2], Clive Wasserfall[2], Mark Atkinson[2]

[1]University of Florida, College of Veterinary Medicine, Gainesville, FL, USA, [2]University of Florida, College of Medicine, Gainesville, FL, USA

Canine diabetes mellitus is a common disease that may have parallels to human type 1 diabetes (T1D). We recently identified variances in serum metabolomic profiles between fasted diabetic and healthy

dogs, some having similarities to those previously identified in human T1D patients. Given that obtaining fasted samples in diabetic dogs can be difficult in practice, the purpose of this study was to expand these efforts to compare untargeted metabolomic profiles in non-fasted diabetic and healthy control dogs.

Serum from diabetic dogs (n=6) and healthy control dogs (n=6) were analyzed by liquid chromatography-mass spectrometry profiling. Dogs were breed and/or body weight matched, and time of sample collection post-feeding was approximately matched between pairs. Metaboanalyst was utilized for data analysis to identify differences in metabolomic profiles between the two groups.

Based on a heat map analysis of both known and unknown metabolites, clear clustering of metabolites between diabetic and control groups was observed. In diabetic dogs compared with healthy control dogs, many metabolites were significantly ($P < 0.01$) downregulated, including those involved in tryptophan metabolism as well as several amino acids. Fewer metabolites were significantly ($P < 0.01$) upregulated and included microbial derived citramalate and the organic acid alpha-hydroxyisobutyric acid. Multiple metabolic perturbations, including those listed above, were similar to those previously found in fasted diabetic dogs.

In sum, metabolomic profiles differ between non-fasted diabetic and healthy dogs, with some parallels to those found in fasted dogs. Metabolomic alterations may give insight into the pathogenesis of canine diabetes. Future studies to confirm these findings and develop targeted assays to detect metabolites that may be used as biomarkers of canine diabetes are warranted.

EN21

Canine Hyperadrenocorticism Enviromental Risk Factors: a Case-Control Study - Sponsored By Dechra Veterinary Products

Tais Nogueira Bockl[1], *Alan Gomes Poppl*[2]
[1]*Faculdade de Veterinária / Departamento Medicina Animal / Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*, [2]*Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil*

Some studies had shown serum cortisol concentration increases, and corticotropic hyperplasia due to environmental stressful agents. This observation explains physiological effects caused by stress. Nevertheless, few studies have tried to correlate chronic exposure to stress with hypothalamic-pituitary-adrenal axis activity in dogs, and hyperadrenocorticism (HAC) development. The present work aimed to clarify whether dogs with pituitary-dependent HAC (PDH) were more exposed to stressful situations throughout their lives when compared to healthy animals. A 25-item questionnaire regarding temperament, socialization, social interaction, level of environmental enrichment and daily habits was developed. Owners of both recently diagnosed PDH and healthy dogs were invited to answer the questionnaire. Twenty newly diagnosed PDH dogs were included in the study. Subjects were matched with forty healthy dogs (control group) by sex, age, and breed (1:2 ratio). Exclusion criteria for the control group were: HAC suspicion, chronic glucocorticoid therapy, and glucocorticoid's exposure over the last month. Odds ratio estimates (OR) and 95% confident intervals (95% CI) were reported, and P-values less than, or equal to 0.05 were considered statistically significant. Dogs with HAC were more than ten times more exposed to neutering (OR = 11.4, 95%CI: 1,38 - 94,06, $P < 0,05$). However, no other significantly correlation was found between social isolation, confinement, exposure to canned foods or plastic toys, phobias or separation anxiety, and HAC. Even variables under study considered potential stress relievers did not suggested any protective effect. These results emphasize neutering as a potential risk-factor to HAC development and instigate further studies on possible mechanism behind.

EN22

Assessment of the Pancreas in Cats with Diabetes Mellitus using Computed Tomographic Angiography - Sponsored By Dechra Veterinary Products

Scott Secrest, Ajay Sharma, Andrew Bugbee
University of Georgia College of Veterinary Medicine, Athens, GA, USA

Diabetes mellitus (DM) is one of the most common endocrine disease in cats. To date, no known diagnostic test or patient characteristic at the time of diagnosis can predict disease course or diabetic remission potential in affected cats. In people, computed tomographic angiography (CTA) is used to evaluate pancreatic endocrine function and predict which patients will become diabetic after pancreatectomy. The purpose of this study was to describe the CTA characteristics of the pancreas in cats with chronic DM and to compare those findings to healthy control cats.

In this prospective cross-sectional study, 15 cats with naturally occurring DM present for > 1 year and 10 age matched, healthy control cats were utilized. Sedated CTA exams of the pancreas were performed in all cats. The time to arterial enhancement, time to peak portal enhancement, pancreatic attenuation and arterial to portal attenuation ratio (A:P) was determined in all cats and compared between groups. A student t-test was used for statistical analyses.

The mean time to arterial enhancement was 6.6 s for both control and diabetic cats with the mean time to peak portal enhancement being 22 s in control cats and 16.7 s in chronic diabetic cats. While there was no statistical difference in time to arterial enhancement ($p=0.9870$), there was in the mean time to peak portal enhancement ($p =0.0006$). Mean pancreatic attenuation in control cats was 49 (pre-contrast), 76.3 (arterial), 167.9 (portal) and 125 HU (delayed) compared to diabetic cats that had an attenuation of 49, 74.25, 157.76 and 117.41 HU respectively. There was no significant difference in pancreatic attenuation between groups with $p =1.0000$, 0.5742, 0.2786 and 0.3981. The mean A:P was not statistically different ($p =0.2612$) between groups with normal cats having a ratio of 0.45 and diabetic cats 0.48.

The time to peak portal enhancement can be used to differentiate normal sedated cats from those with chronic DM using CTA. This CTA finding warrants further investigation to identify possible associations with endocrine function and diabetic remission in cats.

EN23

Canine Mesenchymal Stem Cells Improve Insulin Resistance by Secreting Fibroblast Growth Factor-1 in vitro - Sponsored By Dechra Veterinary Products

Woo-Jin Song, Qiang Li, Jin-Ok Ahn, Min-Ok Ryu, Ju-Hyun An, Aryung Nam, Hwa-Young Youn
Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea

In the field of diabetes research, many studies on cell therapy have been conducted using mesenchymal stem cells. This research was

intended to shed light on the influence of canine adipose-tissue-derived mesenchymal stem cell conditioned medium (cAT-MSC CM) on *in vitro* insulin resistance models that were induced in differentiated 3T3-L1 adipocytes and the possible mechanisms involved in the phenomenon.

In vitro-induced insulin resistance model was developed in differentiated 3T3-L1 adipocytes. Then the cells were co-cultured with conditioned media (CM) of cAT-MSCs for 48 hours. The insulin receptor substrate-1 (IRS-1) and glucose transporter type 4 (GLUT4) were examined as markers of insulin resistance. Anti-FGF1 neutralizing antibody was added to CM-treated insulin-resistance models for blocking the biological activities of FGF1 contained in cAT-MSCs CM. Quantitative reverse transcriptase PCR and western blot analysis were used to assess mRNA and protein levels of these markers. Data were compared by one-way ANOVA using the GraphPad Prism v.6.01 software.

Relative protein expression levels of IRS-1 and GLUT4 were augmented in the cAT-MSC CM treatment group compared to insulin-resistance model controls, indicating beneficial effects of cAT-MSC to DM, probably by actions of secreting factors. With reference to previous studies on fibroblast growth factor-1 (FGF1), we proposed FGF1 as a contributing factor to the mechanism of action. We added anti-FGF1 neutralizing antibody to the CM-treated insulin resistance models. As a result, significantly diminished protein levels of IRS-1 and GLUT4 were observed, supporting our assumption.

Accordingly, this study advocated the potential of MSC CM as an alternative insulin sensitizer and discovered a signalling factor associated with the paracrine effects of cAT-MSC.

EN24

Microbiota-Related Changes in Fecal Bile Acid Metabolism are associated with Diabetes Mellitus in Dogs - Sponsored By Dechra Veterinary Products

Albert E. Jergens[1], Blake Guard[2], Karin Allenspach[3], Lawrance Chandra[4], So Young Park[5], Albert E. Jergens[6], Jan S. Suchodolski[7]
[1]Iowa State University, College of Veterinary Medicine, Ames, IA, USA, [2]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [3]Iowa State University, Veterinary Clinical Sciences, Ames, IA, USA, [4]Iowa State university, Veterinary Clinical Sciences, Ames, IA, USA, [5]Texas A&M University, College Station, TX, USA, [6]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, Ames, IA, USA, [7]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

Diabetes mellitus (DM) has recently been associated with altered intestinal microbiota. The consequences of intestinal dysbiosis such as increased intestinal permeability and altered microbial metabolites are suspected to contribute to the host inflammatory state and insulin resistance. Human diabetics have been shown to have changes in bile acid (BA) metabolism which may be detrimental to glycemic control. The purpose of this study was to examine BA metabolism in dogs with naturally-occurring DM and to relate these findings to changes in the intestinal microbiota.

Adult dogs with a clinical diagnosis of DM were prospectively enrolled along with age-matched healthy control dogs. The fecal microbiota were analyzed by 16S rRNA gene next-generation (Illumina) sequencing. Fecal concentrations of primary (cholic/chenodeoxycholic) and secondary (lithocholic/deoxycholic/ursodeoxycholic) BA were measured using gas chromatography and mass spectrometry. Non-parametric Mann-Whitney U tests compared median values between healthy controls and dogs with DM. Statistical significance was set at $P < 0.05$.

Ten diabetic dogs and 10 healthy controls were enrolled. Fecal microbial diversity was lower in DM dogs when compared to healthy controls. PCA based on unweighted Unifrac distance metric did not reveal significant clustering between dog groups. However, linear discriminate analysis effects size (LEfSe) detected 27 differentially abundant bacterial taxa (a= 0.01, LDA score >2.0). While Gammaproteobacteria was overrepresented, Erysipelotrichia, Clostridia, and Bacteroidia were underrepresented in DM dogs compared to healthy controls ($P < 0.05$ for all). Total primary BA were increased in DM dogs compared with healthy controls ($P = 0.03$) and lithocholic acid was decreased ($P < 0.05$) in dogs with DM.

Results indicate that dogs with DM have both intestinal dysbiosis and associated BA alteration. The pattern of dysbiosis and altered BA composition is similar to that seen in humans with DM. Further investigation is necessary to determine whether dysbiosis is a cause or consequence of DM and whether it is associated with poor glycemic control.

GI01

Comprehensive Comparison of Upper and Lower Endoscopic Small Intestinal Biopsies in Cats with Chronic Enteropathy

Betty Chow[1], Steve L. Hill[1], Keith P. Richter[2], Sina Marsilio[3], Mark R. Ackermann[4], Jonathan A. Lidbury[5], Jan S. Suchodolski[6], Sarah E. Cocker[1], Joerg M. Steiner[5]
[1]Veterinary Specialty Hospital, San Diego, CA, USA, [2]Veterinary Specialty Hospital/Ethos Veterinary Health, San Diego, CA, USA, [3]Gastrointestinal Laboratory, College Station, TX, USA, [4]Oregon Veterinary Diagnostic Laboratory, Corvallis, OR, USA, [5]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [6]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

Differentiating inflammatory bowel disease (IBD) and alimentary small cell lymphoma (LSA), which are the predominant forms of chronic enteropathy in cats, is often challenging. Performing immunohistochemistry (IHC) and clonality testing (i.e., by PCR for antigen receptor rearrangement; PARR) as an adjunct to routine histopathological assessment may improve the diagnostic accuracy of endoscopic biopsy (EB) samples for making this differentiation. There is evidence to suggest that there is poor agreement between EB samples collected from the upper small intestines (USI) and the lower small intestines (LSI), and that both areas should be sampled in all patients. However, performing lower gastrointestinal endoscopy (LGE) along with upper gastrointestinal endoscopy (UGE) results in increased costs and anesthesia time compared to UGE alone. The goals of this study were to evaluate the diagnostic utility of IHC and clonality testing for the diagnosis of IBD and LSA in cats, to assess the level of agreement between EB samples from the USI and LSI, and to determine the diagnostic utility of procuring LSI samples.

A total of 62 cats with CE (gastrointestinal signs of > 3 weeks duration) were retrospectively (n = 19) and prospectively (n = 43) enrolled at the Veterinary Specialty Hospital, San Diego, CA. All cats had UGE and LGE performed with EB samples obtained from both sites. All cases were retrospectively or prospectively reviewed by a single board-certified pathologist (MRA) and were then categorized as "IBD", "possible LSA", "probable LSA", or "LSA". Samples were also submitted for IHC and clonality testing, as previously described.

Based on HE staining alone, 41/62 cats (66.1%) were classified as having IBD and 21/62 (33.9%) as having LSA. After consideration of IHC and clonality, 13/62 cats were classified as having IBD (21.0%) and 49/62 as having LSA (79.0%). Thirteen of 26 cases (50.0%) diagnosed as "IBD" and 15/15 cases (100%) diagnosed as "possible LSA" or "probable LSA" based on HE staining alone were subsequently diagnosed with LSA after the addition of IHC and clonality testing. Using Cohen';s Kappa statistic (k), agreement between USI and LSI samples was moderate (k = 0.65) based on HE staining alone, and also moderate (k = 0.68) after including IHC and clonality testing. For LSA cases diagnosed based on HE alone, there were 4/21 (19.0%) cases diagnosed from USI biopsy samples alone and 3/21 (14.3%) cases diagnosed from LSI biopsy samples alone. For LSA cases diagnosed after IHC and clonality, LSA was diagnosed in 7/49 cases (14.3%) from USI biopsy samples alone, but only 1/49 cases (2.0%) from LSI biopsy samples alone.

Our results show that the addition of IHC and clonality testing to HE staining increases the number of cases diagnosed with LSA. Further research is warranted to assess the diagnostic accuracy of IHC and clonality testing and to determine if a change in diagnosis correlates with patient outcome. Additionally, there was moderate agreement for the diagnosis of IBD and LSA in the USI and LSI. Samples from the LSI rarely added additional diagnostic information.

GI02

Effects of a Combination Anthelmintic (Febantel, Pyrantel, Praziquantel) on the Fecal Microbiota of Dogs Infected with Giardia and Cryptosporidium

Madeline A. Fujishiro[1], *Jonathan A. Lidbury*[2], *Joerg M. Steiner*[2], *Jan S. Suchodolski*[3], *Michael Lappin*[4]
[1]*Texas A&M, College Station, TX, USA,* [2]*Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA,* [3]*Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA,* [4]*Colorado State University, Fort Collins, CO, USA*

The gastrointestinal microbiota is a complex ecosystem that plays an important role in host health and immunity. Concerns regarding the effects of pharmaceuticals on the bacterial microbiota in humans and veterinary species have been raised, more specifically concerning the potential deleterious effects of antimicrobials. The effect of anthelmintic therapy on the bacterial microbiota in dogs has not yet been evaluated and little information is available concerning the effect of *Giardia* species or *Cryptosporidium canis* infectionon the gastrointestinal microbiota. The purpose of this study was to evaluate the fecal microbiota in adult research beagles with chronic, subclinical *Giardia* spp. and *C. canis* infections before and after administration of a commercially available preparation of febantel combined with pyrantel and praziquantel (FPP; Drontal®Plus; Bayer Animal Health, Shawnee, KS).

Six healthy adult research beagles with a subclinical *Giardia* spp. infection and a *C. canis* co-infection were administered FPP as directed by the manufacturer by mouth, daily, for 3 days. Fecal samples were collected 7 and 3 days before FPP (days -7 and -3), on the first day of administration (day 0), and 4, 14, and 21 days after the start of administration (days 4, 14, 21). Fecal DNA was extracted and qPCR assays were performed to assess the abundance of total bacteria, *Faecalibacterium*, *Turicibacter*, *E. coli*, *Streptococcus*, *Blautia*, *Fusobacterium*, and *C. hiranonis* and to calculate a previously published dysbiosis index (DI). Bacterial groups and DI were compared across time points using the Friedman test and Dunn';s post-test as appropriate. Statistical significance was set as $p < 0.05$.

The abundance of *Fusobacterium* increased between days 0 and 4 ($p < 0.01$), otherwise there were no statistically significant changes in the DI or abundance of bacterial groups before and after treatment with FPP. Additionally, the DI in this group of dogs was similar to that of previously evaluated healthy dogs; only 2 of 6 dogs had transiently increased DI results (DI > 2 at 1 pre-treatment time point for each).

FPP administration did not considerably alter the fecal bacterial microbiota in this group of research beagles. Further studies are necessary to evaluate the effect of this and other anthelmintic agents on the gastrointestinal microbiota of client-owned dogs.

GI03

TNF-inducible Gene/Protein-6 (TSG-6) Released by Canine Mesenchymal Stem cells Alleviate Inflammatory Bowel Disease in Mice

Ju-Hyun An, Woo-Jin Song, Jin-Ok Ahn, Qiang Li, Ms. Min-Ok Ryu, Aryung Nam, Hwa-Young Youn
Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Previous studies have revealed that mesenchymal stem cells (MSCs) alleviate inflammatory bowel disease (IBD) by modulating inflammatory cytokines in the inflamed intestine. However, the mechanisms underlying these effects are not completely understood. We sought to investigate the therapeutic effects of canine adipose tissue-derived (cAT)-MSCs in an inflammatory bowel disease mouse model and to explore the mechanisms of the regulation of inflammation.

Dextran sulfate sodium-induced colitis mice were infused with cAT-MSCs intraperitoneally and colon tissues were collected on day 10. Data were compared by one-way ANOVA using the GraphPad Prism v.6.01 software.

cAT-MSCs were shown to induce the expression of M2 macrophage markers and to regulate the expression of pro- and anti-inflammatory cytokines in the colon. Quantitative real time-PCR analyses demonstrated that less than 20 cAT-MSCs, 0.001% of all intraperitoneally injected cAT-MSCs, were detected in the inflamed colon. To investigate the effects of cAT-MSC-secreted factors in vitro, transwell co-culture system was used, demonstrating that tumour necrosis factor-α-induced gene/protein 6 (TSG-6) released by cAT-MSCs induces M2 macrophages. In vivo, cAT-MSCs transfected with TSG-6 small interfering RNA, administered intraperitoneally, were not able to

induce M2 macrophage phenotype switch in the inflamed colon and had no significant effects on IBD severity.

In conclusion, cAT-MSC-produced TSG-6 can ameliorate IBD by inducing M2 macrophage switch in mice.

GI04

Serum IL-2, IL-6, IL-8, and TNF-α Concentrations in Dogs with Chronic Enteropathies

Agostino Buono[1], Tomomi Minamoto[1], Yasushi Minamoto[1], Jonathan A. Lidbury[2], Jan S. Suchodolski[1], Joerg M. Steiner[2]
[1]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [2]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA

Canine chronic enteropathies (CE) are associated with an excessive release of proinflammatory mediators (e.g., cytokines). In human and animal studies, cytokines such as interleukin (IL) -2, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) have all been reported to be involved in the induction and maintenance of CE. The aim of this study was to describe serum concentrations of IL-2, IL-6, IL-8, and TNF-α in dogs with CE.

Twenty-five healthy dogs (CTRL) and 68 dogs with CE were included in this study. Serum IL-2, IL-6, IL-8, and TNF-α concentrations were measured using previously analytically validated electrochemiluminescence immunoassays (Canine Proinflammatory Panel 3 Ultrasensitive kit, Meso Scale Discovery). Data were analyzed using nonparametric statistics, receiver operating characteristic analysis (ROC), and principal component analysis (PCA). Significance was set at $p < 0.05$.

The CE group was composed of 46 males and 22 females, the median age was 6.3 years (min-max: 1-16 years). No significant differences for age or sex were identified between CE and CTRL dogs.

Serum IL-2, IL-6, and TNF-α concentrations were significantly increased in the CE group when compared to CTRL dogs. Results for both groups are shown in the table below. No correlation was found between age, sex, and any of the serum cytokine concentrations. Using a cutoff value of > 14 pg/mL for IL-6, sensitivity for discriminating CE and CTRL dogs was 84% and specificity was 72%. Using a cutoff value of > 2.2 pg/mL for TNF-α, sensitivity was 76% and the specificity was 80%. The areas under the curves for IL-6 and TNF were 0.81 and 0.84, respectively. The first principal component of PCA explained 85.5% of the variance where TNF-α was the major contributor.

In conclusion, dogs with CE have increased concentrations of IL-2, IL-6, and TNF-α, but we were unable to identify any significantly altered concentrations for IL-8. Further studies are needed to assess the utility of these cytokines as diagnostic or prognostic markers in dogs with chronic enteropathies.

(GI04)

	IL-2		IL-6		IL-8		TNF-α	
	CTRL	CE	CTRL	CE	CTRL	CE	CTRL	CE
Median (pg/mL)	11.3	23.4	9.5	25.1	1,021.4	1,534.7	1.4	3.9
min-max	0.7-90.3	0-166.8	1.5-40.8	0-184.7	97.8-4,498	7-10,308.8	0.6-5.4	0.5-39.3
p-value	**p = 0.01**		**p < 0.0001**		p = 0.11		**p < 0.0001**	

GI05

Enterococcus Inhibit Growth and Adhesion of Feline Tritrichomonas foetus

Rachel Dickson[1], Maggie Daves[2], David Bemis[2], Thomas Cecere[3], Jody Gookin[4], Julie Vose[2], M. Katherine Tolbert[2]
[1]University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA, [2]University of Tennessee, Knoxville, TN, USA, [3]Virginia - Maryland College of Veterinary Medicine, Blacksburg, VA, USA, [4]North Carolina State University, Raleigh, NC, USA

Enterococcus inhibit growth and adhesion of feline *Tritrichomonas foetus* Rachel E. Dickson[1], Maggie Daves[1], David A. Bemis[1], Thomas Cecere[2], Jody Gookin[3], Julie Vose[1], M. Katherine Tolbert[11]University of Tennessee College of Veterinary Medicine, Knoxville, TN [2]Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA[3]North Carolina State College of Veterinary Medicine, Raleigh, NC

Tritrichomonas foetus (Tf) causes chronic diarrhea in cats. Limited treatment options and increasing resistance support investigation of alternative treatments. Probiotics have emerged as adjunct therapies for many intestinal pathogens; however, little work has been done to investigate their efficacy against Tf infection.

Commercial *Enterococcus faecium* (Efm) and a novel probiotic, *Enterococcus hirae* (Eh), were used to evaluate efficacy in the inhibition of Tf growth and adhesion in vitro. The effect of *Enterococcus* on Tf proliferation was evaluated throughout log phase growth in co-culture media. The potential of probiotics to reduce Tf adhesion to intestinal epithelium was analyzed with a previously validated co-culture model using porcine intestinal epithelial cells (IPEC-J2). Tf adhesion and cytotoxicity were evaluated using fluorescent microscopy and crystal violet spectrophotometric analysis. SEM and IFA were used to visualize the interactions of Tf, *Enterococcus*, and IPEC-J2 monolayers. Data were analyzed using SigmaStat.

Enterococcus-induced inhibition of Tf growth was observed at concentrations as low as 10:1 Tf:Efm ($p < 0.001$) and was determined to be largely pH dependent ($p < 0.01$). Inhibition of Tf replication was not observed when co-cultured with heat-killed *Enterococcus*, indicating viable *Enterococcus* organisms were required for inhibition. Pretreatment of IPEC-J2 monolayers with *Enterococcus* significantly reduced Tf- induced cytopathogenicity ($p = 0.002$). This benefit was not observed when *Enterococcus* was introduced simultaneously or following Tf infection. These findings suggest that pre-treatment of at-risk cats with probiotics containing Enterococci may alleviate clinical illness resulting from subsequent Tf infection. Eh more effectively decreased Tf adhesion to the intestinal epithelium as compared to Efm ($p < 0.001$), suggesting its superiority as a novel probiotic in Tf infection. Preliminary results also showed Eh was effective at reducing growth and adhesion of a ronidazole-resistant strain of Tf. These results support further investigation of the efficacy of probiotics as an adjunct treatment to combat Tf infection in cats.

GI06

Association between Serum Calprotectin Concentrations and Insulin Resistance in Miniature Schnauzers with Idiopathic Hyperlipidemia

Romy M. Heilmann[1], Panagiotis G. Xenoulis[2], Miss Jasmin Gneipel[3], Miss Denise Riggers[3], Miss Eva Stavroulaki[2], Jan S. Suchodolski[4], Joerg M. Steiner[5]
[1]College of Veterinary Medicine, University of Leipzig, Leipzig, Sachsen, Germany, [2]Clinic of Medicine, Faculty of Veterinary Science, University of Thessaly, Karditsa, Greece, Karditsa, Kardhitsa, Greece, [3]University of Leipzig, College of Veterinary Medicine, Leipzig, Sachsen, Germany, [4]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [5]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA

Miniature Schnauzers are predisposed to idiopathic hyperlipidemia (IH), which is characterized by fasting hypertriglyceridemia (HTGL) with or without hypercholesterolemia (HCHOL). Our previous data suggest that IH is associated with subclinical inflammation in this breed. IH is considered a risk factor for several pathologic conditions including pancreatitis, reduced gallbladder motility, proteinuria, and might also lead to insulin resistance (IR), which can be evaluated by the homeostatic model assessment for insulin resistance (HOMA-IR) score. Increased serum concentrations of calprotectin were associated with IR in people, but such an association has not been reported for dogs. Thus, the aim of this study was to evaluate indicators of IR in a population of Miniature Schnauzers.

Serum samples from 150 clinically healthy Miniature Schnauzers were used for this study. A study questionnaire was completed for each dog to confirm the health status and medication history. Serum triglyceride, cholesterol, glucose, and calprotectin concentrations were measured in all samples. Paired serum samples were available from 16 of the dogs with IH 14–26 weeks after starting an ultra-low fat diet. In addition to serum triglyceride, cholesterol, glucose, and calprotectin, the concentration of serum insulin was measured in all samples, and the pre-treatment HOMA-IR was calculated as [(insulin [mU/L] x glucose [mmol/L])/22.5]. Statistical analyses were performed using non-parametric (paired or unpaired) group comparisons and likelihood ratio tests.

A total of 71 dogs (47%) were diagnosed with IH, of which 51 had HTGL, 7 HCHOL, and 13 had combined IH. Serum glucose concentrations did not differ between dogs with normal vs. increased serum calprotectin concentrations in either the hyperlipidemic or the normolipidemic group (all $P > 0.05$). In the 16 dogs with IH included in the ultra-low fat dietary trial (n = 6 with isolated HTGL, n = 9 with combined IH), the HOMA-IR was significantly higher in dogs with increased vs. normal serum calprotectin concentrations ($P = 0.0430$), but no significant difference was seen in serum insulin or glucose concentrations. Dogs with combined IH had significantly higher HOMA-IR ($P = 0.0216$), serum insulin concentrations ($P = 0.0443$), and a higher proportion of dogs with increased serum calprotectin concentrations ($P = 0.0249$) than dogs with isolated HTGL, but no difference in serum glucose concentration was detected. Resolution of IH through dietary intervention with an ultra-low fat diet was associated with a decrease in serum insulin concentrations ($P = 0.0472$) but increased serum calprotectin concentrations ($P = 0.0229$).

These results suggest that the presumed subclinical inflammatory phenotype in Miniature Schnauzers with IH is associated with an increased IR. The findings lend further support to the ambivalent pro- vs. anti-inflammatory properties of calprotectin.

GI07

Assessment of Gastrointestinal Injury in Racing Alaskan Sled Dogs

Matthew Tanner[1], Tracy L. Hill[1], Robert Gogal[1], Michael Davis[2]
[1]University of Georgia, Athens, GA, USA, [2]Oklahoma State University, Stillwater, OK, USA

Changes in gastrointestinal (GI) permeability and injury are caused by a breakdown of the protective gastrointestinal epithelial barrier and commonly occurs in humans, horses, and dogs following strenuous exercise. Gastrointestinal injury and increased permeability raises the risk of ulceration and translocation of intestinal bacteria causing sepsis or systemic inflammatory response syndrome (SIRS). Current methods to assess intestinal injury and permeability in dogs are either invasive, difficult to preform, or have high variability. Intestinal fatty acid binding protein (I-FABP) and diamine oxidase (DAO) are two enterocyte proteins absorbed from the epithelium with intestinal injury. We hypothesized that I-FABP and DAO concentrations will increase with strenuous exercise and be correlated with previously obtained assessments of intestinal permeability and injury.

Serum was collected after various distances of a racing trial in conditioned Alaskan sled dogs. I-FABP and DAO levels were quantified using commercially available canine-specific ELISA assays. Protein concentrations were correlated with 5-sugar gastrointestinal permeability and severity of gastroduodenal ulceration, previously obtained by one of the authors.

DAO was significantly increased (P = 0.002) with strenuous exercise. There was no relationship between I-FABP and exercise. There was no correlation to previously collected GI permeability. This study establishes the potential utility of serum DAO in the assessment of gastrointestinal permeability and injury in dogs. Future research will continue to validate these easily quantified biomarkers and investigate their potential both as a prognostic marker for acute gastrointestinal injury in racing sled dogs.

Changes in gastrointestinal (GI) permeability and injury are caused by a breakdown of the protective gastrointestinal epithelial barrier and commonly occurs in humans, horses, and dogs following strenuous exercise. Gastrointestinal injury and increased permeability raises the risk of ulceration and translocation of intestinal bacteria causing sepsis or systemic inflammatory response syndrome (SIRS). Current methods to assess intestinal injury and permeability in dogs are either invasive, difficult to preform, or have high variability. Intestinal fatty acid binding protein (I-FABP) and diamine oxidase (DAO) are two enterocyte proteins absorbed from the epithelium with intestinal injury. We hypothesized that I-FABP and DAO concentrations will increase with strenuous exercise and be correlated with previously obtained assessments of intestinal permeability and injury.
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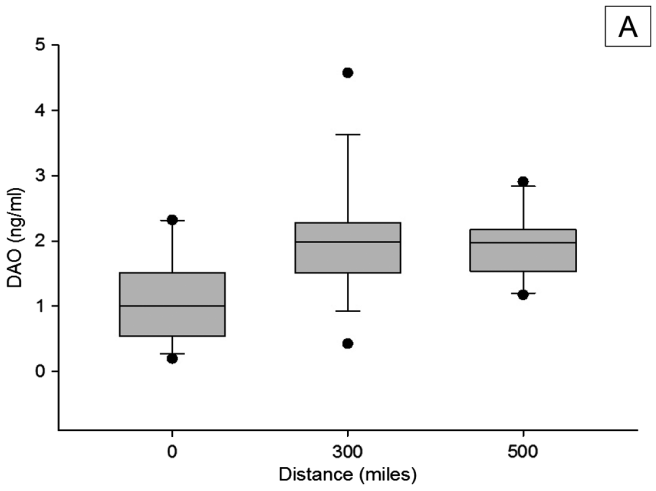


permeability and severity of gastroduodenal ulceration, previously obtained by one of the authors.

DAO was significantly increased (P = 0.002) with strenuous exercise, while there was no relationship between I-FABP and exercise discovered. There was no correlation to previously collected GI permeability and injury. This study establishes the utility of serum DAO in the assessment of gastrointestinal permeability and injury in dogs. Future research will continue to validate these easily quantified biomarkers and investigate their potential both as a prognostic marker as well as assessment of therapies in diseases of gastrointestinal injury in dogs, including sepsis/SIRS, exercise, obesity, and decompensated heart disease.

GI08

Effect of Metoclopramide, Erythromycin and Exenatide on Solid Phase Gastric Emptying in Healthy Cats

Roman Husnik[1], *Jon M. Fletcher*[2], *Lorrie Gaschen*[2], *Frederic Gaschen*[2]
[1]*Gastrointestinal Laboratory, Texas A&M University, College Station, TX, Baton Rouge, LA, USA,* [2]*Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, Baton Rouge, LA, USA*

Feline GI motility disorders present both a diagnostic and therapeutic challenge. The available data on the effect of various prokinetic drugs in cats is tenuous. Therefore most recommendations for drug usage and dosage are based on collective clinical experience. Recently, incretins have been a focus of interest because of their beneficial role in glucose homeostasis and the treatment of type 2 diabetes mellitus. However, their impact on GI motility has not been evaluated. This study assesses the effects of metoclopramide, erythromycin and exenatide on gastric emptying (GE) and gastric motility in comparison to placebo.

In a randomized, double-blind, 4-way crossover design, 8 healthy cats were administered placebo (saline PO or SC), metoclopramide (0.5 mg/kg SC q8h), erythromycin (1 mg/kg PO q8h) or exenatide (1.2 µg/kg SC q12h) for 2 consecutive days followed by a minimum 5-day washout period. Cats were randomized to a treatment group. Sonographic assessment of GE was performed in dorsal recumbency at 0, 15, 30 and 60 minutes following a solid test meal (20% of daily energy requirements), and at 30-minute intervals thereafter for 8 hours. Mean cross-sectional area of transverse images of the relaxed antrum was obtained for each time point, and expressed as a percentage of the maximal antral area. The area under the curve (AUC) was calculated and 25% - 90% GE times (GET) determined. The motility index (MI) of antral contractions was plotted against time. A mixed model ANOVA with cat as a random effect and treatment as fixed effect was used to assess the difference in each fractional GET and MI AUC between treatments. Posthoc pairwise comparisons were examined with the Tukey';s test as appropriate.

The rate of GE following metoclopramide or erythromycin treatment was significantly faster compared to that after placebo or exenatide. There was a statistically significant difference at all fractional GE between GET following metoclopramide and erythromycin treatments when compared to placebo ($p = 0.002 – 0.049$ and $p = 0.001 – 0.015$ for metoclopramide and erythromycin respectively) and exenatide ($p < 0.001$ and $p < 0.001$ for metoclopramide and erythromycin respectively). The rate of GE following administration of exenatide was significantly slower compared to placebo during the first half of the GE curve (25 - 55% fractional GE; $p = 0.013 – 0.046$). The total AUC of MI following administration of erythromycin (1857 ± 415 [mean ± SD]) and metoclopramide (1847 ± 253 [mean ± SD]) was significantly larger ($p = 0.034$ and 0.042 respectively) than the total AUC of MI following administration of placebo (1555 ± 286 [mean ± SD]), indicating an increase in the MI of antral contractions. However, the total AUC of MI following exenatide administration (1509 ± 296 [mean ± SD]) was not different from that obtained after placebo was given.

The results indicate that metoclopramide and erythromycin have a positive gastric prokinetic effect in healthy cats: they shorten GE times and increase the MI of antral contractions. In contrast, exenatide administered to healthy cats delays GE.

GI09

Esophageal Lumen Ph and Lavage in Dogs with Gastroesophageal Reflux

Francesco Lotti[1], *Pedro Boscan*[1], *David Twedt*[1], *Kanawee Warrit*[2], *Heather Weir*[1], *Pamela Vogel*[1], *Emory Wilcoxen*[1], *Shilo Bryan*[1], *Cindy Vaca*[1]
[1]*Colorado State University/Veterinary Teaching Hospital, Fort Collins, CO, USA,* [2]*Colorado State University/Veterinary Teaching Hospital, Fort Collins, CO, USA*

Gastroesophageal reflux (GER) during general anesthesia is reported in 15-60% of dogs and can in some cases lead to esophagitis or esophageal stricture formation. The pH of the GER and the time it remains in the esophageal lumen are thought to play an important role in the development of esophageal disease. However, the incidence of esophagitis or stricture formation from GER is unknown. There are as yet no studies in dogs preventing GER from causing esophageal damage. The aim of this study is to determine esophageal pH and if esophageal lavage affects lumenal pH following GER.

Sixty dogs anesthetized for elective ovariectomy were evaluated for GER and 27 dogs identified with GER and having contents with a pH of < 4 were included in the study. An esophageal pH/impedance catheter (Divarsatek™, Milwaukee) was placed shortly following

anesthesia using endoscopic guidance with the catheter tip placed approximately 3 cm cranial to the lower esophageal sphincter. A GER episode was defined as an orally progressing decrease in impedance (50% decrement in ohms) from the pre-episodic esophageal baseline recording. When esophageal pH < 4 remained at the conclusion of the surgery esophageal lavage was performed. Tap water in 60 mL aliquots was instilled through a gastric tube and carefully suctioned. If after an interval of 2 minutes post lavage esophageal pH was still under 4, another aliquot was instilled and again suctioned until pH > 4. Paired t test, Wilcoxon matched pairs test and Spearman';s rank correlation coefficient were used to analyze parametric and non-parametric data.

Of the 27 dogs having strongly acidic GER (pH < 4), 16 dogs were able to neutralize their esophageal lumen pH before the end of the procedure. These 16 dogs increased their esophageal lumen and GER pH from 2.1 ± 0.8 to 5.4 ± 0.9 during the anesthesia (p < 0.0001). 14/27 dogs did not neutralize their esophageal lumen and GER pH having esophageal lumen and GER pH of 2.9 ± 0.9 at the end of the procedure. Esophageal lavage with tap water increased the lumen and GER to 4.1 ± 1 in 11/14 (78%) of dogs (p = 0.003). The volume of water used for lavage was not associated with the changes in lumen and GER pH (r = -0.19, p = 0.50).

In the present study, we show for the first time that some dogs are capable of neutralizing strongly acidic GER. The dogs that were unable to clear acidic GER are possibly the ones more predisposed to esophagitis or stricture formation. Esophageal lumen lavage with water in dogs having strongly acidic pH increased the esophageal lumen and GER pH in a majority of dogs suggesting esophageal lavage might be beneficial in preventing GER complications.

GI10

Contrast Videofluoroscopy Can Help Manage Dogs with Congenital Idiopathic Megaesophagus

Janne G. Lyngby[1], *Jillian Haines*[2]
[1]*University of Copenhagen, Frederiksberg C, Hovedstaden, Denmark,* [2]*Washington State University, Pullman, WA, USA*

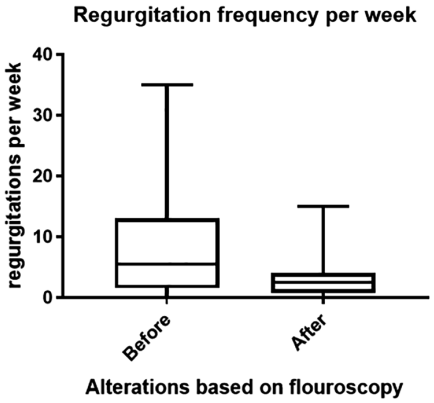
Megaesophagus (ME) has a high mortality rate associated with the difficulty of managing patients' feeding. Currently there are no evidence-based guidelines available for determining the best diet consistency in dogs with ME.

The aim of this study was to compare esophageal clearance times (ECT) of food with different consistencies in dogs with congenital idiopathic ME, and to assess if esophageal contrast videofluoroscopy can be used to guide management of dogs to improve clinical signs and quality of life.

Twenty-one dogs with congenital idiopathic ME were included. For evaluation, each dog was placed in a Bailey chair and administered barium sulfate orally in three forms: liquid, slurry and within canned food meatballs. The amount of barium and food was determined by bodyweight and resting energy requirement. Contrast videofluoroscopy of the esophagus was obtained at baseline and every 5 minutes for up to 30 minutes or stopped sooner if esophageal content had cleared. Each patient received specific recommendations for management based on ECT of the different diet consistencies, frequency of regurgitation, presence of esophageal reflux, decreased gastric motility, and body condition score. The follow up communication was performed to assess compliance and response to management changes.

No clearance was observed in 29% of dogs fed liquid, in 43% of dogs fed slurry and 67% of dogs fed meatballs. There was a significant difference (p < 0.001) between groups with the ECT of liquid being faster compared to both meatballs and slurry. There was no significant difference in ECT between slurry and meatballs, but the median percentage of esophageal clearance was 50% (interquartile range 0-79%) for slurry and 0% (interquartile range 0-60%) for meatballs.

The median number of regurgitations per week (RPW) decreased significantly from 5.5 (interquartile range 1.5-13) at the time of the first assessment to 2.5 (interquartile range 0.75-4) at the time of follow up (P = 0.0004) In addition, 16/21 (76%) of clients perceived their dogs quality of life to be much better, 4/21 (19%) to be somewhat better and 1/21 (5%) saw no change in quality of life at the time of recheck.



GI11

Utility of Capsule Endoscopy in the Assessment of Microcytosis in Dogs

Kasey Mabry[1], *Tracy Hill*[2]
[1]*Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA,* [2]*University of Georgia, Athens, GA, USA*

Video capsule endoscopy (VCE) is a non-invasive imaging modality with reported applications including identification of mucosal lesions not detected with traditional endoscopy or abdominal imaging and evaluation of anthelmintic efficacy. In people, VCE is used in diagnostic and management protocols of various gastrointestinal disorders, particularly in obscure gastrointestinal bleeding or unexplained iron deficiency anemia (IDA). The objective of this study was to evaluate the use of VCE in dogs with microcytosis.

Medical records were reviewed to identify dogs with microcytosis that received VCE without a defined etiology based on other imaging. Signalment, history, physical examination findings, clinicopathologic data, and capsule endoscopy findings were recorded.

Dogs were 8 to 12 years old, 6.9-40 kg, and a mix of sexes and breeds. Clinical signs included pica, melena, hematochezia, vomiting, diarrhea, and hyporexia. Abdominal radiographs and abdominal ultrasound were unremarkable in all dogs. Esophageal transit time, gastric emptying time, and small intestinal transit time was: 5-14 minutes,

18-451.30 minutes, and 67.5-228 minutes, respectively. Mucosal lesions were identified in 8/9 dogs. Gastric lesions included erosion/ulcers (7), hemorrhage (2), mucosal irregularity (5), and a polypoid lesion (1). Intestinal lesions included erosions (3), hemorrhage (1), irregular mucosa (5), and a mass in the duodenum (1) and jejunum (1). Colonic lesions included an irregular mucosa (1).

VCE identified lesions not detected with other diagnostic modalities in 8/9 dogs with microcytosis of unknown origin. VCE is a non-invasive diagnostic tool that could assist in the identification of gastrointestinal lesions in dogs presenting with microcytosis when other diagnostic modalities are inconclusive.

GI12

Histopathology, Immunohistochemistry, and Clonality of Intestinal Biopsies from Healthy Cats

Sina Marsilio[1], Jonathan A. Lidbury[2], Jan S. Suchodolski[3], Mark R. Ackermann[4], Joerg M. Steiner[2]
[1]Gastrointestinal Laboratory, College Station, TX, USA, [2]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [3]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [4]Oregon Veterinary Diagnostic Laboratory, Corvallis, OR, USA

Cats with chronic enteropathy frequently undergo endoscopy and assessment of intestinal biopsies using histopathology, immunohistochemistry, and clonality testing (PCR for antigen receptor rearrangements (PARR)). However, there is a paucity of data on findings for these modalities in a cohort of healthy cats. Thus, the aim of this study was to describe results of histopathology, immunohistochemistry, and PARR in endoscopically derived duodenal biopsy specimens from clinically healthy, client-owned cats.

Twenty clinically healthy, client-owned cats underwent gastroduodenoscopy at the Veterinary Medical Teaching Hospital at Texas A&M University (Animal Care and Use Protocol 2015-0276 CA). Tissue specimens were collected from the stomach (18 cats) and the duodenum (20 cats) and were evaluated according to the WSAVA scoring system by a board-certified pathologist (MRA) who was blinded to the health status of the cats. In addition, samples were evaluated by routine immunohistochemistry and clonality testing without disclosure of the clinical status of the cats.

Cats had a median age of 9.5 years (min-max: 3 to 18 years), body weight of 5.0 kg (min-max: 2.9-8.6 kg), and body condition score of 6 (min-max: 5 to 9). Sample quality was reported as very good in all cases. Histologic evaluation of the stomach revealed a mean gastric score of 1.77 (± 1.59) and a mean duodenal score of 3.50 (± 1.89). Immunohistochemistry of duodenal samples revealed epitheliotropic and/or lamina propria infiltrates that were CD3+ in all cases. Results of clonality testing in duodenal samples revealed clonal rearrangements in 8 cats and polyclonal rearrangements in 6 cats. In 5 cats, results were suggestive of clonal rearrangements in a polyclonal background. Results for one cat were reported to be consistent with pseudoclonality due to a low quantity of target DNA. An interpretation of small cell lymphoma based on immunohistochemistry and clonality testing was reached in 12 of these healthy cats. In one additional cat, results were interpreted as emerging small cell lymphoma, and an interpretation of enteritis was given for 6 cats. Results from one cat were deemed uninterpretable due to pseudoclonality.

In conclusion, intestinal biopsies from clinically healthy cats commonly show abnormal findings using histopathology, immunohistochemistry, and clonality testing without any apparent clinical significance. While the sensitivity of clonality tests is reported to be high, these results imply that further assessment of the specificity of this diagnostic modality is warranted.

GI13

Characterization of the Intestinal Proteome of Cats with Inflammatory Bowel Disease or Alimentary Lymphoma

Sina Marsilio[1], Lawrence J. Dangott[2], Betty Chow[3], Steve L. Hill[3], Jonathan A. Lidbury[4], Jan S. Suchodolski[5], Joerg M. Steiner[4]
[1]Gastrointestinal Laboratory, College Station, TX, USA, [2]Director of the Protein Chemistry Laboratory, Texas A&M University, Dept of Biochemistry & Biophysics, College Station, TX, USA, [3]Veterinary Specialty Hospital, San Diego, CA, USA, [4]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [5]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

Inflammatory bowel disease (IBD) and alimentary small cell lymphoma (SCL) are the most common types of chronic enteropathy in cats. Diagnosis and differentiation requires invasive and expensive procedures, namely gastrointestinal endoscopy with histopathological assessment of biopsies. The aim of this study was to characterize the intestinal mucosal proteome of cats with IBD or SCL and to identify potential biomarkers for the differentiation of these disease entities.

The mucosal proteome was characterized in endoscopically-derived duodenal biopsies from 6 cats with IBD and 8 cats with SCL, using two-dimensional difference gel electrophoresis (2D DIGE) (Animal Care and Use Protocol 2014-0369 CA). Endoscopically-derived duodenal biopsies of 6 healthy client-owned cats served as controls (Animal Care and Use Protocol 2015-0276 CA). Prior to analysis all cats underwent a comprehensive work-up including histopathology, immunohistochemistry, and clonality testing (PCR for antigen receptor rearrangements) of intestinal biopsy samples. Fluorescent gel images were analyzed using the DeCyder™ 2D 6.5 Differential Analysis Software. Spot volumes were compared by ANOVA. Differences in spot volumes between individual groups were compared by student';s t-tests. Statistical significance was set at $p < 0.05$. Protein spots with a significant difference between groups and a ≥ 2-fold change were analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS).

A total of 2,349 spots were identified, of which 9 were differentially expressed with a ≥ 2-fold change between healthy cats and cats with IBD and SCL ($p \leq 0.01$). Eight of these 9 spots were also differentially expressed between cats with IBD and those with SCL ($p \leq 0.04$). LC-MS/MS identified proteins of the annexin and apolipoprotein families, and malate dehydrogenases in the differentially expressed spots.

Our results show differences between the mucosal proteome of healthy cats, cats with IBD, and cats with SCL. These proteins might hold potential for the development of minimally-invasive biomarkers for the differentiation of IBD and SCL in cats with chronic enteropathy. Further studies to validate these findings are warranted.

GI14

Effects of Gastrointestinal Diets on Feline Fecal Occult Blood Testing

Kate E. Spies, Jennifer E. Slovak
Washington State University College of Veterinary Medicine, Pullman, WA, USA

The goal of this study was to determine the prevalence of positive fecal occult blood testing in cats fed veterinary prescribed gastrointestinal formulated diets. Fecal occult blood testing identifies microscopic blood in the stool. Trace bleeding in the gastrointestinal tract can occur from pathologies such as gastrointestinal lymphoma, chronic enteropathies, and gastric ulcers/erosions. Guaiac-based fecal occult blood testing (gFOBT) relies on heme peroxidase activity. It is widely used in human medicine as a non-invasive and inexpensive diagnostic tool. For veterinary patients, gFOBT has been presumed to cross-react with dietary peroxidases but has been incompletely researched, especially in cats.

Eleven clinically healthy, indoor-only cats aged 1-8 years old were initially fed their normal diets, then transitionally fed 2 gastrointestinal diets over a 7-week period: hydrolyzed protein Purina HA diet® and gastroenteric Purina EN diet®. Two fecal samples were tested per cat each week using human point-of-care Hemoccult® tests.

Two cats produced positive stool samples during the first 2 weeks of the study when fed their normal, liver-based diet and when mixed with the HA diet. Stool from a single cat fed the canned EN diet, containing liver (unlike the dry form), produced positive results during weeks 4, 5, and 6 of the study. Additionally, 2 other cats produced positive stool results during week 4 when fed mixed HA/EN diets. This study suggests that dietary protein sources can interfere with feline gFOBT, especially when there is liver in the diet. Additionally, it suggests that gFOBT should occur 1 week following a diet change to a gastrointestinal formulated diet to avoid potential transitional diet inference.

GI15

Fecal Metabolites from the Tryptophan-Serotonin-Indole Pathway in Dogs with Intestinal Disease

Rachel Pilla[1], Anna-Lena Ziese[2], Melanie Werner[2], Linda Toresson[3], Ms. Hannah L. Klein[4], Jonathan A. Lidbury[5], Joerg M. Steiner[5], Stefan Unterer[2], Jan S. Suchodolski[6]
[1]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX., College Station, TX, USA, [2]Clinic of Small Animal Medicine, Ludwig Maximilian University, Munich, Germany., Munich, Bayern, Germany, [3]Evidensia Specialist Animal Hospital, Helsingborg, Sweden/Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, Helsinki University, Helsinki, Finland, Helsingborg, Skane Lan, Sweden, [4]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, COLLEGE STATION, TX, USA, [5]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [6]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

The essential amino acid tryptophan and its degradation products (e.g., serotonin) are important in the regulation of T-cell response within the intestine as well as intestinal motility. Furthermore, bacteria metabolize tryptophan into various indole-derivatives, which also serve as signaling molecules, activating pathways in other organ systems (e.g., brain, liver, kidney). Alterations in tryptophan metabolism is associated with inflammatory bowel disease (IBD) in humans, and dietary supplementation with tryptophan has been shown to have anti-inflammatory effects in experimental colitis models. The aim of this study was to evaluate changes in the tryptophan-serotonin-indole pathway in dogs with intestinal disease.

Fecal samples from 8 dogs with idiopathic IBD and 10 dogs with acute hemorrhagic diarrhea (AHD) were collected. Fecal samples from 10 healthy dogs were included as a control group. All 28 samples were extracted using methanol-chloroform before targeted analysis by TSQ Altis Triple Quadrupole liquid chromatography-mass spectrometry. Chromatograph peaks were compared with a standard curve for quantification, and adjusted for initial fecal weight. The following metabolites were measured: acetylcholine, anthranilic acid, indole, indole-3-acetaldehyde, indole-3-acetamide, indole-3-acetic acid, indole-3-carboxaldehyde, indole-3-lactic acid, serotonin, tryptamine, tryptophan, and tyramine. Fecal metabolites were compared amongst groups using Kruskal-Wallis tests, followed by Dunn's multiple comparisons tests. Significance was set as $p < 0.05$.

In the AHD group, anthranilic acid, indole, indole-3-acetamide, and indole-3-lactic acid were significantly increased (p=0.036; 0.004; 0.029; and 0.023, respectively), and serotonin, and tryptamine were decreased (p=0.037; and 0.178, respectively) compared to healthy controls. Fecal tryptophan was increased 2-fold in dogs with IBD (not significant, p=0.524), and 8.5-fold in dogs with AHD ($p < 0.001$) compared to healthy control dogs.

Significant differences in fecal metabolites from the tryptophan-serotonin-indole pathway were found in dogs with AHD compared to healthy control dogs. Further studies are needed to determine the clinical implications of these differences.

GI16

Longitudinal Characterization of the Fecal Microbiome in Dogs with Idiopathic Inflammatory Bowel Disease

Rachel Pilla[1], Blake Guard[2], Craig Webb[3], Steve L. Hill[4], Jonathan A. Lidbury[5], Joerg M. Steiner[5], Albert E. Jergens[6], Jan S. Suchodolski[7]
[1]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX., College Station, TX, USA, [2]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [3]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [4]Veterinary Specialty Hospital, San Diego, CA, USA, [5]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [6]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, Ames, IA, USA, [7]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

Idiopathic inflammatory bowel disease (IBD) is characterized by intestinal mucosal inflammation and is associated with alterations in the gut microbiome or intestinal dysbiosis. Treatment is directed at the pathophysiologic mechanisms and often includes dietary modification, antibiotics, and/or immunosuppressants. Treatment responses are often suboptimal, and relapses are common, making management of IBD a challenge. Intestinal dysbiosis is present at the time of diagnosis

in dogs with IBD, however changes in the microbiome over time in dogs undergoing standard immunosuppresssive therapy have not been well characterized. The purpose of this study was to evaluate longitudinal changes of the intestinal microbiome in dogs with IBD being treated with prednisone.

Thirteen dogs diagnosed with idiopathic IBD, that previously failed to respond to treatment with elimination diets and metronidazole, were enrolled. Stool samples were collected from all dogs before initiating therapy with prednisone, after 3 and 8 weeks, and more than one year after beginning treatment. Thirteen healthy dogs were enrolled in the study as a control group. Stool samples were kept frozen at -80°C until DNA extraction. The microbiota was characterized using Illumina sequencing of 16S rRNA genes. Data were analyzed using Quantitative Insights Into Microbial Ecology (QIIME). Beta diversity was evaluated with the phylogeny based unweighted UniFrac distance metric, and statistics were performed with the Analysis of Similarities (ANOSIM).

In the IBD group, clinical disease severity (CIBDAI) at baseline was scored as moderate to severe. All dogs achieved partial or complete remission of clinical signs by 3 or 8 weeks of treatment, and CIBDAI scores were significantly reduced after 8 weeks of treatment ($p < 0.001$). At baseline, dogs with IBD showed differences in microbial composition when compared to healthy dogs, as unweighted UniFrac distances demonstrated significantly different beta diversity between groups ($p=0.001$, $R=0.462$). Differences in the IBD group included increased Firmicutes ($p < 0.001$) and Actinobacteria ($p=0.027$), and reduced Bacteroidetes ($p < 0.001$), Fusobacteria ($p < 0.001$) and Proteobacteria ($p=0.006$). Within the IBD group, beta diversity was still significantly changed at 8 weeks ($p < 0.001$) compared to healthy controls. At the 1 year follow up, diversity metrics placed the microbiome of treated dogs closer to that of healthy dogs, but they were still significantly different from those of healthy controls ($p=0.003$, $R=0.258$).

Our results suggest that, while treatment can be effective in improving clinical signs, the dysbiosis associated with IBD is still present after more than one year after the initiation of immunosuppressive therapy, even when dogs were clinically well controlled.

GI17

Prevalence of Clostridium perfringens Encoding netF Gene in Dogs with Acute and Chronic Gastrointestinal Diseases

Fatima Sarwar[1], Anna-Lena Ziese[2], Kathrin Busch[2], Yasushi Minamoto[3], Amanda B. Blake[4], Jonathan A. Lidbury[5], Joerg M. Steiner[5], Stefan Unterer[2], Jan S. Suchodolski[3], Melanie Werner[2]
[1]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, Texas, College Station, TX, USA, [2]Clinic of Small Animal Medicine, Ludwig Maximilian University, Munich, Germany., Munich, Bayern, Germany, [3]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [4]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, College Station, TX, USA, [5]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA

The virulence of *Clostridium perfringens* is attributed to various toxins. Recently, the newly described pore-forming toxin NetF, that is associated with *Clostridium perfringens* type A, has been reported in dogs with acute hemorrhagic diarrhea syndrome (AHDS), but limited information is available about the role of this toxin in other intestinal diseases of dogs. The aim of this study was to evaluate the prevalence of *Clostridium perfringens* encoding the *netF* gene in fecal samples from dogs with acute and chronic gastrointestinal diseases.

Fecal samples from 324 dogs were analyzed for the abundance of the *netF* gene by quantitative real time PCR. Dogs were divided into the following groups: healthy (HC, n=130), acute hemorrhagic diarrhea (AHDS, n=42), acute non- hemorrhagic diarrhea (AD, n=41), chronic enteropathy (CE, n=95), and dogs with exocrine pancreatic insufficiency (EPI, n=16). The proportions of dogs that had fecal samples positive for the *netF* gene were statistically analyzed using a Chi-square test (Graphpad Prism v7.0), and significance was set at $p < 0.05$.

A total of 20 fecal samples from dogs with gastrointestinal diseases were positive for *Clostridium perfringens* encoding the *netF* gene: 19/42 (45%) dogs with AHDS and 1/16 (6%) dogs with EPI. Fecal samples from all healthy dogs, dogs with CE, and dogs with acute non- hemorrhagic diarrhea were below the detection limit of the PCR assay. There was a significant association between the presence of *Clostridium perfringens* encoding *netF* gene and AHDS ($p < 0.0001$).

In this study, fecal samples of dogs with acute hemorrhagic diarrhea were commonly positive for *Clostridium perfringens* encoding the *netF* gene. Also, *Clostridium perfringens* encoding the *netF* gene was not detected in healthy dogs, dogs with acute non-hemorrhagic diarrhea, or dogs with chronic enteropathy.

GI18

Microbiota analysis-detected dysbiosis in dogs with cancer is unaffected by Enterococcus faecium NCIMB 10415 therapy

Linda A. Matthewman[1], Jan S. Suchodolski[2], Dirk Werling[3]
[1]Royal Veterinary College, Hatfield, England, United Kingdom, [2]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [3]Pathobiology and Population Sciences Royal Veterinary College, Hatfield, England, United Kingdom

Dysbiosis of the gastrointestinal microbiota is associated with an increasing number of disease conditions, including cancer in humans and has just been described in 12 dogs with B-cell lymphoma. Indeed, chemotherapeutic agents have clearly been shown to directly affect the microbiota, as do antibiotics, which are often prescribed during chemotherapy. However, treatment with probiotics may stabilise and support the functions of the microbiota during dysbiosis.

A randomised, double-blinded, placebo-controlled, crossover trial was performed to investigate the effect of treatment for 14 days with the probiotic bacterium *Enterococcus faecium* NCIMB 10415 (2×10^9 CFU) on the microbiota of dogs with sarcoma (n = 22) receiving chemotherapy with doxorubicin. Faecal samples were collected before administration of doxorubicin and during and after treatment with the probiotic or the placebo. Faecal DNA was extracted and submitted for Illumina sequencing, followed by analysis of the microbiota, using the QIIME platform. In addition, 12 healthy dogs were fed the probiotic preparation for 14 days, faecal samples were collected on the same days as for the trial dogs and processed identically. A quantitative

polymerase chain reaction was developed to detect and quantify the probiotic bacterium in the faeces.

Analysis of the faecal microbial communities revealed that dogs with sarcoma had severe dysbiosis, characterised by a significant reduction in the number of observed species ($p < 0.001$) and the species richness and diversity (Chao1 $p < 0.001$; Shannon index $p < 0.001$), by comparison with healthy dogs. Treatment with the probiotic preparation did not impact the species richness and diversity of the faecal microbial communities of these dogs and there was a significant increase in the amount of *Enterobacteriaceae* ($p < 0.05$) in the faecal microbial communities over the duration of the clinical trial. The probiotic bacteria were significantly increased in the faeces whilst both the healthy and sick dogs took the probiotic formulation ($p < 0.01$), but were only detectable in small amounts in some dogs ($n = 11$), seven days after the end of the treatment. Concurrent antibiotic therapy did not affect the dysbiosis present in the faeces of dogs with sarcoma (mixed model analysis $p > 0.05$).

Dogs with sarcoma also have dysbiosis which is not affected by treatment with the probiotic bacterium *E. faecium* NCIMB 10415. Clinicians should be aware that chemotherapy and antibiotic therapy may lead to an increase in *Enterobacteriaceae* in the faeces of their immunosuppressed patients

GI19

Linearity, Precision, and Reproducibility of the VetScan cPL Rapid Test

Joerg M. Steiner[1], *Phillip Guadiano*[2], *Robynne R. Gomez*[3], *Jan S. Suchodolski*[4], *Jonathan A. Lidbury*[1]
[1]*Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA,* [2]*GI Lab, College Station, TX, USA,* [3]*Texas A&M University, College Station, TX, USA,* [4]*Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA*

Measurement of serum concentrations of pancreatic lipase immunoreactivity (PLI) has become the standard for the diagnosis of pancreatitis in both dogs and cats. Recently, a new patient-side test for measurement of canine PLI (cPLI), the VetScan cPL rapid test for use with the VetScan VUE analyzer, has become available. Marketing material as well as the analyzer read-out suggest that this new assay provides accurate serum cPLI concentrations within a margin of ±65 µg/L compared to the Spec cPL® assay, but analytical or clinical validation data for this assay have not been published. The goal of this study was to evaluate linearity, precision, and reproducibility of the VetScan cPL rapid test.

Leftover serum samples from clinical submissions to the Gastrointestinal Laboratory at Texas A&M University were used for this study. For assessment of linearity, 7 good quality (i.e., not lipemic, hemolyzed, or icteric) serum samples throughout the working range of the cPLI assay as measured by Spec cPL® (Idexx Laboratories, Portland, Maine) were analyzed using the VetScan cPL rapid test assay (Abaxis, Union City, CA) undiluted and diluted at 1:2, 1:4, and 1:8, and observed/expected ratios were calculated. Intra-assay variability was assessed with 3 good quality serum samples analyzed 10 times on 4 different VUE analyzers at the same time. For assessment of inter-assay variability, 10 good quality serum samples were analyzed on 8 different days on the same machine (7 samples were analyzed on a single machine, while 2 samples were analyzed on 4 machines each).

Observed to expected ratios for dilutional parallelism ranged from 77.4 to 162.9%, with a mean of 119.3%. Intra-assay variability ranged from 16.9 to 36.7% (mean %CV for all samples and machines: 25.1%). Inter-assay variability ranged from 14.1% to 51.2% (mean %CV for all samples and machines: 31.8%) and most samples resulted in two (7 samples) or even three (5 samples) diagnostic bins (i.e., within the reference interval; gray range; suggestive of pancreatitis) during the experiment.

The new VetScan cPL rapid test for the VetScan VUE shows poor linearity. This is of clinical significance as the working range of this assay is very narrow, making dilution necessary for samples with higher cPLI concentrations. Also, the assay is not very precise (mean %CV: 25.1%). Finally, inter-assay variability is unacceptably high (mean % CV: 31.8%), affecting the actual diagnostic bin in many cases. In conclusion, the VetScan cPL rapid test for the VetScan VUE lacked linearity, precision, and reproducibility.

GI20

Effects of Malnutrition on the Mortality Rate in Dogs with Protein-losing Enteropathy

Aarti Kathrani, *Edward Hall*
University of Bristol, Bristol, England, United Kingdom

Malnutrition is associated with increased mortality in humans with inflammatory bowel disease. Therefore, the aims of our study were to determine if historical, clinical, and laboratory parameters of malnutrition at the time of diagnosis in dogs with protein-losing enteropathy (PLE) due to chronic enteropathy (CE) or lymphangiectasia affect mortality following treatment failure.

The medical records between 2010 and 2017 were retrospectively searched for canine cases of PLE, diagnosed with CE or lymphangiectasia on histopathology of intestinal biopsies. Neoplastic causes, including alimentary lymphoma, were excluded. For each case, historical, clinical, and laboratory parameters at the time of diagnosis was recorded and follow-up obtained by telephone contact with the referring veterinary practice. Dogs with a body condition score (BCS) below 4/9 were categorized as under-conditioned, dogs with a BCS of 5/9 as ideal-conditioned, and dogs with a BCS of above 5/9 as over-conditioned. Dogs with a BCS of 4/9 were placed in the under-conditioned or ideal-conditioned group based on the clinician';s written assessment.

Seventy-one dogs were identified; 47 (66 %) were under-conditioned (median: 3; range: 1 to 4), 18 (25 %) ideal-conditioned (median: 4; range: 4 to 5) and 6 (8 %), over-conditioned (median: 6 to 6 - 7; range: 5 - 6 to 8). Sixty-one dogs (88 %) had documented weight loss at the time of diagnosis; 30 (43 %) had severe (≥10 %), 22 (32 %) had moderate (5 - 9.9 %), and 9 (13 %) had mild (0.1-4.9%) loss. Two dogs were lost to follow-up and 29 out of 69 dogs (42 %) died or were euthanized following treatment failure. There were no significant effects of body condition score, percentage weight loss, body weight, appetite, serum albumin, cholesterol, cobalamin, and folate concentrations, canine chronic enteropathy activity index, and duration of signs on

mortality (P>0.05). However, dogs that had higher caloric intake at diagnosis had increased number of days to death or euthanasia following treatment failure (P = 0.002, correlation coefficient = 0.571).

Parameters associated with malnutrition at the time of diagnosis of PLE due to CE or lymphangiectasia in dogs in this study were not a predictor of mortality following treatment failure and therefore cannot be used to predict response to treatment. However, alternative measures of malnutrition may be required to definitively investigate the role of malnutrition in the prognosis of canine PLE.

GI21

Usefulness of Blood Urea Nitrogen/Creatinine Ratio (BUN/Cr) in Confirming and Localizing Gastrointestinal Bleeding in Dogs

Jenny Stiller[1], Alice Defarges[2], Brigitte Brisson[2], Alexa Bersenas[2], Brittany Lang[2], Jill Pomrantz[3]
[1]Ontario Veterinary College - University of Guelph, Guelph, ON, Canada, [2]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [3]Infiniti Medical, LLC, Redwood City, CA, USA

This study aimed to assess the usefulness of BUN/Cr to confirm occult gastrointestinal (GI) hemorrhage and to localize lesions along the GI tract.

Medical records from 230 dogs were retrospectively reviewed and BUN/Cr recorded. Patients presenting with visible GI hemorrhage were classified as overt GI bleeders (n = 60). Dogs presenting without visible GI hemorrhage were classified as occult GI bleeders (n = 60) if signs of GI hemorrhage developed while hospitalized and/or if bleeding was confirmed via capsule endoscopy, laparotomy, or necropsy. Healthy blood donors acted as controls (n = 60). GI lesions were classified as upper (n = 45), lower (n = 15) or upper and lower (n = 6) when the location of bleeding was identified. Median values were compared among groups using Kruskal-Wallis tests. Receiver operating characteristic (ROC) curves were used to identify parameter cut-points and their respective sensitivity and specificity. Significance was set at $P < 0.05$.

BUN/Cr was significantly higher in dogs with overt and occult GI bleeding compared to healthy dogs. BUN/Cr of overt GI bleeders was significantly higher than that of occult GI bleeders. There was no significant difference in BUN/Cr between dogs with lesions in the upper, lower and in both parts of the GI tract. Cut-off values for BUN/Cr of 18.2 had 51.7% sensitivity and 78.3% specificity to distinguish between occult GI bleeders and healthy dogs while a cut-off > 24.8 had 100% specificity.

Measuring BUN/Cr may be useful to predict occult GI bleeding in dogs.

GI22

Increased Fecal Fatty Acid Concentrations in Dogs with Chronic Enteropathy Normalize with Treatment

Julia B. Honneffer[1], Linda Toresson[2], Blake Guard[3], Sara Wennogle[4], Albert E. Jergens[5], Jonathan A. Lidbury[6], Joerg M. Steiner[6], Jan S. Suchodolski[7]
[1]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, Corvallis, OR, USA, [2]Evidensia Specialist Animal Hospital, Helsingborg, Sweden/Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, Helsinki University, Helsinki, Finland, Helsingborg, Skane Lan, Sweden, [3]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [4]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [5]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, Ames, IA, USA, [6]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [7]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

The clinical signs of canine chronic enteropathy (CE) indicate gastrointestinal dysfunction, including malabsorption. This results in abnormal composition of material within the gastrointestinal tract, which may perpetuate or exacerbate the clinical signs. Compared to healthy dogs, altered concentrations of fecal fatty acids (FFA) in dogs with various acute and chronic gastrointestinal diseases have been reported previously. This study aimed to identify a subset of dogs with CE characterized by increased FFA concentrations and assess those concentrations after one month of treatment.

Fecal samples were collected from dogs with active signs of chronic enteropathy (CE, n = 57) and from healthy dogs (n = 28), and were analyzed by gas chromatography/mass spectrometry (GC/MS) using an in-house assay. The healthy reference interval was calculated as the mean ± 0.96 SD. Samples taken approximately one month after baseline from the subset of dogs with increased FFA concentrations (n = 25) were also analyzed. A Mann-Whitney U test or Wilcoxon signed rank were used to compare groups, with statistical significance set at $p < 0.05$.

Total FFA concentrations at baseline were not significantly increased in dogs with CE relative to healthy dogs (p = 0.099). The healthy canine FFA reference interval was found to be 12-26 mg/mg, and 44% (25/57) of dogs with CE had increased FFA (median ± SD, [min-max]: 66 ± 37 mg/mg, [28-160], $p < 0.001$). The FFA concentrations for these dogs after one month of treatment were significantly decreased (19 ± 35 mg/mg, [4-165], $p < 0.001$) and were no longer statistically different from the healthy dogs (p = 0.64), with 60% (15/25) no longer exceeding the healthy dog reference interval.

This study identified increased FFA as a subtype of CE and demonstrated normalization of FFA concentrations after one month of treatment. Identifying patients with increased FFA concentrations and treating those patients with a highly digestible, moderate to low fat diet may improve response to therapy in dogs with CE. However, further studies are required to correlate treatment with clinical outcome.

GI23

A Retrospective Study of Granulomatous Gastritis in Miniature Dachshunds: 11 Cases

Hirotaka Igarashi[1], Koichi Ohno[2], Masaya Tsuboi[3], Kenjiro Fukushima[2], Nozomu Yokoyama[2], Ryohei Nishimura[2], Kazuyuki Uchida[2], Hajime Tsujimoto[2]
[1]Azabu University, 1-17-71, Fuchinobe, Sagamihara, Kanagawa, Japan, [2]The University of Tokyo, Tokyo, Tokyo, Japan, [3]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Tokyo, Japan

Granulomatous gastroenteritis is a rare disease in dogs, and it usually occurs in association with an infectious disease or a reaction to endogenous substances and foreign objects. Recently, a study reported that Miniature Dachshunds (MDs) were predisposed to

developing suture-associated granuloma and were likely to develop granulomatous or steroid-responsive gastroduodenitis within a few months after surgical resection. However, little information has been reported on this disease in MDs. Therefore, the purpose of this study was to describe the clinical characteristics of granulomatous gastroenteritis in MDs.

Medical records of dogs histopathologically diagnosed as having granulomatous gastritis, enteritis, and/or colitis at the Veterinary Medical Center of the University of Tokyo, between January 2007 and July 2015, were retrospectively reviewed. Information on the signalment, clinical signs, clinicopathological data, treatment protocol, response to treatment, and prognosis were collected.

Twenty dogs, comprising 11 MDs, 2 French Bulldogs, a Boston Terrier, a Miniature Schnauzer, a Toy Poodle, a Pembroke Welsh Corgi, a Pomeranian, a Shi-Tzu, and a mixed breed, were included in the study. All eleven MDs had granulomatous lesion at the pylorus and/or duodenum. In other breed dogs, 2 dogs had idiopathic granuloma at the pylorus and/or duodenum, 4 dogs had idiopathic granulomatous lesion at jejunum, ileum, and/or colon, and remaining 3 dogs were diagnosed as granulomatous colitis (i.e. histiocytic ulcerative colitis). Therefore, further analysis on the granulomatous gastritis in 11 MDs was performed.

Median age was 75 months (range, 20–118 months); there were 6 male (3 castrated) and 5 female (all spayed) dogs. Six dogs had a history of suture-associated granuloma, which were surgically resected 1–25 months prior to their inclusion in the study. Fever was commonly observed (median 39.0°C, range 37.5–40.1°C), and elevated serum C-reactive protein (>1.0 mg/dl) was observed in 9 dogs (median 10 mg/dl, range 0.1–20 mg/dl). On histopathologic examination, no foreign material was detected in all cases. Four dogs initially underwent pyloroplasty or gastroduodenostomy to resect the granuloma. Subsequently, they received immunosuppressive therapy within 2 months after the surgery due to recurrence of the lesion (n = 2) or based on the veterinarians'; decision (n = 2); the other 7 dogs were initially treated with immunosuppressive drugs. As a result, all dogs received immunosuppressive therapy with prednisolone (1–3 mg/kg/day) and/or cyclosporine (5–6 mg/kg/day). After the initiation of immunosuppression, clinical signs were resolved and serum C-reactive protein concentration decreased to the reference range in all dogs within 2 months. Nine of the 11 dogs showed recurrence of the lesion when prednisolone was tapered (median duration from the initiation of immunosuppression: 55 days, range: 14–394 days); they responded well to re-induction of immunosuppression with higher-dose prednisolone (2–4 mg/kg/day) or with addition of cyclosporine or azathioprine. Median follow-up period was 462 days (range, 28–2,343 days), and 3 dogs died at 40, 322, and 1,450 days after initial presentation. The causes of death were aspiration pneumonia (n = 2) and hepatic tumor (n = 1).

In conclusion, immunosuppressive therapy was effective in the management of granulomatous gastritis in MDs, and prevention of vomiting (i.e. aspiration pneumonia) may be important for ensuring a good prognosis. As described in a previous study, granulomatous gastritis was likely to occur in MDs, and a history of suture-associated granuloma seemed to be a risk factor. However, there were several cases of dogs that had never undergone surgery. Future studies on the innate immune systems of dogs in these cases are warranted.

GI24

Distribution of Regulatory T Cells in Inflammatory Colorectal Polyps in Miniature Dachshunds

Hirotaka Igarashi[1], Keisuke Konishi[2], Eri Uchida[3], Takashi Tamamoto[2], Koichi Ohno[3], Kazuyuki Uchida[3], Daiji Endoh[2]
[1]Azabu University, 1-17-71, Fuchinobe, Sagamihara, Kanagawa, Japan, [2]Rakuno Gakuen University, Ebetsu, Hokkaido, Japan, [3]The University of Tokyo, Tokyo, Tokyo, Japan

Inflammatory colorectal polyp (ICRP) is an emerging disease in Miniature Dachshunds (MDs). MDs with ICRP exhibit multiple polyps with severe neutrophil infiltration that respond to immunosuppressive therapy. Macrophages in polypoid lesions have been described to play an important role in neutrophil infiltration in the lesion by producing interleukin (IL)-8. On the contrary, IL-10, an anti-inflammatory cytokine, has also been reported to be upregulated in polypoid lesions, but its significance in the pathogenesis of ICRP has not been clarified. Mucosal regulatory T cell (Treg) is one of the cellular sources of IL-10 and has been described to maintain gut health by regulating mucosal inflammation in humans and dogs. Therefore, the objective of this study was to compare the distribution of Tregs in polypoid lesions of ICRP with that in non-inflamed colonic mucosa.

This study comprised 28 MDs diagnosed with ICRP and 21 dogs as control subjects (control dogs). Tissue biopsy specimens of polypoid lesions obtained from the 28 ICRP-affected MDs were collected. Tissue samples of macroscopically non-inflamed colonic mucosa from 24 ICRP-affected MDs and 21 control dogs were further included as controls. Of these, samples obtained from 24 ICRP-affected MDs and all 21 control dogs were used in a previous study (Igarashi et al., 2014). Real-time quantitative polymerase chain reaction was used to quantify gene expression of IL-1β, IL-4, IL-6, IL-8, IL-10, IL-17, IL-22, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, transforming growth factor-β, and forkhead boxprotein P3 (Foxp3) in each tissue sample. Gene expression data of IL-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α presented in our previous study (Igarashi et al., 2014) were included in this analysis. The numbers of Foxp3 positive cells (Tregs) and ionized calcium binding adapter molecule 1 (Iba1) positive cells (macrophages) were determined using immunohistochemistry.

The expression levels of all tested genes, with the exception of IL-4, were significantly up-regulated in the polypoid lesion. When the rate of increase in the expression of each gene was compared, the rates of increase of IL-1β, IL-6, IL-8, and IL-17 were significantly higher than that of IL-10, whereas the rates of increase of IFN-γ and TNF-α were significantly lower than that of IL-10. The number of Tregs in the polypoid lesion was significantly increased compared to that in the non-inflamed colonic mucosa of ICRP-affected MDs and control dogs. The number of macrophages was not significantly different between the groups. There was a significant positive correlation between the expression level of IL-10 gene and that of Foxp3 gene ($r = 0.749$, $P < 0.001$). In addition, a significant positive correlation was observed between the expression level of IL-10 gene and the number of Tregs ($r = 0.527$, $P < 0.01$).

In summary, the upregulation of IL-10 was significantly correlated with the distribution of Tregs in polypoid lesions of ICRP-affected MDs. However, the rate of increase in the expression level of IL-10 was relatively low compared to that of proinflammatory and Th17 cytokines. Therefore, the increase in numbers of Tregs and anti-inflammatory cytokines in polypoid lesions was considered to be a reactive change and was insufficient to regulate the development of inflammation. Further analysis on the association between Treg distribution in the lesion and the prognosis, including the response to immunosuppressive therapy, are warranted.No

GI25

Feeding a High Fiber Diet for Management of Acute Large Bowel Diarrhea in Shelter Dogs - Sponsored By Purina

Michael R. Lappin[1], Claire Hovenga[1], Amy Zug[1], Patrick Khalife[1], Lisa Kaminski[2], Jason Gagne[3]
[1]Colorado State University, Fort Collins, CO, USA, [2]Weld County Humane Society, Greeley, CO, USA, [3]Nestle Purina, St. Loius, MO, USA

Clinical signs of large bowel diarrhea are common in dogs housed in animal shelters and is often due to parasitism and stress. Use of diets with increased levels of dietary fiber is thought to be beneficial in the management of these cases. The purpose of this study was to determine whether feeding a commercially available veterinary diet Purina® Pro Plan® Veterinary Diets EN Gastroenteric Fiber Balance® would be associated with improved outcomes when compared to a diet with typical fiber levels (Purina® Pro Plan® Savor® Adult Shredded Blend Chicken & Rice Formula).

The affected dogs were housed in a single shelter and the design was approved by the shelter and by the Clinical Review Board. A fecal score was determined by 1 of 4 trained observers that was masked to the diet groups on each sample by comparing to a standardized score sheet: 0 = no stool; 1 = very hard and dry; 2 = firm but not hard; 3 = normal, little or no segmentation, moist; 4 = very moist, log shaped; 5 = very moist, piles; 6 = texture but no defined shape; and 7 = watery puddle. To qualify for the study, each dog had to present with a fecal score > 4 and have hematochezia, mucus, or straining. The dogs were randomized to be fed 1 of the 2 diets and all were administered fenbendazole at 50 mg/kg, PO, daily for 5 days and metronidazole at 10 - 15 mg/kg, PO, twice daily for 5 days. A dog had to be in the study for at least 4 days to be included in the data analysis. The proportions of dogs with a fecal score of less than 5 on the day of adoption or the last study day (Day 9) and the proportions of stools in each group with a fecal score of greater than 3 were compared by Fisher's exact test with significance defined as $P < 0.05$.

A total of 52 dogs were entered into this pilot study, with 22 dogs (11 per diet) completing the protocol to date. All of the dogs fed the high fiber diet had a fecal score less than 5 on the last day of the study which was statistically different than dogs fed the control diet (6 of 11 dogs; $P < 0.035$). The proportions of stools with a fecal score > 3 was significantly greater ($P = 0.0001$) in the dogs fed the control diet (39 of 48 stools; 81.3%) compared to the high fiber diet (18 of 58 stools; 31.0%).

The results suggest a therapeutic effect induced by the Fiber Balance® food and support the feeding of this diet to dogs with acute large bowel diarrhea.

GI26

Metabolomic Markers in Fecal Samples from Cats with Chronic Enteropathy

Sina Marsilio[1], Betty Chow[2], Steve L. Hill[2], Jonathan A. Lidbury[3], Joerg M. Steiner[3], Jan S. Suchodolski[4]
[1]Gastrointestinal Laboratory, College Station, TX, USA, [2]Veterinary Specialty Hospital, San Diego, CA, USA, [3]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [4]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

Chronic enteropathy (CE) is common in cats, especially in the senior cat population. The metabolome is the complete set of small-molecule compounds found in a biological sample. Changes in the metabolome have been documented in different species and for various conditions. The metabolome holds potential for the discovery of disease biomarkers as well as therapeutic targets. The aim of this study was to perform an untargeted analysis of the fecal metabolome of cats with different forms of chronic enteropathy.

The fecal metabolome was characterized in 30 cats with CE using Ultrahigh Performance Liquid Chromatography - Tandem Mass Spectroscopy (UPLC-MS/MS). Prior to analysis, all cats underwent a comprehensive work up including histopathology, immunohistochemistry, and clonality testing (PCR for antigen receptor rearrangements) of endoscopically-derived biopsies to determine their phenotype. Fifteen healthy cats of similar age served as a control group. Differences between healthy cats and cats with CE were evaluated using Welch';s two-sample t-test adjusted using the False Discovery Rate and expressed as q-values. Statistical significance was set at $q < 0.05$. Differences between cats with idiopathic inflammatory bowel disease (IBD) and small cell lymphoma (SCL) were evaluated using the Mann-Whitney U test.

Based on histopathology, immunohistochemistry, and clonality testing a diagnosis of IBD was reached in 7 cats and intestinal small cell lymphoma (SCL) in 11 cats. In 12 cats, histopathology was consistent with inflammatory lesions, while clonality testing of intestinal biopsies revealed clonal rearrangements. UPLC-MS/MS identified 865 named biochemicals of which 85 showed a decreased concentration, while 235 were found to be increased in fecal samples from cats with CE compared to healthy controls. Metabolic pathways found to be significantly altered in cats with CE included: tryptophan metabolism, glycolysis, polyunsaturated fatty acid metabolism, and secondary bile acid metabolism. Random Forest analysis revealed a predictive accuracy of 80% for differentiating controls from cats with CE. Arachidonic acid showed a trend towards differentiation of cats with IBD from those with SCL ($p = 0.0853$). Principal Component Analysis showed separate clustering of healthy cats and cats with CE. However, no separate clusters were visible between different types of CE.

Our results revealed disruptions in several major metabolic pathways and pathways associated with inflammation. Further studies are warranted to determine whether the identified compounds hold

discriminatory power for the differentiation of IBD from SCL or may serve as therapeutic targets for cats with CE

GI27

Evaluation of Duodenal Perfusion by Contrast-Enhanced Ultrasonography in Dogs with Chronic Enteropathy and Intestinal Lymphoma

Khoirun Nisa[1], Sue Yee Lim[1], Masayoshi Shinohara[1], Tatsuyuki Osuga[1], Nozomu Yokoyama[1], Noboru Sasaki[1], Keitaro Morishita[1], Kensuke Nakamura[2], Hiroshi Ohta[1], Mitsuyoshi Takiguchi[1]
[1]Department of Veterinary Clinical Sciences, Hokkaido University, Sapporo, Hokkaido, Japan, [2]Organization for Promotion of Tenure Track, University of Miyazaki, Miyazaki, Miyazaki, Japan

Contrast-enhanced ultrasonography (CEUS) was reported to be feasible in evaluating intestinal perfusion of healthy dogs. It has been considered to support diagnosis and monitoring human patients with inflammatory bowel disease, thus it is also expected to be as valuable for dogs with chronic intestinal diseases. A prospective study was conducted to examine the duodenal perfusion of dogs with chronic enteropathy (CE, n = 26) and intestinal lymphoma (n = 7) in comparison to control involving dogs with histopathologically normal duodenum (n = 14). Dogs in CE group were further classified based on canine chronic enteropathy clinical activity index (CCECAI) at CEUS into remission (CCECAI 0-3, n = 16) and symptomatic (CCECAI > 3, n = 10). The duodenum was imaged after an intravenous bolus injection of the contrast agent (Sonazoid®, 0.01 ml/kg). Perfusion parameters derived from quantitative analysis of CEUS images including the time-to-peak (TTP), peak intensity (PI), area under the curve (AUC), wash-in and wash-out rates (WiR and WoR, respectively) were evaluated. PI was significantly higher in symptomatic CE (median(range): 105.4 (89.3-128.8)) mean pixel value (MPV) compared to control (89.9 (68.5-112.2) MPV). AUC was significantly higher in symptomatic CE (4847.9 (3824.3-8462.8) MPV.sec) when compared to control (3448.9 (1559.5-4736.9) MPV.sec) and remission CE (3862.3 (2094.5-6899.0) MPV.sec). Positive correlation was detected between PI and CCECAI in CE dogs. No significant difference in any perfusion parameter was detected between intestinal lymphoma and CE as well as control groups. In conclusion, PI and AUC could detect a change of duodenal perfusion in associated with CE that make these parameters provide useful information for diagnosis and monitoring CE dogs.

GI28

Dysbiosis in Dogs with Acute Diarrhea Treated with a Fecal Microbial Transplantation or an Antibiotic

Jennifer Chaitman[1], Yasushi Minamoto[2], Jonathan A. Lidbury[3], Joerg M. Steiner[3], Jan S. Suchodolski[4]
[1]Animal Emergency and Specialty, Duvall, WA, USA, [2]Animal Emergency and Specialty, College Station, TX, USA, [3]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [4]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA

Dysbiosis plays a role in the pathogenesis of gastrointestinal (GI) diseases in humans and animals. Medical interventions have a potential to alter the GI microbiota and fecal microbial transplantation (FMT) has been utilized to treat human patients with GI diseases. The efficacy of FMT has been demonstrated in several studies involving human patients with GI disease. Also, potential adverse effects of the use of antibiotics on the GI microbiota have been reported in recent studies in humans and dogs. The aim of this study was to evaluate the effects of FMT or antibiotic therapy on GI microbial communities in dogs with acute diarrhea.

A total of 17 fecal samples from dogs with acute diarrhea treated with either FMT (2.5 to 5 g of donor stool/kg diluted in 60 cc saline via enema; n = 10) or metronidazole (MTZ at 15mg/kg PO BID for 7 days; n = 7) were collected before (T0), after 1 week (T2), and after 4 weeks (T3) of each intervention. Fecal DNA was evaluated for quantitative PCR (qPCR) analysis for selected bacterial groups (total bacteria, *Faecalibacterium* spp., *Turicibacter* spp., *Streptococcus* spp., *E. coli*, *Blautia* spp., *Fusobacterium* spp., and *C. hiranonis*) and results were used to calculate the Dysbiosis Index (DI). Feces were evaluated using a fecal scoring system (Nestle PURINA fecal scoring system). DI and the fecal score were compared between both groups using a Wilcoxon rank-sum test. The presence of dysbiosis (DI > 0) was compared between both groups using a fisher's exact test. A $p < 0.05$ was considered to be statistically significant.

The fecal score did not differ significantly between the 2 groups at either T0 or at T2 ($p = 0.200$ and $p = 0.116$, respectively). At T3, the FMT group had a significantly lower fecal score than the MTZ group ($p = 0.020$). DI did not differ significantly at T0 between the two groups ($p = 0.961$). At T2 and T3, the FMT group had a significantly lower DI than the MTZ groups ($p = 0.001$ and $p = 0.002$, respectively). Recovery from dysbiosis was also evaluated, and the FMT group had a significantly lower rate of dysbiosis (30% [3/10]) than the MTZ group (100% [7/7], $p = 0.009$) at T2. Likewise, the FMT group had a significantly lower rate of dysbiosis (20% [2/10]) than the MTZ group (85% [6/7], $p = 0.015$) at T3.

In this study, dogs with acute diarrhea treated with FMT showed a significantly lower fecal score and dysbiosis index than dogs treated with metronidazole after 4 weeks of treatment. Further studies are required to determine whether this difference is clinically relevant.

GI29

Serum Fatty Acid Binding Protein 2 and 6 Concentrations in Dogs with Chronic Enteropathy

Yasushi Minamoto[1], Tomomi Minamoto[2], Robert Kyle. Phillips[2], Jonathan A. Lidbury[3], Joerg M. Steiner[3], Jan S. Suchodolski[2]
[1]Animal Emergency and Specialty, Duvall, WA, USA, [2]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [3]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA

Fatty acid-binding protein (FABP) is a 14 to 15 kDa cytosolic protein that plays a role in intracellular lipid transport. FABP isoforms 2 and 6 are found in the epithelium of the gastrointestinal tract and are released in response to increased intestinal permeability and/or enterocyte damage. Studies in humans and rodent models have shown increased serum FABP concentrations in several gastrointestinal disorders. The aim of this study was to evaluate serum concentrations of FABP 2 and 6 in healthy pet dogs and dogs with chronic enteropathy.

Serum samples were collected from 13 dogs with chronic enteropathy (CE) and 9 healthy control dogs. Serum concentrations of FABP 2 and 6 were measured using commercially available canine specific ELISA kits (canine FABP 2 and 6 ELISA kit, BlueGene Biotech, China). The serum FABP concentrations were compared between both groups using a Wilcoxon rank-sum test. Correlation of serum FABP concentrations and disease severity in dogs with CE (evaluated using the canine chronic enteropathy clinical activity index [CCECAI]) were evaluated using Spearman's ρ. A $p < 0.05$ was considered to be statistically significant.

Serum FABP 2 concentrations did not differ significantly between dogs with CE (median: 0.55 ng/ml; min-max: 0.04-9.41 ng/ml) and healthy control dogs (median: 0.80 ng/ml; min-max: 0.29-3.81 ng/ml; $p = 0.974$), serum FABP 6 concentrations did not differ significantly between dogs with CE (median: 0.92 ng/ml; min-max: 0.01-21.93 ng/ml) and healthy control dogs (median: 0.85 ng/ml; min-max: 0.21-21.68 ng/ml); $p = 0.815$). Within the CE group we did not observe any significant correlation between either serum FABP 2 (Spearman's $\rho = 0.037$, $p = 0.904$) or FABP 6 (Spearman's $\rho = 0.028$, $p = 0.926$) and CCECAI.

In this study, serum FABP 2 and 6 concentrations in dogs with chronic enteropathy were not significantly different compared to those in healthy control dogs. Also, serum concentrations of FABP 2 and 6 did not correlate with disease severity in dogs with CE.

GI30

Retrospective Study of 157 Cats with Pancreatitis Requiring Hospitalization: Clinicopathological Findings, Prognostic Markers and Outcome

Ran Nivy, Alina Kaplanov, Sharon Kuzi, Michal Mazaki-Tovi, Einat Yas, Gilad Segev, Jennifer Ben-Oz, Eran Lavy, Itamar Aroch
The Koret school of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, HaMerkaz, Israel

Feline pancreatitis (FP) is being increasingly diagnosed in recent years, but large-scale clinical studies are scarce. This retrospective study characterized a large cohort of 157 client-owned cats diagnosed with FP that required hospitalization. Inclusion criteria included ultrasonographic evidence of pancreatitis and/or positive SNAP fPLI test and/or increased DGGR-lipase activity and/or histopathological diagnoses, vis-à-vis compatible clinical signs.

The death rate was 22% (35 cats,) including death (16 cats) and euthanasia owing to clinical deterioration (19 cats) during hospitalization. The median time from onset of clinical signs until presentation to the hospital was longer ($P = 0.003$) in the non-survivors vs. the survivors. Etiologies of FP, identified in 13.4% of the cats, included recent general anesthesia, trauma, hemodynamic compromise and organophosphate intoxication. Weight loss ($P = 0.034$) and depression at presentation ($P = 0.003$) were more frequent in the non-survivors, while fever was more frequent ($P = 0.042$) in the survivors. Occurrence of pleural effusion ($P = 0.003$) and acute kidney injury ($P = 0.045$) was associated with death. At presentation, median serum glucose ($P = 0.022$) and total CO_2 ($P = 0.027$) concentrations were lower, while median serum creatinine concentration ($P = 0.048$) was higher in the non-survivors vs. the survivors. Hypoglycemia and ionized hypocalcemia at presentation, parenteral nutrition administration and persistent anorexia during hospitalization were associated with death ($P < 0.017$ for all), while antibiotics administration ($P = 0.023$) was associated with survival. These unreported prognostic factors warrant future prospective evaluation to ascertain their validity.

GI31

Effect of Fat-Loading on Gastrointestinal Transit Time and Mucosal Appearance as assessed by Capsule Endoscopy

Jean-Sébastien Palerme[1], Auri Silverstone[2], Elizabeth Riedesel[2], Jill Pomrantz[3], Kristina Simone[4]
[1]Iowa State University College of Veterinary Medicine, Ames, IA, USA, [2]Iowa State University, Ames, IA, USA, [3]San Francisco SPCA, San Francisco, CA, USA, [4]Cypress Veterinary Imaging, LLC, Charleston, SC, USA

Though definitive diagnosis of lymphangectasia requires histopathologic assessment, visual assessment has been shown to be a sensitive indicator of disease. In an effort to increase the conspicuity of the characteristic dilated lacteals seen in lymphangectasia, many endoscopists recommend enteral administration of high fat meals (fat-loading) prior to endoscopy in spite of a lack of any controlled studies reporting the efficacy of this technique. Using capsule endoscopy (ALICAM®), we investigated the effect of high fat meals on gastrointestinal transit time and endoscopic as well as ultrasonographic appearance of the small intestines.

In this randomized prospective crossover study, four healthy dogs had baseline capsule endoscopies and abdominal ultrasounds performed. Thereafter, they were administered 2 different high fat meals (corn oil or dairy cream) at 2 different time points (1 and 2 hours) prior to undergoing capsule endoscopy and abdominal ultrasound. Outcomes assessed included gastrointestinal transit time and appearance of the intestinal mucosa, as assessed by capsule endoscopy. In addition, intestinal wall thickness and the presence of mucosal hyperechoic speckling were assessed in a blinded manner by ultrasound.

Fat-loading resulted in significantly longer gastric transit times when compared to controls ($p < 0.01$). Also, gastric retention of the capsules occurred significantly more commonly in dogs fed either corn oil or dairy cream ($p < 0.01$). While 10/16 fat-loading studies resulted in gastric retention of the capsule for more than 12 hours, none of the control studies had gastric retention. In addition, gastric retention occurred significantly more often with dogs fed corn oil than dairy cream ($p < 0.05$). Though gastric retention limited the number of cases where visual assessment of the small intestinal mucosa could be performed, 5/6 fat-loaded studies were subjectively judged to have moderate to marked prominence of villi with increased numbers of dilated lacteals when compared to control studies.

Small intestinal wall thickness did not differ significantly between control and fat-loaded dogs. However, mucosal speckling scores of the jejunum were significantly greater in dogs fed cream or oil than in control dogs ($p < 0.05$).

Though fat-loading might increase prominence of duodenal villi, gastric transit times were significantly prolonged resulting in the inability of capsule endoscopy to visually assess the small intestines in over half the cases in this study. Future studies investigating fat-loading techniques should include the use of traditional push endoscopy to overcome this limitation and allow consistent assessment of the intestinal mucosal appearance.

GI32

Immunolocalization of S100A12 and its association with Helicobacter spp colonization in canine gastric mucosa

Rafael R. Santisteban-Arenas[1], Joerg M. Steiner[2], Carlos A. Sanchez[3], Carlos Hernandez[4], Francisco Pedraza[3], Jan S. Suchodolski[5], Jonathan A. Lidbury[2], Sergio Linares[6]
[1]UNISARC, UD-Veterinary Medicine Hospital, Santa Rosa de Cabal, Colombia, Manizales, Caldas, Colombia, [2]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA, [3]Laboratorio de Patología Animal, Universidad de Caldas, Manizales, Manizales, Caldas, Colombia, [4]Facultad de Medicina Veterinaria y Zootecnia, Universidad CES, Medellín, Colombia, Envigado, Antioquia, Colombia, [5]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [6]Departamento de Salud Animal, Universidad de Caldas, Manizales, Manizales, Caldas, Colombia

S100A12 is a novel inflammatory marker that has been evaluated in both humans and animals. Increased concentrations of S100A12 in the gastric mucosa of humans with Helicobacter pylori infection have previously been reported. The gastric mucosa of dogs is frequently colonized by several Helicobacter species, however there is a poor association between Helicobacter organism density and mucosal inflammation. The purpose of this study was to evaluate gastric tissue localization of S100A12 by immunohistochemistry (IHC) in dogs and determine correlations between the density of Helicobacter organisms and S100A12 containing cells in the lamina propria of dogs with gastric disease and in healthy control dogs.

Localization of S100A12 positive cells and Helicobacter organisms was studied using paraffin-embedded gastric biopsies, 11 from dogs with chronic gastritis and 9 from healthy control dogs (5 of these were obtained post-mortem after the patient had presented to the emergency service at the Veterinary Teaching Hospital at Texas A&M University, none of which had gross or histopathological lesions suggesting gastrointestinal disease and 4 were research dogs from Universidad de Caldas). No dog had received antibiotic or corticosteroid treatment in the last two weeks prior to the collection of gastric biopsies. IHC was performed using antibodies directed against canine S100A12 (cS100A12) and Helicobacter spp., respectively. cS100A12 staining in the lamina propria was quantified and classified into absent, mild, moderate, or severe and Helicobacter density was graded using similar criteria. Histopathological evaluation was conducted following the World Small Animal Veterinary Association (WSAVA) grading scheme. Spearman';s rank correlation was used to evaluate any possible associations between cS100A12 with Helicobacter density and histopathology findings. A Mann-Whitney U test was used to compare ordinal variables between healthy and sick dogs.

cS100A12 staining was found predominantly in neutrophils and monocytes, and less frequently observed in fibroblasts and some epithelial cells. Infiltration of the lamina propria by cS100A12 containing cells was considered mild in 44% of healthy animals and absent in 56%, cS100A12 expression was correlated with lamina propria neutrophils (P= 0.04). In dogs with chronic gastritis, cS100A12 infiltration was considered mild in 33% and absent in 67%. Of all dogs 80% had mild to moderate mononuclear infiltration and 4 dogs had a normal gastric mucosa. Helicobacter organisms were identified in 80% of all dogs, in 14 dogs, gastric colonization was considered mild to moderate, only two dogs had severe colonization. Helicobacter distribution included superficial epithelium, lumen of gastric glands, and within parietal cells. No significant differences were found between healthy and sick dogs in Helicobacter organism density or cS100A12 staining.

Contrary to Helicobacter pylori infection in humans, colonization of the stomach with Helicobacter spp. was not associated with infiltration of cells expressing cS100A12. Also, expression of cS100A12 is limited to neutrophils and monocytes in the canine gastric mucosa and correlates with infiltrating neutrophil counts, the potential causes of which requires further study. IHC cS100A12 expression in dogs with chronic gastritis was not different from the mucosa of healthy dogs.

GI33

Increased Immunoglobulin Binding Fecal Bacteria: Role of Response to Dysbiosis in Dog Inflammatory Bowel Disease

Sirikul Soontararak[1], Valerie Johnson[1], Lyndah Chow[2], Jonathan Coy[1], William Wheat[1], Tracy Webb[3], Sara Wennogle[1], Craig Webb[1], Steven Dow[1]
[1]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA, [2]Department of Cell and Molecular Biology, Colorado State University, Fort Collins, CO, USA, [3]Department of Clinical Sciences Colorado State University, Fort Collins, CO, USA

Canine inflammatory bowel disease (IBD) results in chronic intestinal inflammation from immune dysregulation in the gut including abnormal immune responses against commensal intestinal flora. Increased Immunoglobulin (Ig)-binding to gut bacteria likely activates immune responses locally and systemically however it has never been investigated in dog IBD yet. Therefore, our study investigated whether the humoral immune response against commensal gut bacteria in IBD is greater than that of healthy dogs. Additionally, whether the Ig-binding bacteria potentially triggers an exacerbated immune response that aggravates gut homeostasis.

Fresh fecal material was collected from 20 IBD dogs and 9 clinical healthy dogs (mean age ± SD = 6.1 ± 3.9 and 6.6 ± 2.1 years respectively). All patients were diagnostic confirmed by endoscopic biopsy and had no history of receiving immunosuppressive medication. This work compares the amount of fecal bacteria binding to IgG and IgA in vivo between IBD and healthy canines by flow cytometric analysis. In addition, the extent of macrophage phagocytosis and macrophage activation by fecal bacteria derived from IBD vs. healthy dogs were investigated by both flow cytometric analysis and ELISA (TNF- α and IL-10). Comparison between groups was evaluated using t-test and statistical significant was set at $P < 0.05$ level.

We found that fecal bacteria from IBD dogs already bound both IgA and IgG subclasses of Ig at significantly higher levels and extensive than bacteria from normal dogs (IgA; P = 0.018, IgG; P = 0.0002). Interestingly, there was no correlation between % Ig-bound bacteria and the degree of scoring index (Canine IBD Activity Index; CIBDAI; 6.8 ± 3.73 (mean ± SD), r = 0.19 and Canine Chronic Enteropathy Clinical Activity Index; CCECAI; 8.2 ± 4.2, r = 0.27). Moreover, our results suggested that the IgG which bound to gut bacteria was mainly derived from local mucosal immune activity; not systemically; since

serum incubation with *E. coli* isolates was not different between IBD and healthy animals.

In addition, when compared to bacteria from normal control animals, IBD fecal bacteria significantly stimulated higher levels of phagocytosis in canine primary macrophage ($P = 0.02$) as well as in DH82 cells; canine macrophage tumor cell line ($P = 0.04$). The upregulation of surface activation markers (MHC II, CD40, CD86 and CD80) in response from both groups was comparable. Phagocytosis of IBD gut bacteria activated macrophages and stimulated higher production of TNF- α than normal gut bacteria ($P = 0.047$). In contrast, IL-10 production from primary macrophages incubated with IBD fecal bacteria was significantly decreased compared to normal flora from healthy dogs ($P = 0.045$).

Overall, the results showed that IBD dogs had higher Ig-bound fecal bacteria than normal healthy controls and potentially stimulates greater immune responses as determined by macrophage phagocytosis and activation. This suggested that the local humoral response against gut bacteria in IBD plays a crucial role in the pathogenesis resulting in chronically active inflammation scenario characteristic of IBD.

GI34

Altered Fecal Fatty Acid, Sterol, and Bile Acid Metabolism in Dogs with Acute Diarrhea

Amanda B. Blake[1], *Blake Guard*[2], *Julia B. Honneffer*[3], *Michelle Jonika*[3], *Bristin A. Rustenbeck*[3], *Jennifer Chaitman*[4], *Jonathan A. Lidbury*[5], *Joerg M. Steiner*[5], *Jan S. Suchodolski*[6]

[1]*Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, College Station, TX, USA,* [2]*Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA,* [3]*Texas A&M University Gastrointestinal Laboratory, College Station, TX, USA,* [4]*Veterinary Internal Medicine and Allergy Specialists, New York, NY, USA,* [5]*Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA,* [6]*Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA*

While many studies of canine gastrointestinal diseases have focused on changes of the fecal microbiota, few have examined functional changes by studying fecal metabolites. Some of these metabolites, such as fatty acids and primary bile acids, have been associated with diarrhea in humans. Therefore, the aim of this study was to quantify several metabolites and associated bacterial groups in feces of dogs with acute diarrhea.

Fecal samples were obtained from healthy dogs (n = 24) and dogs with acute diarrhea (n = 11). DNA was extracted for analysis of major bacterial groups by qPCR, and a targeted gas chromatography-mass spectrometry assay was used to quantify fatty acid, sterol, and bile acid concentrations in feces. A dysbiosis index was calculated based on the abundances of major bacterial taxa (DI; reference limit < 0 with values ≥ 0 indicating dysbiosis). Statistical analysis was performed using Mann Whitney tests with significance set at $p < 0.05$.

Fecal concentrations of stearic acid ($p = 0.018$), arachidonic acid ($p < 0.001$), and nervonic acid ($p = 0.037$) were increased in dogs with acute diarrhea compared to healthy dogs. Cholesterol ($p = 0.018$) concentration was increased, while coprostanol ($p = 0.047$), campesterol ($p = 0.002$), stigmasterol ($p = 0.029$), fucosterol ($p < 0.001$), β-sitosterol ($p < 0.001$), and sitostanol ($p < 0.001$) concentrations were all decreased in dogs with acute diarrhea. The ratio of secondary bile acids to total bile acids concentration was decreased in dogs with acute diarrhea ($p = 0.039$). The dysbiosis index was significantly increased in dogs with acute diarrhea ($p < 0.001$) compared to healthy dogs.

In conclusion, our results suggest that numerous metabolic changes occur concurrently with alterations of the microbiota in dogs with acute diarrhea. Fecal metabolite patterns in some dogs with acute diarrhea resemble those found in humans with bile acid or fatty acid diarrhea.

GI35

Characterization of Paneth-like Cells in the Canine Small Intestine

Karin Allenspach[1], *Jonathan P. Mochel*[2], *Dawn D. Kingsbury*[3], *Lawrance Chandra*[3], *Todd Atherly*[3], *Rachel Phillips*[4], *Jesse Hostetter*[5], *Michael Wannemuehler*[6], *N Matthew Ellinwood*[7], *Elizabeth Snella*[7], *Albert E. Jergens*[8]

[1]*Iowa State University, Veterinary Clinical Sciences, Ames, IA, USA,* [2]*Iowa State University College of Veterinary Medicine, Ames, IA, USA,* [3]*Iowa State university, Veterinary Clinical Sciences, Ames, IA, USA,* [4]*Iowa State university, Deprtment of Pathology, Ames, IA, USA,* [5]*Iowa State university, Department of Pathology, Ames, IA, USA,* [6]*Iowa State University, Department of Veterinary Microbiology and Population Health, Ames, IA, USA,* [7]*Iowa State university, Department of Animal Science, Ames, IA, USA,* [8]*Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, Ames, IA, USA*

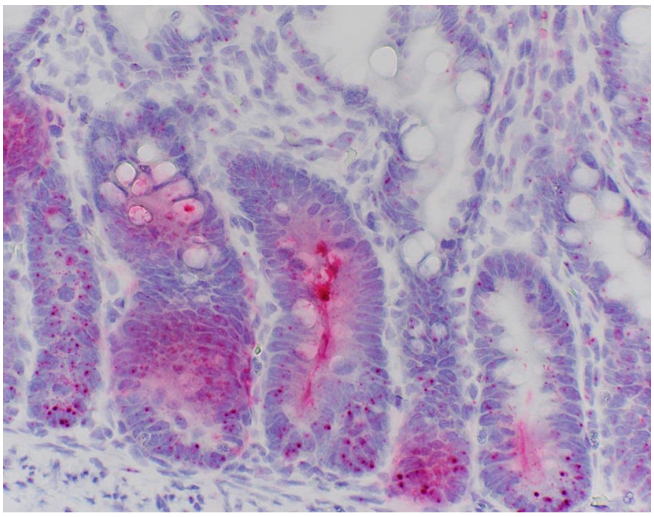
In humans, mice and pigs, Paneth cells locate in crypts close to intestinal stem cells (ISC), and contain large granules staining positively for lysozyme. These cells are involved in the production of growth factors necessary for ISC function, and produce antimicrobial peptides. Defects of Paneth cell function are involved in the pathogenesis of different intestinal diseases in humans, such as inflammatory bowel disease and colorectal cancer. In dogs and cats, the small intestine consistently stains negative for lysozyme, and cells that morphologically resemble Paneth cells have not been identified by light microscopy. We therefore sought to characterize cells that functionally represent Paneth cells in the dog small intestine, using full thickness biopsies as well as primary 3D cultures of epithelial cells (so-called enteroids).

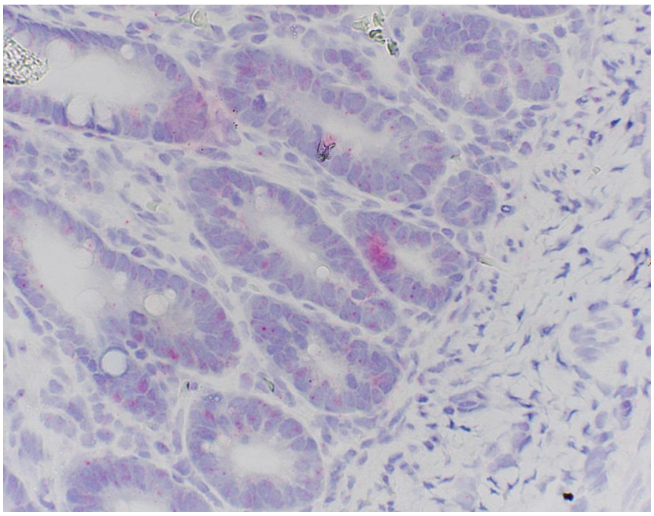
Ten-centimeter tissue pieces were acquired from the jejunum of healthy dogs which had been euthanized for an unrelated project. Full thickness tissues were fixed in 10% formalin saline, routinely processed and embedded in paraffin. For enteroid culture, minced samples were washed and crypts were enriched, using EDTA chelation, embedded in matrigel, and grown in intestinal stem cell media. RNA in situ hybridization (RNA ISH) and immunohistochemistry (IHC) was used to identify cells staining positive for 7 previously reported markers of Paneth cells for mouse and human: lysozyme (LYS), canine interleukin 17 (IL-17), canine beta defensin 103 (CBD 103), canine cathelicidin (CATH), frizzled class receptor 5 (FZD5), neurogenin 3 (NEUROG3) and ephrin receptor tyrosine kinase B2 (EPHB2). In addition, ISC markers were used to characterize the stem cell niche:

leucine rich repeat G protein coupled receptor-5 (LGR5), and SRY-related HMG Box Transcription Factor 9 (SOX9).

Full thickness sections of canine jejunum stained negative for LYS and IL-17 by IHC, and positive for CBD103, CATH, FZD5, EPHB2 and NEUROG3 by RNA ISH. Fully differentiated enteroids (day 6-9) stained negative for LYS, IL-17, CBD103 and CATH, and positive for FZD5, EPHB2 and NEUROG3. Cells expressing Paneth cell markers were highly positive in the crypts of both full thickness sections and enteroids, however, positive staining was also found throughout the differentiated cells of the villus tip, although with less intense staining. Markers for ISC (LGR5 and SOX9) were positive and clearly defined to 3-4 cells at the bottom of crypt areas in both full thickness sections and enteroids. These staining patterns differ from those of humans and mice, in that Paneth-like cells seem to be more dispersed throughout the epithelium in the canine small intestine as compared to localizing within the defined crypt in other mammals.

We have, for the first time, identified cells that functionally represent Paneth-like cells in the jejunum of dogs. These data lay the foundation for further investigation of the role of Paneth-like cells in canine intestinal diseases, such as inflammatory bowel disease and colorectal cancer.





GI36

Sequencing of Chromosome 11 Identifies SNPs Associated with Inflammatory Bowel Disease in German Shepherd Dogs

Atiyeh Peiravan[1], Mazdak Salavati[2], Albert E. Jergens[3], Elisabeth Mueller[4], Kenneth W. Simpson[5], Dirk Werling[6], Karin Allenspach[7]
[1]Iowa State University, Veterinary Clinical Sciences, Ames, IA, USA, [2]Pathobiology and Population Sciences, Hatfield, England, United Kingdom, [3]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, Ames, IA, USA, [4]Collaborator Royal Veterinary College, Bad Kissingen, Bayern, Germany, [5]Cornell University, Ithaca, NY, USA, [6]Pathobiology and Population Sciences Royal Veterinary College, Hatfield, England, United Kingdom, [7]Iowa State University, Veterinary Clinical Sciences, Ames, IA, USA

We have previously identified 10 candidate genes on Chromosome 11 Using Genome Wide Association Study that are potentially associated with Inflammatory Bowel Disease (IBD) in German Shepherd Dogs (GSD).

In the present study, we performed a targeted re-sequencing of the two Mb region on Ch11, including all 10 of the newly identified candidate genes, using a custom-designed sequence capture array (SelectSure XT custom 0.5-2.9 Mb, Agilent).

In brief, a hybridization DNA library was prepared using the selectSure XT Library Prep Kit (Agilent) following the manufacturer instructions. The targeted region on Ch11, 1Mb up- and downstream of the most significant SNPs, was captured using the custom-designed sequence capture array (SelectSure XT custom 0.5-2.9 Mb, Agilent). Subsequently, captured libraries were indexed and subjected to DNA sequencing on Illumina platform, followed by alignment/mapping to the canine genome (CanFam3.1). Haplotypecaller was used to call variants, followed by hard-filtering extracted SNPs and Indels as recommended in GATK's best practices. SNPs were annotated using the SNPEff and Variant Effect Predicting (VEP) tools. The variants were divided into different groups based on genomic coordinates. The SNPs present only in cases (diagnosed with IBD), controls as well as overlapping SNPs with different alternative alleles in case vs control groups were further investigated.

In the control group, 1 known missense SNP was identified in exon 1/10 of the *SLC22A5* gene, which has been identified to be associated with IBD using both approaches. This SNP was present in 7/20 control animals. In addition, one new missense SNP was detected in *IL-4* with moderate impact (1/20 controls showed the variant). Furthermore, 21 SNPs (1 known and 20 new ones) upstream and 46 SNPs (4 known and 42 new ones) downstream were identified only in the control group, however, the number of variants is 9 for one of them and 1 for the rest of the SNPs. In the overlap group with different alternative alleles, no missense SNPs, SNPs in splice regions, or 1k up- and downstream of genes were found.

In the case only group, we found one missense SNP (moderate impact, deleterious based on SIFT score) and one in the splice region (low impact, with 1/28 cases sequenced). Seventeen SNPs (5 known and 12 new ones) were identified within 1kb upstream of gene TSS coordinates and 32 SNPs (7 known and 25 new ones) downstream. Two of these SNPs with modifier impact were located within 1kb upstream of *IL-3*, a hematopoetic cytokine driving the development of myeloid stem cells, which was previously identified to be associated with IBD in both approaches that we used (enrichment and list of genes

involved in human IBD). Two SNPs were found downstream of *PDLIM* (a protein with cysteine-rich double zinc fingers involved in protein-protein interaction and cytoskeletal organisation) and *IL-13* (involved in IgE synthesis, chitinase up-regulation and hyperresponsiveness of mucosal surfaces) and one new SNP was found downstream of *IL-4*. Interestingly, all these genes have also been associated with a higher risk of development of human IBD.

In summary, we have identified several SNPs in the genes for canine *IL-3*, *IL-4*, *IL-13 and PDLIM*, which, based on the known function of their corresponding proteins, further our insight into the pathogenesis of IBD in GSDs.

HM01

Prevalence of Naturally Occurring non-AB Red Blood Cell Alloantibodies in Cats

Megan E. McClosky[1], *Dorothy Cimino. Brown*[2], *Nicole Weinstein*[3], *Nicole Chappini*[3], *Michael Taney*[4], *Kym Marryott*[1], *Mary Beth Callan*[4]
[1]*University of Pennsylvania, Philadelphia, PA, USA,* [2]*Eli Lilly/Elanco Animal Health/University, Greenfield, IN, USA,* [3]*Department of Pathobiology, University of Pennsylvania, Philadelphia, PA, USA,* [4]*Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania, Philadelphia, PA, USA*

Recognition of the feline red blood cell (RBC) antigen *Mik* and the presence of naturally occurring anti-*Mik* antibodies resulting in acute hemolytic transfusion reactions prompted the recommendation to perform a blood crossmatch prior to a cat';s first RBC transfusion. The objectives of this retrospective study were: 1) to determine the prevalence of naturally occurring non-AB RBC alloantibodies detectable by tube crossmatch, and 2) to compare transfusion outcomes in feline patients with and without a tube crossmatch performed. In addition, a limited prospective study was undertaken to compare results of the gel and tube methods for feline crossmatch.

Medical records of 300 cats that received a RBC transfusion and/or major crossmatch between January 1, 2013 to December 31, 2016 were reviewed retrospectively. Information recorded included previous transfusion history, major and minor crossmatch results (tube method), volume of pRBCs administered, pre- and post-transfusion PCV, adverse events, and patient outcome. For 10 cats, a crossmatch was performed using both the gel and tube methods for comparison.

RBC compatibility data was available for 154 transfusion naïve cats, 23 (14.9%) of which showed some degree of incompatibility (1+ to 3+ hemagglutination) on the major crossmatch to 1 or more donors. Of 55 cats previously transfused, 15 cats (27%) were incompatible (1+ to 2+ hemagglutination) on the major crossmatch to 1 or more donors, significantly more than in the transfusion naïve group ($P = 0.042$). Packed RBC transfusions were administered to 249 cats, and a tube crossmatch was performed for 167 cats (67%) prior to their first pRBC transfusion; the remaining 82 cats received type-compatible, non-crossmatched pRBCs. The median volume of pRBCs administered during the first transfusion was 5.3 mL/kg (range, 2.4-18). The median increase in PCV post-transfusion was 5%; cats receiving crossmatch-compatible pRBCs did not have a greater increase compared to those without a crossmatch. Febrile transfusion reactions occurred more often in cats that received typed, non-crossmatched pRBCs (n = 8) than cats administered crossmatch-compatible pRBCs (n = 4) ($P = 0.022$). Of the 246 cats receiving pRBC transfusions, 188 (76.4%) survived to hospital discharge. A pre-transfusion crossmatch was not associated with improved survival to discharge or at 30 or 60 days post-transfusion.

A crossmatch was performed using both the tube and gel method for 10 cats (total of 31 crossmatches). For 9 recipient cats (24 crossmatches), there was agreement between results of the tube and gel methods, with the exception of 1 cat having a 2+ and 3+ major incompatibility to the same donor with the tube and gel methods, respectively, likely a result of the subjectivity of grading. Differing results observed for 1 cat, with a negative tube but 4+ mixed field agglutination gel result for autocontrol and major crossmatch tests with 3 donors, were attributed to marked rouleaux. Microscopic evaluation and saline replacement during the tube crossmatch supported an interpretation of rouleaux rather than hemagglutination.

RBC incompatibility noted in 15% of transfusion naïve cats suggests that the prevalence of naturally occurring non-AB RBC alloantibodies is sufficiently high to justify the recommendation to perform a blood crossmatch prior to all (including the first) RBC transfusions in cats.

HM02

The Use of High-Dose IgM-Enriched Human Immunoglobulin in Canine Immune-Mediated Hemolytic Anemia

Jason P. Bestwick[1], *Mellora J. Sharman*[1], *Nathaniel T. Whitley*[2], *Caroline Kisielewicz*[3], *Barbara J. Skelly*[4], *Simon Tappin*[5], *Lindsay Kellett-Gregory*[6], *Mayank Seth*[1]
[1]*Animal Health Trust, Newmarket, England, United Kingdom,* [2]*Davies Veterinary Specialists, Higham Gobion, England, United Kingdom,* [3]*Pride Veterinary Centre, Derby, England, United Kingdom,* [4]*The Queen's Veterinary School Small Animal Hospital/University of Cambridge, Cambridge, England, United Kingdom,* [5]*Dick White Referrals, Six Mile Bottom, England, United Kingdom,* [6]*The Royal Veterinary College, Hatfield, England, United Kingdom*

The use of human intravenous immunoglobulin (hIVIG) in cases of canine primary immune-mediated hemolytic anemia (IMHA) has been previously described, but investigations have been limited by cost and availability. The aim of this study was to investigate the administration of high-dose IgM-enriched hIVIG (Pentaglobin®) at the time of diagnosis of canine primary IMHA.

Dogs diagnosed with primary IMHA at UK specialty hospitals were prospectively enrolled. All dogs received prednisolone or dexamethasone alongside clopidogrel. Patients were randomized to receive Pentaglobin® at 1g/kg on up to two occasions, or a control group. No additional immunosuppressive drugs were allowed within the first 7 days of treatment and other supportive treatments were given at the discretion of the attending clinician. Remission was defined as a stable PCV for 24 hours.

Ten of 11 dogs from the treatment group and 2 of 3 dogs from the control group achieved remission and survived to hospital discharge. Survival and time to remission were not significantly different between groups. The volume of packed red blood cells transfused, normalized for body weight, was not significantly different between groups. Potential adverse reactions to Pentaglobin® occurred in two patients but clinical signs may have been related to the patients'; underlying disease.

Treatment with high-dose Pentaglobin® was well tolerated by patients but no significant advantage was found in this small study. Larger studies are warranted to identify any potential benefits.

HM03

Educating Veterinary Students in an Intensive Care Unit: Impact of a Transfusion Reaction Learning Module

Jillian Haines[1], K. Jane Wardrop[1], Christopher Lindberg[2], Kira Carbonneau[1], Tandi Ngwenyama[1], Linda Martin[1]
[1]Washington State University, Pullman, WA, USA, [2]Oregon State University, Corvallis, OR, USA

Blood product transfusions are used commonly in veterinary medicine. Similar types of transfusion reactions are seen in veterinary patients as those seen in humans. The incidence of reactions in dogs and cats is considered higher than in people and the severity of reactions is similar with the potential for some reactions to be fatal. In human medicine, lack of education in transfusion medicine has been shown to lead to inappropriate blood product use, which in turn can lead to an increased incidence of transfusion reactions. Most veterinary college core curricula provide limited education in the area of small animal transfusion reactions. Improving veterinary student education could lead to earlier recognition of reactions as well as improved knowledge in the areas of prevention and treatment. This may ultimately lead to a reduction in patient morbidity and mortality.

The objectives of this randomized controlled study were to develop and test the instructional efficacy of an online learning module on transfusion reactions in small animals and to evaluate participants'; satisfaction of the module. Our hypothesis was that students who completed the module would perform better on two post module assessments compared to students who received information about transfusion reactions through traditional methods (i.e. verbal instruction, clinical case rounds).

Content for the module was developed by veterinary specialists in the areas of internal medicine, critical care, and clinical pathology with guidance from an educational specialist and designed by an instructional design coordinator. The interactive module covered recognition, treatment, prevention, case examples, and self-assessment questions for 6 common transfusion reactions. Fourth year veterinary students on their critical care rotation were randomly selected to either receive the instructional module (treatment group) or only receive standard rotation instruction on transfusion reactions (control group). Two tests covering the same concepts were developed and students randomly received 1 as a pretest at the start of rotation and the other as a posttest at the end of the 2 week rotation. Immediately following the pretest, the treatment group received the learning module and module satisfaction survey. The survey asked students to rate their level of agreement to 10 statements on a 1-5 scale (1= strongly disagree to 5=strongly agree). All students were asked to complete an optional retention test, composed of questions from the pre and posttests, 1 month later.

A total of 45 students completed both the pre and posttests and were included in the study. Of these students, 23 were in the treatment group and received the module and 22 were in the control group. Thirty-three of the 45 students (73%) also completed the retention test (18 from the treatment group and 15 from the control group). Students in the treatment group scored significantly higher on the posttest ($p < 0.001$) and retention test ($p = 0.002$) than those students that did not receive the learning module. Mean post test scores were 73.8% and 56.1% and mean retention test scores were 71.2% and 46.6% for the treatment and control groups respectively. Nineteen of the 23 students (83%) in the treatment group completed the satisfaction survey. Students taking the survey indicated that the module was easy to use (mean 4.89), that information was presented in a clear manner (mean 5), and 89% of students agreed that the module was a good use of their educational time (mean 4.73).

In conclusion, a transfusion reaction instructional module can be delivered successfully to veterinary students on clinical rotations and is considered beneficial by the students. Administration of the learning module resulted in significantly improved transfusion reaction knowledge and improved knowledge retention over conventional clinical instruction methods in 4th year veterinary students.

HM04

Evaluation of Two Apheresis Techniques for Plateletpheresis in the Dog

Anne S. Hale[1], Theresa Westfall[2], Ed Sullivan[2]
[1]BodeVet, Inc, Rockville, MD, USA, [2]Northwest Veterinary Blood Bank, Bellingham, WA, USA

Apheresis collections have become much more common in the last five years within the veterinary community. Evaluation of two standard human apheresis devices for collection of canine platelet concentrate was performed. It was hypothesized that platelet concentrate quality would not be influenced by apheresis collection technique in the dog.

Three canine donors were chosen from a volunteer blood donor population in Washington state. Three canine donors were chosen from a purpose bred blood donor population in New York state. All donors had been previously screened by PCR and IFA for infectious disease as defined by the ACVIM consensus statement. Donors demonstrated a normal CBC at the time of donation. Only donors with platelet counts in excess of 200,000/ul were selected. Apheresis was performed utilizing the Terumo BCT Trima device or the Haemonetics MCS plus device utilizing standard “human” protocols provided by the manufacturer. Briefly, the Trima device and protocol utilizes a continuous separation technique for the collection of leukoreduced platelet concentrates. The Haemonetics device utilizes a discontinuous separation technique for the collection of leukoreduced platelet concentrates. Variances between protocols included the use of supplemental intravenous calcium gluconate with the Trima protocol and supplemental intravenous saline with the Haemonetics protocol. Donor groups were provided with sedation and/or anesthesia appropriate for donor comfort. Approximately 1.0×10^{11} platelets/100ml of concentrate were collected. Residual plasma and all red blood cells were returned to the donor. No serious adverse reactions in the donors were noted during or immediately after the event. Platelet concentrates were shipped via independent courier in a novel platelet shipper to the laboratory in Maryland. Platelet concentrates were evaluated by automated coulter counter on site prior to shipment, after arrival

and after pooling. pH and lactate were measured via IStat handheld analyzer. If pH was less than 6.5, then pH was measured by standard pH meter. All units were evaluated for "swirl" and evidence of visual clumping. Platelet size distribution was evaluated by Flow cytometry.

Both apheresis protocols were managed without adverse reaction to the donors. Immediate post apheresis counts confirmed a consistent harvest of 1.0 x 10^{11}+/- 1.2 x 10^{6} by both devices. Red blood cell contamination was less than 0.6 x 10^{6}/ul. White blood cell counts were less than 6.0 x 10^{2}. Platelets prepared by the Haemonetics technique had a decrease in pH and increase in lactate when compared to platelets prepared by the Terumo technique. However, this difference was not statistically significant. Flow cytometric evaluation suggested a higher population of platelets in the 2.53- 5.0 micron size range in the concentrates prepared by the Haemonetics technique. There was no significant difference between techniques using standard methodology for evaluation. Both techniques provided platelet concentrates that met acceptance criteria for standard apheresis units.

HM05

Comparison of Haemoglobin and Haematocrit Measurements Using Ear Prick and Venous Blood Samples in Dogs

Maria Lyraki[1], *Miss Alice Sloan*[2], *Ian Ramsey*[2]
[1]*Small Animal Hospital, University of Glasgow, Langford, Bristol, England, United Kingdom,* [2]*Small Animal Hospital, University of Glasgow, Glasgow, Scotland, United Kingdom*

To evaluate ear prick sampling as a possible alternative to venous sampling using a point-of-care (POC) meter for the assessment of haemoglobin (Hb) and haematocrit (Hct) in dogs.

Simultaneous venous and ear prick blood samples were collected from 53 dogs that were considered to be anaemic (n = 17), non-anaemic (n = 30), or polycythaemic (n = 6) and were undergoing blood sampling as part of their clinical investigation. Hb and Hct were measured on both samples using a POC meter that requires a 2 microliter sample volume and which has been previously validated in our hospital and shown to have a correlation (r) of 0.96 with standard laboratory analysers and a co-efficient of variation of less than 5 %. Hb and Hct measurements were also obtained from the venous samples using one of two reference analyzers in 40 of the dogs. Pearson';s correlation co-efficient was calculated for the two sets of POC results and the bias calculated (and expressed as mean +/- standard deviation).

In agreement with previous studies, the POC results for Hb and Hct from the venous samples were significantly correlated with the laboratory analysers (0.98 and 0.97 respectively). The results for Hb and Hct from the ear prick samples when measured using the POC device were significantly correlated with the POC results of the venous samples (r = 0.93 for both). The calculated biases were 0.17g/l (+/- 1.56) for the Hb and 0.36% (+/- 4.6) for the Hct respectively, which is consistent with the small degree of peripheral haemoconcentration. However, in 8 of the 53 (15 %) samples the peripheral sample was more than 15 % different from the venous sample, a difference that could have been clinically significant (see Figure 1).

The measurement of Hb and Hct using a simple ear prick test with a POC meter that requires a small sample volume may be a useful alternative to conventional venous blood sampling for the evaluation of these parameters in dogs, however there is a small positive bias and some clinically significant variation.

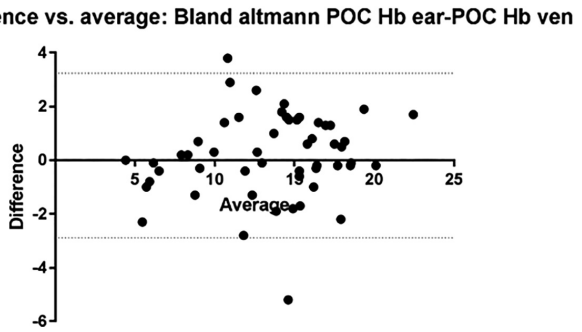


FIGURE 1 A Bland-Altman plot comparing the average Hb concentration (g/dl) with the difference between the Hb concentration when measured using venous and peripheral blood

HM06

Therapeutic Monitoring of Rivaroxaban in Dogs Using Thromboelastography and Prothrombin Time

Junwoo Bae[1], *Woosun Kim*[2], *Hyunwoo Kim*[3], *Hayoung Yang*[4], *Soomin Ahn*[4], *Jinho Park*[5], *Dong-In Jung*[6], *Do-Hyeon Yu*[6]
[1]*College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea/May Animal Medical Center, Jeonju, Cholla-bukto, Republic of Korea,* [2]*College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea/May Animal Medical Center, Jeonju-si, Cholla-bukto, Republic of Korea,* [3]*College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea, Gwangju, Cholla-namdo, Republic of Korea,* [4]*College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju, Kyongsang-namdo, Republic of Korea,* [5]*College of Veterinary Medicine, Chonbuk National University, Iksan, Cholla-bukto, Republic of Korea,* [6]*College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju-si, Kyongsang-namdo, Republic of Korea*

The newly developed oral direct factor Xa anticoagulant, rivaroxaban, was recently approved for use in human patients for thrombosis. Clinicians need to appropriately prescribe drugs for correct indications, but the guidelines for rivaroxaban have not been established in veterinary medicine. The gold standard for monitoring the anti-Xa effect of rivaroxaban is the chromogenic anti-Xa assay, but it is not clinically applicable. Therefore, the goal of this study was to evaluate clinical modalities for measuring the anticoagulant effects of rivaroxaban using point-of-care test of prothrombin time (PT) and thromboelastography (TEG) in healthy dogs.

Four different doses of rivaroxaban (0.5, 1, 2, and 4 mg/kg) were orally administered to six healthy Beagle dogs. Single oral and three consecutive dosing regimens were also assessed using citrated blood samples collected 0 and 3 h after single oral dosing and 0, 3, 8, and 12 h after consecutive trials. The plasma rivaroxaban concentration was determined using a chromogenic anti-Xa assay, and point-of-care PT and TEG analysis with four activators, RapidTEG, 1:100 tissue factor (TF100), 1:3700 tissue factor (TF3700), and kaolin, were compared. Spearman correlation coefficients were calculated between ratios (peak to baseline PT; peak reaction time (R) of TEG to baseline R of TEG) and anti-Xa concentration.

Oral rivaroxaban administration exhibited predictable anticoagulant effects and was well tolerated by healthy dogs. There were no clinical

signs of minor or major hemorrhage and gastrointestinal side effects in any dosing groups. The highest anti-Xa value and longest delayed R of TEG and PT were observed 3 h after the administration of rivaroxaban; however, the anticoagulant effect in TEG and plasma rivaroxaban concentration decreased significantly from 8 h after administration. Inter-individual differences in drug effects were observed as expected, and further experiments to monitor the anticoagulant effects were performed. The results showed that the anti-Xa concentration and point-of-care PT ratio were strongly correlated (R = 0.82, $P < 0.001$). R ratios of RapidTEG-TEG, TF100-TEG, and TF3700-TEG showed a significant correlation with rivaroxaban concentration measured using the anti-Xa assay (R = 0.76, $P < 0.001$; R = 0.82, $P < 0.001$; and R = 0.83, $P < 0.001$, respectively).

Overall, 1.5–1.9 times delay of the PT and R values of TEG 3 h after rivaroxaban administration is required to achieve therapeutic anti-Xa concentrations of rivaroxaban in canine plasma. TEG using tissue factors as activators and point-of-care PT validation for rivaroxaban can be used for therapeutic monitoring of rivaroxaban and determining individual rivaroxaban doses in dogs.

HM07

Influence of Canine Donor Plasma Hemostatic Protein Concentration on Quality of Cryoprecipitate

Macy Drinkhouse[1], Marjory Brooks[2], Darko Stefanovski[3], Kym Marryott[1], Mary Beth Callan[4]
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Cornell University, Ithaca, NY, USA, [3]University of Pennsylvania, Kennett Square, PA, USA, [4]Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania, Philadelphia, PA, USA

Cryoprecipitate (CRYO) is a plasma component containing high concentrations of factor VIII (FVIII), von Willebrand factor (VWF), and fibrinogen. Due to wide inter-individual variations in plasma FVIII and VWF levels among healthy dogs, there is concern about uniformity and standard potency of CRYO to treat dogs with hemophilia A or VWD. While Greyhounds are commonly used as blood donors, previous studies have documented lower plasma VWF and fibrinogen content in Greyhounds compared to non-Greyhounds. Greyhound plasma, therefore, may not yield high potency CRYO. The objectives of this study were to determine if: 1) plasma hemostatic protein content is a good predictor of CRYO potency; 2) there is a difference in quality of CRYO prepared from Greyhounds versus non-Greyhounds; and 3) canine CRYO produced by our protocol meets human blood banking standards.

A 450 mL unit of blood was collected from 20 Greyhounds and 20 non-Greyhounds enrolled in a blood donor program. CRYO was prepared from fresh frozen plasma (FFP) using standard methods; all blood component volumes were recorded. Aliquots of FFP and CRYO from each unit were analyzed for FVIII, VWF, and fibrinogen content. Recovery, the percentage of total factor content in FFP retained in CRYO unit, was calculated for each factor.

There was a positive correlation between FVIII, vWF and fibrinogen concentration in FFP and their respective factor content in CRYO ($P < 0.0001$, σ 0.723-0.763). Median recovery was greatest for VWF (65%), followed by fibrinogen (49%) and FVIII (33%), with no differences between Greyhounds and non-Greyhounds. There was no difference in median FVIII (95 and 94 IU/unit) or vWF (210 and 264 IU/unit) content of CRYO units when comparing Greyhounds and non-Greyhounds, respectively. However, median fibrinogen content in CRYO was less in Greyhounds (223 mg/unit) compared to non-Greyhounds (332 mg/unit) ($P = 0.0005$). Nevertheless, there was no difference between Greyhounds and non-Greyhounds for the number of CRYO units meeting human blood banking standards for any of the 3 hemostatic proteins: VWF, 19 Greyhounds and 18 non-Greyhounds (CRYO from 2 VWF-deficient Dobermans did not meet standard); fibrinogen, 17 Greyhounds and 20 non-Greyhounds; and FVIII, 8 Greyhounds and 11 non-Greyhounds.

In conclusion, the factor content in donor FFP is strongly associated with CRYO potency, suggesting that pre-screening of blood donors may enhance CRYO quality. CRYO prepared from Greyhounds is not inferior to that from other breeds, justifying use of their plasma for preparation of CRYO. While most CRYO units met human blood banking standards for VWF and fibrinogen, variable recovery of FVIII resulted in approximately 50% of the CRYO units having FVIII content below human standards. Control of bleeding in dogs with hemophilia A may require transfusion to effect due to nonuniform FVIII potency of single CRYO units.

HM09

Nucleated Erythrocytes and Anemia in Dogs with Systemic Inflammatory Response Syndrome: Could they Affect Outcome?

Eleonora Gori[1], Alessio Pierini[2], Gianila Ceccherini[2], George Lubas[3], Veronica Marchetti[2]
[1]University of Pisa, Dept. Veterinary Sciences, San Piero A Grado, Pisa, Toscana, Italy, [2]University of Pisa, Dept. of Veterinary Sciences, Veterinary Teaching Hospital, San Piero a Grado, Pisa, Toscana, Italy, [3]University of Pisa, Dept. of Veterinary Sciences, Veterinary Teaching Hospital, San Piero A Grado, Pisa, Toscana, Italy

During Systemic Inflammatory Response Syndrome (SIRS) a release of inflammatory mediators occurs and hematological modifications are common. The study's aim was to evaluate anemia and nucleated RBC (NRBCs) in canine SIRS compared to the severity of illness and outcome.

This retrospective study included the following dogs: 90 with SIRS, 50 healthy, 50 with chronic diseases. SIRS grading was based on how many criteria were fulfilled. $APPLE_{fast}$ score was allocated in SIRS dogs. Mortality rate was assessed at 7 and 15 days after admission. Hemolytic or hemorrhagic disorders were excluded. SIRS grading and $APPLE_{fast}$ score groups were compared to the outcome. Types of anemia and NRBCs counts were evaluated in three study populations and to the outcome.

$APPLE_{fast}$ scores >25 (p = 0.03) and SIRS grading >2 (p = 0.001) were associated with poor outcome. In SIRS group, anemia was present in 56/90 dogs. The most frequent types of anemia were mild (45%) or moderate (43%), microcytic (55%) or normocytic (41%), and normochromic (93%). Anemia and its severity were associated with poor outcome (p = 0.0197). SIRS group showed worse anemia patterns than the other two groups (p < 0.001). The presence of NRBCs occurred in 22/90 of SIRS dogs and was associated with poor outcome (p = 0.005). NRBCs count were significantly higher in the SIRS group than healthy dogs (p = 0.0007).

Mild-moderate, micro-normocytic normochromic anemia is a frequent finding in canine SIRS. Our results suggest that circulating NRBCs and their amount could be an additional negative prognostic value.

HM10

Rapid Decrease in Prednisolone Dosage Can Cause Early Recurrence of Immune-Mediated Thrombocytopenia in Dogs

Keitaro Morishita, Daichi Takamura, Tatsuyuki Osuga, Noboru Sasaki, Hiroshi Ohta, Mitsuyoshi Takiguchi
Department of Veterinary Clinical Sciences, Hokkaido University, Sapporo, Hokkaido, Japan

Primary immune-mediated thrombocytopenia (pIMT) is a common hematologic disorder in dogs. Although immunosuppressive doses of prednisolone can normalize the platelet count in most cases, the high recurrence rate is still a problem. This study was performed to clarify the relationship between tapering of the prednisolone dosage and the time to relapse.

Sixteen dogs treated for pIMT at Hokkaido University Veterinary Teaching Hospital from March 2013 to May 2017 were retrospectively evaluated. A relapse episode was defined as a therapeutic course from remission to relapse. A non-relapse episode was defined as a therapeutic course from remission to cessation of prednisolone. Concurrent immunosuppressive medications were not considered.

In total, 17 relapse episodes and 18 non-relapse episodes occurred while tapering the prednisolone. The median dosage of prednisolone at the time of remission was 2.4 mg/kg/day (range, 1.0–3.7 mg/kg/day). The median dosage of prednisolone at the time of relapse was 0.9 mg/kg/day (range, 0.2–2.5 mg/kg/day). The median remission period was 69 days (range, 8–221 days). A significant negative correlation was present between the tapering rate of prednisolone ($P<0.01$, $r=-0.74$) and the remission period. When the 17 relapse episodes were divided into acute relapse (remission period of ≤60 days, n=7) and delayed relapse (remission period of >60 days, n=10), the tapering rate of prednisolone was significantly higher for the acute relapse episodes than for both the delayed relapse and non-relapse episodes ($P=0.04$ and $P=0.01$, respectively).

These results suggest that a rapid decrease in the prednisolone dosage can cause early recurrence of pIMT.

HM11

Comparison of Fibrinolysis via Thromboelastography in Greyhounds versus Non-Greyhounds

Sarah Shropshire, Christine Olver
Colorado State University, Fort Collins, CO, USA

Greyhounds have several hematologic differences from other breeds of dogs, including higher hematocrit and hemoglobin concentration and lower neutrophil and platelet counts. It has also been observed that some Greyhounds have a propensity to bleed excessively 36-48 hours following minor traumas or routine surgeries. The exact etiology behind this bleeding is unknown however several studies have postulated this may occur due to hyperfibrinolysis. Dynamic viscoelastic testing using thromboelastography (TEG) allows for not only assessment of coagulation but also fibrinolysis. TEG has been performed previously in Greyhounds but no study has investigated fibrinolysis variables via TEG in the presence of tissue plasminogen activator (tPA) in healthy Greyhounds. Therefore, the purpose of this study was to evaluate if Greyhounds appear hyperfibrinolytic as compared to non-Greyhounds.

Tissue factor-activated TEG with tPA added (TF + tPA-TEG) was performed in six healthy client-owned Greyhounds and six healthy laboratory Beagles. For TF + tPA-TEG, reaction time (R), clotting time (K), rate of clot formation (α), maximum amplitude (MA), percent clot lysis 30 and 60 minutes after MA is reached (LY30 and LY60), amount of clot lysis 30 and 60 minutes after MA is reached (CL30 and CL60), maximal rate of thrombus generation (MRTG), time to maximum rate of thrombus generation (TMRTG), total thrombus generated (TG), maximum rate of lysis (MRL), time to maximal rate of lysis (TMRL) and clot lysis time (CLT) were recorded. The hematocrit, platelet count, and fibrinogen were also recorded. An unpaired t-test was performed on R, K, α, MA, LY30, LY60, MRTG, TMRTG, TG, MRL, TMRL, CLT, hematocrit, platelet count, and fibrinogen and a Mann-Whitney *U* test was performed on CL30 and CL60. For all tests, $p < 0.05$ was considered significant.

The α, MA, MRTG, TG, platelet count and fibrinogen were significantly lower and the K and hematocrit were significantly higher in the Greyhounds. There were no statistical differences for R, LY30, CL30, LY60, CL60, TMRTG, MRL, TMRL and CLT between the groups.

As noted in previous studies, the Greyhounds had higher hematocrits and lower platelet counts as compared to non-Greyhounds. Greyhounds appeared significantly hypocoagulable (increased K and decreased α, MA, MRTG and TG) but did not appear hyperfibrinolytic as compared to non-Greyhounds. It is possible that hyperfibrinolysis in Greyhounds may not be related to responsiveness to tPA or that hyperfibrinolysis is not detected via TF + tPA-TEG in the preoperative setting and cannot be used to predict bleeding. Additional studies are warranted to further investigate the mechanism of bleeding appreciated in this breed.

HM12

Viability of Two Platelet Agonist Reagents in Whole Blood Impedance Platelet Aggregometry in Dogs

Sarah Shropshire, Christine Olver
Colorado State University, Fort Collins, CO, USA

Whole blood impedance platelet aggregometry can be performed with several different agonists to evaluate platelet function. Although the manufacturer recommends disposal of stored reagent after 1 month, the viability after reconstitution of these reagents under different storage conditions is unknown. If the reagent viability is stable for long periods of time, assay costs could be decreased dramatically. Therefore, the purpose of this study was to determine the viability of reconstituted arachidonic acid (AA) and adenosine diphosphate (ADP) platelet agonists stored under two conditions up to 6 months.

60µl aliquots of reconstituted AA and ADP were stored at -20°C and -80°C monthly for six months. Six healthy staff-owned dogs were enrolled for the study. A physical examination, complete blood count (CBC), diagnostic panel, and urinalysis were performed in all enrolled dogs. Platelet aggregometry was performed on all dogs using fresh and stored aliquots of AA and ADP reagents on the same day. The

area under the curve (AUC) was recorded from each platelet aggregometry analysis. Repeated measures (RM) analysis (one-way ANOVA) was performed and subsequent time points (1, 2, 3, 4, 5, 6 months) were compared to fresh AUC results.

All dogs appeared clinically healthy and the CBC, diagnostic panel, and urinalysis were normal. There were no differences in the AUC obtained from fresh samples at any time point or at either temperature for AA or ADP.

In dogs, the whole blood impedance platelet aggregometry reagents AA and ADP are viable for up to six months when stored at -20°C or -80°C. We conclude that these reagents can be stored up to 6 months, obviating the need to discard viable reagents, and decreasing assay cost.

HM13

Effects of Irradiation and Leukoreduction on Down-regulation of CXCL-8 and Storage Lesion in Canine Blood

Hayoung Yang[1], Woosun Kim[2], Junwoo Bae[3], Hyunwoo Kim[4], Soomin Ahn[1], Jinho Park[5], Dong-In Jung[6], Do-Hyeon Yu[6]

[1]College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju, Kyongsang-namdo, Republic of Korea, [2]College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea/May Animal Medical Center, Jeonju-si, Cholla-bukto, Republic of Korea, [3]College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea/May Animal Medical Center, Jeonju, Cholla-bukto, Republic of Korea, [4]College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea, Gwangju, Cholla-namdo, Republic of Korea, [5]College of Veterinary Medicine, Chonbuk National University, Jeonju 54596, Republic of Korea, Iksan-si, Cholla-bukto, Republic of Korea, [6]College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju-si, Kyongsang-namdo, Republic of Korea

Blood transfusions are commonly used to manage anemia conditions. However, potential adverse effects include febrile nonhemolytic transfusion reaction (FNHTR), transfusion-associated graft versus host disease (TA-GvHD), immunologic or non-immunologic hemolysis, transfusion-related acute lung injury, transfusion-associated circulatory overload, and storage lesions (i.e., biochemical and biomechanical changes depending on the red blood cells storage time). Because white blood cells (WBCs) and storage period are the main factors for causing adverse reactions, several ways to prevent these transfusion reactions were proposed. These include leukoreduction (LR), which removes leukocytes and platelets by filtration, and irradiation (IR) of blood to prevent TA-GvHD by inactivating lymphocytes and close monitoring of storage lesion.

The objective of this study was to measure cytokine/chemokine concentrations after LR and IR in canine stored whole blood. Red blood cell (RBC) storage lesion caused by irradiation and LR were compared.

Blood samples were obtained from 10 healthy Beagle dogs. The collected blood samples were divided into four groups of 10 samples each (no treatment, LR, IR, and LR + IR). Leukocytes were removed by filtration in the LR group and gamma radiation (25Gy) was applied in the IR group. Immunologic factors (WBCs, interleukin-6 [IL-6], C-X-C motif chemokine ligand 8 [CXCL-8], and tumor necrosis factor-alpha [TNF-α]) and storage lesion factors (blood pH, potassium and hemolysis) were evaluated on days 0, 7, 14, 21, and 28 from aliquots of each group.

IL-6 and CXCL-8 concentrations in control (no treatment of stored whole blood) were significantly higher during storage, which indicates a high probability of transfusion reactions such as FNHTR. On the other hand, the LR group did not show changes in cytokine/chemokine concentrations, and the storage lesion was relatively mild compared to that in other groups. Irradiation significantly increased CXCL-8 after 14 days of storage, but irradiation of leukoreduced blood did not increase CXCL-8 during 28 days of storage. However, storage lesions such as hemolysis, increased potassium, and low pH were observed 7 days after IR of blood, regardless of LR.

In conclusion, LR is beneficial in preventing transfusion reactions and storage lesion. However, IR of canine whole blood was not suitable for long-term storage because of increasing factors of CXCL-8 and storage lesion. IR of leukoreduced blood is beneficial to avoid immune reaction, however, storage lesion should be considered upon storage.

HP01

Bacterial Community Composition of Bile from Healthy Dogs and Dogs with Suspected Cholangitis or Mucocele

Ashley Hartley[1], Andrea Azcarate-Peril[2], Kathleen Aicher[3], Megan E. Jacob[1], Devorah Stowe[1], Gabriela Seiler[1], Rachel Cullen[1], John Cullen[1], Kyle Mathews[1], Jason Arnold[4], Stephen Stauffer[1], Jody Gookin[1]

[1]North Carolina State University, Raleigh, NC, USA, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [3]Veterinary Specialty Hospital of the Carolinas, Cary, NC, USA, [4]Microbiome Core Facility UNC Chapel Hill, Chapel Hill, NC, USA

Bacterial cholecystitis and mucocele formation are considered the most common causes of gallbladder disease in dogs. Positive bacterial growth of bile samples from these patients is less common than is our suspicion for an infectious culprit. Studies to determine if bacteria reside in the gallbladder of healthy dogs or dogs with culture-negative gallbladder disease has not been reported. The present study performed next-generation sequencing of gallbladder bile and mucus from dogs with and without gallbladder disease to characterize the bacterial community composition for presence of a commensal microbiome or unrecognized pathogenic bacteria.

Bile samples were obtained by cholecystocentesis from 22 healthy dogs (CTRL) and 36 dogs with suspected bacterial cholecystitis (SBC). Gallbladder mucus was obtained after cholecystectomy in 22 dogs with mucocele formation (GBM). Presence of Eubacteria was examined by aerobic and anaerobic culture, cytology, and fluorescence *in situ* hybridization (FISH). All samples underwent DNA extraction, PCR amplification of the V3-V4 region of the 16S rRNA gene, and Illumina-based sequencing.

Dogs in the CTRL group were significantly younger (median 3, range, 1-4 yrs) than dogs in SBC (9.6, 1.2-15 yrs) or GBM (10.9, 4.8-16 yrs) groups ($p < 0.001$). GBM dogs had higher neutrophil counts ($p = 0.006$) and higher total serum bilirubin ($p = 0.043$) compared to SBC dogs. No CTRL dogs were positive for the presence of bacteria by means of culture, cytology, or FISH. Similar numbers of SBC and GBM dogs had positive results of bacterial culture, cytology, or FISH. Compared to conventional means to identify bacteria, a majority of CTRL (13/22; 59%), SBC (14/36; 39%) and GBM dogs (14/22; 64%) harbored a diverse microbiome. PCR targeting of the 16S rRNA gene

was highly effective in identifying the same bacteria (i.e. *E. coli* and *Enterococcus* spp.) as were diagnosed by culture or revealing their presence in culture negative dogs with recent history of antibiotics. The remaining 16S rRNA gene positive, culture negative dogs demonstrated a diverse microbiome consisting predominantly of Proteobacteria (Neisseriaceae, *Acinetobacter*), Firmicutes (*Staphylococcus*, *Streptococcus*, *Lactococcus*), Actinobacteria (*Corynebacterium*, *Propionibacterium*), Cyanobacteria (*Streptophyta*), and Bacteroidetes. Obvious differences in the between CTRL and GBM dogs were not observed.

Results of these studies identify that a majority of normal and diseased dogs harbor a rich and diverse bile microbiome. This microbiota is likely to contain previously unrecognized beneficial as well as deleterious bacteria. Tailoring bile culture media to routinely identify these bacteria is likely to improve our understanding and diagnosis of canine hepatobiliary disease.

HP02

Potential Serum Biomarkers of Hepatic Fibrosis and Necroinflammatory Activity in Dogs with Liver Disease

Chantel Raghu[1], *Joanne Ekena*[2], *John Cullen*[3], *Craig Webb*[4], *Lauren Trepanier*[5]

[1]*CareCenter Cincinnati, Cincinnati, OH, USA,* [2]*UW School of Vetmed, Madison, WI, USA,* [3]*North Carolina State University, Raleigh, NC, USA,* [4]*Colorado State University, Fort Collins, CO, USA,* [5]*University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI, USA*

Serum interleukin 6 (IL-6), chemokine ligand 2 (CCL2), C-reactive protein (CRP) and the ratio of aspartate transaminase to alanine transaminase (AST:ALT) have been correlated with fibrosis and necroinflammatory activity in humans with various hepatopathies. The objective of this study was to determine whether increases in these potential biomarkers were associated with moderate to severe fibrosis or necroinflammatory activity in dogs with various hepatopathies. Forty-four client-owned dogs with clinical evidence of liver disease and 10 healthy purpose-bred dogs were enrolled, all undergoing liver biopsies by laparoscopy or laparotomy. Serum IL-6, CCL2, CRP, AST and ALT were measured within one week before scheduled liver biopsy; liver histopathology was evaluated using the METAVIR scoring system used in human medicine, blinded to clinical presentation. Median serum IL-6 was approximately twice as high in dogs with high fibrosis scores (15.5 pg/ml; range, 1.4 to 235 pg/mL) compared to dogs with low fibrosis scores (7.6 pg/mL; range, 1.4 to 148.1 pg/mL), with marginal significance (P = 0.05). Median serum CCL2 was significantly higher in dogs with active necroinflammation (444 pg/mL; range, 144 to 896 pg/mL) compared to dogs without detectable necroinflammation (326 pg/mL; range, 59 to 1692 pg/mL; P = 0.008), but with considerable overlap between groups. Neither serum CRP nor AST:ALT ratios were significantly different based on fibrosis or necroinflammatory scores. Because of substantial variability among dogs, single measurements of IL-6 and CCL2 have limited diagnostic utility for identifying fibrosis or necroinflammation, respectively, in dogs with various chronic liver diseases. The value of these biomarkers should be explored further in monitoring response to treatment in individual dogs with chronic hepatopathies.

HP03

The Evaluation of Cyclosporine in the Treatment of Chronic Hepatitis in Dogs

Tarini V. Ullal[1], *Yoko Ambrosini*[2], *Sangeeta Rao*[1], *Cynthia Webster*[2], *David Twedt*[1]

[1]*Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO, USA,* [2]*Cummings School of Veterinary Medicine,Tufts University, North Grafton, MA, USA*

Previous studies have reported a response to corticosteroids suggesting that some cases of chronic hepatitis (CH) in the dog are likely immune mediated. However, corticosteroid therapy is associated with significant side effects, including a steroid hepatopathy that confounds interpretation of serum liver enzymes during therapy. Thus, alternative immunosuppressive therapies are needed for the management of CH in dogs.

The aim of this retrospective, collaborative study was to evaluate the ability of cyclosporine (Cys) to induce remission (defined by normalization of alanine aminotransferase [ALT]) in dogs with presumed immune mediated CH as well as to assess tolerability. A secondary aim was to evaluate if previously reported prognostic factors influenced the ability to attain remission with Cys therapy.

Fifty dogs diagnosed with CH between 2010-2017 based on World Small Animal Veterinary Association criteria and treated with Cys for at least 2 weeks met inclusion criteria. Dogs with positive hepatic bacterial cultures or hepatic copper > 400 ppm dry weight were included if copper chelation or antibiotic therapy failed to achieve remission. Concurrent corticosteroid therapy was permitted if remission was not achieved prior to Cys initiation.

Descriptive statistics were used to analyze categorical variables (age, gender, breed, side effects to Cys) and continuous variables (Cys dose, time to remission, ALT concentration). Means, medians, ranges, and standard deviations were calculated for continuous data. Wilcoxon, Student t-test, Fisher';s exact test, or survival analysis was used to evaluate the impact of prognostic factors (pre-treatment clinical scores, hypoalbuminemia, hyperbilirubinemia, ascites, fibrosis on histopathology, prolonged PT/aPTT) on remission.

Twenty-five different breeds were represented. Ages ranged from 0.7-14 years (median 8.0 years). Fifty-two percent were female spayed and 43% were male castrated. The most common treatment side-effects were gastrointestinal signs, which ranged from mild inappetence to vomiting and diarrhea in 44% (22/50) and gingival hyperplasia in 26% (13/50). Seventy six percent (38/50) achieved remission with a median time to remission of 2 months (0.75-12 months). The median dose at the time of remission was 7.8 mg/kg/day (1.5-12.7 mg/kg/day). No association was found between prognostic factors and remission with the exception of hypoalbuminemia. There was an increased likelihood of remission ($p < 0.05$) and a decreased time to remission in dogs with hypoalbuminemia ($p < 0.05$).

In conclusion, Cys therapy was tolerated and effective in achieving normalization of ALT in dogs with presumed immune mediated CH. Previously reported prognostic factors did not negatively impact the ability to achieve ALT normalization with Cys therapy.

HP04

Serum Vitamin D Status in Cats with Cholestatic Hepatobiliary Disease

Lesli Kibler, Cailin Heinze, Cynthia Webster
Cummings School of Veterinary Medicine,Tufts University, North Grafton, MA, USA

The liver performs an essential role in metabolism of vitamin D (VD); deficiency of vitamin K, another fat-soluble vitamin, is common in cats with cholestatic hepatobiliary disease (CHD). We hypothesized that VD levels in cats with CHD would be significantly lower than in normal cats or sick cats with non-hepatobiliary disorders. A prospective case control study was done comparing cats with CHD disease (defined by hyperbilirubinemia and increased alanine aminotransferase), sick cats without hepatobiliary disease, and normal cats represented by the validated reference range for 25(OH)VD. Thirty-six cats with CHD and 23 cats with non-hepatobiliary disease were enrolled. Parathyroid hormone (PTH), ionized calcium (iCa) and 25(OH)D were measured in all cats. Mean 25 (OH)VD levels were significantly lower in sick cats (89 nmol/L +/-24 nmol/L) and cats with cholestatic disease (88 nmol/L +/- 33 nmol/L) compared to the reference range (117 nmol/L +/- 26.2 nmol/L). Median iCa and PTH in cats with CHD was significantly higher (1.32 mmol/L and 0.95 pmol/L respectively) than in sick cats (1.26 mmol/L and 0.65 pmol/L, respectively). Six of 36 (17%) of CHD cats and 1/24 (4%) of sick cats had high PTH levels. Overall 13/36 (36%) CHD cats and 7/24 (29 %) sick cats were 25(OH) VD deficient or insufficient (< 74 nmol/L). There was no correlation between 25(OH)VD and serum bilirubin. As inadequate 25(OH)VD may negatively impact overall health and low levels were common in all the sick cats in this study. Future studies assessing the impact of insufficient VD levels in cats are necessary.

HP05

Treatment of Canine Congenital Extrahepatic Portosystemic Shunts - A Systematic Review and Meta-Analysis

Gonçalo Serrano, Marios Charalambous, Ms. Nausikaa Devriendt, Hilde De Rooster, Ms. Femke Mortier, Dominique Paepe
Faculty of Veterinary Medicine - Ghent University, Merelbeke, Oost-Vlaanderen, Belgium

The assessment of the most optimal treatment option for congenital extrahepatic portosystemic shunts (cEHPSS) is a topic of interest in veterinary medicine. A combined systematic review and meta-analysis was designed to evaluate the existing evidence with regards to the effectiveness of different cEHPSS management modalities. Electronic databases of PubMed, CAB Direct and Google Scholar were searched without date or language restrictions. Conference proceedings from 1990 to 2017 of major congresses were also searched. Peer-reviewed full-length studies describing the outcome for at least one treatment modality in dogs with cEHPSS were included. All studies were assessed for quality of evidence (study design, study group sizes, subject enrolment quality and overall risk of bias) and outcome measures reported (percentage of perioperative outcome, clinical and surgical/interventional outcome, all concomitantly reported with 95 % confidence interval).

Sixty-nine studies, including seven conference abstracts, reporting perioperative and/or clinical and/or surgical/interventional outcomes of dogs managed with one surgical/interventional technique and/or medical management for cEHPSS were identified. Only 19 studies were designed as prospective studies, including one non-blinded randomized controlled clinical trial, one non-blinded non-randomized controlled clinical trial and 17 uncontrolled clinical trials. The majority of the studies showed overall high risk of bias and evaluated low to very low numbers of cases per treatment group (67%) but with clearly characterized subject enrolment criteria (97%).

Direct comparison of ameroid constrictor versus thin film band revealed a statistically significant difference between the two techniques with regards to the surgical outcome, with ameroid constrictor being superior. Direct comparisons also suggested that ameroid constrictor placement might have a better perioperative, clinical, and surgical/interventional outcome than ligation. However, none of these comparisons were statistically significant. Direct comparison of other techniques was not possible due to lack of data. Indirect comparison suggested that ameroid constrictor placement and complete ligation were the techniques with better perioperative, clinical, and surgical/interventional outcome, followed by thin film band, coil embolization, and partial ligation. The outcome assessment for Amplatzer vascular plug and medical management was based on small numbers of cases to allow accurate interpretations.

In conclusion, this combined systematic review and meta-analysis provides objective evaluation of the treatment options of the cEHPSS. Ameroid constrictor was shown to be the technique with the higher likelihood of providing a good outcome. Complete ligation, thin film band, coil embolization and partial ligation were shown to be effective techniques in the treatment of cEHPSS, although less than ameroid constrictor. Blinded randomized studies with low overall risk of bias and good number of cases comparing different treatment modalities that routinely include postoperative imaging to assess cEHPSS closure and/or acquired portosystemic shunts development are essential.

HP06

Is the Common Bile Duct Truly Obstructed or Not?

Chung-In Wang[1], Yu Hung Hsieh[2], Shao-Han Chang[3], I-Hsuan Liu[3], Hsin Yu Ho[4]
[1]Fleur Animal Hospital, Toufen City, Miaoli, Taiwan (Republic of China), [2]The LifeCentre, Leesburg, VA, USA, [3]Fleur Animal Hospital, Toufen, Miaoli, Taiwan (Republic of China), [4]The veterinary cancer center, Norwalk, CT, USA

Extrahepatic bile duct obstruction (EHBDO) is usually tentatively diagnosed based on the clinical findings. However, definitely diagnosing obstructed common bile duct (CBD) is challenging without certain diagnostics. The goal of this study is to evaluate the feasibility of ultrasound-guided percutaneous cholangiography (UPC) to determine if CBD is truly obstructed and to re-evaluate the term, EHBDO, used in clinical application.

Four dogs and two cats, tentatively diagnosed with “infected EHBDO”, were enrolled in this study. UPC was performed, (1) cholecystocentesis, removing most of the bile to decompress the gallbladder and for cytology and culture/sensitivity; (2) less than 5 mL of Iodixanol 320 was injected into the gallbladder. Ventrodorsal, left

oblique ventrodorsal, left lateral and right lateral projections of the abdomen were made 2 minutes after UPC, every 10 minutes for the first hour and every 30 minutes after until contrast medium was noted in the duodenum.

All patients had CBD dilation (4.2 - 14.4 mm in diameter). The contrast was noted in the duodenum in five patients. Only one dog was diagnosed with completely obstructed CBD given no contrast in the duodenum 4 hours after UPC. The rest was diagnosed with CBD dilation and gallbladder delayed emptying. The time for UPC was from 2 to 240 minutes.

UPC is a feasible technique in patients with dilated CBD and can be used to confirm complete obstruction of CBD. Based on this study, the term "extrahepatic bile duct disease" should be used instead of EHBDO before further diagnostics, such as UPC.

HP07

Demographic Features, Characteristics and Risk Factors in a Retrospective Study of Hepatocellular Carcinoma in Dogs

Rommaneeya Leela-arporn[1], *Hiroshi Ohta*[2], *Noriyuki Nagata*[2], *Kazuyoshi Sasaoka*[2], *Tatsuyuki Osuga*[2], *Keitaro Morishita*[2], *Noboru Sasaki*[2], *Kensuke Nakamura*[2], *Mitsuyoshi Takiguchi*[2]
[1]*Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Hokkaido University, Sapporo, Hokkaido, Japan,* [2]*Department of Veterinary Clinical Sciences, Hokkaido University, Sapporo, Hokkaido, Japan*

Hepatocellular carcinoma (HCC) is the most common primary liver tumor in dogs. However, the clinical features and risk factors of HCC have not been confirmed. Therefore, the aim of this study was to investigate the clinical features and risk factors of canine HCC.

Medical records of forty-one dogs diagnosed with HCC at Hokkaido University Veterinary Teaching Hospital (HUVTH) between May 2013 and May 2017 were retrospectively reviewed. To examine the breed, age, or sex predisposition of HCC, all cases that came to HUVTH during the study period were used as the reference population. Other possible related factors including clinicopathological findings and concurrent disorders of HCC were determined by comparison of two breed- and age matched control dogs and each HCC case.

Prevalence of histologically confirmed HCC was 0.89% (41/4,607 cases). Dogs diagnosed with HCC was significantly older (median 11 years; range 9-15 years; $P < 0.001$) than reference population (median 9 years; range 0-20 years). Welsh Corgis were significantly predisposed to HCC (odd ratio [OR]: 4.13; 95% CI: 1.81-9.41; $P = 0.0032$). Breed- and age matched case control analysis revealed that thrombocytosis (27/40 cases; 67.5%), increased ALT (38/41 cases; 92.7%), increased ALP (39/41 cases; 95.1%), and hypercalcemia (12/32 cases; 37.5%) were significantly associated with HCC. In addition, 27 out of 41 dogs with HCC had at least one concurrent disorder. The most common concurrent disorder was hyperadrenocorticism (12 dogs), and the odds of hyperadrenocorticism in dogs with HCC were 3.36 times that of controls (95% CI: 1.28-8.81; $P = 0.020$).

These findings indicated that aging increase the risk of development of HCC, and Welsh Corgis were predisposed to HCC. In addition, a significant association between HCC and hyperadrenocorticism was presented, suggesting that hyperadrenocorticism might play a role in the development of HCC in dogs.

HP08

Serum Gastrin Concentrations in Dogs with Hepatic Disease

Yuri A. Lawrence[1], *Joerg M. Steiner*[2], *Jan S. Suchodolski*[3], *Jonathan A. Lidbury*[2]
[1]*Gastrointestinal Laboratory, Texas A&M University, College station, TX, USA,* [2]*Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA,* [3]*Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA*

Gastroduodenal ulceration has been reported to occur as a sequela to hepatobiliary disease in dogs. Reduced mucosal blood flow due to portal hypertension and increased gastric acid secretion secondary to hypergastrinemia have been suspected as potential pathogenetic mechanisms causing gastroduodenal ulceration. We hypothesized that dogs with hepatobiliary disease would have hypergastrinemia. Our study aimed to compare serum gastrin concentrations among dogs with chronic hepatitis, dogs with a congenital portosystemic shunt, and healthy control dogs.

After withholding food for 12 hours, blood samples were collected from 20 dogs with chronic hepatitis, 8 dogs with a congenital portosystemic shunt, and 39 healthy client owned control dogs. Serum was separated, frozen immediately, and stored at −80°C until analysis. Gastrin was measured using a chemiluminescent, enzyme-labeled immunometric assay (IMMULITE® 2000 Serum Gastrin Assay, Siemens). The assay working range is 10 - 1,000 ng/L and our previously developed reference range is The median (min - max) serum gastrin concentrations was ≤10 (≤10 - 39) for dogs with chronic hepatitis, ≤10 (≤10 - 17) for dogs with a portosystemic shunt, and ≤10 (≤10 - 30) ng/L for healthy control dogs. There was no significant difference among groups ($P = 0.153$).

We found no significant difference in serum gastrin concentration among dogs with chronic hepatitis, a congenital portosystemic shunt, and healthy control dogs. Further studies are warranted to determine the etiology of gastroduodenal ulceration in dogs with hepatic disease.

IM02

Efficacy of the Canine Lyme Vaccine in North America: A Systematic Review and Meta-analysis

Nadine Vogt[1], *Jan Sargeant*[1], *Melissa MacKinnon*[1], *Ali Versluis*[2]
[1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Research and Scholarship Team, McLaughlin Library, University of Guelph, Guelph, ON, Canada*

Lyme disease is endemic to some parts of North America, and is an emerging disease in others. Although tick preventives are available, vaccination is an increasingly common though controversial method used in the prevention of canine Lyme infection; reported efficacies of canine Lyme vaccines are highly variable, ranging from 50% to 100%. The objective of our research was as follows: to determine the efficacy of vaccines for *Borrelia burgdorferi* in North American dogs by comparing vaccinated dogs to those not receiving the vaccine. We used a systematic review and meta-analysis to address this objective.

Monovalent and multivalent vaccines were considered eligible interventions. Our main outcome of interest was the reduction of clinical

illness following exposure to *B. burgdorferi*. Outcome data were extracted as a binary outcome for the following clinical signs (assessed separately): lameness, anorexia, pyrexia, depression, lymphadenopathy. Experimental and analytical observational studies were considered eligible for inclusion. In addition to grey literature searches, the following electronic databases were searched with no language restrictions: MEDLINE, Web of Science, CAB Abstracts. The last search was performed on November 29, 2016.

Thirteen challenge trials and three observational studies were identified as eligible, and twelve challenge trials contained sufficient data to be included in our meta-analysis. A meta-analysis could not be performed for observational studies due to an insufficient number of studies. None of the challenge trials assessed lymphadenopathy, but for each of the remaining four clinical signs a separate random effects meta-analysis was performed. With the exception of anorexia, all summary odds ratios were less than the null value of 1. Overall, the findings of our meta-analysis suggest that North American Lyme vaccines reduce the odds of clinical illness in dogs following exposure to *B. burgdorferi*. However, these results should be interpreted with caution since a number of issues related to small sample size, study quality, and publication bias were identified. No experimental field trials were identified, highlighting a major gap in the literature on this topic. Future studies should focus on larger sample sizes in field conditions.

IM03

Neutrophil Myeloperoxidase Index in Dogs with Babesiosis

Anri Celliers[1], Emma H. Hooijberg[2], Mary M. Christopher[3], Yolandi Rautenbach[2], Amelia Goddard[2]
[1]Onderstepoort Veterinary Academic Hospital, Pretoria, Gauteng, South Africa, [2]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa, Pretoria, Gauteng, South Africa, [3]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA, Davis, CA, USA

Babesiosis caused by the more virulent tick-borne hemoprotozoan, *Babesia rossi*, leads to a marked systemic inflammatory host response. Neutrophils form part of the innate immune system and myeloperoxidase is the predominant component of the neutrophil lysosomal protein in azurophilic granules. Neutrophil myeloperoxidase index (MPXI) is a reflection of the intracellular myeloperoxidase content and a recognized marker of neutrophil activation. The aims of this study were to (a) determine whether MPXI is correlated with outcome in dogs with babesiosis caused by *B. rossi*; and (b) determine correlation with the severity of the host response using cytokine concentrations.

Data for 140 dogs, naturally infected with *B. rossi*, and 20 healthy control dogs were retrospectively evaluated. Owner consent was obtained for enrolment of each case, together with approval from the University';s Animal Ethics committee. MPXI was generated on an automated cell counter, ADVIA 2120, and cytokine concentrations were determined using a canine-specific multiplex assay.

Fifteen of the *Babesia*-infected dogs died (14 %). MPXI was significantly higher in the *Babesia*-infected dogs ($P = 0.033$), and in the non-survivors ($P = 0.009$), compared to the controls. For the dogs that died, significant correlations were found between MPXI and interleukin-2 (IL-2; $r = 0.616$, $P = 0.033$), IL-6 ($r = 0.615$, $P = 0.033$), IL-18 ($r = 0.613$, $P = 0.034$), granulocytic-macrophage colony stimulating factor ($r = 0.630$, $P = 0.028$) and monocyte chemo-attractant protein-1 ($r = 0.713$, $P = 0.009$).

These findings suggest that significant neutrophil activation is present in dogs with *B. rossi* infection. In addition, increased MPXI was associated with disease outcome and was correlated with the severity of the cytokine-driven pro-inflammatory host response.

IM04

Analytical Validation of an Immunoturbidimetric Assay for the Measurement of Serum CRP Concentrations in Dogs

Marshal Covin[1], Robynne R. Gomez[1], Jan S. Suchodolski[2], Joerg M. Steiner[3], Jonathan A. Lidbury[3]
[1]Texas A&M University, College Station, TX, USA, [2]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [3]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA

C-reactive protein (CRP) is an acute phase protein synthesized by the liver. Measurement of serum CRP is used increasingly to monitor the progression of inflammatory diseases in canine patients. The purpose of this study was to analytically validate a commercially available immunoturbidimetric assay for the measurement of serum CRP concentrations in dogs.

Leftover serum samples from dogs were used for the analytical validation. Serum CRP concentrations were measured using a commercially available immunoturbidimetric canine CRP assay (Gentian cCRP, Gentian AS) on an automated platform (Beckman Coulter AU480). The following validation variables were assessed: linearity by dilutional parallelism, precision by intra-assay variability, reproducibility by inter-assay variability, and accuracy by recovery (mixing samples of known concentration). CRP concentrations were compared to those from a previously analytically validated commercially available immunoassay (EIA Canine CRP, Tridelta Development).

Observed to expected ratios for dilutional parallelism (5 samples) ranged from 108.0% to 137.6% with a mean ± SD of 117.7% ± 8.3%. Intra-assay (6 samples) and inter-assay (7 samples) coefficients of variation (%CVs) ranged from 1.4% to 2.7%, and 3.4% to 5.8%, respectively. Observed to expected ratios for mixtures of two serum samples of known concentrations (15 pairs), ranged from 91.5% to 128.6% with a mean ± SD of 101.3% ± 8.3%. Comparison with the Tridelta assay showed a non-linear relationship (R-squared = 0.46). This was especially apparent for values greater than 30 mg/L (reference interval < 7.6 mg/L) when measured by the Tridelta assay, where the Gentian results were higher than expected). Using the suggested cut-off values of 10.0 mg/L for the Gentian assay and 7.6 mg/L for the Tridelta assay, agreement between results was moderate (Cohen'; s kappa statistic = 0.52).

The evaluated assay (Gentian) was shown to be precise, reproducible, and accurate. A non-linear relationship with results from the comparison assay (Tridelta), especially for high concentration samples was shown. In conclusion, the Gentian assay appears to be sufficient for use but further work is needed to determine the optimal diagnostic cut-off value when using this assay.

IM05

Validation of a Clinically Applicable Flow Cytometric Assay for Detection of Immunoglobulin-Associated Platelets in Dogs

Sarah Shropshire[1], *Steven Dow*[2], *Michael Lappin*[1]
[1]*Colorado State University, Fort Collins, CO, USA,* [2]*Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA*

Direct flow cytometry is a technique that can be used to detect immunoglobulin-associated platelets in dogs. However, the present requirement is that samples must be processed within 24 hours for assessment in currently available assays which has limited its wide spread use. The purpose of this study was to develop a clinically applicable direct flow cytometric assay for detection of canine IgG-associated platelets expressed as percent IgG (%IgG).

The direct flow cytometry assay was first optimized and a cut-off value of ≤ 10% to differentiate negative and positive classifications was determined by serial direct flow evaluation of platelets from a healthy dog. The assay was then performed on samples from 9 healthy beagles and 12 client-owned thrombocytopenic dogs at four time points: fresh and 24, 48, and 72 hours after storage at 4°C and the % IgG was recorded. A repeated measures analysis (one-way ANOVA) was performed and storage time points were compared to fresh samples using Dunnett';s method. The average coefficient of variation (CV) was calculated for samples from thrombocytopenic dogs to evaluate intra-assay variability.

Using samples from healthy dogs, there were no differences among fresh and 24 and 48 hour samples but there was a difference between fresh and 72 hour samples. There were no differences among fresh and 24, 48, or 72 hour samples in thrombocytopenic dogs. Based on the cut-off value, healthy and thrombocytopenic dogs were consistently categorized at every time point. The average intra-assay CV for thrombocytopenic dogs was 4.32%.

Since samples can be processed and evaluated within 72 hours, the assay can be used for samples that require shipping to a central laboratory. This direct flow cytometric assay is reproducible and represents an accessible and potentially clinically useful test for the detection of IgG-associated platelets in dogs.

IM06

Comparing RNA Quality and Quantity Extracted from Canine Blood Using Commercially Available RNA Extraction Kits

Dahlia H. Tesfamichael, Jessica C. Pritchard, Michael W. Wood
UW-Madison School of Veterinary Medicine, Madison, WI, USA

Quantitative reverse transcription PCR (RT-qPCR) is increasingly used to study and diagnose disease in small animals. The performance of these assays relies upon first obtaining quality RNA in sufficient quantities. There are many commercially available kits optimized to extract RNA from human blood and one designed specifically for animal blood. The purpose of this study was to use stored canine blood to assess the performance of these commercially available RNA extraction kits using RNA yield and purity as benchmarks.

The RNA extraction performance of the RiboPure (ThermoFisher, Carlsbad, Calif.), TRIzol (ThermoFisher, Carlsbad, Calif.), RNeasy Protect Animal Blood (Qiagen, Germantown, Md.), and RNeasy Mini (Qiagen, Germantown, Md.) kits were assessed in this study. Whole EDTA anticoagulated blood was obtained from a healthy dog, and stored for a minimum of one week at -30°C in the RNA stabilizing buffer (RNAlater, TRIzol, and Protect Stabilizing Reagent) recommended by each manufacturer. RNA isolation also utilized the manufacturer';s protocol for each kit. DNase treatment was performed on all samples where indicated in the manufacturer';s protocol or if not included, by using a commercially available kit (Qiagen, Germantown, Md.). Extracted RNA was stored at -80C until further analysis. Resultant RNA yield and purity were calculated using spectrophotometric absorbance peak measurements at 260nm and 280nm to determine nucleic acid concentration and assess for protein contamination. Median RNA concentration and A260/280 were compared amongst groups with a Kruskal-Wallis test with Dunn';s test for multiple comparisons. Significance was defined as $p < 0.05$.

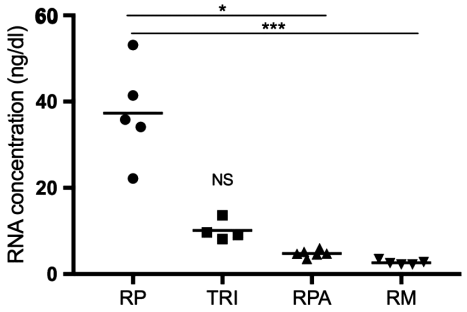


FIGURE 1 Comparison of mean RNA concentration amongst commercially available extration kits. Ribopure (RP) had significantly higher concentration of RNA extracted compared to RPA and RM but not TRI. *RP = TRIzol, RPA = RNeasy Protext Animal, RM = RNeasy Mini, *p<0.05, ***p<0.001, NS = not significant*

RiboPure extraction resulted in significantly higher nucleic acid concentrations than the RNeasy Protect Animal Blood ($p < 0.05$) and RNeasy Mini ($p < 0.001$) kits (Figure 1). There was no difference in median RNA concentration between RiboPure and TRIzol ($p > 0.99$). There was no difference in the median A260/280 amongst the kits, indicating no significant differences in protein contamination during extraction. The mean A260/280 for each kit was 1.88, 1.89, 2.08, and 1.87; RiboPure, TRIzol, RNeasy Protect Animal Blood, and RNeasy Mini kits, respectively.

These results indicate that the RiboPure kit isolated the greatest quantity of genetic material. Further evaluation utilizing RT-PCR with no reverse transcriptase controls to assess for genomic DNA contamination of extracts is necessary to fully evaluate RNA purity. Researchers can use these findings to appropriately select an RNA extraction kit based on their priorities.

ID01

Risk Factors for Candida Urinary Tract Infections in Dogs and Cats

Krystle L. Reagan[1], *Jonathan Dear*[2],*Philip H. Kass*[2], *Jane E. Sykes*[2]
[1]*University of California, Davis, Woodland, CA, USA,* [2]*University of California, Davis, Davis, CA, USA*

Candida infections have been described in both dogs and cats. Limited data is available regarding risk of development of these infections.

The objective in this study was to investigate risk factors associated with development of *Candida* urinary tract infections in dogs and cats. Eighteen dogs and eight cats with culture-confirmed candiduriawere evaluated in a retrospective case-control study to identify risk factors associated with candiduria. Control dogs and cats had bacterial cystitis or cutaneous *Malassezia* infection (dogs only). Univariate exact logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals. P-values < 0.05 were statistically significant.

Administration of antibacterial drugs in the 30 days before diagnosis was associated with candiduria in dogs using controls with bacterial cystitis (OR 14.5; 95% CI 3.1-66.9) and with *Malassezia* infection (OR 26.4; 95% CI 3.4-206.7). Antibacterial drug administration was also associated with candiduria in cats (OR 15.7; 95% CI 1.9-132.3). Immunosuppression was associated with candiduria in dogs when compared to controls with *Malassezia* infection (OR 4.2, 95% CI 1.4-12.8) but not significantly with bacterial cystitis controls (OR 2.7, 95% CI 0.9-8.0). Lower urinary tract diseases other than infection were associated with candiduria in cats (OR 6.7, 95% CI 1.6-27.9), but not significantly in dogs (OR 2.5, 95% CI 0.7-8.7). Neither diabetes mellitus nor history of hospitalization was significantly associated with candiduria in either species.

Recent administration of antibacterial drug therapy was a potential risk factor for development of candiduria in this population; their judicious use may help prevent this fungal infection.

ID02

Clinical Evaluation of a Commercial Hyperimmune Plasma Product in Dogs with Parvoviral Enteritis

Rachel Acciacca[1], *Lauren Sullivan*[2], *Tracy Webb*[2], *Valerie Johnson*[3], *Steven Dow*[3]
[1]*Colorado State University, Fort Collins, CO, USA,* [2]*Department of Clinical Sciences Colorado State University, Fort Collins, CO, USA,* [3]*Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA*

This randomized, placebo-controlled clinical trial prospectively evaluated the safety and clinical efficacy of a single infusion of hyperimmune plasma (HIP) when administered to dogs with canine parvovirus (CPV). Client-owned CPV dogs were randomized to receive either placebo [n =16, 0.9% NaCl (10 mL/kg IV)] or the study drug [n =16, HIP (10 mL/kg IV)] within the first six hours of hospital admission. Supportive care was standardized for both groups throughout the duration of hospitalization.

Dogs within the HIP group demonstrated a lower shock index at the 12-hour mark (p =0.046), and this difference was still observable at the 24-hour mark (p =0.04). Blood lactateconcentration was lower at the 24-hour mark in the HIP dogs when compared to the placebo dogs (p =0.049), although this was not statistically different at the 48-hour mark (p= 0.10). There was no difference in duration of hospitalization between groups (p= 0.35). Overall survival was 16/16 (100%) for the HIP group, compared to 14/15 (93.3%) for the placebo group (p =0.48). HIP was well tolerated with no adverse events noted during drug administration.

Results of this study indicate that hyperimmune plasma improves cardiovascular parameters during the first 24 hours of hospitalization. This study did not identify a difference in clinical severity improvement, duration of hospitalization, or mortality when comparing HIP and placebo dogs. Future studies evaluating HIP dose, and timing of HIP administration relative to disease onset, are needed to better determine the clinical benefit of this product.

ID03

Prevalence of Babesia spp. and Clinical Characteristics of Babesia Microti-Like Infections in North American Dogs

Nanelle R. Barash[1], *Adam J. Birkenheuer*[2], *Ms. Erica Lemler*[3], *Edward B. Breitschwerdt*[4], *Barbara A. Qurollo*[1]
[1]*North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA,* [2]*North Carolina State College of Veterinary Medicine, Raleigh, NC, USA,* [3]*NCSU-College of Veterinary Medicine, Raleigh, NC, USA,* [4]*North Caroline State University College of Veterinary Medicine, Fuquay Varina, NC, USA*

Babesiosis is an important cause of fever, splenomegaly, and hemolytic anemia in dogs. *Babesia microti*-like infection has been reported in domesticated European dogs and wild North American foxes; however, infection in domestic dogs from North America has only been rarely reported. Due to the genetic differences from other *Babesia* organisms that cause canine babesiosis, detection of *B. microti*-like DNA requires use of species-specific PCRs or broader *Piroplasma* PCR primers. The North Carolina State University-College of Veterinary Medicine- Vector-borne Disease Diagnostic Laboratory (NCSU-CVM-VBDDL) recently validated and implemented a novel PCR assay designed to amplify a wide range of *Babesia* spp. Using the new PCR assay, dog blood samples (n = 5214) submitted to the NCSU-CVM-VBDDL for PCR testing between June 2015 and December 2017 were analyzed for *Babesia* DNA prevalence.

Babesia spp. were detected in 195/5214 (3.74%) dogs, including *B. gibsoni* (2.26%), *B. vogeli* (0.44%), *B. microti*-like (0.42%), *B. canis* (0.33%) and *B. coco* (0.29%). *B. microti*-like specimens were further evaluated by amplification and sequencing of multiple mitochondrial and nuclear genes. Geographical location and available clinical data were collated. All *B. microti*-like infected dogs (n = 22) resided in North America, primarily in the eastern and southern US (predominant source of NCSU-CVM-VBDDL accessions), with no known travel to Europe.

Clinicopathologic abnormalities in 7 *B. microti*-like infected dogs included regenerative anemia (n = 6), thrombocytopenia (n = 3), proteinuria (n = 2), splenomegaly (n = 2), and hyperglobulinemia (n = 2). 81.8% (18/22) of *B. microti*-like infected dogs were American Pitt Bull Terrier-type breeds. Of 9 *B. microti*-like PCR positive dogs tested by *B. vogeli* and *B. gibsoni* IFA, only 6 (66.7%) were seroreactive, further suggesting that antibody detection is inadequate to fully screen for babesiosis in dogs. *B. microti*-like coinfections included *B. gibsoni* (n = 12) and *Mycoplasma* spp. (n = 2); one dog was *E. canis* IFA seroreactive. We conclude that: 1) *B. microti-like* infection occurs in dogs in North America. 2) Infection with these organisms induce clinicopathological abnormalities consistent with babesiosis. 3) Clearance of the infection (based upon seroreversion and negative PCRs) correlates with clinical improvement. 4) Dogs suspected of vector-borne infections in North America should be screened using PCR assays that detect *B. microti*-like parasites.

ID04

Differences in Clinicopathologic Variables between Borrelia C6 Antigen Seroreactive and Borrelia C6 Seronegative Canine Glomerulopathy

Moria A. Borys[1], Philip H. Kass[2], F. Charles Mohr[2], Jane E. Sykes[2]
[1]Sage Centers for Veterinary Specialty and Emergency Care, Dublin, CA, USA, [2]University of California, Davis, Davis, CA, USA

Rapidly progressive glomerulonephritis has been described in dogs that seroreact to *Borrelia burgdorferi*; however, no studies have compared clinicopathologic differences in Lyme-seroreactive dogs with protein-losing nephropathy (PLN) to dogs with *Borrelia*-seronegative PLN. In this study, we hypothesized that dogs with *Borrelia* C6 antigen-seroreactive PLN have distinct clinicopathologic findings when compared to dogs with *Borrelia* seronegative PLN. Specifically, we hypothesized that seroreactive dogs were more likely to have polyarthritis and thrombocytopenia.

This study was a retrospective prevalence case-control study. One hundred and eighteen dogs with PLN and *Borrelia* C6 antigen testing between 2002 and 2015 were identified (40 seroreactive dogs, and 78 temporally-matched seronegative dogs). Clinical information was retrieved from the University of California, Davis Veterinary Medical Teaching Hospital. Histopathology of renal tissue procured by biopsy and/or necropsy of dogs with PLN was reviewed.

Retrievers and retriever mixes were overrepresented in seroreactive dogs ($p < 0.001$). Seroreactive dogs were more likely to have polyarthritis ($p = 0.044$), thrombocytopenia ($p < 0.001$), azotemia ($p = 0.002$), hyperphosphatemia ($p < 0.001$), anemia ($p < 0.001$), and neutrophilia ($p = 0.003$). Hematuria, glucosuria and pyuria (despite a negative urine culture) were more likely in seroreactive dogs (all $p \leq 0.002$). Histopathology was consistent with immune-complex glomerulonephritis in 16/16 case dogs and 7/23 control dogs ($p = 0.056$).

This study demonstrates that *Borrelia* C6 seroreactivity in dogs with PLN is associated with a clinicopathologically distinct syndrome when compared with other types of PLN. Early recognition of this syndrome based on clinical findings has the potential to improve outcomes through specific aggressive and early treatment.

ID05

Primary-Care Practice Clinical Trial Provides Evidence That Reduced Antibiotic Use Does Not Affect Clinical Outcome

Sarah L. Caddy[1], Victor Ortiz[2], Rachael Day[2], Laura Klein[2]
[1]University of Cambridge, Cambridge, England, United Kingdom, [2]Cromwell Veterinary Group, Huntingdon, England, United Kingdom

Antimicrobial resistance (AMR) is a major problem in human and veterinary populations. A key way veterinarians can limit AMR is through reducing overall antimicrobial use (ACVIM consensus statement 2015). Culture and sensitivity testing (C&S) is recommended prior to commencing any antibiotics, but in primary-care practice where time and finances are frequently limited, this is often not possible.

We therefore aimed to evaluate the clinical need for antibiotics in a common condition where it is suspected primary-care veterinarians overuse antibiotics. We chose to study haemorrhagic diarrhoea in dogs, where two antibiotics are often administered as standard. Both amoxicillin-clavulanic acid and metronidazole are typically prescribed to severe cases of haemorrhagic diarrhoea, but there is no evidence that this is beneficial to the patient.

A clinical trial was designed to determine whether dogs with haemorrhagic diarrhoea had an improved clinical outcome when receiving both amoxicillin-clavulanic acid and metronidazole, or whether administration of amoxicillin-clavulanic acid alone was sufficient. Dogs presenting to a private primary-care veterinary hospital with haemorrhagic diarrhoea < 3 day duration were recruited to an ethically approved, prospective, placebo controlled, blinded treatment trial with owner consent. Cases were randomised to receive either metronidazole or saline in a blinded manner, in addition to receiving standard supportive therapy; amoxicillin-clavulanic acid, intravenous fluid therapy, analgesia and a gastroprotectant. Diagnostics were performed in accordance with the usual primary-care practice protocol; routine haematology and biochemistry but no faecal C&S. The efficacy of treatment was assessed by the duration of hospitalisation, and daily clinical progress was measured by a clinical scoring system.

Between Feb 2016 and Jan 2018, 34 dogs successfully completed the clinical trial. For cases receiving metronidazole, the average duration of hospitalisation was 29.6hr (SD 15.4hr). For the saline placebo this was 26.3hr (SD 11.5hr). There was no significant difference between the two patient groups. All dogs in the trial showed a significant improvement in daily clinical score between day 1 (admission) and day 2 ($p < 0.001$), but there was no statistically significant difference between the daily clinical scores when comparing the patient groups.

In summary, this clinical trial has two valuable conclusions. First, by demonstrating that two antibiotics are not necessary for treatment of haemorrhagic diarrhoea we have shown that antibiotic usage can be safely reduced in these patients. Second we have proven that rigorous clinical trials can be performed in primary-care practice in the absence of extensive diagnostics. It is hoped that similar trials examining possible overuse of antibiotics in other common conditions will be conducted in the future.

ID06

Feline Retroviral Prevalence in Feral Cats on the San Francisco Peninsula, 2001-2003, 2005-2007, and 2014-2016

Charlotte H. Edinboro
Exponent, Inc. Health Sciences, Menlo Park, CA, USA

Peninsula Humane Society & SPCA';s [PHS';s] spay/neuter clinic provides discounted or free services for feral cats in San Mateo County, the suburban and rural county south of San Francisco. Feral cats receiving free services must be tested for feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), and if positive, they are euthanized. Over the years, feral cat caretakers sought spay/neuter services elsewhere to avoid this requirement. This study describes feline retroviral prevalence among feral cats presented to PHS';s spay/neuter clinic for the past 15 years.

Between 2001 and 2003, 28 of 1765 (1.6%) and 85 (4.8%) cats tested positive for FeLV and FIV, respectively. From 2005 through 2007, 22 of 1846 (1.2%) and 107 (5.8%) cats tested positive for FeLV and FIV, respectively. Between 2014 through 2016, 2 of 696 (0.3%) and 42 (6.0%) cats tested positive for FeLV and FIV, respectively.

Over the three 3-year periods, prevalence of FIV increased from 4.8% to 6.0% (p = 0.31) and the prevalence of FeLV decreased significantly from 1.6% to 0.3% (p < 0.03). For each year of the study, there were significantly more FIV-positive males than females (p < 0.01). Despite a 60% decrease in admissions in the third period, the prevalence of FIV for males remained similar across the years (p < 0.08), and increased for females, suggesting that FIV testing should continue among free-living community cats in San Mateo County.

ID07

Canadian K9 Lifetime (Lyme) Study: Descriptive analyses of Year 1 Data

Michelle Evason[1], *Jason Stull*[2], *David Pearl*[3], *Andrew Peregrine*[4], *Claire Jardine*[4], *J. Scott Weese*[5]
[1]*Atlantic Veterinary College University of Prince Edward Island Charlottetown, PE Canada, Charlottetown, PI, Canada,* [2]*Depart of Veterinary Preventive Medicine Ohio State University Columbus, Ohio USA;Dept of Companion Animal Atlantic Veterinary College University of Prince Edward Island Charlottetown, PE Canada, Charlottetown, PI, Canada,* [3]*Department of Population Medicine Ontario Veterinary College, University of Guelph,, Guelph, ON, Canada,* [4]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [5]*Dept of Pathobiology Ontario Veterinary College University of Guelph, Guelph, ON, Canada*

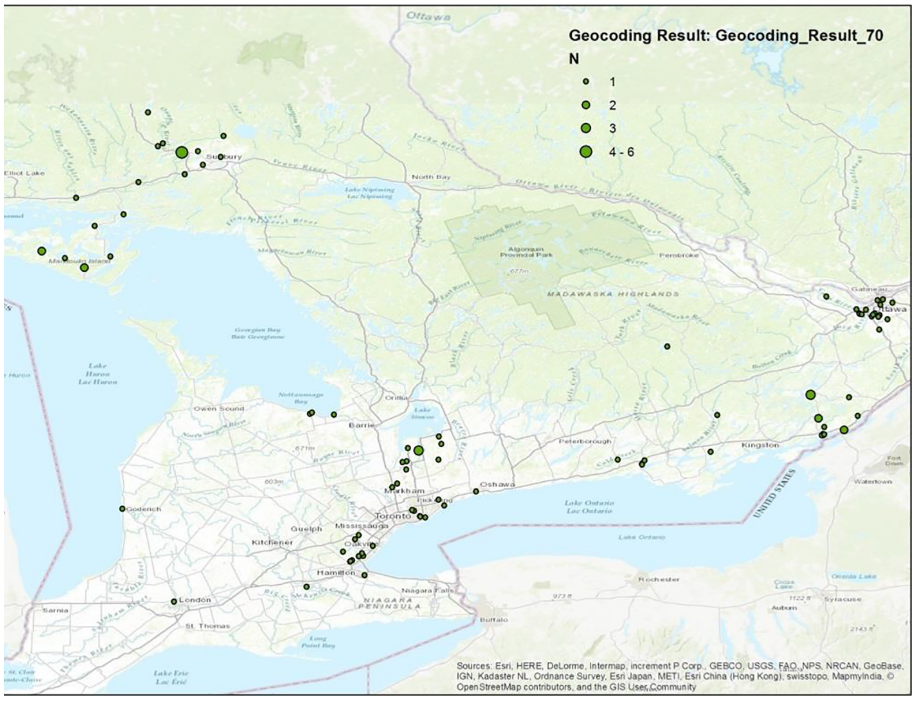
Screening for *Borrelia burgdorferi* seropositivity is commonly performed in dogs in conjunction with annual heartworm testing. Widespread canine testing provides seroprevalence data which are useful in identifying emerging and established Lyme disease risk areas, such as regions of Eastern, Atlantic and Central Canada. However, point prevalence data from uncharacterized populations have limitations. One example being that absence of clinical canine data precludes determination of true clinical disease prevalence and significance. Such clinical knowledge gaps can lead to confusion and potential mismanagement of dogs.

Given the current dearth of evidence to guide important clinical decisions regarding *B. burgdorferi* exposure and infection (along with myriad other infectious and non-infectious canine health concerns), a concerted effort beyond cross-sectional or case-control studies from referral facilities (with limited generalizability to primary practice) is needed. These types of veterinary longitudinal study efforts, such as the Morris Animal Foundation';s Golden Retriever Lifetime Study, are reliant on the development of educational partnerships with general practice veterinary clinics and recruitment of engaged study participants for their ongoing success.

Specific objectives of the study were to: 1) develop educational partnerships between veterinary clinics and veterinary schools in Canada; 2) enroll and engage dog owners in order to perform a long-term Lyme disease and canine health study; 3) evaluate the seroconversion rate of dogs to *B. burgdorferi*, *Ehrlichia* and *Anaplasma* spp. over a two-year period; 4) assess the incidence of clinical signs of Lyme disease compared to seroconversion; 5) identify factors associated with seroconversion, such as vaccination, tick prevention, dog demographics and environment; and 6) evaluate client perceptions, awareness and education on vector-borne disease and prevention.

General practice veterinary clinics in Ontario, Canada were invited to participate. Healthy dogs, 7 months of age and younger, were eligible for inclusion. At baseline visit, blood was tested for heartworm, *B. burgdorferi*, *Ehrlichia* and *Anaplasma* spp. (SNAP 4Dx® Plus® Test). An on-line questionnaire was completed after enrolment. Descriptive statistics were used to assess questionnaire data and baseline test results.

Pet-owners live in emerging and established Lyme disease risk areas of Ontario, Canada (Figure). Over 63% (62/98) of owners completed the questionnaire. Respondents (n=62) reported the majority of dogs were obtained from local breeders (65%), with 30% being first-time



puppy owners. Most owners lived in the suburbs of cities (50%) and almost all were female (97%).

All participants reported being aware of Lyme disease; however, none reported that a family or household member had been diagnosed with Lyme disease. The majority of respondents stated that they knew the cause of Lyme disease (92%) and correctly identified a tick as the vector of Lyme disease. However, only half of respondents actively tried to prevent tick attachment on themselves (56%), with 29% sometimes doing so, and 23% not trying to prevent tick attachment. Similarly, only 21% of respondents undertook non-chemical efforts (e.g. trimming of brush or shrubs) to reduce ticks on their property. Despite a high awareness of Lyme disease, few of the respondents recalled their human health care provider ever discussing Lyme disease (7%). Most (97%) did not recall ever receiving information about Lyme disease from any human health care source.

The majority of respondents recalled tick prevention being discussed by their veterinarian (95%) and were administering a tick prevention product to their dog (85%). Puppies were reported to be vaccinated for Lyme disease by 40% of respondents, while 15% were not certain if vaccination had occurred.

Baseline vector-borne disease testing (SNAP 4Dx® Plus® Test) for all 4 pathogens has been negative for all enrolled puppies to date (n =120).

This evaluation of baseline test results and confirmation of high pet-owner knowledge of Lyme disease will form the basis for ongoing and future research efforts utilizing this unique study population.

ID08

Investigation of the Association Between Lyme Seroreactivity and Chronic Kidney Disease in Dogs

Corie Drake[1], Jennifer S. Ogeer[1], Melissa J. Beall[2], Jesse S. Buch[2], Celeste A. Clements[2], Donald J. McCrann[2], Roberta L. Relford[2]
[1]IDEXX Laboratories, Inc., Markham, ON, Canada, [2]IDEXX Laboratories, Inc., Westbrook, ME, USA

Borrelia burgdorferi, the causative agent of Lyme disease, is a common tick-borne pathogen known to infect dogs in the United States. While infection with *B. burgdorferi* has a recognized, albeit uncommon, association with the onset of acute kidney disease, termed acute Lyme nephritis, less is known about associations between infection by this pathogen and the onset of chronic kidney disease in dogs. A retrospective cohort study was performed to determine if dogs with detectable antibodies to the VlsE C6 peptide of *B. burgdorferi* could be associated with an increased risk of kidney disease. Lyme serology data were obtained from the IDEXX Reference Laboratory (IRL) and in-clinic databases from 2003 through 2017. Chemistry and urinalysis results were obtained from the IRL database from July 2015 through January 2017. Patients exposed to VBD were defined as having a *B. burgdorferi* positive test result recorded at any point in time, while non-exposure was defined as having a negative result for all test events. For this study, chronic kidney disease (CKD) was defined as concurrent increased SDMA (> 14 μg/dL) and creatinine (> 1.5 mg/dL) for a minimum of 25 days with inappropriate urine specific gravity (USG B. burgdorferi, as evident by positive antibody to *B. burgdorferi*, within the defined Lyme region was found to be 1.43 with 95% confidence intervals [(1.27, 1.61), P < 0.0001]. This study identified an association between dogs with positive Lyme test results and a 43% increased risk for CKD.

ID09

Feline Vector-Borne Disease in Cats with Acute-Onset Fever

Barbara Qurollo[1], Ms. Elizabeth Walsh[2], Ms. Erica Lemler[2], Jeffrey Tyrrell[3], Jesse S. Buch[4], Ramaswamy Chandrashekar[5], Edward B. Breitschwerdt[6]
[1]NCSU-CVM, Raleigh, NC, USA, [2]NCSU-College of Veterinary Medicine, Raleigh, NC, USA, [3]NCSU-College of Veterinary Medicine, 1060 William Moore Drive, NC, USA, [4]IDEXX Laboratories, Inc., Westbrook, ME, USA, [5]IDEXX, Inc., Westbrook, ME, USA, [6]North Caroline State University College of Veterinary Medicine, Fuquay Varina, NC, USA

Acute-onset fever (AOF) is a common finding in cats and may be an indicator for infection with a vector-borne pathogen. To better understand the prevalence of feline vector-borne pathogens (FVBP), serological and molecular diagnostic assays were used to prospectively screen cats with AOF for FVBP. PCR was used to detect infection with *Anaplasma*, *Bartonella*, *Babesia*, *Cytauxzoon*, *Ehrlichia*, *Hepatozoon*, *Mycoplasma*, and *Rickettsia* spp. *Anaplasma*, *Borrelia burgdorferi* and *Ehrlichia* seroreactivity was tested by the SNAP®4DX®Plus Test; and *Bartonella henselae*, *B. koehlerae*, and *B. vinsonii* subsp. *berkhofii* seroreactivity was assessed by IFA testing. AOF cat blood and serum samples with corresponding physical examination findings, CBCs and biochemistry panels were submitted between June 2015 and December 2017 by private practice veterinarians. Inclusion criteria included flea and/or tick exposure, AOF illness (103-106◦F) without an obvious historical (trauma) or physical examination (abscess) cause, and owners had to agree to return for follow-up blood collection for convalescent serology. Study objectives included: 1) determine the prevalence of specific FVBP in cats with AOF: 2) identify differences in clinicopathological abnormalities between FVBP-infected and uninfected cats. Eighty four cats met the inclusion criteria. Corresponding convalescent samples were submitted from 40/84 (47.6%) cats. Cats resided primarily in Florida (27/84; 32%) and North Carolina (32/84; 38%) and included 49 (57.6%) males, 35 (41.7%) females, 14 (16.5%) pure breeds and 70 (83.3%) mixed breeds; the average age was 4.4 years. The majority (64%) of cases occurred in the summer (29/84; 34.5%) and fall (25/84; 29.8%) seasons. FVBP were detected by PCR in 38/84 (45.2%) cats and included *B. henselae* (25/84; 29.8%), *Mycoplasma haemominutum*. (7/84; 8.3%), *Hepatozoon* spp. (2/84; 2.4%), *M. haemofelis* (1/84; 1.2%), *A. phagocytophilum* (1/84; 1.2%), *C. felis* (1/84; 1.2%) and *R. felis* (1/84; 1.2%). Cats were seroreactive to *Bartonella* spp. (57/84; 67.9%), *B. burgdorferi* (2/84; 2.4%), *Ehrlichia* spp. (1/84; 1.2%) and *Anaplasma* spp. (1/84; 1.2%). When comparing acute with convalescent samples (n = 40), 1 *B. clarridgeae/B. henselae* co-infection and 2 *B. henselae* infections were newly diagnosed by PCR. Most convalescent samples were Bartonella seroreactive (27/40; 67.5%) with 17 (42.5%) having been seroreactive with the corresponding acute sample; 7 (17.5%) cats had a ≥ 4 fold increase in Bartonella titers between acute and convalescent samples. One acute *A. phagocytophilum* PCR+ cat seroconverted to *Anaplasma* by SNAP®4DX®Plus Test. Clinicopathologic data was collected only at the time of acute sample collection. The average rectal temperature

for all 84 AOF cats was 104.6°F. Other predominant clincopathological abnormalities included heart murmurs (17/84; 20.2%), ocular discharge (14/84; 16.6%), neutrophilia (38/84; 45.2%), anemia (24/84; 28.6%), thrombocytopenia (24/84; 28.6%), hyperbilirubinemia (15/84; 17.6%) and hyperglobulinemia (11/84; 13.1%). Of the 65 cats tested for FeLV/FIV, all were FeLV antigen negative and 4 were FIV seropositive. To evaluate potential FVBP-AOF clinicopathological risk factors, cats were grouped by FVBP PCR+ (n = 38), *B. henselae* PCR+ (n = 25) and PCR− (n = 48) results. In cats with AOF, neutrophilia was significantly associated with a FVBP (p = 0.037; Odds Ratio (OR) 2.6; 95% CI: 1.050 – 6.205) or *B. henselae* (p = 0.003; OR 4.7; 95% CI: 1.633 – 13.461) PCR+ result compared to PCR− cats. Based on our study, FVBPs are common in cats with AOF. *Bartonella* spp. were the most prevalent FVBP; however, prevalence rates were comparable to rates reported in cats without AOF. *Hepatozoon* spp. may be a more frequent cause of AOF in cats in the United States than previously determined. Clinicians should routinely promote the use of flea and tick-borne preventive strategies in cats and consider FVBP in the etiology of AOF, particularly if associated with neutrophilia.

ID10

Three-Year Longitudinal Seroprevalence of Vector-borne Pathogens in Mid-Missouri Dogs

Leah A. Cohn[1], *Jennifer Braff*[2], *Brendon Thatcher*[2], *Melissa J. Beall*[2], *Ramaswamy Chandrashekar*[2], *Kaylyn M. McDaniel*[1]
[1]*University of Missouri, Columbia, MO, USA,* [2]*IDEXX Laboratories, Inc., Westbrook, ME, USA*

Dogs are commonly infected with vector borne pathogens, and seropositive dogs without clinical evidence of disease are frequently identified. This study followed the serological and clinical status of dogs in mid-Missouri over the course of 3 years in regard to *Ehrlichia*, *Anaplasma*, and *Borrelia* spp. A convenience population of dogs belonging to staff and students at the University of Missouri College of Veterinary Medicine was used. Dogs aged in range from 1 to 10 years at the start of the study. Preliminary findings regarding change in serologic status over the first two years have previously been presented in abstract form; this is an update with more complete data.

Dogs not suspected of having vector borne disease were enrolled in a three-year prospective study. Dogs underwent physical exam and owners completed a history questionnaire periodically, and at the same visit blood samples were submitted to IDEXX Laboratories for testing. Plasma was separated and analyzed using a multi-analyte SNAP® research ELISA for antibodies to Bb (Lyme), E-genus (*E. canis/E. chaffeensis*), Ech (*E. chaffeensis*), Ew (*E. ewingii*), Ec (*E. canis*), A-genus (*Anaplasma* genus), Apl (*A. platys*), and Aph (*A. phagocytophilum*). If dogs were found to be seronegative for all tested pathogens, testing was repeated q 6 months. If dogs were positive at any time point for any tested pathogen, testing was repeated q 4 months. Seropositive dogs were not treated with antibiotics unless a clinical illness developed during the three years that the treating veterinarian (unrelated to our study) believed warranted antibiotics. Tick prevention was used only at the owner's discretion.

A total of 858 samples were collected from 118 dogs; 101 participated in every planned sampling point while 10 dogs were euthanized, one died, and five moved out of state. Antibodies to *Ehrlichia* spp. were recognized in as many as 33 of dogs on at least one occasion. Antibodies to *Anaplasma* spp. were recognized in only 4 dogs, and no dog was ever positive for *Borrelia* C6 peptide. Serologic status of individual dogs varied from time point to time point, with both seroconversion and reversion to a seronegative status.

Dogs in this mid-Missouri population were frequently seropositive for *E. ewingii* and *E. chaffeensis*, however very few dogs had clinical evidence consistent with ehrlichiosis that prompted treatment. Evidence of seroconversion and intermittent seropositive results were common without clinical disease. Dogs may remain constantly or intermittently seropositive for *Ehrlichia* spp. at least 3 years without clinically obvious disease.

ID11

Clinical and Laboratory Findings in Cats Fed Toxoplasma gondii Sporulated Oocysts

Janelle Scott, Michael Lappin
Colorado State University, Fort Collins, CO, USA

It is now known that people commonly acquire *Toxoplasma gondii* infection by ingestion of sporulated oocysts passed in cat feces. Since cats are obligate carnivores, it has been assumed that most are infected with *T. gondii* by ingesting tissue cysts in prey species. However, the incidence, clinical sequelae, and laboratory findings associated with sporozoite-induced *T. gondii* infection in cats are poorly understood. The primary purpose of this study was to optimize the human *T. gondii* sporozoite antibody assay for use with cat sera for evaluation in subsequent epidemiological studies. To generate positive control sera against sporozoites for use in the assay titrations, 3 young adult cats were each fed 100,000 sporulated oocysts of the ME49 strain of *T. gondii* and monitored for systemic and ocular signs of toxoplasmosis. The cats were group housed during the day and enclosed in separate cages at night to enable collection of feces for sugar centrifugation and microscopic examination for oocysts. *Toxoplasma gondii* IgM and IgG antibodies against *T. gondii* tachyzoites were measured on Day 3, 7, 10, 14, 17, 21, 28, 35, 42, 49, and 56. Ocular lesions or systemic signs of disease were not recognized. Oocysts were seen in the feces of one cat on Day 46. One cat developed both IgM and IgG antibodies on Day 17; one cat developed IgG antibodies alone, first detected on Day 28, and one cat developed both IgM and IgG antibodies on Day 28. The IgG antibodies persisted through the experiment. The results confirm that cats that ingest sporulated T. gondii oocysts can become infected but shed lower levels of oocysts and have delayed seroconversion when compared to historical controls who were fed tissue cysts. The samples collected will be used in the sporozoite antibody assay titration and an ongoing study of naturally exposed cats.

ID12

Comparison of Immediate versus Delayed Streak Plate Inoculation of Urine Bacterial Culture and Sensitivity Testing

Emily Coffey[1], *Kim Little*[1], *Davis Seelig*[1], *Aaron Rendahl*[2], *Jennifer Granick*[1]
[1]*University of Minnesota, Saint Paul, MN, USA,* [2]*University of Minnesota, Minneapolis, MN, USA*

Quantitative bacterial culture and sensitivity testing is the gold standard for diagnosing bacterial urinary tract infections. Samples are

commonly transported to external reference laboratories prior to inoculation of culture media. The objective of this study was to compare the results of bacterial culture and sensitivity testing of canine and feline urine samples when streak plate inoculation is performed immediately after sample collection versus when streak plate inoculation is delayed until arrival at a reference laboratory.

This was a prospective, observational study that included urine samples from 194 canines and 45 felines submitted for routine urinalysis and urine culture and sensitivity testing. Streak plate inoculations of urine samples were performed immediately after sample collection, and again after receipt by an outside reference laboratory. Culture and sensitivity results were compared. Additional data collected included signalment, comorbidities, presence of lower urinary tract signs, urinalysis results, and antimicrobial history.

Overall agreement between immediate and delayed culture was 87%. While 16% of samples were collected via free-catch, they represented 41% of discrepant culture results. However only 8% of free-catch, versus 50% of cystocentesis, culture discrepancies were deemed to have a clinical impact. Pyuria was significantly associated with positive culture results.

Use of external reference laboratories for urine culture and sensitivity testing is generally reliable if samples are stored and transported according to pre-established guidelines. Though discordant results of immediate versus delayed culture of cystocentesis samples were more likely to alter clinical decisions, these discrepancies were uncommon.

ID13

Prospective Observational Study: Serum Intercellular Adhesion Molecule 1 (sI-CAM-1) in Canine Leptospiral Pulmonary Hemorrhage Syndrome

Franziska Sonderegger, Thierry Francey, Ariane Schweighauser, Eliane Marti, Jelena Mirkovitch, Simone Schuller
University Bern, Bern, Bern, Switzerland

Leptospiral pulmonary hemorrhage syndrome (LPHS) is a severe manifestation of leptospirosis, which affects both humans and animals, and is associated with high mortality. The pathogenic mechanisms of LPHS are poorly understood. Aim of this prospective observational clinical study was to examine whether soluble I-CAM-1 (sI-CAM-1), a serum marker of endothelial cell activation and dysfunction, is altered in dogs with leptospirosis and whether this marker is correlated with the occurrence of pulmonary hemorrhage and outcome.

Ethical approval was obtained for the study. Prospectively collected day 1 from healthy dogs and day 1 and 3 serum samples from dogs with leptospirosis with and without pulmonary hemorrhage and dogs with acute kidney injury (AKI) due to other causes were included. Dogs were deemed healthy based on an uneventful history and normal physical examination. Dogs with leptospirosis presented consistent clinical, laboratory and imaging findings and at least one microscopic agglutination test (MAT) titre ≥1:800 or *Leptospira*-PCR positivity. Dogs with AKI due to other causes showed negative MAT on one or paired samples and a convincing alternative diagnosis. Serum sI-CAM-1 was measured using a commercial ELISA-kit (SEA548Ca; Brunschwig) and results compared between groups using the Kruskal-Wallis Test. Associations between sI-CAM-1, disease group and outcome were tested using logistic regression analysis. The diagnostic accuracy of day 1 sI-CAM-1 to predict the occurrence of LPHS was tested using ROC curve analysis.

Median sI-CAM-1 serum concentrations were significantly higher in dogs with AKI (43.6 ng/ml, IQR 18.9-72.1; n = 18) and in dogs with LPHS (64.2 ng/ml, IQR 42.4-107.1; n = 14) compared to heathy controls (15.8 ng/ml, IQR 8.8-29.9; n = 31; $p < 0.001$). Median sI-CAM-1 was higher in dogs with leptospirosis without LPHS (37.6 ng/ml, IQR 20.9-53.4, n = 10) compared controls, and lower than in the LPHS group, but these differences did not reach statistical significance. Few day 3 samples were available for analysis, but showed similar trends with highest sI-CAM-1 in dogs with LPHS (87.5 ng/ml, IQR 44.1-101.7; n = 5) compared to healthy controls, dogs with AKI (21.9 ng/ml, IQR 10.5-71.3; n = 5) and leptospirosis without LPHS (26.2 ng/ml, IQR 23.2-29.3; n = 2). However, only the difference between healthy and LPHS group was statistically significant ($p = 0.015$). There was no significant association between sI-CAM-1, disease group and outcome. Day 1 sI-CAM-1 predicted the development of LPHS with reasonable accuracy (AUC 0.79; sensitivity 79%, CI 49-95; specificity 73%, CI 60-84).

These findings suggest, that in the context of leptospirosis, overexpression of endothelial ICAM-1 may be associated with the development of pulmonary hemorrhage.

ID14

Antimicrobial Resistance: Comparison of Canine and Pediatric Escherichia coli Urinary Isolates in Washington State

Cheryl Adler[1], Peter Rabinowitz[2], Sally Trufan Vegso[2], Marguerite Pappaioanou[2], Laren Frisbie2, Scott J. Weissman[3]
[1]University of Washington Center for One Health Research/ Phoenix Central Labs/ Washington State Reserve Veterinary Corps, Seattle, WA, USA, [2]University of Washington Center for One Health Research, Seattle, WA, USA, [3]Seattle Children's Hospital, Seattle, WA, USA

Antimicrobial resistance is a growing problem within both veterinary and human medicine. While much attention and publication has been dedicated to the resistance to and use of antimicrobial agents in human medicine and in food producing animals, less is known about the potential for exchange of antimicrobial resistant bacteria between companion animals and their humans. The Washington State Integrated Surveillance for Antimicrobial Resistance (WISAR) project is a statewide collaboration spanning human medicine, human public health surveillance, veterinary medicine, and veterinary medical surveillance. Within these collaborative efforts we examined the relative frequencies of antimicrobial resistance phenotypes of select organisms across the close interface of dogs and children.

We examined data from a regional human pediatric medical center (2010-2016), and from a veterinary clinical laboratory and a veterinary reference laboratory (2002-2017). We focused on urinary isolates from pediatric and canine populations to compare resistance patterns most common in each and to examine for the emergence of similar resistance patterns. We examined the resistance of *E. coli* urinary tract isolates to five major classes of antibiotics from dogs and children. We further examined the prevalence of phenotypic resistance patterns to combinations of drug classes.

E. coli isolates, 2002-2017 (urinary and non-urinary)		% Non-susceptible (#isolates testing non-susceptible)							
		Penicillins	Cephems		Fluoroquinolones		Aminoglycosides	Folate Inhibitors	Tetracyclines
Host Species	# Isolates tested	Ampicillin	Ceftiofur	Ceftriaxone	Ciprofloxacin	Enrofloxacin	Gentamicin	Trimethoprim/ Sulfamethoxazole	Tetracycline
Human (*E.coli*)	4938	**0.0% (1)**		**5.2% (4938)**	**9.9% (4939)**		**6.8% (4938)**	**29.6% (4938)**	**0.0% (1)**
Canine (*E. coli*)	3483	**27.8% (3465)**	**15.2% (3374)**			**7.4% (3480)**	**7.4% (3490)**	**7.6% (3483)**	**6.7% (1945)**

 # Isolates in the second column indicates the total possible isolates for human *E .coli*
(#n) = the denominator # of isolates used to calculate the percentage

We found that in canine urinary isolates, the prevalence of *E. coli* resistance to the class representatives of third generation cephalosporins (ceftiofur) was higher than resistance of human urinary isolates to ceftriaxone. We did not detect, however, an increasing rate of resistance in the canine isolates over the study period. The prevalence of the phenotype commonly associated with *E. coli* isolates of the ST131 clonal lineage, featuring resistance to both cephalosporins and quinolones, was also more common in the companion animal isolates compared to the human isolates.

Our findings suggest a higher rate of *E. coli* resistance to third generation cephalosporins is found in canine patients compared to human pediatric patients. While this difference could be due to differences in sampling and analysis, (including the use of different class representatives), it is deserving of further investigation since there are implications both for veterinary practice as well as public health. Our findings also highlight the importance of developing and validating integrated databases of antimicrobial in humans and animals in a particular region.

ID15

Distribution of the Feline Lungworm Aelurostrongylus abstrusus in the United States Based on Fecal Testing - Sponsored By IDEXX

Ariel J. Carruth[1], Jesse S. Buch[1], Jennifer Braff[1], Ramaswamy Chandrashekar[1], Dwight D. Bowman[2]
[1]IDEXX Laboratories, Inc., Westbrook, ME, USA, [2]College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Aelurostrongylus abstrusus is a globally-distributed lungworm known to infect the lower respiratory tract of cats that can result in bronchitis or pneumonia. Although *A. abstrusus* is considered endemic in parts of Europe, its distribution in the United States is largely unknown due to the utilization of insensitive diagnostic test methods and a lack of widespread prevalence studies. More epidemiological data about this parasite's presence within the United States is needed to raise awareness among veterinary professionals and identify at-risk regions. The objective of the present study was to compile commercial reference laboratory data over a seven-year period to determine the distribution of *A. abstrusus* within the United States based on widespread fecal testing in cats.

The results of 3,136,542 feline ova and parasite (O&P) zinc sulfate centrifugation fecal flotation tests performed at IDEXX Reference Laboratories in the United States from January 2010 through September 2017 were compiled and sorted for tests positive for *A. abstrusus* larvae. The results of 2,790 Baermann tests, currently considered the gold standard diagnostic for this feline lungworm, were also retrieved from the same time period.

Of the feline fecal flotation tests performed, 3,949 (0.13%) were positive for the presence of *A. abstrusus* larvae. In addition, 65 (2.3%) of the Baermann tests conducted were also positive. As reported in other studies, age was a significant risk factor with cats 1 to 12 months having a relative risk of 5.72 (95% confidence interval: 5.30-6.18, P < 0.0001) compared to cats older than 12 months. In contrast, there was no association observed between infection status and sex. Significant variation in

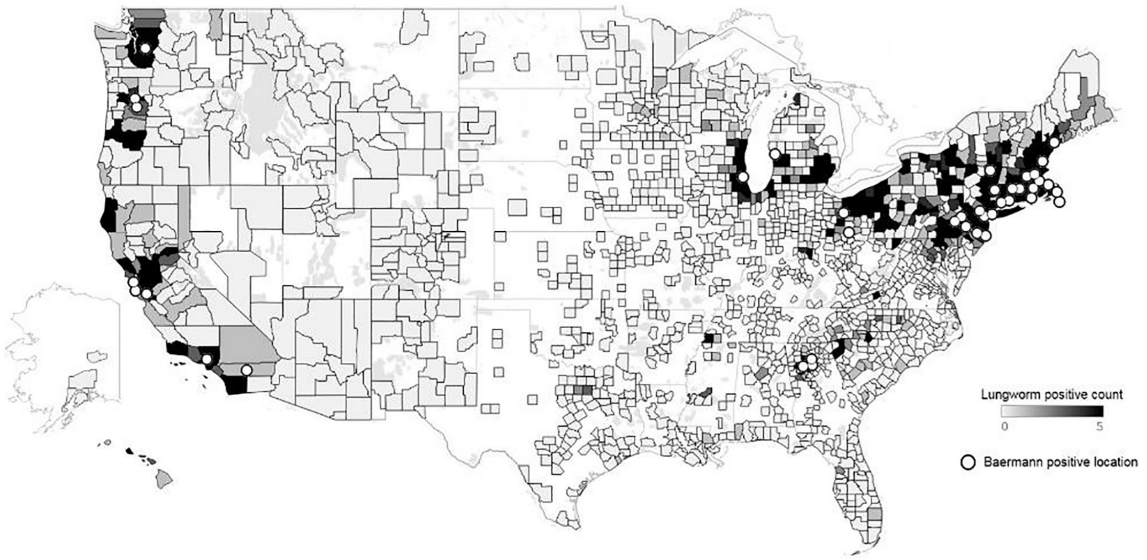


FIGURE 1 Map showing the US distribution by country of samples positive for *Aelurostronglylus abstrusus* via fecal flotation along with the distribution of Baermann positive samples (based on postal code)

positive rates were observed by region (Northeast = 0.19%, 95% confidence interval: 0.18-0.20%, n=1,245,592; Midwest = 0.13%, 0.12-0.14%, n=749,510; West = 0.06%, 0.05-0.07%, n=642,108; South = 0.04%, 0.03-0.04%, n=499,332) and the majority of positive cases were clustered in the Northeast, Great Lakes, and West Coast areas of the United States, as shown in Figure 1.

This study highlights the emergence of feline lungworm in the United States and the importance of utilizing appropriate testing to accurately diagnose this disease. Compared to the gold standard Baermann method, fecal flotation is significantly less sensitive for detecting *A. abstrusus* larvae [Traversa et al., Parasites & Vectors 2010, 3:62] and likely underestimates the true infection rate in this population of cats. Recent epidemiological surveillance of a canine lungworm, *Angiostrongylus vasorum*, and its emergence into new areas of Europe has been greatly enhanced by the introduction of new high-throughput serological tests [Liu et al., Veterinary Parasitology: Regional Studies and Reports 2017, 7:45-47]. Similarly, the introduction of higher throughput and less labor-intensive diagnostic methods could help increase awareness of this parasite among veterinary professionals, achieve a greater understanding of epidemiological factors, and improve the care and treatment for clinically ill feline patients.

ID16

ELISA Seroreactivity to Three Bartonella Species in Dogs in the United States - Sponsored By IDEXX

Anton Mestek[1], Brendon Thatcher[1], Ariel J. Carruth[1], Nandhakumar Balakrishnan[2], Pradeep Neupane[3], Barbara A. Qurollo[3], Barbara C. Hegarty[4], Jancy Hanscom[1], Jesse S. Buch[1], Edward B. Breitschwerdt[5], Ramaswamy Chandrashekar[1]
[1]IDEXX Laboratories, Inc., Westbrook, ME, USA, [2]Virginia Commonwealth University, Henrico, VA, USA, [3]North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, [4]North Carolina State University College of Veterinary Medicine, Cary, NC, USA, [5]North Caroline State University College of Veterinary Medicine, Fuquay Varina, NC, USA

The genus *Bartonella* consists of pleomorphic Gram negative bacteria that induce long-lasting intraerythrocytic and endotheliotropic infections within the vasculature of dogs, humans and other animals. Infection with at least 10 *Bartonella* species have been documented in pet dogs worldwide. In particular, infection with *B. henselae*, *B. koehlerae* and *Bartonella vinsonii* subsp. *berkhoffii* have been reported in dogs with endocarditis, vasoproliferative pathology, vasculitis, myocarditis, polyarthritis, granulomatous lymphadenitis and hepatitis, epistaxis, and neurologic symptoms.

Previous reports have evaluated *Bartonella* seroreactivity in large canine populations using either indirect immunofluorescent antibody (IFA) assays [Lashnits, E. *et al*. (2018) J. Vet. Intern. Med. DOI: 10.1111/jvim.14890], or ELISAs [Henn, J.B. *et al*. (2005) Am. J. Vet. Res. 66:688]. The current study expands on previous work by using whole cell protein lysates from each of three clinically relevant *Bartonella* species as antigens in three separate ELISA assays. Archived dog sera (n = 5957) submitted to IDEXX Reference Laboratories from across all 9 census regions of the United States between May and August 2016 were used to evaluate each of the three *Bartonella* ELISAs.

Overall 5.0%, 6.8%, and 3.9% of sera were ELISA reactive to *B. henselae* San Antonio 2 (*Bh*SA2), *B. vinsonii* subsp. *berkhoffii* genotype II (*Bvb*T-II), and *B. koehlerae* (*Bk*) whole cell lysates, respectively. Further, 3.1% of dog sera were reactive in all three *Bartonella* sp. ELISAs, whereas 0.9%, 2.4%, and 0.3% of sera were only reactive to *Bh*SA2, *Bvb*T-II, or *Bk* whole cell lysates, respectively. Seroreactivity to two *Bartonella* sp. whole cell lysates in one of three following combinations, included *Bh*SA2 / *Bvb*T-II (0.9%), *Bk* / *Bvb*T-II (0.4%), or *Bh*SA2 / *Bk* (0.05%).

By Chi-squared analysis, dogs from the South Atlantic, East and West South Central regions had higher overall *Bartonella* seroreactivity rates relative to dogs in other US census regions. Breed comparisons indicate that Toy dogs were less likely to be *Bartonella* seroreactive compared to Mix, Sporting and Working dogs. Gender (male vs female) did not have significant impact to *Bartonella* seroreactivity. Adult dogs (aged 1 to 5 years) were more likely to be *Bartonella* seroreactive relative to younger dogs (aged 7 to 12 months).

Our whole cell ELISA results are concordant with previously published dog seroprevalence studies. We again found substantially lower *Bartonella* seroprevalence rates in dogs as compared to cats from the same geographic region. Because of variability among individual dogs (seroreactivity detected to only one, two or to all three ELISAs), our results indicate that multiple antigens are required for serologic documentation of exposure to or infection with these three *Bartonella* species.

ID17

Highly Specific Screening Tests Maintain Actionable Positive Predictive Values in Areas with Low Heartworm Prevalence - Sponsored By IDEXX

Jennifer Braff[1], Jesse S. Buch[1], Andrei Rakitin[1], Melissa J. Beall[1], Ramaswamy Chandrashekar[1], Dwight D. Bowman[2]
[1]IDEXX Laboratories, Inc., Westbrook, ME, USA, [2]College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Canine heartworm disease is a globally-distributed and potentially fatal form of dirofilariosis caused by *Dirofilaria immitis* infections transmitted via mosquito vectors. In the United States, the overall prevalence of canine heartworm disease has declined significantly since the late 20th Century due to widespread usage of commercially-available chemoprophylactic drugs. Despite this progress, autochthonous transmission persists nationwide and the risk of heartworm infection for unprotected dogs remains. Therefore, dogs are screened annually using rapid tests that detect the presence of heartworm antigen in blood as recommended by the American Heartworm Society.

At low prevalence, the positive predictive value of a diagnostic screening test is directly proportional with disease prevalence within the region of use. Consequently, in areas where the prevalence of heartworm infection has dropped below the claimed false positive rate (1 - specificity) for a screening test, rare positive test results may be suspected as false positives. The aim of this study was to re-evaluate the field-observed specificity of the SNAP® 4Dx® Plus Test for heartworm (HW) antigen and estimate the local positive predictive value (PPV) for specific California counties.

To better understand the diagnostic value of positive HW screening results in low-prevalence areas such as California, in-clinic SNAP® 4Dx® Plus Test HW antigen test results collected from 2013 to 2016 were compared for both Scandinavia, where virtually no autochthonous transmission of HW is believed to occur, and California. Although positive test results in Scandinavian clinics may be due to infections acquired elsewhere, to be conservative we considered all positive HW test results from Scandinavian clinics to be false positives. The observed specificity was therefore defined as the proportion of Scandinavia SNAP® 4Dx® Plus HW tests that returned a negative result. Duplicate tests for a single patient within a given year were removed from the dataset prior to analysis. Using the observed Scandinavian specificity, prevalence data from the Companion Animal Parasite Council (www.capcvet.org), and the stated test sensitivity, we calculated the positive predictive values in California counties using standard methodology based on Bayes theorem. Confidence intervals for all proportions were calculated using the USDA-recommended Clopper-Pearson exact method.

SNAP® 4Dx® Plus HW antigen test results from 190 veterinary clinics in Finland, Norway, and Sweden were analyzed to determine the observed test specificity. Of the 14,778 Scandinavian tests results analyzed, 18 were positive for HW antigen giving a positive rate of 0.12% (95% CI: 0.07% to 0.19%) and an observed specificity of 99.88% (95% CI: 99.81% to 99.93%). For the same period, 301,997 SNAP® 4Dx® Plus HW antigen test results were identified from 476 California veterinary clinics representing 46 counties. Of these, 1,742 were positive for HW antigen for a positive rate of 0.58% (95% CI: 0.55%-0.60%). The positive rate for the SNAP® 4Dx® Plus HW test in California was found to be significantly higher than the rate observed in Scandinavia by chi-square test ($P < 0.001$). Therefore, the higher than expected rate of SNAP® 4Dx® Plus HW test positives cannot be solely attributed to a lack of specificity (false positives) but strongly suggests the actual presence of *Dirofilaria immitis* in California. This aligns with recent studies which reported HW infection among coyote and mosquito populations within California [Huang et al., J Med Entomol. 2013 Nov;50(6):1315-23].

Additionally, significant variation in HW positive rates were observed between counties within California. Of 53 California counties with at least 30 results reported to CAPC during the study period, all had at least one dog test positive to HW. From 2013-2016, Madera County had the highest SNAP® 4Dx® Plus Test HW positive rate (9.06%, 95% CI: 6.06-12.91%, n=298) while San Benito County had the lowest (0.00%, 95% CI: 0.00-0.63%, n=588). The overall PPV of the SNAP® 4Dx® Plus Test for HW in California was estimated at 82.02% and was well above 50% for all counties with a between-county range of 67.32-98.04%.

The results from this study support that any HW-positive test results on the SNAP® 4Dx® Plus Test, due to its high specificity, are highly probable to be true positives. Therefore, it is important for veterinarians in low prevalence regions of California and other areas of the United States to adhere to American Heartworm Society guidelines and confirm unexpected test results by retesting with an ancillary rapid test (such as the SNAP® Heartworm RT Test) or reference laboratory test (such as the PetChek® Heartworm PF Antigen Test).

ID18

Experimental Infection of Dogs with H3N2

Michael R. Lappin[1], Louana Cheung[1], Julia Veir[1], Jennifer R. Hawley[2], Eileen Ball[2], Sharon Wappel[3]
[1]Colorado State University, Loveland, CO, USA, [2]Zoetis Animal Health, Parsippany, NJ, USA, [3]Zoetis, Kalamzoo, MI, USA

There are 2 canine influenza A viruses in the United States, the H3N8 strain that originated from horses in the USA (2004) and the H3N2 strain which is an avian strain from Asia first detected in dogs in the USA in 2015. Field experiences with H3N2 suggests the agent is shed intermittently and longer than H3N8. The purpose of this study was to define the duration and magnitude of H3N2 shedding in experimentally inoculated dogs.

In this descriptive pilot study, 8 dogs were inoculated in a chamber with a USA field strain of H3N2 on Day 0. Each primarily inoculated dog was paired with another dog (secondary exposure) on Day 1 (1 dog), Day 2 (2 dogs), Day 3 (2 dogs), Day 4 (2 dogs) and Day 5 (1 dog). The 16 dogs were housed in 2 rooms, each containing 4 pens. A clinical scoring rubric was applied to each dog by 2 masked observers for 45 minutes twice daily for 42 days and oropharyngeal swabs were collected once daily for qualitative and quantitative PCR assay.

All 16 dogs developed clinical signs, as early as 30 hours after exposure, including the dog that was not exposed until Day 5 after the primary inoculation of the pen mate. In the first 21 days after primary inoculation, 3 of the 8 secondary exposure dogs had total clinical scores and cough scores greater than the paired primarily inoculated dog. For the 7 of the 8 direct contact dogs, the last PCR positive day was 1 day (1 dog), 2 days (3 dogs), and 5 days (3 dogs) after the first positive result. One dog was clinically ill, but never positive for H3N2 nucleic acids in the qualitative assay. Clinical signs were minimal in all dogs after Day 21 and PCR assay results were negative in all dogs on Day 21. Quantitative PCR assay results are pending.

The results to date suggest that dogs with primary exposure to this dose and strain of H3N2 will be contagious for up to 5 days.

ID19

Use of Vancomycin in Life-Threatening Infection in Dogs and Cats, 41 Cases (2003-2017)

Ian M. DeStefano[1], Annie Wayne[1], Elizabeth Rozanski[1], Jonathan Babyak[1], Jason Mallada[2]
[1]Tufts University, North Grafton, MA, USA, [2]Beth Israel Deaconess Medical Center, Brookline, MA, USA

Vancomycin is an antibiotic reserved for Gram-positive infections not susceptible to other antimicrobials. There are minimal published data of vancomycin use in naturally-occurring infections in small animals. The goal of this study was to evaluate the indications, dosing, potential adverse effects, and survival to discharge in dogs and cats treated with vancomycin. An additional goal was to evaluate if vancomycin treatment was based on current recommendations and best practices. Records were retrieved using the keyword “vancomycin” and retrospectively reviewed.

Records from thirty-three dogs and eight cats were reviewed. The most common indications for vancomycin included infections

involving the integument (10/41, 24.4%) or hepatobiliary systems (8/41, 19.5%), endocarditis (6/41, 14.6%), and infected orthopedic implants (6/41, 14.6%). The most common culture isolates included *Enterococcus sp.* (27/92, 29.3%), *E. coli* (19/92, 20.7%), and methicillin-resistant *S. aureus* (MRSA) (13/92, 14.1%). The most common dosing interval was 15 mg/kg IV q6h (24/41, 58.5%). Acute kidney injury as defined by IRIS guidelines occurred after starting vancomycin in 6/41 cases (14.6%). Neutropenia or allergic reaction was not documented in any animal. There were negative culture results or lack of culture evidence for vancomycin use in 8/41 cases (19.5%). In 22/41 cases (53.6%), culture results documented the availability of an alternative antimicrobial. Most patients survived to discharge (30/41, 73.2%).

Adverse effects attributable to vancomycin use were infrequent in dogs and cats. Most patients in this study survived to discharge. In most cases, there were potential alternative effective antimicrobials or lack of culture data to support treatment with vancomycin.

ID20

Interleukin (IL)-2, IL-6, IL-8, IL-10 and Tumor Necrosis Factor-Alpha in Dogs Infected with Angiostrongylus vasorum

Bernhard Gerber[1], *Felicitas Boretti*[2], *Luca Aresu*[3], *Nadja Sieber-Ruckstuhl*[2], *Manuela Schnyder*[4]
[1]*Clinic for Small Animal Internal Medicine, Vetsuisse Faculty University of Zurich, Zurich, Zurich, Switzerland,* [2]*Clinic for Small Animal Internal Medicine, Vetsuisse Faculty University of Zurich, Switzerland, Zurich, Zurich, Switzerland,* [3]*Department of Veterinary Science, Università degli Studi di Torino, Italy, Torino, Piemonte, Italy,* [4]*Institute of Parasitology, Vetsuisse Faculty University of Zurich, Switzerland, Zurich, Zurich, Switzerland*

Infection with *Angiostrongylus vasorum* (Av) is an emerging disease in dogs. The disease may stimulate pro-inflammatory and regulatory cytokines in dogs. The aim of the study was to evaluate cytokine changes in dogs experimentally infected with Av.

Sixteen beagle dogs were orally infected with third stage larvae. Eight dogs were treated with an anthelmintic against Av after 30 days and the other eight were left untreated. Blood samples were collected before the study and 56 to 59 after the infection from each dog. IL-2, IL-6, IL-8, IL-10 and TNF-α were measured with dog specific sandwich immunoassays using electrochemiluminiscence.

There was no significant difference of IL-2, IL-6, IL-8, IL-10 and TNF-α between treated and untreated dogs before infection. The median serum levels of IL-2, IL-6, IL-8, IL-10 and TNF-α in all dogs before the infection were 6.2, 5.3, 2'318.0, 1.5 and 0.7 pg/ml respectively. At the end of the study median IL-2, IL-6, IL-8, IL-10 and TNF-α were 7.7, 7.0, 472.9, 4.1 and 0.3 pg/ml respectively in untreated dogs and 3.2, 4.4, 1'381.6, 1.6, and 0.6 pg/ml respectively in treated dogs. IL-8 decreased significantly in both, treated and untreated dogs. IL-10 increased significantly only in untreated dogs. IL-2, IL-6 and TNF-α did not change significantly after infection in both, treated and untreated dogs.

The survival of Av in the host might be partly due to an increase of the regulatory cytokine IL-10. Avoiding of an increase or even decreasing of pro-inflammatory cytokines might be another survival strategy of the parasite.

ID21

Assessment for associations Between Bartonella Spp. and Select Complete Blood Cell Count Abnormalities

Michael R. Lappin[1], *Christian M. Leutenegger*[2], *Jennifer Braff*[3], *Jesse S. Buch*[3], *Ramaswamy Chandrashekar*[3]
[1]*Colorado State University, Fort Collins, CO, USA,* [2]*IDEXX Laboratories, Inc., West Sacramento, CA, USA,* [3]*IDEXX Laboratories, Inc., Westbrook, ME, USA*

There are multiple *Bartonella* spp. strains that infect cats. Most cats have subclinical *Bartonella* spp. infections but a variety of clinical and laboratory abnormalities like fever, endocarditis, and hyperglobulinemia have been reported. *Bartonella henselae* is one of the most common species that infects cat, has an intra-erythrocyte phase, and has been associated with vasculitis. While there are many studies of *Bartonella* spp. infections of cats, most have low sample sizes. The purpose of this study was evaluate for associations between *Bartonella* spp. DNA in the blood of cats and select complete blood cell count abnormalities.

The medical records data base (IDEXX) was searched for feline cases that had blood evaluated in a PCR assay that amplifies DNA of *Bartonella* spp. and also had results available from a complete blood cell count completed concurrently. The cases were first classified as those being tested as blood donors and those that were suspected to be clinically ill and then samples from states with low risk for *C. felis* were excluded (AK, AZ, CO, MT, NM, NV, UT). Associations among clinical status, select complete blood cell abnormalities (yes or no) and *Bartonella* spp. PCR assay results (positive or negative) were assessed by calculating odds ratios and 95% confidence intervals.

Clinically ill cats were more likely (OR = 9.0315; 95%CI = 3.9658 to 20.5675; P < 0.001) to be positive for *Bartonella* spp. DNA in blood (120 of 2,567 samples = 4.7%) than blood donor cats (6 of 1111 samples = 0.54%). Cats with platelet counts of Bartonella spp. DNA in blood (16 of 310 samples = 4.9%) than cats with >200,000/μl (120 of 2,834 samples = 2.6%). Cats with a hematocrit of 28 were less likely (OR = 0.6074; 95%CI = 0.3872 to 0.9528; P = 0.036) to be positive for *Bartonella*spp. DNA in blood (25 of 782 samples = 3.2%) than cats with a hematocrit > 28% (92 of 1,784 samples = 5.2%).

Since other clinical information like use of flea control or antibiotics are not available, these results should be interpreted carefully. In addition, the estimated *Bartonella* spp. prevalence data from the blood donor cats was likely lowered by selection of cats of low risk. However, the results seem to confirm those of other smaller studies that failed to link *Bartonella* spp. to anemia in cats. Further studies will be required to confirm the weak association between *Bartonella* spp. DNA in blood and the presence of thrombocytopenia.

ID22

Pradofloxacin for Treatment of Bartonella henselae in Experimentally Inoculated Cats

Michael R. Lappin[1], *Ronan Fitzgerald*[2]
[1]*Colorado State University, Fort Collins, CO, USA,* [2]*Bayer Animal Health, Leverkusen, Bayern, Germany*

Bartonella henselae is the most common cause of cat scratch disease and other clinical syndromes in people. Cats develop high levels of

B. henselae bacteremia and are associated with human infections, particularly in the presence of *Ctenocephalides felis*. Several antibiotic protocols used for the treatment of *B. henselae* infection in cats have failed to clear bacteremia. The purpose of this study was to assess the safety and efficacy of a high dose pradofloxacin protocol to eliminate *B. henselae* bacteremia in experimentally inoculated cats.

Infection with *B. henselae* was initiated in 8 cats by intravenous inoculation of infected feline blood. Each of the cats had been positive for *B. henselae* DNA in blood and positive for *Bartonella* spp. IgG in serum for 12 weeks prior to entering this study. Pradofloxacin was administered at 7.5 mg/kg, PO, twice daily for 28 days. Complete blood cell counts were performed prior to pradofloxacin administration and then every 2 weeks for 10 weeks. *Bartonella* PCR assay and *Bartonella* spp. IgG serology were performed prior to pradofloxacin administration and approximately every 2 weeks for 10 weeks and then weekly for 4 weeks. Methylprednisolone acetate was administered at 5 mg/kg by intramuscular injection to all cats on week 10.

There were no clinical side effects noted during the pradofloxacin administration period. None of the cats developed anemia or a neutrophil or lymphocyte count lower than the reference range. After pradofloxacin administration, all cats were negative for *B. henselae* DNA in blood on all sample dates. One cat with a maximal IgG titer of 1:128 was intermittently negative after starting pradofloxacin administration (7 of 11 samples). Another cat with a maximal IgG titer of 1:64 was negative the last 5 sample collections of the study.

The pradofloxacin protocol used in this study was well tolerated. *Bartonella henselae* bacteremia was not detected in any cat after administration of pradofloxacin even after the administration of a dose of methylprednisolone acetate. The results suggest an antibiotic effect or that the organism was cleared spontaneously. The study should be repeated with cats infected with *B. henselae* by exposure to infected *C. felis* and cats infected in the field.

ID23

Efficacy and Safety of Novel isonitrile Compounds for Treatment of Staphylococcus pseudintermedius

Ashley Hopkins, Lynn Guptill, Abhijit Mukhopadhyay, Haroon Mohammad
Purdue University, West Lafayette, IN, USA

This study tested the hypothesis that aryl isonitrile compounds will significantly reduce *Staphylococcus pseudintermedius* replication and survival in planktonic culture and biofilm, and are not toxic for mammalian cells. Aryl isonitrile compounds were recently shown to have potent activity against drug-resistant *Staphylococcus aureus*.

A panel of 67 compounds synthesized in Dr. Mingji Dai's laboratory at the Purdue Institute for Drug Discovery was screened against two *S. pseudintermedius* isolates; 16 compounds with minimum inhibitory concentration (MIC) ≤ 2 μM were evaluated further against a panel of 8 isolates. *Staphylococcus pseudintermedius* isolates from animals treated at Purdue University's Veterinary Teaching Hospital were obtained from the Indiana Animal Disease Diagnostic Laboratory. Minimum bactericidal concentration (MBC) and MIC were tested using Clinical and Laboratory Standards Institute methods. Efficacy against biofilm was tested using the 96 well plate method. Toxicity for mammalian cells (Madin-Darby canine kidney (MDCK) cells) was assessed via MTS (3-[4,5,dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4- sulfophenyl]-2H-tetrazolium, inner salt) assay. MIC and MBC ranges where 50 percent of *S. pseudintermedius* isolates were inhibited were 4 to 18 and 4 to 28 μM respectively. Compounds were not toxic to MDCK cells at concentrations well above MIC (64 μM). Four compounds were tested against established biofilms; all disrupted over 50 percent of biofilm at 32 μM; three disrupted over 90 percent of biofilm at 64 μM. Aryl isonitrile compounds had potent activity against planktonic *S. pseudintermedius*, were not toxic for mammalian cells, and effectively disrupted established biofilms. These compounds hold great promise for further development as treatments for *S. pseudintermedius*.

ID24

Genetic Characterization of Canine Urinary Pathogenic Escherichia coli

Megan E. Jacob[1], Jessica M. Gilbertie[1], Anna Rogers[1], Keri Norman[2], Gizem Levent[2], H. Morgan Scott[2]
[1]North Carolina State University, Raleigh, NC, USA, [2]Texas A&M University, College Station, TX, USA

Urinary tract infections (UTIs) are a common morbidity of canines, affecting approximately 14% of the companion population at least once in their lifetime. The most commonly isolated bacterial species associated with canine UTIs is *Escherichia coli*. Treatment of uncomplicated UTI is typically antimicrobial therapy. In the small subpopulation of dogs that develop chronic urinary tract infections, repeated, prolonged, and increasingly aggressive antimicrobial therapies are often used. Genetic characterization of *E. coli* associated with canine UTIs would be valuable to optimize empiric antimicrobial therapy choices and to understand association between clinical presentation, pathogenesis and persistence. Our hypothesis is that urinary pathogenic *E. coli* (UPEC) associated with canine UTIs are diverse, yet predictable associations between specific virulence (e.g. toxin, biofilm) and antimicrobial resistance genes can be elucidated.

A collection of 79 *E. coli* isolates collected from canine urine samples were previously characterized for O and H antigen types, antimicrobial susceptibility, and ability to form biofilm. Here, a subset of these isolates (n = 16) were characterized using whole genome sequencing on the MiSeq platform.

Thirteen different *E. coli* sequence types were recovered; two sequence types (127 and 156) were found among multiple isolates. *E. coli* sequence type 156 was associated with multiple samples collected from the same patient three months apart. *E. coli* sequence type 127 was found in three different dogs. A number of different antimicrobial resistance genes were identified, including *aac(6′) lb-cr*, *aad*A, *aad*B, *aph(3′)-I*, bla_{CMY}, bla_{CTX-M}, bla_{OXA}, bla_{TEM}, *cat*, *cml*, *cml*A, *dfr*A, *mph*(A), *str*A, *str*B, *sul*1, *sul*2, *sul*3, and *tet*(B). The presence of several of these genes has importance in classifying these isolates as extended-spectrum beta lactamase (ESBL) producers. Sequence type 127 which represented three isolates lacked associated resistance genes. All sequenced isolates had at least one fimbriae-associated gene, and *eae*H, a gene associated with *E. coli* attachment was found

in 15 (94%) isolates. Previously, the presence of one fimbriae-associated gene, *fim*H has been strongly associated with the ability to form biofilm, and was detected in 15 of 16 isolates here.

In conclusion, *Escherichia coli* causing canine UTIs appear diverse; still, the presence of consistent attachment and virulence genes was noted across sequence types. Antimicrobial resistance genes and phenotypic resistance to cephalosporins were detected in several samples and are concerning for therapeutic choice and animal handling considerations. Further work to explore associations among virulence, antimicrobial resistance, and biofilm potential is underway to better understand UPEC presentation and pathogenesis in dogs.

NU01

Increase in Serum Symmetric Dimethylarginine Level Following Anesthesia in Dogs

Shinichi Namba[1], Masahiko Befu[2], Masahiko Befu[2], Ryo Kitamura[2], Takao Amaha[2], Tomone Zama[2], Taku Moriwaki[2], Shohei Kumono[2], Saki Shichijo[2]

[1]Laboratory of Veterinary Biochemistry, College of Bioresource Sciences, Nihon University/Marble Veterinary Medical Center, Fujisawa, Kanagawa, Japan, [2]Marble Veterinary Medical Center, Fujisawa, Kanagawa, Japan

Serum levels of creatinine (sCr) and blood urea nitrogen (BUN) have been routinely used as biomarkers of kidney function. However, they are insufficient for the early detection of kidney dysfunction, because their serum levels rise only after severe histopathological damage has occurred in the kidneys. Symmetric dimethylarginine (SDMA) is a structural isomer of the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine. Asymmetric dimethylarginine is metabolized by dimethylarginine dimethylaminohydrolase, whereas SDMA is eliminated by renal excretion. In this study, we investigated the serum levels of SDMA before and after anesthesia in dogs.

Client-owned dogs were anesthetized with Propofol (n = 80) or Alphaxalone (n = 20). Blood samples were collected 2-4 hours before initiation of anesthesia and immediately after completion of the surgical procedure. Levels of SDMA were measured at IDEXX Laboratories, and measurements of sCr and BUN were performed at the Marble Veterinary Medical Center.

In dogs that were anesthetized with Propofol, serum levels of SDMA measured after anesthesia (15.95 ± 4.03 μg/dL) were significantly higher than those measured before anesthesia (11.85 ± 3.06 μg/dL, $P < 0.01$). Levels of sCr and BUN, traditional markers of renal function, also increased. Additionally, BUN and sCr levels were significantly and positively associated with SDMA levels ($r = 0.23$ and $r = 0.42$, respectively). In the Alphaxalone-treated dogs, the average serum level of SDMA before anesthesia was 12.15 ± 1.79 μg/dL, which increased to 17.70 ± 4.64 μg/dL after anesthesia, whereas smaller increases were detected in sCr and BUN.

In this study, an increase in serum SDMA was observed in both Propofol- and Alphaxalone-treated dogs, whereas the levels of sCr and BUN only increased in Propofol-treated dogs. Taken together, these data suggest that serum SDMA concentration is likely to be a sensitive biomarker for kidney dysfunction.

NU02

Evaluation of Serum Symmetric Dimethylarginine in Dogs Naturally Infected by Leishmania infantum

Felipe dos Santos Muniz[1], Vitor Márcio Ribeiro[2], Pedro Paulo Abreu Teles[3]

[1]Santo Agostinho Veterinary Hospital Belo Horizonte-MG, Belo Horizonte, Minas Gerais, Brazil, [2]Pontifical Catholic University of Minas Gerais/ Santo Agostinho Veterinary Hospital Belo Horizonte-MG, Belo Horizonte, Minas Gerais, Brazil, [3]Federal University of Minas Gerais (UFMG) /Santo Agostinho Veterinary Hospital Belo Horizonte-MG, Belo Horizonte, Minas Gerais, Brazil

Canine visceral leishmaniasis (CVL) is a zoonotic disease caused by the protozoa *Leishmania sp.* Among the clinical alterations observed in dogs infected by the protozoa, symptoms associated with decrease of renal function are often described. Serum symmetric dimethylarginine (SDMA) has recently been evaluated as a renal biomarker that can efficiently detect early renal dysfunction in dogs. The level of serum SDMA has shown a significant relationship with the glomerular filtration rate because it is only excreted through renal clearance in dogs.

This study evaluated serum SDMA in 60 dogs naturally infected by *L. infantum.*

The inclusion criteria of the dogs in the study were to have either positive Elisa, Rifi above 1:160 or positive reaction on Snap Leishmania Idexx. All dogs, regardless of their renal staging by the IRIS (International renal interest society), were submitted to the following exams: serum SDMA, complete blood count, reticulocytes, serum creatinine, serum blood urea nitrogen (BUN), serum phosphorus, urinalysis, urinary protein creatinine ratio (UP/C), systemic blood pressure (BP) and abdominal ultrasonography. The dogs were divided into the IRIS stages: At Risk Stage, Stage 1, Stage 2, Stage 3 and Stage 4. The correlation between renal markers and SDMA was determined by Pearson';s coefficient of correlation analysis.

Serum SDMA has shown a 99.9% relationship with serum creatinine ($R = 0.89$; $P = 0.001$), serum BUN ($R = 0.81$, $P = 0.001$) and serum phosphorus ($R = 0.7$, $P = 0.001$). The relationship of serum SDMA with the UP/C was 95% ($R = 0.31$, $P = 0.05$). The serum SDMA presented a 99% negative relationship with the hematocrit ($R = -0.46$, $P = 0.01$) and urinary density ($R = -0.39$, $P = 0.01$). With reticulocytes the negative relationship was 95% ($R = -0.306$, $P = 0.05$). Serum SDMA had 60% relationship with BP ($R = 0.09$, $P = 0.47$). Dogs in IRIS at Risk stage had a serum SDMA increased on 13.3% (4/30), in IRIS stage 1 31.2% (5/16), IRIS stage 2 50% (3/6), IRIS stage 3 83.3% (5/6) and IRIS stage 4 100% (2/2). All dogs with hyperphosphatemia showed increased serum SDMA

The present data shows that serum SDMA has a strong relationship with several parameters used in the evaluation of renal function, such as serum creatinine, serum phosphorus, serum BUN, hematocrit, UP/C, urinary density and reticulocytes. Serum SDMA had a weak relationship with BP. Serum SDMA increased prior to image alteration on kidney ultrasonography in 13.3% (3/30) of the dogs. Serum SDMA also increased in 15% (9/60) of dogs before values of serum creatinine were above 1.4mg / dl. The differences found in IRIS staging considering serum SDMA were: of the dogs in IRIS stage 2, 33.3% (2/6) had serum SDMA above 25μg / dl, so these dogs were reclassified from IRIS stage 2 to IRIS stage 3. Dogs in IRIS stage 3, 16.6% (1/6) had

serum SDMA above 45µg / dl so this dog was reclassified from IRIS stage 3 to IRIS stage 4. So we conclude that serum SDMA is an important tool for the evaluation of renal function and should be used routinely in the evaluation of dogs infected with *L. infantum*.

NU03

Validation of an Point-of-Care Immunoassay for Measurement of Symmetric Dimethylarginine in Feline Serum

Graham E. Bilbrough[1], *Barbara Evert*[1], *Karen Hathaway*[1], *Gina Panagakos*[1], *Maha Yerramilli*[1], *Jane E. Robertson*[2]
[1]*IDEXX LABORATORIES INC, Westbrook, ME, USA,* [2]*IDEXX Laboratories, Inc., Westbrook, ME, USA*

Measurement of symmetric dimethylarginine (SDMA) is used for diagnosis and monitoring of kidney disease in veterinary medicine. Recently, IDEXX introduced a novel immunoassay to measure SDMA on the Catalyst® chemistry analyzers. The assay performance for feline samples has not been published elsewhere.

This study evaluates the in-clinic Catalyst SDMA Test (CatalystSDMA) for SDMA measurement in feline samples by comparing results with those obtained by liquid chromatography–mass spectrometry (LC-MS; technique previously validated for feline samples).

Serum samples from 113 cats were obtained from IDEXX Reference Laboratories (RefLab). The samples were originally submitted for clinical purposes and, after all requested testing was completed and the standard retention period (7 days; 4 ˚C), the RefLab typically disposes of remaining material. Instead, under the RefLab Terms and Conditions, the residual samples were frozen until use in this study.

Samples were thawed in batches and analyzed once using CatalystSDMA, on a Catalyst One® Chemistry Analyzer, and once using LC-MS (RefMethod). The study was completed over several days, however, for each sample, all testing was completed within four hours. All testing was completed at IDEXX.

Results are reported with 95 % confidence limits in parentheses.

RefMethod median SDMA was 21.5 µg/dL; interquartile range (IQR) of 11.7 to 32.5 µg/dL; range: 5.1 to 85.3 µg/dL. CatalystSDMA median SDMA was 21.7 µg/dL; IQR: 11.3 to 32.9 µg/dL; range: 1.9 to 79.5 µg/dL. No statistical difference existed between SDMA concentrations obtained by the two methods (P = 0.88; Mann-Whitney U test).

Passing-Bablok regression analysis: intercept 1.0 µg/dL (0.0 to 2.0); slope 1.0 (0.9 to 1.1); Tau 0.84. The Pearson';s correlation coefficient (*r*) was 0.94. The mean difference (Bland-Altman plot) was 0.0 µg/dL; standard deviation: 5.7 µg/dL (4.9 to 6.4). Limits-of-agreement (LOA) plot showed no fixed or proportional bias, with increased variation at the higher end of the dynamic range where unlikely to impact clinical decisions. LOA upper limit 11.1 µg/dL (9.4 to 13.0). LOA lower limit -11.1 µg/dL (-13.0 to -9.3).

For each assay, results to the nearest whole number were assigned to one of three categories: ≤ 14 µg/dL; 15 to 19 µg/dL; ≥ 20 µg/dL. The percentage agreement (concordance) between the two assays was calculated. There was good agreement (85 %) on results classification.

The minimal bias, good concordance and excellent correlation to the reference method provide confidence that CatalystSDMA can be used for in-clinic measurement of feline SDMA.

NU04

Complications of Transurethral Ectopic Ureter Correction

Timothy C. McCarthy
VetMIST(Veterinary Minimally Invasive Surgery Training), Beaverton, OR, USA

The purpose of this presentation is to report a complication in three cases of transurethral ectopic ureter correction(TUCLCUE). Transurethral cystoscopy (TUC) is the procedure of choice for correction of ectopic ureters in female dogs. Since the first correction was performed in 2004 this procedure has been performed at multiple institutions with reports on technique and success rates. Significant complications other than failure to completely correct incontinence have not been reported.A 2 year old spayed female Golden Retriever was presented for lethargy and abdominal pain 18 months after bilateral TUCLCUE. Ultrasound revealed a large hydronephrotic right kidney with perirenal fluid and right hydroureter. TUC revealed complete occlusion of the right neostoma. Right nephrectomy and ureterectomy were performed at the owners insistence. Communication was received from a remote client that their 2 year old female Australian Shepherd was diagnosed with right side hydronephrosis and hydroureter 18 months after bilateral TUCLCUE. Communication was received from a remote client 2 years after TUCLCUE was performed on a 4 month old female Newfoundland with ultrasound showing early hydronephrosis and hydroureter that was monitored with ultrasound. Transurethral cystoscopy revision of the neostoma has been recommended in all cases. The last case was reexamined with TUC after an inadequate response to TUCLCUE and a portion of the left vaginal ectopic ureter had not been completely corrected. Revision of the neostoma and residual vaginal ectopic ureter was performed. Spontaneous closure of the neostoma after TUCLCUE surgery is a significant complication of the procedure. Postoperative monitoring TUCLCUE cases with ultrasound is recommended to diagnose hydronephrosis and hydroureter early and allow early stoma revision to preserve the involved kidneys.

NU05

Serum Calcification Propensity In Cats with Chronic Kidney Disease

Henk van den Broek[1], *Ruby Chang*[1], *Jonathan Elliott*[1], *Rosanne Jepson*[2]
[1]*Royal Veterinary College, London, England, United Kingdom,* [2]*Royal Veterinary College, Hertfordshire, England, United Kingdom*

Vascular calcification is an important risk factor in human chronic kidney disease (CKD) and associated with dysregulated mineral metabolism. Precipitation of calcium and phosphate starts by formation of soluble colloidal primary calciprotein particles (CPPs) which transform to crystalline secondary CPPs. Shorter duration of this transformation step (i.e. increased serum calcification propensity) is associated with increased mortality in human CKD patients. Although vascular calcification is under recognized in cats with CKD, mineral disturbances similar to those observed in human CKD are common. Plasma fibroblast growth factor 23 (FGF23) concentration is a marker of mineral disturbances in feline CKD. Here, we examined serum calcification propensity in a cross-section of cats with varying degrees of renal dysfunction, and its relationship with plasma FGF23, phosphate, total

calcium, magnesium, and total protein concentrations, and calcium phosphate product.

Blood samples were prospectively collected from 5 euthyroid non-azotemic cats ≥9 years old, 12 cats with International Renal Interest Society (IRIS) stage 2 CKD, and 3 cats with IRIS stage 3 CKD, and stored at -80 C. Serum calcification propensity was measured with a functional assay that detects the transformation time (T_{50}) of spherical primary CPPs to elongated secondary CPPs by a diagnostic company blinded to the clinical data (Calciscon, Nidau-Biel, Switzerland). Intact FGF23 was measured in EDTA plasma using a validated ELISA assay (Kainos Laboratories, Tokyo, Japan). Serum T_{50} values among the three groups were compared using one-way ANOVA with post-test for linear trend. Correlations between serum T_{50} and plasma FGF23, phosphate, total calcium, magnesium, and total protein concentrations, and calcium phosphate product were assessed with Spearman's rank correlation (r_s).

The mean T_{50} value was 250 (standard deviation [SD], 58.4) minutes in non-azotemic cats, 204 (SD, 66.5) minutes in cats with IRIS stage 2 CKD, and 119 (SD, 51.5) minutes in cats with IRIS stage 3 CKD. Mean T_{50} was different among groups (P = 0.037) with a significant linear trend (P = 0.011), indicating that serum calcification propensity increased in groups with worsening kidney function. Serum T_{50} was inversely correlated with plasma FGF23 (r_s = -0.63; P = 0.003) and total protein (r_s = -0.53; P = 0.017), but did not correlate significantly with plasma creatinine (r_s = -0.42; P = 0.069), phosphate (r_s = -0.34; P = 0.146), total calcium (r_s = -0.23; P = 0.341), calcium phosphate product (r_s = -0.27; P = 0.250), or magnesium (r_s = 0.27; P = 0.258).

These preliminary data suggest serum of cats loses its intrinsic properties to inhibit calcification with declining renal function. The velocity with which serum calcified was inversely associated with plasma FGF23 and total protein concentrations, which may suggest that dysregulation of mineral metabolism and chronic inflammation are underlying mechanisms of soft tissue calcification in cats. Further research in a larger population of cats is needed to confirm our findings and assess the prognostic value of serum calcification propensity in cats with CKD.

NU06

Prospective Evaluation of the Incidence of Canine Hypercalciuria and iCa Concentration with Calcium Oxalate Urolithiasis

Joao Felipe. de Brito Galvao[1], *Cristiane C. Otoni*[1], *Dennis J. Chew*[2], *Jessica A. Hokamp*[2], *Maxey Wellman*[2]
[1]*VCA Arboretum View Animal Hospital, Downers Grove, IL, USA,* [2]*The Ohio State University, Columbus, OH, USA*

Hypercalciuria has been suggested to be a predisposing cause for the development of calcium oxalate (CaOx) urolithiasis in dogs. The purpose of the study was to identify the incidence of hypercalciuria via fasting urine calcium to creatinine ratio (uCa:Cr) in dogs that were evaluated for CaOX urolithiasis.

Fasting uCa:Cr that was collected at initial evaluation was considered increased if the ratio were >0.06 based on previously published data. Dogs were included in this study if CaOx urolithiasis was confirmed via quantitative calculi analysis and ionized calcium (iCa), routine serum chemistry, CBC, and urinalysis were available for evaluation. Dogs were divided into hypercalciuric andnonhypercalciuricstone-forming groups. Mann-Whitney test was used to compare groups.

Thirty-two dogs were evaluated between 6/2016 and 1/2018. Sixteen dogs (50%) had increased UCa:Cr. Dogs with hypercalciuria had a higher median ionized calcium (1.33 mg/dL) than nonhypercalciuric (1.24 mg/dL) dogs. Sixteen dogs had an iCa in the upper half of the reference (>1.3 mg/dl). Urine specific gravity (median 1.018 vs. 1.032; *P=0.04*) was lower and total calcium (11.4 vs. 10.2 mg/dl; *P=0.002*) was higher in the hypercalciuric group. More than half (56%) of the hypercalciuric dogs had recurrent calculi compared to 38% of the non-hypercalciuric group. Fractional excretion of calcium was 4.2 times *(P<0.0001)* and 3.8 times *(P<0.0001)* greater in hypercalciuric dogs compared to nonhypercalciuric when using iCa and TCa respectively.

Hypercalciuria may be an additional predisposing factor for calcium oxalate urolithiasis. It is possible that correction of hypercalciuria will decrease the recurrence rate of canine CaOx urolithiasis.

NU07

Clinical Presentation and Prognosis of 77 Dogs Diagnosed with Focal Segmental Glomerulosclerosis by Renal Biopsy

Sarah K. Lorbach, Rachel E. Cianciolo, Jessica M. Quimby, Jessica A. Hokamp
The Ohio State University, Columbus, OH, USA

Focal segmental glomerulosclerosis (FSGS), a disease caused by irreversible podocyte injury, is an important cause of proteinuria in dogs and humans. It is the most common non-immune complex glomerulopathy in dogs and has a previously reported prevalence of approximately 20 % in dogs biopsied for suspected glomerular disease, with females being over-represented. The purpose of this study was to retrospectively characterize the clinical presentation and prognosis of FSGS in dogs.

Clinical and histopathological data and cause of death were obtained by reviewing the records of dogs diagnosed with FSGS on renal biopsies submitted to the International Veterinary Renal Pathology Service (IVRPS) between January 2015 and May 2017. FSGS is a disease characterized by an absence of immune - complex deposits and the presence of a segmental consolidation of the glomerulus by extracellular matrix; it is often associated with adhesions to Bowman';s capsule and hyalinosis. Kaplan Meier survival analysis with log - rank test was used to determine whether International Renal Interest Society (IRIS) stage, urine protein : creatinine ratio (UPC), presence of severe hypoalbuminemia (< 2 g / dL), ascites or edema at biopsy, history of hypertension (HT), or percentage of global glomerulosclerosis (GS) on biopsy were associated with death from renal disease or any cause. A two - sample test of proportion was used to assess the significance of the male : female distribution.

Of 299 dogs with renal biopsies submitted to IVRPS with proteinuria during the study period, 77 (26 %) were diagnosed with FSGS. Median age was 9.5 years at biopsy (range 2.3 - 14.8 years), and 48 (62 %) of dogs were female (p = 0.04). Median serum creatinine concentration was 1.2 mg / dL (range 0.3 - 8.7 mg / dL). Median serum albumin concentration (Alb) was 2.8 g / dL (range 1.1 - 4.6 g / dL). HT was documented prior to or at the time of biopsy in 44 / 72 (61 %) of dogs. Median UPC was 5.9 (range 1.4 - 22) and 6 dogs were reported to

have ascites / edema at biopsy. At the time of data collection, 23 dogs (30 %) were alive, 38 (49 %) were deceased and 16 (21 %) were lost to follow up. Of the 38 dogs that died (all cause death, AC), 18 (47 %) were renal-related deaths (RD). RD and AC dogs in IRIS stage 3 or 4 at biopsy had significantly shorter survival post - biopsy (SPB) than those in IRIS stage 1 or 2 (RD $p < 0.001$; AC $p < 0.001$). RD and AC dogs with Alb < 2 g / dL had significantly shorter SPB than dogs with Alb > 2 g / dL (RD $p = 0.01$; AC $p = 0.004$). RD and AC dogs with ascites / edema at time of biopsy had significantly shorter SPB compared to dogs without (RD $p = 0.001$; AC $p = 0.03$). AC dogs with history of HT had significantly shorter SPB compared with AC dogs without a history of HT ($p = 0.03$). RD dogs with GS affecting ≥ 25 % of glomeruli had significantly shorter SPB than RD dogs with < 25 % GS ($p = 0.03$). UPC was not significantly associated with SPB in RD or AC dogs.

In this retrospective study, FSGS had a similar prevalence to what has been previously reported. Females continued to be over-represented. Severe hypoalbuminemia and IRIS stages 3 or 4 were associated with a poorer prognosis in all dogs with FSGS. Hypertension was associated with a shorter survival in dogs that died of any cause, whereas biopsy samples with at least 25 % global glomerulosclerosis were associated with a poorer prognosis in dogs that were known to have renal-related deaths.

NU08

Pre and Postprandial Urine Calcium-to-Creatinine Ratio to Identify Calcium Oxalate Urolithiasis in Miniature Schnauzers

Susan Carr, David Grant, Stefanie DeMonaco, Megan Shepherd
Virginia Polytechnic and State University, Blacksburg, VA, USA

The intent of this research is to identify a simple diagnostic test to detect abnormal calciuresis and predict calcium oxalate (CaOx) urolith presence in Miniature Schnauzers. We are investigating the impact of postprandial time on the specificity of urine calcium:creatine (UCa:Cr) in identifying affected dogs.

The hypotheses are: 1) Significant differences exist in fasted and postprandial UCa:Cr between urolith-forming and control schnauzers. 2) UCa:Cr increases significantly from fasted baseline at one or more postprandial time point(s).

Urine samples are being collected from Miniature Schnauzers with (urolith-formers) and without (controls) CaOx uroliths in a fasted state and 1, 2, 4, and 8 hours after feeding a standardized diet. The change in UCa:Cr from baseline was calculated for each postprandial time. Urolithiasis status and the time point were assessed for impact on the UCa:Cr and change in UCa:Cr using a mixed model ANOVA.

Based on 7 urolith-forming and 12 control dogs enrolled thus far, urolith-forming Miniature Schnauzers have significantly higher mean UCa:Cr at 1 hour, 4 hours and 8 hours postprandial timepoints indicating altered calciuresis. The change in UCa:Cr was not significant at any post-prandial time point between or within groups.

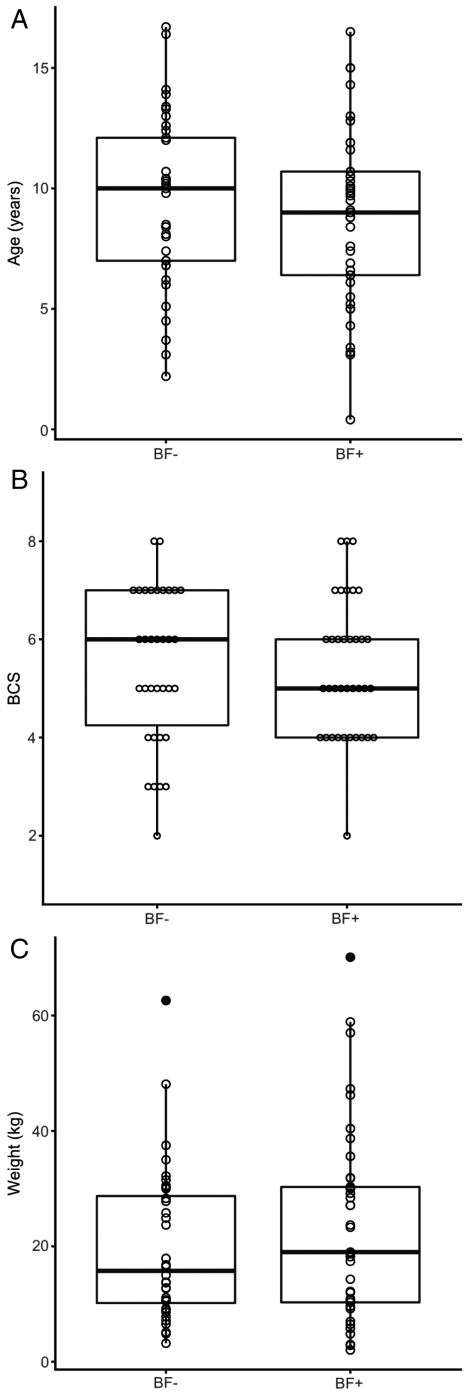
Urolith forming Miniature Schnauzers have excessive calciuresis, providing insight into the mechanism behind their formation of CaOx uroliths. If using the Ca:Cr ratio, the postprandial sampling time is not critical. This simple urine measurement has potential as a marker of urolith presence and possibly risk of urolith formation.

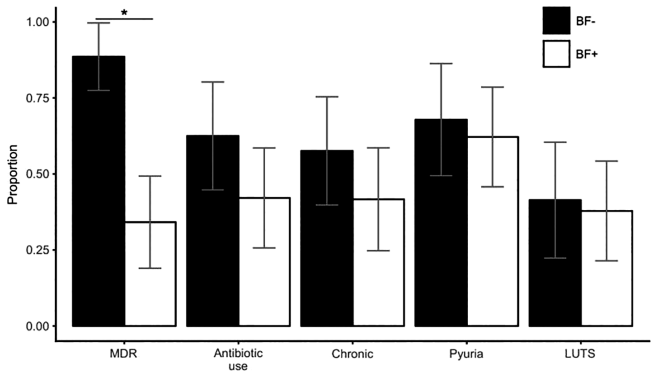
NU09

Population Characteristics and Clinical Presentation of Dogs with Biofilm-forming Escherichia coli Urinary Tract Infection

Zachary Kern, Megan E. Jacob, Jessica M. Gilbertie, Shelly L. Vaden, Sara K. Lyle
North Carolina State University, Raleigh, NC, USA

The purposes of this study were (1) to describe the population of dogs with biofilm-forming *Escherichia coli* (*E. coli*)UTIs, and (2) to determine whether there were clinical differences between dogs with biofilm-forming *E. coli* UTIs and dogs with non-biofilm-forming *E. coli* UTIs. We hypothesized there would be no difference in the two population characteristics of dogs whereas biofilm-formation would be more prevalent in dogs whose UTIs are chronic, complicated, and/or asymptomatic.





This was a retrospective cross-sectional study in which we evaluated 76 client-owned animals with *E. coli* UTIs, divided into two groups based on the *in vitro* biofilm-forming capability of the *E. coli* isolates. Biofilm formation was established using a crystal violet assay. Medical records of the dogs were reviewed and their population characteristics (age, sex, breed, weight, body condition score) and infection characteristics (infection class, chronicity, exposure to antibiotics, pyuria, multi-drug resistance, clinical signs) were compared.

The majority (52.6%) of our isolates showed biofilm-forming capability. Dogs with biofilm-forming *E. coli* UTIs had a lower likelihood of multi-drug resistance ($p < 0.001$) than those with non-biofilm-forming *E. coli* UTIs. There were no other statistically significant differences between the population characteristics or infection characteristics of the two groups.

We concluded that because there are no reliable clinical indices by which biofilm-formation can be ruled out, consideration should be given to the possibility of biofilm-formation whenever *E. coli* UTIs are diagnosed. Additionally, the association of MDR and non-biofilm-forming *E. coli* may antimicrobial tolerance conferred by biofilm formation.

NU10

The Fecal Microbiome, Indoxyl Sulfate, and P-Cresol Sulfate in Cats with Stable Chronic Kidney Disease

Stacie C. Summers[1], *Jessica M. Quimby*[2], *Anitha Isaiah*[3], *Jan S. Suchodolski*[4], *Craig Webb*[5], *Daniel Gustafson*[6]
[1]*Colorado State University, College of Veterinary Medicine, Fort Collins, CO, USA,* [2]*The Ohio State University, Columbus, OH, USA,* [3]*Texas A&M Gastrointestinal Laboratory, College Station, TX, USA,* [4]*Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA,* [5]*Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA,* [6]*Colorado State University, Fort Collins, CO, USA*

The uremia associated with chronic kidney disease (CKD) has been shown to profoundly affect the composition of the gut microbiome in people and rat models. Toxic products generated by dysbiosis may contribute to morbidity and progression of CKD.Indoxyl sulfate (IS) and p-cresol sulfate (pCS) are uremic toxins produced by colonic bacteria. The first study objective was to compare the fecal microbiome of healthy, older (> 8 years) cats (n = 11) and cats with stable CKD (IRIS stage 2-4) (n = 30). The second objective was to measure serum IS and pCS in healthy, older cats (n = 10) and compare to CKD cats (n = 28).

In this cross-sectional study, all cats had a complete blood count, chemistry panel, total T4, urinalysis, blood pressure, and fecal flotation at enrollment. CKD cats had creatinine > 1.6 mg/dL and USG < 1.035 or an elevated creatinine at at least two time points in addition to an elevated SDMA > 14 ug/dL. Healthy cats were > 8 years of age and had a creatinine < 1.6 mg/dL and USG > 1.035. Exclusion criteria were a history of antibiotic, probiotic, or antacid administration < 6 weeks prior to enrollment or a history of uncontrolled hyperthyroidism and known or suspect gastrointestinal disease. A fresh fecal sample was collected by the owner and placed on ice until frozen within 24 hours of collection. For microbiome analysis, extracted fecal DNA was used for Illumina sequencing of the bacterial 16S rRNA gene. Differences in the proportions of bacterial taxa between the species were evaluated using Kruskal-Wallis tests. Serum IS and pCS were measured using LC/MS/MS. Median values between CKD cats and healthy control cats were compared using the Mann Whitney test and groups were compared using Kruskal Wallis with Dunn';s post hoc analysis.

The number of observed species and Chao 1 were significantly decreased in CKD cats when compared to healthy cats, p = 0.026 and p = 0.0284 respectively. The Shannon diversity index was decreased in CKD cats compared to healthy cats, however it was not significant (p = 0.0617). There was no significant difference in overall clustering of microbial communities between CKD cats and healthy cats (p = 0.72). However, when individual bacterial groups were analyzed based on LDA effect size (LEfSe) several bacterial taxa were identified as being significantly different among the groups. When comparing healthy cats to CKD cats, CKD cats had significantly decreased bacterial populations belonging to the genera *Holdemania*, *Adlercreutzia*, *Eubacterium*, *Slackia*, and *Mogibacterium*. No significant differences in the functional potential of the microbiota were found between CKD cats and healthy cats after correcting for multiple comparisons. IS levels were found to be significantly higher in CKD cats compared to healthy cats (p < 0.0001). Healthy control cats had significantly lower IS levels compared to stage 2 (p = 0.01) and stage 3 & 4 (p = 0.0006) CKD cats. No significant difference was found between Stage 2 and Stage 3 & 4 CKD cat groups. pCS levels were not significantly different between CKD and healthy controls.

In conclusion, feline CKD is associated with decreased diversity of the gut microbiome. IS is significantly elevated in feline CKD and merits exploration as a potential therapeutic target. Additionally, IRIS stage 2 cats may suffer from a similar uremic toxin burden as cats with later stage disease. Additional work is needed to further understand the interplay between the fecal microbiome and uremic toxins.

NU11

Assessment of Symmetric Dimethylarginine and Creatinine Concentrations in Cats with Urethral Obstructions

Kendall E. Wilson[1], *Allyson Berent*[1], *Donald A. Szlosek*[2], *Jane E. Robertson*[2]
[1]*The Animal Medical Center, New York, NY, USA,* [2]*IDEXX Laboratories, Inc., Westbrook, ME, USA*

The purpose of the study was to evaluate symmetric dimethylarginine (SDMA) and creatinine concentrations in cats with urethral

obstructions (UO), and to determine if pre-decompression values correlate with post-decompression values.

Sixty-one cats admitted for treatment of a UO were eligible for inclusion. Serum SDMA and creatinine concentrations were assessed at presentation, 24 hours post-decompression, and 5-20 days post-decompression. Urinalysis and culture were assessed at presentation and at final follow-up. Cats were excluded if they had: bacterial growth on culture, evidence of re-obstruction, or failure to obtain all samples.

Twenty percent of cats (n = 12) were excluded due to a urinary tract infection (UTI) at presentation. The mean SDMA and creatinine were significantly higher in these cats (59.6 μg/dL and 11.7 mg/dL, respectively), compared with those without a UTI (27.6 μg/dL and 4.76 mg/dL, respectively) ($p < 0.002, < 0.001$).

Twenty-six cats met the inclusion criteria. There was a significant positive correlation between SDMA and creatinine at the time of UO ($\rho = 0.732$, $p < 0.0001$). There was no association between initial SDMA and SDMA post-decompression at 24 hours ($p = 0.817$) or 5-20 days ($p = 0.744$). Mean SDMA and creatinine at presentation (24.7 μg/dL and 4.4 mg/dL, respectively) were significantly higher compared with final values (13.7 μg/dL and 1.7 mg/dL, respectively) ($p < 0.017, < 0.016$).

SDMA appears to be a useful marker of post-renal azotemia in cats with UO, and pre-decompression SDMA does not reflect underlying kidney disease. UTIs may be more frequent in cats with UO than historically suggested, and screening should be considered, especially if SDMA and creatinine concentrations are elevated.

NU12

A Multi-Institutional Retrospective Study of 17 Cases of Histopathologically Confirmed Feline Pyelonephritis

Adrienne Cheney[1], Jean-Sébastien Palerme[2], Laura R. Van Vertloo[1], Jessica Pritchard[3], Shelly L. Vaden[4], Wingert Allie[5], Kate KuKanich[5]
[1]Iowa State University, Ames, IA, USA, [2]Iowa State University College of Veterinary Medicine, Ames, IA, USA, [3]University of Wisconsin, Madison, WI, USA, [4]North Carolina State University, Raleigh, NC, USA, [5]Kansas State University, Manhattan, KS, USA

Despite pyelonephritis being commonly suspected in hospitalized patients, its definitive diagnosis remains challenging and reports of this disease in cats are limited to a small number of case reports.

With the goal of characterizing the clinical signs as well as clinicopathologic and ultrasonographic changes in cats with pyelonephritis, necropsy reports from 4 veterinary teaching hospitals were searched for cats with histopathologically diagnosed pyelonephritis. For inclusion in the study, all cats had to have undergone a full physical examination prior to death in addition to a complete post-mortem.

Seventeen cats were identified for inclusion in the study. The median age was 12 years (range 3-17 years). Spayed females (n = 14) were more prevalent than castrated males (n = 3). Anorexia (7/17), lethargy (6/17) and vomiting (5/17) were the most common presenting complaints. Common physical exam abnormalities included muscle wasting (8/17), hypothermia (6/17), and cardiac murmurs (6/17). Clinical signs classically associated with pyelonephritis, such as renal pain and pyrexia were present in only 3 and 2 cats, respectively. Serum chemistries and complete blood counts were available for 11 and 10 cats, respectively. Common clinical pathologic abnormalities included azotemia, hyperphosphatemia, and non-regenerative anemia in 11, 8 and 7 cats, respectively. Of the 5 cats for which urinalysis data was available, all had bacteriuria and 4 had isosthenuric urine. Of the cats that had a urinalysis performed, four also had urine samples submitted for culture. An additional cat had urine submitted for culture without a concurrent urinalysis. Four of the 5 urine samples submitted for culture yielded bacterial growth, with *E. coli* isolated in 3/4 of cultures. Of the cats that had abdominal radiographs performed (n = 3) all had renal abnormalities including atypically sized kidneys (n = 2) or mineralization (n = 3). Ultrasonographic appearance of the kidneys was abnormal in all cats for which this information was available (n = 4). Common changes included mineralization (n = 4) and pyelectasia (n = 2). Histopathology of post-mortem samples revealed purely neutrophilic cellular infiltrates in 7/17 cats, with bacteria being present in 3 cases. Lymphocytic-plasmacytic pyelonephritis was diagnosed in 6/17, none of which had intralesional bacteria noted. Concurrent lymphocytic-plasmacytic and neutrophilic pyelonephritis was present in the remaining four cats, with intralesional bacteria present in 2 cases.

These findings suggest that cats with pyelonephritis are far less likely to exhibit classical physical exam findings such as renal pain and pyrexia than is commonly assumed. In addition, they are likely to have renal azotemia and may have evidence of an active infection on urine sediment.

NU13

Use of a Subcutaneous Ureteral Bypass Device for Treatment of Benign Ureteral Obstructions in Dogs

Melissa Milligan[1], Allyson Berent[1], Chick Weisse[1], Kenneth Lamb[2], Tozier Erik[2]
[1]The Animal Medical Center, New York, NY, USA, [2]Lamb Consulting, St. Paul, MN, USA

Subcutaneous ureteral bypass (SUB)™ device placement is an alternative to traditional ureteral surgery. However, outcomes have not been described for treatment of benign ureteral obstructions in dogs. The purpose was to evaluate pre-operative, peri-operative (< 7 days), short (7-30 days) and long-term (> 30 days) parameters in dogs treated with SUBs™ for benign ureteral obstructions. The hypothesis was SUBs™ were associated with favorable technical outcomes when compared with alternatives.

SUBs™ were placed using fluoroscopic- and surgical-assistance. Medical records were reviewed for pre-, intra-, and post-operative data.

Twelve SUBs™ were placed in nine dogs (3 bilateral). Causes of obstruction included: ureterolithiasis (9/12;75%), extraluminal compression (2/12;17%), and stricture (1/12;8%). Eleven of 12 ureters had a stent placed prior and needed a SUB™ for: recurrent stricture (4/11;36%), ureteritis (4/11;36%), or stent migration (3/11;27%). Placement was successful in all ureters.

The median creatinine pre-operative and 3 months post-operative was 2.1 mg/dL and 1.2 mg/dL, respectively. Seven dogs (7/9;78%) had a history of urinary tract infection(s) prior to SUB™ placement.

Long-term complications included infection (5/9;55%) and mineralization (6/12;50%). Dogs that mineralized their device had a history of urolithiasis. Historical pre-operative infections commonly had post-operative infections (5/7). There were no peri-operative or procedure-related deaths. No dog had worsening azotemia in the

short-term. The median survival time was > 774 days, with 5/9 still alive.

Use of the SUB™ device in dogs is a safe and effective treatment option for benign ureteral obstructions and associated with a good prognosis. The high rate of mineralization and infections should be considered in the long-term.

NU14

Characterization of Hypoxia-Induced, Profibrotic Pathways in an ischemic Model of Feline Chronic Kidney Disease

Bianca N. Lourenco[1], *Amanda E. Coleman*[1], *Chad W. Schmiedt*[1], *Cathy A. Brown*[2], *Daniel R. Rissi*[2], *James B. Stanton*[2], *Steeve Giguere*[3], *Scott A. Brown*[4], *Jaime L. Tarigo*[2]

[1]*Department of Small Animal Medicine and Surgery, University of Georgia College of Veterinary Medicine, Athens, GA, USA,* [2]*Department of Pathology, University of Georgia College of Veterinary Medicine, Athens, GA, USA,* [3]*Department of Large Animal Medicine, University of Georgia College of Veterinary Medicine, Athens, GA, USA,* [4]*Department of Small Animal Medicine and Surgery and Pharmacology & Physiology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA*

Recent work has emphasized the critical role of tubulointerstitial hypoxia in the development and progression of renal fibrosis and CKD. Increased genetic expression of hypoxia-induced mediators of fibrosis has been demonstrated in CKD of non-feline species. The objective of this study was to characterize the expression of hypoxia-induced mediators of fibrosis in banked renal tissues from cats with experimental CKD. We hypothesized that cats with experimental CKD would have increased intrarenal expression of these mediators when compared to control cats. This pilot study included bilateral renal tissue samples from 6 cats having previously undergone temporary unilateral renal ischemia as a model of CKD and from 8 healthy cats (control group). For the CKD model group, tissues from both the ischemic kidney (IK group) and the contralateral, non-ischemic kidney (NIK group) were evaluated. Reverse transcription-quantitative polymerase chain reaction was used to characterize the tissue expression of hypoxia-inducible factor-1α, vascular endothelial growth factor-A (VEGF-A), matrix metalloproteinases 2 and 7 (MMP-2 and MMP-7), tissue inhibitor of metalloproteinase 1 (TIMP-1), and transforming growth factor β1 (TGF-β1). Statistically significant differences in gene expression among groups were observed (all *P*-values < 0.045). VEGF-A was downregulated in both the IK and NIK groups, compared to the control group. Conversely, the MMPs, TIMP and TGF-β1 were upregulated in CKD tissues: MMP-2, MMP-7 and TGF-β1 were upregulated in the IK group only, and TIMP-1 was upregulated in both the IK and NIK groups. Dysregulation of major pathways of renal fibrosis was identified in experimental feline CKD. Future studies investigating these pathways in naturally occurring CKD may identify new therapeutic targets for this highly prevalent disease.

NU15

Early Clinical Evaluation of Urethral Thermoplasty In The Treatment of Urinary Incontinence in Dogs

Carrie A. Palm[1], *William Culp*[1], *Jeffrey Solomon*[2], *Simon Thomas*[3]

[1]*UC Davis School of Veterinary Medicine, Davis, CA, USA,* [2]*Infiniti Medical, LLC, Redwood City, CA, USA,* [3]*Infiniti Medical, LLC Consultant, Redwood City, CA, USA*

Urinary incontinence (UI) is a common problem in dogs. Effective treatments are lacking, and many dogs demonstrate clinical signs throughout life resulting in subsequent complications and owner frustration. In woman, a minimally invasive procedure, low temperature urethral thermoplasty (LTUT), restores continence by reducing the compliance of the periurethral tissue through controlled application of heat.

Goals of this study were to evaluate the outcomes and complications associated with LTUT in female dogs with incontinence. Dogs underwent extensive medical evaluation and LTUT was performed. In short, the thermoplasty probe was positioned in the proximal urethra via fluoroscopic guidance. Heating of the periurethral tissue was performed in 4 different planes for a total of 64 treatment sites per dog. The treating clinician and owner completed serial questionnaires and incontinence diaries.

Three large breed (23-29 kg) female dogs with naturally occurring UI have been treated. LTUT was performed successfully in each case without significant complication (no leakage or stricture). Follow-up time for the 3 dogs ranges between 3-12 months. Owners reported pre-procedure incontinence scores of 3.5-5 (0 = severely incontinent; 10 = no incontinence), with improvements in score post-LTUT to 9-10 in all dogs. The impact of UI on owner quality of life decreased from between 7-9 (0 = no impact; 10 = constant source of concern) to 0-3 post-LTUT.

LTUT was well tolerated in treated dogs, with improvement in both incontinence and owner quality of life scores. Further evaluation of LTUT in larger case numbers is ongoing.

NU16

Calcium Oxalate Urolithiasis in Juvenile Dogs

Alexander Saver, Jody Lulich, Eva Furrow

University of Minnesota College of Veterinary Medicine, Saint Paul, MN, USA

Canine calcium oxalate (CaOx) urolithiasis typically occurs as a mature-onset disease, and the features of juvenile-onset disease have not been previously reported.

The objective of this study was to identify patient and stone characteristics of juvenile dogs with CaOx urolithiasis compared to mature urolith-formers.

Information on 232 juvenile (≤ 1 year) and 39,093 mature (7-9 years) dogs with CaOx (≥ 70%) urolithiasis was obtained from submissions to the Minnesota Urolith Center between 2012 and 2016. Fisher';s exact tests were used to identify breeds overrepresented in the juvenile group, and chi-squared tests were performed to determine whether the sex, stone location (upper vs lower urinary tract) and salt type (monohydrate vs dihydrate) differed compared to the mature group.

English (OR = 8.6, P = 0.0070) and French (OR = 7.5, P = 0.012) Bulldogs were overrepresented in the juvenile group. While no difference in sex was observed between the juvenile and mature groups, all juvenile and > 90% of adult English and French Bulldogs were male. Stone location did not differ between juvenile and mature dogs, however < 2% of the stones in both groups were from the upper urinary tract. Juvenile dogs were more likely to form dihydrate stones compared to

mature dogs (OR = 1.7, P < 0.001), although monohydrate were predominant in both groups (79% and 87%, respectively).

This study identified two related breeds at risk for juvenile CaOx urolithiasis. Inherited risk factors, particularly X chromosome mutations, should be investigated due to the strong breed and sex predispositions identified.

NU17

Transcutaneous Assessment of Renal Function in Hyperthyroid Cats pre and post Radioiodine Treatment

Sarah ML. Steinbach[1], Johanna Rainer[2], Daniel Schock-Kusch[3], Reto Neiger[4]
[1]Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA, [2]Small Animal Clinic - Internal Medicine, Justus-Liebig University, Giessen, Germany, Giessen, Hessen, Germany, [3]Medibeacon GmbH, Mannheim, Baden-Wurttemberg, Germany, [4]Tierklinik Hofheim, Hofheim am Taunus, Hessen, Germany

Assessment of glomerular filtration rate (GFR) is important in veterinary and human medicine. Hyperthyroid cats have an increased GFR, which will decrease after successful radioiodine treatment and can hence be used as a model to investigate new and minimally invasive techniques to assess renal function.

The objective of this prospective study was to investigate the usefulness of a minimally invasive transcutaneous GFR (tGFR) monitor (NIC-Kidney) to assess renal function in cats with naturally occurring hyperthyroidism pre (t0) and 2 weeks post radioiodine treatment (t2).

Eight hyperthyroid cats were included of which 5 were euthyroid at t2. Three cats remained hyperthyroid, but had significantly reduced serum T4 concentrations. Plasma GFR (pGFR) and plasma half-life (pHL) were assessed by plasma sinistrin clearance. Transcutaneous half-life (tHL) was measured in parallel using a fluorescent marker (FITC-sinistrin) and the NIC-kidney device. Plasma data was used to calculate a species-specific conversion factor to determine tGFR from tHL. Overall pGFR and tGFR were significantly correlated (Spearman's correlation r = 0.73, $P = 0.0019$). In 7/8 cats pGFR decreased by 26% (mean); 13-59% (range). It remained unchanged in 1 cat. Using tGFR, a decrease of GFR was identified in 6/8 cats with a decrease (25%; 5-40%). The remaining 2 cats showed a mild increase (9% and 32%, respectively).

Although results are not directly comparable, transcutaneous GFR assessments is a promising minimally invasive technique to allow for GFR estimation in small animals. Further research in a larger cohort is necessary to evaluate the full potential of this method.

NU18

Owner Survey of Amoxicillin-Clavulanic Acid Side Effects in Cats with and without Azotemic CKD

Kellyi Benson[1], Jessica M. Quimby[2], Liberty Sieberg[1], Catherine Langston[2], Kristy Dowers[1]
[1]Colorado State University, Fort Collins, CO, USA, [2]The Ohio State University, Columbus, OH, USA

Cats with chronic kidney disease (CKD) have an anecdotal increased frequency of side effects with amoxicillin- clavulanic acid such as decreased appetite, vomiting and diarrhea in comparison to cats with normal kidney function. In human patients with decreased kidney function, it has been shown that potentiated penicillins, like amoxicillin-clavulanic acid, have impaired clearance (by as much as 90% in severe renal impairment). This impaired clearance results in higher serum concentrations in comparison to patients with normal kidney function and reduction of dosing interval is recommended to avoid side effects. The purpose of this study was to determine the frequency of side effects in cats with and without azotemia, with the hypothesis that cats with azotemic CKD will have an increased frequency of side effects.

Owners of cats prescribed amoxicillin- clavulanic acid at Colorado State University or The Ohio State University were sent a survey regarding the occurrence of side effects (yes / no), specific type of side effect (vomiting, diarrhea, decreased appetite, none) and whether treatment was altered as a result. Signalment and clinicopathologic data were obtained from the medical record for cats for which surveys were returned. Cats included in the azotemic CKD group had a creatinine > 2.0 mg/dL, USG < 1.035 and a clinical designation of CKD. Cats included in the without azotemic CKD group were those had a creatinine < 2.0 mg/dL. A cut off between groups of creatinine 2.0 mg/dL was chosen to define cohorts as impaired clearance of amoxicillin- clavulanic acid is more common in humans with a greater degree renal impairment. Chi square was utilized to assess distribution of presence of side effects (yes / no) between groups, prevalence of specific side effects and changes in therapy.

73 surveys were returned with 12 being excluded for incomplete data or no serum creatinine available. 11 cats were categorized as azotemic CKD and consisted of 4 males and 7 females, with a median age of 14 years (range 4-20 years) and a median creatinine of 2.4 (range 2.0-6.0 mg/dL). 50 cats were categorized as not having azotemic CKD and consisted of 17 males and 33 females, with a median age of 11 years (range 1-18 years) and a median creatinine of 1.4 (range 0.7-1.9 mg/dL). Owners reported 6 / 11 cats (55 %) with azotemic CKD had side effects. Specific side effects included diarrhea 5 / 11 cats (45 %), vomiting 2 / 11 cats (18 %), and decreased appetite 3 / 11 cats (27%), with 5 / 11 cats (45 %) having more than one side effect. Owners reported 20 / 50 cats (40 %) cats without azotemic CKD had side effects. Specific side effects for these cats included diarrhea 11 / 50 cats (22 %), vomiting 10 / 50 cats (20 %), and decrease appetite 7 / 50 cats (14 %), with 6 / 50 cats (12 %) having more than one side effect. The difference in distribution of side effects (yes / no) or distribution of specific type of side effects was not statistically significant, however significantly more azotemic CKD cats had more than one side effect (p = 0.009). Additionally, significantly more owners of azotemic CKD cats reported that an alteration in treatment plan was necessitated by side effects with 55 % indicating the antibiotic was stopped, changed or supportive care was added due to side effects, while only 14 % of owners of cats without azotemic CKD reported alteration in treatment plan (p = 0.003).

Side effects such as diarrhea, vomiting and decreased appetite were common with amoxicillin- clavulanic acid administration in both cohorts, but may be of increased incidence in cats with azotemic CKD and result in alterations of treatment plan. Investigations are currently underway to determine if these findings are associated with a higher drug serum concentration.

NU19

Feline Urinary Incontinence: A Retrospective Study of 39 Cases (2006-2017)

Kaitlin M. Lonc, J M. Kruger
Michigan State University, East Lansing, MI, USA

Clinical observations indicate that the frequency and causes of urinary incontinence in cats are considerably different from those of dogs. Since comparative studies in cats have not been reported, the purpose of this study was to determine the proportional morbidity of causes and assess outcomes of cats with urinary incontinence presented to the Michigan State University Veterinary Medical Center (MSUVMC). Medical records of cats presented to the MSUVMC from 2006 to 2017 were searched electronically using 47 keywords related to urinary incontinence. Cats were included if they had a diagnosis of urinary incontinence based on owner history or physical examination. Cats with insufficient information to confirm a diagnosis were excluded. Thirty-nine of 22,646 (0.17%) cats met inclusion criteria.

Affected cats included 20 castrated males, 1 intact male, and 18 spayed females. Median age at onset of clinical signs was 4.0 years (range 1 month to 17 years). Most affected cats were mixed breed (35/39). Spinal cord disease was identified in 18 of 39 (46%) affected cats (9 trauma, 3 congenital lumbosacral malformations, 2 intervertebral disk disease, 2 neoplasia, 1 fibrocartilaginous embolus, 1 Manx syndrome). Urethral disease was identified in 14/39 (36%) affected cats (4 urethral stricture, 3 urethritis, 2 urethral stent, 1 perineal urethrostomy, 1 congenital urethral sphincter mechanism incontinence, 3 unclassified urethral disease). Bladder disorders were identified in 6/39 (15%) cats (3 cystitis, 1 ureteral stent, 1 neoplasia, 1 bladder atony). Bilateral ureteral ectopia was identified in one cat.

Outcomes were available in 28/39 cases; 8 cats were euthanized, 5 cats regained continence, 15 cats remained incontinent despite therapy. Most euthanized cats had spinal cord disease (5/8). Conditions in recovered cats included cystitis (1 bacterial UTI, 1 idiopathic cystitis), bladder atony (1), urethral stricture (1), and urethritis (1). In conclusion, feline urinary incontinence is a rare condition most commonly associated with acquired or congenital spinal cord disorders. Urinary incontinence in cats is generally associated with a poor prognosis, with many having persistent incontinence despite therapy.

NU20

Outcome in Dogs with Non-Steroidal Anti-Inflammatory Drug Toxicity Treated by Therapeutic Plasma Exchange

Melisa G. Rosenthal[1], Mary Anna Labato[2]
[1]Tufts University, North Grafton, MA, USA, [2]Cummings School of Veterinary Medicine, Tufts University, Shrewsbury, MA, USA

Non-steroidal anti-inflammatory drug (NSAID) overdose can have severe, life-threatening toxicity in dogs. Due to the heavily protein-bound nature of NSAIDs, conventional hemodialysis techniques are ineffective, but therapeutic plasma exchange (TPE) can be utilized. The purpose of this study was to evaluate this technique, with specific goals including: (1) identification of variables at presentation that predicted outcome, (2) evaluation of therapeutic complications, and (3) determination of the effect of adjunctive therapies.

All canine patients that underwent TPE for NSAID overdose at the Tufts Foster Hospital for Small Animals between January 2015 and May 2017 were included in the study, and data regarding their presentation, hospitalization, treatments, and outcome was collected.

Eleven cases were included in this study. Of these, the NSAID ingested was ibuprofen in six, naproxen in four, and deracoxib in one. Six patients had complications associated with the TPE treatment, with the most common complication being hemorrhage. All cases survived to discharge with three developing acute kidney injury during hospitalization, one of which went on to have chronic kidney disease. The final outcome was correlated with dose of NSAID ingested.

This study identifies plasma exchange as an effective treatment for NSAID overdose in that none of the patients had lethal outcomes despite high NSAID dose ingestion. No presenting factor determined the likelihood of acute kidney injury apart from the initial NSAID dose. Complications were common, but were managed with supportive care and did not affect final outcome. TPE should be considered in patients presenting for NSAID ingestion.

NU21

Vitamin D Metabolites in Chronic Kidney Disease Cats with Nephrolithiasis

Fernanda Chicharo. Chacar[1], Marcia Mery Kogika[1], Dennis Chew[2], Joao Felipe de Brito Galvao[3], Helio Autran de Moraes[4], Ramiro Toribio[5], Andrew Joseph Makowski[6], Marcio Antonio. Brunetto[1], Archivaldo Reche Jr.[7]
[1]School of Veterinary Medicine and Animal Science - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil, [2]College of Veterinary Medicine - The Ohio State University, Columbus, OH, USA, [3]VCA Arboretum View Animal Hospital, Downers Grove, IL, USA, [4]College of Veterinary Medicine - Oregon State University, Corvallis, OR, USA, [5]The Ohio State University College of Veterinary Medicine, Columbus, OH, USA, [6]Heartland Assays LLC & Metabolic Technologies, Inc., Ames, IA, USA, [7]University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil

Calcium oxalate accounts for up to 98% of the upper urinary tract stones of cats, and most have concurrent chronic kidney disease (CKD). In human beings, calcium kidney stone formers have changes in vitamin D metabolism. The role of vitamin D metabolism in CKD cats with nephrolithiasis remains unclear. Thus, our study aimed to assess serum $1,25(OH)_2D_3$, $25(OH)D_3$, $24,25(OH)_2D_3$ metabolites, and ionized calcium in CKD cats with nephrolithiasis. CKD cats with IRIS stage 1 (n= 1; hypercalcemic), stage 2 (n= 3; 1 of 3 hypercalcemic), and stage 3 (n= 2; 1 of 2 hypercalcemic) that had nephrolithiasis based on ultrasound were enrolled. Hypercalcemia was defined as an ionized calcium concentration higher than 1.4 mmol/L. Hypercalcemic cats (n= 3) had 1.50 mmol/L, 1.64 mmol/L and 1.53 mmol/L of ionized calcium (heparinized whole blood). Serum concentrations of $1,25(OH)_2D_3$ were lower in hypercalcemic cats (73 pg/mL; 53 pg/mL; 122 pg/mL) in comparison with normocalcemic cats (181.4 pg/mL; 264.6 pg/mL; 292.7 pg/mL). Hypercalcemic cats also had lower serum $25(OH)D_3$ (27.5 ng/mL; 20.6 ng/mL; 34.2 ng/mL) and $24,25(OH)_2D_3$ (4.1 ng/mL; 2.8 ng/mL; 10.0 ng/mL) than normocalcemic cats (38.1 ng/mL, 54.3 ng/mL, 44.1 ng/mL of $25(OH)D_3$ and 18.9 ng/mL, 27.1 ng/mL, 19.6 ng/mL of $24,25(OH)_2D_3$). Serum PTH (reference range 0.40 to 2.50 pmol/L) was measured in 2 out of 3 hypercalcemic cats, and it was suppressed (0.01 pmol/L) in the cat (stage 2) with 1.64

pmol/L of blood ionized calcium, and normal (PTH= 1.5 pmol/L) in the cat with 1.53 mmol/L (stage 3). Hyperphosphatemia (5.17 mg/dL) was detected in only one hypercalcemic cat (stage 1). Proteinuria was detected in one cat (stage 3) with hypercalcemia (UP/C= 2.0; SDS-PAGE =tubular pattern). Our results suggest that CKD cats with nephrolithiasis and hypercalcemia have differences in vitamin D metabolism compared to normocalcemic cats characterized by decreased in $1,25(OH)_2D_3$, $25(OH)D_3$, and $24,25(OH)_2D_3$. Hyperparathyroidism was ruled out in two hypercalcemic cats. It is possible that the cause of hypercalcemia may be associated with decreased degradation of $25(OH)D_3$ as supported by the lower $24,25(OH)_2D_3$ concentrations. Alternatively, low $24,25(OH)_2D_3$ could result from less substrate provided for catabolism since hypercalcemic cats had lower concentrations of $25(OH)_2D_3$ than normocalcemic cats. Based on traditional understanding of normal calcium regulation, it seems paradoxical that hypercalcemia would be associated with lower $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations than that in cats with normocalcemia. Further studies with larger numbers of cats are needed to support these findings as well as to investigate whether proteinuria may lead to a greater loss of vitamin D-binding protein (VDBP) in urine that could contribute to $25(OH)D_3$ deficiency.

NU22

Evaluation of an Herbal Compound Used to Manage Lower Urinary Tract Disease in Healthy Cats

Cook F. English[1], Ashley Shaw[1], Sherry Cox[2], Joe Bartges[1]
[1]The University of Georgia, Athens, GA, USA, [2]The University of Tennessee, Knoxville, TN, USA

Lower urinary tract disease occurs commonly in cats. While conventional therapy involves modified diet and pharmacological intervention, traditional Chinese and Western herbs have been recommended as alternative and complementary treatments. The purpose of this study was to evaluate efficacy of an herbal supplement. We hypothesized that the herbal supplement would be associated with increased urine volume and decreased urine saturation for calcium oxalate and struvite when compared with placebo.

Seven healthy, male cats, aged between 8 months and 5 years, were evaluated using a randomized placebo cross-over study in a pairwise fashion, each cat receiving treatment every 12 hours for a two-week period. An approximate 24-hour voided urine sample was collected at the end of each treatment period. Samples were analyzed for pH, sodium, potassium, chloride, calcium, phosphorous, magnesium, citrate, oxalate, ammonia, and creatinine. Relative supersaturation for calcium oxalate and struvite was estimated using an iterative computer program (EQUIL 93b, University of Florida, Gainesville, FL). Data were assessed for normal distribution using Shapiro-Wilk. Paired t-test (normal distribution) or Wilcoxon signed rank (non-normal distribution) were used for statistical analysis. Data were analyzed using 2-tailed statistical testing except for relative supersaturation and urine volume where 1-tailed testing was used. A p-value < 0.05 was considered significant.

Data from 6 cats were used due to incomplete urine collection from 1 cat. Urine saturation for struvite was significantly lower when cats received supplement compared with placebo (supplement = 0.364 ± 0.193, placebo = 1.565 ± 1.284; p = 0.04) and 24-hour urinary excretion of phosphorous was lower when cats received supplement compared with placeb0.o (supplement = 44.89, range = 24.4 – 61.3 mg/kg/24h; placebo = 50.75, 41.4 – 63.5 mg/kg24h; p = 0.04). There were no differences in other analytes, body weight, or urine volume.

There are several limitations of this study. The study was of short duration and included a small number of healthy, non-urolith forming cats. There may not have been complete urine collection despite use of modified litter boxes.

There was a significant decrease in struvite supersaturation even with these limitations suggesting determination of relative supersaturation is a better determinant of risk of urolith formation than electrolyte & mineral concentrations or excretions. The herbal supplement may be beneficial for managing struvite-associated urinary disease in cats.

NU23

The Prognostic Factors in Cats with Big Kidney Little Kidney Syndrome

Po-Yao Huang[1], Wan-Chu Hung[2], Ya-Jane Lee[3]
[1]Institute of Veterinary Clinical Science National Taiwan University, Taipei, Taipei, Taiwan (Republic of China), [2]National Taiwan University Veterinary Hospital, Taipei, Taipei, Taiwan (Republic of China), [3]Institute of Veterinary Clinical Science National Taiwan University/National Taiwan University Veterinary Hospital, Taipei, Taipei, Taiwan (Republic of China)

"Big kidney little kidney (BKLK) syndrome," which was known as the asymmetric size of 2 kidneys under radiography, is due to the atrophy or fibrosis after complete or partial ureteral obstruction of one side kidney and the compensatory hypertrophy of the contralateral kidney in cats. The patients are typically stable and show no azotemia in clinical practice. At present, there is no consistent definition or evaluation factors of BKLK syndrome in cats.

Although a steady state can last for long, it can be a lethal situation when a new obstruction of the hypertrophied kidney occurs. In ureteral obstruction of feline, medical and surgical treatments can be optional. Research indicated one-month survival rates were 70% in surgical treatment group with ureterotomyureterotomy, ureteroneocystostomy or ureteral resection and anastomosis, and 58 % in medical treatment group individually. One study revealed that with stents and subcutaneous ureteral bypass (SUB) for the treatment in cats with ureteral obstruction can increase the survival rate but no parameter was associated with disease outcome. However, prognostic factors in cats with obstructive and non-obstructive BKLK syndrome remain unclear.

The aim of this study is to characterize cats with BKLK syndrome and to determine the prognostic/progression factors for cats with BKLK syndrome in obstructive and non-obstructive ureter state. First sixteen healthy cats without any abnormality in physical, blood, radiography examinations were collected to define BKLK syndrome. The divergence between kidneys on abdominal ventrodorsal radiograph in these cats ranged from 0.01 to 0.69 cm with a mean of 0.23 ± 0.20 cm. We defined the mean plus two standard deviations (0.63 cm) as the cut-off value. However, considering the observational error during the measurement, exceeding 0.8cm and with the history of CKD more than 2 months was set as the criteria for BKLK syndrome in this study.

Forty-four cats with BKLK syndrome was further divided into obstruction group (29) and non-obstruction group (15) based on their

Table 2

The differences in variables between normal and non-obstructive BKLK group

	Normal			Non-obstructive BKLK			P value
variable	Median	IQR	n	Median	IQR	n	
Hemoglobin (g/dL)	13.3	12.7-14.0	16	11.2	9.5-13.3	14	0.001
Hematocrit (%)	36.9	35.3-39.6	16	29.2	25.5-37.7	14	0.003
RBC (10^6/μL)	8.6	7.7-8.9	16	6.9	5.7-7.7	14	0.002
White blood cell (/μL)	6800.0	5900.0-10950.0	16	12000.0	7750.0-30450.0	14	0.013
Segmented cell (/μL)	4640.0	3015.0-7767.5	16	11520.0	5716.0-23899.0	13	0.023
ALKP (U/L)	44.0	35.00-47.50	16	34.0	23.5-52.5	14	0.010
BUN (mg/dL)	23.0	21.0-26.0	16	30.0	25.5-52.5	15	0.001
Creatinine (mg/dL)	1.4	1.3-1.5	16	2.1	1.6-2.5	15	<0.001
Urine pH	6.7	6.2-7.2	9	6.0	5.6-6.2	11	0.004
USG	1.049	1.043-1.057	9	1.020	1.012-1.026	11	0.001

1. ALKP, Alkaline phosphatase; BUN, Blood urine nitrogen; USG, Urine specific gravity.

Table

The differences in various variables between survival and not in obstructive BKLK group

	Survival			Non-survival			P value
variable	Median	IQR	n	Median	IQR	n	
Body temperature (°C)	38.6	38.3-38.9	16	38.0	37.8-38.2	5	0.009
2rd lumber length (cm)	1.9	1.8-2.0	20	2.0	1.9-2.1	8	0.079
White blood cell (/μL)	9280.0	6482.5-12265.0	12	12570.0	10365.0-25670.0	5	0.073
Neutrophil (/μL)	7690.0	4682.5-9130.0	12	11440.0	9260.0-22165.0	5	0.027
Hematocrit (%)	30.9	28.5-37.3	19	23.0	17.4-30.4	8	0.034
Neutrophil/Lymphocyte	3.9	2.4-9.0	12	16.3	7.3-20.8	5	0.015
Hemoglobin (g/dL)	11.2	10.7-13.2	19	8.9	6.6-11.7	8	0.036
RBC (10^6/μL)	7.0	6.2-8.4	19	5.3	3.9-6.7	8	0.026
MCHC (g/dL)	37.0	35.8-37.6	19	38.2	36.4-39.2	8	0.041
Albumin (g/dL)	3.3	3.0-3.4	19	2.9	2.7-3.2	8	0.061

1. MCHC, Mean corpuscular hemoglobin concentration.

ultrasound finding. There was no significant difference between these 2 groups with regard to the body weight and gender. All patient in obstruction group was neutered, but 25.8% were intact in non-obstruction group (P = 0.01). The length of right kidney under X-ray was longer (P = 0.034), and the ratio of the big kidney to 2nd lumber was higher in obstruction group (P = 0.002). Presence of urolith, nephrolith and the length difference between two kidneys did not show significant difference between two groups (P = 0.977, P = 0.241, P = 0.085). In blood examinations, the obstruction group had significant lower values of eosinophil (P = 0.004), reticulocyte (P = 0.029), Cl (P = 0.044), and higher value of MCHC (P = 0.027), BUN (P < 0.001), serum creatinine (P < 0.001).

Comparing the non-obstruction group with control group, male proportion was significantly higher in non-obstruction group (66.7% to 50%). The clinical pathological variables revealed that the values of Hb (P = 0.001), HCT (P = 0.003), RBC (P = 0.002), ALKP (P = 0.010), Urine pH (P = 0.004), USG (P = 0.001) were found to be significantly lower in the non-obstruction group. By contrast, the value of WBC (P = 0.013), segmented cells (P = 0.023), BUN (P < 0.001) and serum creatinine (P < 0.001) were higher in non-obstruction group.

The obstruction group was further divided into survival group (20) and non-survival group (8) (within 30 days). The survival group had significantly higher body temperature (P = 0.009), HCT (P = 0.058), Hb (P = 0.036), RBC (P = 0.026) and lower neutrophil (P = 0.027), ratio of neutrophil to lymphocyte (P = 0.015) and MCHC (P = 0.041). However, the surgery intervention, renal index including BUN, serum creatinine and phosphorus between the two groups did not meet any significant difference with the exception of serum creatinine to increase the odds

ratio of death. Additionally all BKLK cats were also divided into survival group (33) and non-survival group (10) (within 60 day). Body temperature (P = 0.005), reticulocyte (P = 0.022), Hb (P = 0.038), RBC (P = 0.030) were higher in survival group. And non-survival group had higher ratio of neutrophil to lymphocyte (P = 0.022), MCHC (P = 0.014), BUN (P = 0.014) and creatinine (P = 0.009). Significant positive relations were found between serum creatinine and the length of the bigger kidneys in obstruction group, while the length of the bigger kidney under X-ray was not significant different between survival and non-survival groups (P = 0.161).

In conclusion, body temperature, Hb, RBC, MCHC, and ratio of neutrophil to lymphocyte are good prognostic factors in both obstructive and non-obstructive BKLK syndrome. Surprisingly, the length of the bigger kidney cannot determine the prognosis.

NU24

Expression Profile of Matrix Metalloproteinase-9, Neutrophil Gelatinase-Associated Lipocalin and Hemojuvelin in Cat with Urinary Diseases

Hwei Jing[1], *Po-Han Wu*[2], *Wei-Li Hsu*[3], *Ya-Jane Lee*[2]
[1]*Institute of Veterinary Clinical Science National Taiwan University/National Taiwan University Veterinary Hospital, Taipei City, Taipei, Taiwan (Republic of China),* [2]*Institute of Veterinary Clinical Science National Taiwan University/National Taiwan University Veterinary Hospital, Taipei, Taipei, Taiwan (Republic of China),* [3]*Graduate Institute of Microbiology and Public Health National Chung Hsing University, Taichung, Taichung, Taiwan (Republic of China)*

Renal diseases are associated with hypoxia and subsequent inflammation. The utilization of oxygen and modulation of tissue oxygenation status mainly rely on iron. Both neutrophil gelatinase-associated lipocalin (NGAL) and the hemojuvelin (HJV)-hepcidin-ferroportin axis are important mechanisms for body iron homeostasis and regulation. NGAL, a 25-kDa glycoprotein belonging to lipocalin superfamily, acts as promising renal biomarker for humans, dogs and cats. Urinary NGAL had been reported with 3 configurations, including monomer, dimer and the complex with matrix metalloproteinase-9 (MMP-9) in human and laboratory animals. It is recently characterized in canine and feline urinary disease in our research group. MMP-9 plays an important role in regulating the degradation of extracellular matrix in process such as angiogenesis, tumor growth and metastasis. Additionally it has been reported that MMP-9 involves in renal fibrosis in chronic kidney disease (CKD). In addition, HJV, the upstream modulator of hepcidin-ferroportin axis, has been identified as a novel renal biomarker of acute kidney injury (AKI) and CKD in human renal diseases. Interestingly, both HJV and NGAL serve as modulators of inflammation, MMP-9 is involved in the expression of NGAL, and association of NGAL prevents MMP-9 auto-degradation; hence it is possible that these proteins work cooperatively in the course of feline renal diseases. To resolve this unknown issue, we aimed to characterize the overall expression profile of three factors in cats with urinary diseases.

The urine samples were collected for study. Control group contained 12 cats, AKI group contained 22 cats and pyuria group included 16 cats. According to IRIS stage, 55 CKD cats were defined with 25 cats in stage 2, 19 cats in stage 3 and 11 cats in stage 4 respectively. First, home-made ELISA for the detection of cat MMP-9 were successfully established. Additionally, with previous reported feline NGAL ELISA, urinary MMP-9 and NGAL concentration were determined.

Results showed that both urinary NGAL and MMP-9 were significantly associated with the elevated levels of serum BUN, creatinine and phosphate. Pyuria group had the highest concentration of urinary MMP-9 (Median [IQR], 13.38 [0-29.72]), followed by AKI group (4.33 [0-11.19]) and CKD group (1.86 [0-6.21]). Three of them were significantly higher than that of control group (0.11 [0-0.73]) (P < 0.05). With urinary MMP-9/creatinine ratio (UMCR), pyuria group still had the highest value (10.52 [0-21.64]), followed with AKI group (8.27 [0-19.85]), CKD group (2.64 [0-8.69]) and control group (0.03 [0-0.42]) sequentially.

In feline CKD, both NGAL and MMP-9 can represent the severity. Urinary NGAL and MMP-9 were significantly higher in CKD stage 3 and CKD stage 4 than that in the control group and CKD stage 2. However, urine MMP-9 may not be appropriate to indicate CKD progression. In CKD group and pyuria groups, both urinary NGAL and MMP-9 concentrations and their ratio to urinary creatinine had significant correlations (The Spearman correlation values were 0.64, 0.59, 0.57, and 0.61 in order). However, these correlations could not be identified in AKI group. Moreover, for detection of feline HJV, recombinant feline HJV was prepared for generation of antibody against feline HJV. At present, the expression of HJV was detected in feline urine with Western blot analysis. To quantify the concentration of HJV, development of an ELISA platform is in process.

In conclusion, urinary MMP-9 is a promising biomarker for renal-urinary diseases in cats and especially for interpreted with urinary NGAL. And the role of hemojuvelin in feline renal disease can be highly expected.

Table 1. The differences in urinary NGAL and MMP-9 concentrations among the different groups.

Parameter	Control (n=12)	CKD (n=55)	AKI (n=22)	Pyuria (n=16)	P value
uNGAL (ng/mL)	0.46 [0.31-0.62] [a]	0.37 [0.19-0.56] [a]	1.78 [0-4.69] [b]	1.21 [0-15.08] [b]	< 0.001
UNCR (10^{-6})	0.17 [0.1-0.29] [a]	0.56 [0-1.14] [b]	2.92 [0-6.2] [c]	1.43 [0-14.34] [c]	< 0.001
uMMP-9 (ng/mL)	0.11 [0-0.73] [a]	1.86 [0-6.21] [b]	4.33 [0-11.19] [b, c]	13.38 [0-29.72] [c]	< 0.001
UMCR (10^{-6})	0.03 [0-0.42] [a]	2.64 [0-8.69] [b]	8.27 [0-19.85] [b, c]	10.52 [0-21.64] [c]	< 0.001

1. Data are median [IQR].
2. The different superscripts (a, b, c) indicate various significant differences.
3. uNGAL, urinary NGAL; uMMP-9, urinary MMP-9; UNCR, urinary NGAL-to-creatinine ratio; UMCR, urinary MMP-9-to-creatinine ratio

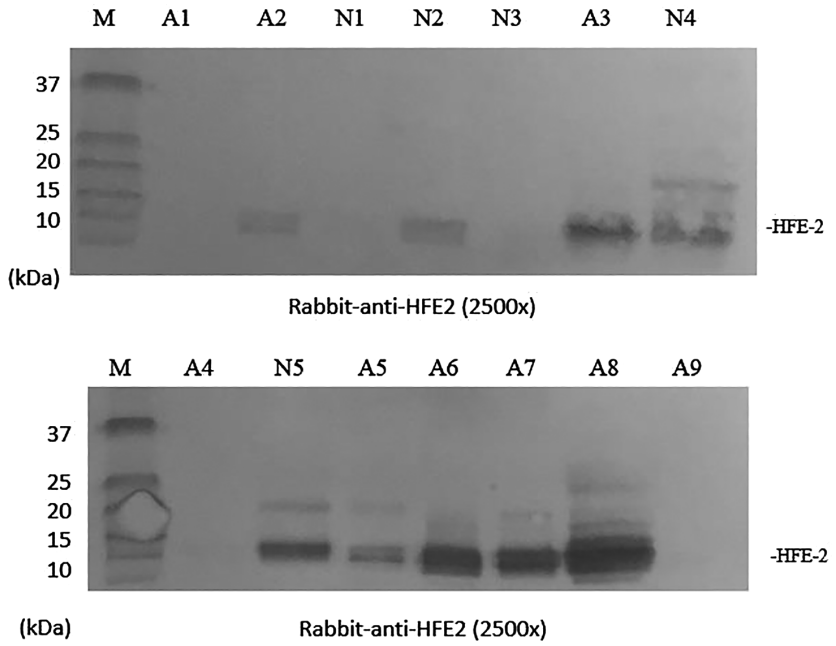


FIGURE 1 Western blot analysis for confirming hemojuvelin in feline urine. HFE-2 represents hemojuvelin. M represents marker. A represents azotemia group. N represents non-azotemia group

NU25

Hyperammonemia in Cats with Renal Azotemia

Mary Anna Labato[1], Denise Kelley[2], Cynthia Webster[3]
[1]Friendship Hospital for Animals, Washington, DC, USA, [2]Friendship Animal Hospital, Washington, DC, USA, [3]Cummings School of Veterinary Medicine,Tufts University, North Grafton, MA, USA

In cats, hyperammonemia has been documented with portal venous anomalies, advanced hepatic disease, and with deficiencies in cobalamin (B12) and arginine. A recent case series reported hyperammonemia in 4 cats with IRIS stage 4 chronic kidney disease (CKD). We hypothesized that in a larger population of cats fasting hyperammonemia would correlate with the blood urea nitrogen (BUN) and creatinine (Cr) and be independent of serum B12.

A fasted blood sample was prospectively collected for ammonia and B12 analysis from 20 client-owned cats with renal azotemia (IRIS Stage ≥ 2: creatinine ≥ 1.6 mg/dL). Blood for ammonia was immediately processed and analyzed in the clinical science laboratory. Cats on medications known to decrease blood ammonia (antibiotics, lactulose, probiotics) or previously diagnosed with disease states known to increase ammonia levels were excluded from the study. Data was analyzed using Pearson's correlation coefficient and ANOVA following log transformation.

Enrolled cats had a median age of 12 years (range 4-19 years) and there were 11 males and 9 females. Five out of 20 (25%), 5/20 (25%) and 10/20 (50%) were staged as IRIS Stage 2, 3, and 4 CKD, respectively.Hyperammonemia was documented in 5/20 (25%) of cats.There was a significant moderate positive correlation between BUN and ammonia ($r = 0.5478$, $p = 0.013$) but no correlation between ammonia and Cr ($r = 0.4306$, $p = 0.058$) or ammonia and B12 ($r = -0.275$, $p = 0.44$). There was no difference between mean ammonia levels between the IRIS Stages (2, 3 and 4) ($p = 0.162$). Hyperammonemic cats did not appear to have overt neurologic signs and did not have urinary tract infections with urease producing bacteria.

In conclusion, in this pilot study of cats with renal azotemia, a correlation existed between BUN and ammonia, but not between ammonia and Cr or B12. Future studies are needed in a larger population of cats to determine the true prevalence, etiology and effect of hyperammonemia on long term prognosis.

NU26

Symmetric Dimethylarginine Concentrations in Dogs with IRIS Stage 4 Chronic Kidney Disease Undergoing Intermittent Hemodialysis

Andre N. V Le Sueur[1], Silvano S. Geraldes[2], Alessandra Melchert[1], Regina K. Takahira[1], Michael Coyne[3], Donald A. Szlosek[3], Rachel Murphy[3], Priscylla T. C. Guimarães-Okamoto[1]
[1]School of Veterinary Medicine and Animal Science, São Paulo State University - UNESP Botucatu, Botucatu, Sao Paulo, Brazil, [2]School of Veterinary Medicine and Animal Science, São Paulo State University - UNESP Botucatu., Botucatu, Sao Paulo, Brazil, [3]IDEXX Laboratories, Inc., Westbrook, ME, USA

Symmetric dimethylarginine (SDMA) is a methylated arginine derived from intranuclear methylation of L-arginine by protein-arginine methyltransferase and released into circulation after proteolysis. SDMA seems to be strictly eliminated by renal excretion, and its concentration highly correlates with GFR in animals and humans; therefore, this biomarker can be an earlier marker of kidney dysfunction than serum creatinine (sCr).

The objective of the prospective study was to evaluate and quantify the effects of intravenous fluid therapy (IF) or intermittent hemodialysis (IH) on renal function in a randomized group of dogs previously diagnosed with IRIS stage 4 Chronic Kidney Disease (CKD).

Serum from 14 dogs treated with IH and 10 dogs treated with IF was submitted for measurement of sCr and IDEXX SDMA. Dogs in each treatment group received 5 treatment sessions, administered 48 hours apart.

Significant differences ($P \leq 0.05$) were seen between treatment groups, however dogs from the hemodialysis group were the most

Table 1. Linear mixed model with Biomarkers as depednant variabes*

Recluded Variable	Dependant Variable	Shift in Dep var	STD error	P-value	Chisq
Treatment (Fluid/ Hemo)	SDMA	-26.13	5.087	<0.001	17.805
Treatment (Fluid/ Hemo)	Creatinine	-3.75	0.667	<0.001	21.589
Treatment (Fluid/ Hemo)	Urea	-116.50	16.712	<0.001	33.122
Treatment (Fluid/ Hemo)	Albumin	-0.25	0.060	<0.001	17.192
Treatment (Fluid/ Hemo)	Sodium	-0.995	0.768	0.196	1.670
Treatment (Fluid/ Hemo)	Hematocrit	-10.251	2.357	<0.01	14.008
Treatment (Fluid/ Hemo)	Phosphorus	-1.300	2.442	0.595	0.283
Treatment (Fluid/ Hemo)	Ionized Calcium	0.011	0.041	0.792	0.070
Treatment (Fluid/ Hemo) * Urea	SDMA	0.024	0.046	0.650	0.206
Treatment (Fluid/ Hemo) * Urea	Creatinine	0.017	0.005	<0.001	11.166
URR (%)	SDMA	-0.621	0.01	0.002	13.56

*Fixed effects: sample time (pre/post), treatment session, breed, age, sex, body weight, neuter status
Random Effects: Patient_ID

affected on the clearance of SDMA (X 2 =17.8, P < 0.001), sCr (X 2 = 21.6, P < 0.001), and blood urea (X 2 = 33.1, P < 0.001). A significant correlation was also found between the urea reduction ratio (URR) and SDMA. For each 10% increase in URR there was a 6.2 µg/dL decrease in SDMA (X 2 = 13.56, P = 0.002) (Table 1).

Although SDMA is a dialysable biomarker, we highly recommend evaluating it in context with the concurrent URR for a better estimate of actual GFR. Despite its removal by IH, SDMA still is a better biomarker than sCr for evaluating GFR in dogs with CKD.

NU27

Evaluation of Commercial ELISA Kits for Quantification of Selected Cytokines in Feline Urine

Sarah Buchholz, Keri Gardner, John M. Kruger
Michigan State University, East Lansing, MI, USA

Feline idiopathic cystitis (FIC) is a common bladder disorder of cats and diagnosis remains one of exclusion. Urine cytokines may be potential biomarkers for FIC. Enzyme-linked immunosorbent assays (ELISAs) are ideal for quantifying cytokines. However, variations in urine matrix characteristics can interfere with ELISA performance. Since few ELISAs have been validated for feline urine, our objective was to evaluate feline-specific ELISA kits for quantification of IL-2, IL-6, and IL-12 in feline urine. Urine was collected from healthy cats evaluated at the Michigan State University Veterinary Medical Center. Normal urine samples were pooled, modified to mimic disease conditions by adding water or hemolyzed blood, and then spiked with high, medium, and low concentrations of recombinant cytokines. Concentrations of IL-2, IL-6, and IL-12 were determined using commercially available feline-specific ELISA kits. The influence of urine matrix variables on test performance was evaluated by spike/recovery tests, linearity testing, and assessment of inter- and intra-assay variation. All assays underestimated high and medium spiked cytokine concentrations in urine as compared to standard assay diluents. Low spiked cytokine concentrations in urine were inconsistently detected. Urine spiked with IL-2, IL-6 and IL-12 achieved maximal quantitative recoveries of 82%, 90%, and 58% respectively. High urine specific gravity contributed significantly to poor IL-2 and IL-12 recovery (P < 0.05). Hematuria had variable effects. Feline urine matrix characteristics appear to significantly impact cytokine quantification by ELISA. Further studies are needed to optimize detection of cytokines in feline urine by ELISA and establish their use as diagnostic biomarkers for feline urinary disorders.

NU28

Investigating Urinary Bladder Transcriptome Alterations in Feline Idiopathic Cystitis Using RNA Sequence Analysis

Taylor Aiello[1], Peter Schall[1], Maciej Parys[2], Vilma Yuzbasiyan-Gurkan[1], John M. Kruger[1]
[1]Michigan State University, East Lansing, MI, USA, [2]Royal (Dick) School of Veterinary Studies and the Roslin Institute, Midlothian, Scotland, United Kingdom

Feline idiopathic cystitis (FIC) is a common lower urinary tract disease in cats that resembles interstitial cystitis/painful bladder syndrome in people. In both conditions the pathogenesis of the disease is unknown and there is no specific treatment. To investigate the molecular pathways and disease functions potentially involved in the pathogenesis of FIC, we evaluated the bladder mucosa transcriptome of affected and healthy cats. Urinary bladder tissues were collected from 3 healthy cats and from 3 FIC cats representing 3 different histological phenotypes (ulcerative, non-ulcerative, and hyperplastic). RNA from bladder tissues was isolated and sequenced using high throughput next generation sequencing. Differential gene expression and molecular pathway and disease factor analysis was performed using Ingenuity Pathway Analysis (IPA) software. A significant level of gene set enrichment (-log [p] > 1.3) was identified in several annotated disease and function categories. The inflammatory response function was up regulated in the non-ulcerative and ulcerative phenotypes (z-scores 1.9 and 2.6 respectively), and down regulated in the hyperplastic phenotype (z-score -3.6). The cellular growth and proliferation category was down regulated in the non-ulcerative and hyperplastic phenotypes (z-scores -2.4 and -3.7) and up regulated in the ulcerative phenotype (z-score 6.5). The cell death and survival pathway was down regulated in the non-ulcerative and hyperplastic phenotype (-2.3 and -2.2) and up regulated in the ulcerative phenotype (6.5). Further investigation of these pathways and other target genes may provide insight into the pathogenesis of FIC and identify new therapeutic targets as well as biomarkers of the disorder for future clinical applications.

NU29

Serum Creatinine vs Serum Symmetric Dimethylarginine on Follow-Up of Dogs with Chronic Kidney Disease

Natalia Garla Nascimento[1], Marcia Mery Kogika[1], Fernanda Chicharo Chacar[1], Douglas Segalla Caragelasco[1], Bruna Maria Coelho Silva[2], Camila Eleuterio Rodrigues[3], Talita Rojas Sanches[4], Lucia Conceicao Andrade[4]
[1]School of Veterinary Medicine and Animal Science - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil, [2]Veterinary Teaching Hospital - School of Veterinary Medicine and Animal Science - USP, Sao Paulo, Sao Paulo, Brazil, [3]School of Medicine - USP, Sao Paulo, Sao Paulo, Brazil, [4]School of Medicine - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil

International Renal Interest Society (IRIS) has also recommended for the chronic kidney disease (CKD) classification the use of serum symmetric dimethylarginine (SDMA) as an adjunct tool for diagnosis and treatment directions as serum creatinine concentration could underestimate the stage. Creatinine concentration may be affected by muscle mass, age, sex and hydration status and may underestimate the degree of kidney dysfunction. SDMA is an endogenous marker of GFR and not influenced by those factors. SDMA concentrations above 25 and 45 µg/dL are considered as stage 3 and stage 4, respectively. The aim of this study was to follow-up CKD dogs with IRIS stages 2 or 3 based on serum creatinine concentration, and also to investigate whether and when SDMA is in disagreement with creatinine concentrations for the classification of CKD stage. The study group was composed of twenty-one CKD dogs. CKD dogs with stage 2 (n= 9) and stage 3 (n= 12) were followed-up for 12 to 15 months or until death. The evaluation by means of serum creatinine concentrations, only two CKD dogs with stage 2 changed the classification to stage 3 during the follow-up, and also in those dogs serum SDMA was increased concomitantly; however other three CKD dogs with stage 2 had the increase only in SDMA (changing the classification to stage 3) and not in serum creatinine, showing the underestimation of the stage based on serum creatinine. In CKD dogs with stage 3, during the follow-up, only two dogs changed the classification to stage 4 based on serum creatinine concentrations, however when taking SDMA concentrations (> 45 µg/dL), 9 out of 12 changed the classification to stage 4 and among them, 6 out 9 dogs that had serum creatinine < 5.0 mg/dL were already in stage 4 since the beginning of the follow-up.
Those 6 CKD dogs with stage 3 had, respectively, creatinine and SDMA concentrations at the first visit of 2.7 mg/dL and 46 µg/dL; 3.1 mg/dL and 60 µg/dL; 4.2 mg/dL and 47 µg/dL; 4.7 mg/dL and 60 µg/dL; 3.2 mg/dL and 61 µg/dL; 3.2 mg/dL and 68 µg/dL. The findings suggested, mainly for stage 3, that serum creatinine concentration is more affected by extrarenal factors and may cause the underestimation of the stage, and it may influence the directions for the treatment and prognosis. Nine CKD dogs with stage 3 had decreased muscle mass score (1/3, n= 3; 2/3, n= 6). The death was observed in 7 CKD dogs with stage 3, and in 5 out of 7, serum SDMA was above 45 µg/dL since the beginning the follow-up (46, 47, 60, 61 and 68 µg/dL). The survival analysis by Kaplan-Meier taking into consideration the stages 2 and 3 showed for SDMA concentrations of 14 to 24 µg/dL the survival time of 373 to 454 days (survival of 100%), and for SDMA 25 to 45 µg/d and > 45 µg/dL the time of 181 to 412 days (60% survival at 258 days) and 29 to 299 days (25% survival at 160 days and 0% at 299 days), respectively. In conclusion, the evaluation of serum SDMA concomitantly with serum creatinine could add important information in order to verify the real renal dysfunction, mainly in those CKD dogs classified as stage 3 by means of serum creatinine concentrations, and that information may help and alert to determine treatment and prognosis.

NU30

Urinary Protein to Creatinine Ratio, Albumin and Retinol-Vitamin D-Binding Proteins in Chronic Kidney Disease Dogs

Douglas Segalla Caragelasco[1], Marcia Mery Kogika[1], Marcia Mery Kogika[1], Talita Rojas Sanches[2], Fernanda Chicharo Chacar[1], Dennis Chew[3], Cinthia Ribas Martorelli[1], Natalia Garla Nascimento[4], Camila Eleuterio Rodrigues[2], Lucia Conceicao Andrade[2]
[1]School of Veterinary Medicine and Animal Science - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil, [2]School of Medicine - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil, [3]College of Veterinary Medicine - The Ohio State University, Columbus, OH, USA, [4]School of Veterinary Medicine and Animal Science - USP, Sao Paulo, Sao Paulo, Brazil

Urinary protein to creatinine ratio (UP/C) measures the total amount of proteins in urine compared to urinary creatinine concentration, but it does not identify the origin of individual proteins. Qualitative evaluation of urinary proteins by immunodetection could add important information as to the nature of a renal disease as albuminuria is associated with glomerular disease, and retinol-binding protein (RBP) and vitamin D-binding protein (VDBP) with tubular damage due to the impairment of tubular reabsorption of low molecular weight proteins that are normally found in glomerular filtrate. The hypothesis of this study was that qualitative methods (e.g. western blotting) in association with quantitative methods (UP/C) may provide better assessment and evaluation of the origin of proteinuria. The aim was to study dogs with chronic kidney disease (CKD) and to determine the UP/C as well as to use immunoassay (western blotting) to detect urinary albumin, RBP and VDBP. The study group was composed of CKD dogs with stage 2 (n=9) and stage 3 (n=13) and they were followed up for 12 to 15 months or until death. Fifteen clinically healthy dogs of different breeds and age comprised the control group. Coomassie brilliant blue (Bradford method) was used for UP/C determination, and anti-albumin (ab112986,1:500; Abcam, Cambridge, MA), anti-VDBP (ab95469,1:500; Abcam) and anti-RBP (ab48624, 1:250; Abcam) for the western blotting. Urinary albumin, RBP and VDBP were not detected in control dogs and UP/C was 0.15±0.02 (mean±SEM) with a min of 0.03 and a max of 0.4. Only one CKD dog with stage 2 had proteinuria based on UP/C (mean of the follow-up, min-max; 1.49, 0.76-3.45) also with loss of VDBP (tubular pattern of renal disease). In the remaining 8 out of 9 CKD dogs with stage 2, the mean of UP/C was normal (UP/C < 0.4) during the follow-up period, however the qualitative evaluation detected loss of tubular proteins (RBP and /or VDBP) in 2/8, loss of glomerular protein (albumin) in 1/8, loss of tubular and glomerular proteins in 2/8, and in 3/8 no level of proteins were found. Proteinuria (UP/C > 0.5) was observed in 7 out of 13 CKD dogs with stage 3, and a tubular pattern characterized by the predominance of low molecular weight proteins loss (RBP and or VDBP) was noticed in 5/7, and the loss of albumin and RBP, both in similar magnitude, in 2/7 (tubular and glomerular pattern). In non-proteinuric (mean

during follow-up, UP/C < 0.5) CKD dogs with stage 3 (n=6), the immunodetection of albumin and RBP or VDBP were observed in 3/6, and in 1/6 only a slight amount of albumin loss was detected. These findings suggest that sequential qualitative evaluation of urinary proteins can add more information to interpretation of UP/C results. Identification of the damaged nephron segment that caused a specific pattern of protein loss could help refine the diagnosis of a specific renal disease as well as to optimize therapy. Importantly, the detection of albumin, RBP and VDBP in urine samples with normal UP/C might serve as an early marker of glomerular and tubular injury.

NU31

Fibroblast Growth Factor-23 association with Vitamin D Metabolites in Dogs with Spontaneous Chronic Kidney Disease

Marcia Mery Kogika[1], Miss Fernanda Chicharo. Chacar[1], Cinthia Ribas. Martorelli[1], Dennis Chew[2], Ramiro Toribio[3], Joao Felipe de Brito Galvao[4], Helio Autran. de Moraes[5], Andrew Joseph. Makowski[6], Douglas Segalla. Caragelasco[1], Camila Eleuterio Rodrigues[7], Talita Rojas Sanches[7], Lucia Conceicao Andrade[7]

[1]School of Veterinary Medicine and Animal Science - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil, [2]College of Veterinary Medicine - The Ohio State University, Columbus, OH, USA, [3]The Ohio State University College of Veterinary Medicine, Columbus, OH, USA, [4]VCA Arboretum View Animal Hospital, Downers Grove, IL, USA, [5]College of Veterinary Medicine - Oregon State University, Corvallis, OR, USA, [6]Heartland Assays LLC & Metabolic Technologies, Inc., Ames, IA, USA, [7]School of Medicine - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil

The kidneys play an important role in mineral and bone disorders. Recent studies in dogs and cats have shown that serum fibroblast growth factor 23 (FGF-23) increases in early stages of chronic kidney disease (CKD). FGF-23 regulates phosphorus through phosphaturic effects on the proximal tubules and inhibition of 1-alpha-hydroxylase activity, decreasing circulating calcitriol [1,25$(OH)_2D_3$]. FGF-23 also increases Cyp24a1 mRNA expression and 24-hydroxylase activity enhancing degradation of calcidiol [25$(OH)D_3$] and calcitriol into their less active metabolites 24,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] and 1,24,25 trihydroxyvitamin D [1,24,25$(OH)_3D_3$], respectively. The ratio 25$(OH)D_3$ to 24,25$(OH)_2D_3$ indirectly estimates 24-hydroxylase enzyme activity. An increased ratio is associated with impaired calcidiol degradation or CYP24a1 inactivating mutations. Our hypothesis was that increased serum FGF-23 concentrations in dogs with CKD IRIS stages 2 or 3 are associated with decreased 25$(OH)D_3$ and 1,25$(OH)_2D_3$, but increased in 24,25$(OH)_2D_3$ concentrations. We recruited 17 CKD dogs (6 with IRIS-Stage 2 and 11 with IRIS Stage 3) aged 2 – 17 years. Fifteen healthy dogs ranging in age from 8 months to 9 years were used as controls. Kruskal-Wallis test was used to compare groups. As expected, serum FGF-23 was highest in dogs with stage 3 (median 1866; range: 957.6-14193 pg/mL) CKD, followed by stage 2 (median 402; range 178.7-1066 pg/mL) and lowest in healthy dogs (median 248.3; range 144.6-422.7 pg/mL) ($p < 0.0001$). Lower serum 1,25$(OH)_2D_3$ concentrations were detected in CKD dogs with stage 3 (median 120.1; range 36.7-376.3 pg/mL; n= 11) when compared to control dogs (median 287.8; range 179.3-392.2 pg/mL) and stage 2 (median 164.1; range 82.4-463.4 pg/mL; n= 6) ($p= 0.02$). Serum 25$(OH)D_2$ and 24,25$(OH)_2D_2$ were below detection limit of the assay. There were no differences in serum 25$(OH)D_3$ among the 3 groups of dogs (control, n= 11, median 41.20 and range: 14.1-74.5 ng/mL; CKD stage 2, n= 5, median 41.8 and range:15.8-74.5 ng/mL; CKD stage 3, n= 8, median 26 and range: 10.9-53.7 ng/mL) ($p= 0.25$). Lowest serum 24,25$(OH)_2D_3$ was observed in CKD dogs with stages 3 (median 4.8; range 1.6-20.8 ng/mL; n= 8), followed by stage 2 (median 16.6; range 7.4-33.2 ng/mL; n= 5) and highest in the control group (median 31.20; range 6.2-49.5 ng/mL; n= 11) ($p= 0.003$). An increase in FGF-23 was negatively correlated with a decrease in 1,25$(OH)_2D_3$ (Spearman, rho = -0.5557, $p= 0.001$) and 24,25$(OH)_2D_3$ (Spearman rho = -0.6365, $p= 0.0008$). Additionally, 1,25$(OH)2D_3$ and 24,25$(OH)_2D_3$ were significantly correlated (Spearman rho= 0.82, $p < 0.0001$). The ratio of 25$(OH)D_3$ to 24,25$(OH)_2D_3$ was highest in CKD dogs with stage 3 (median 5.1; range 1.3-7.4; n= 8), followed by stage 2 (median 2.2; range 2.1-3.46; n= 5) and lowest in the control group (median 1.3; range 0.9-2.27; n= 11) ($p= 0.0008$). Our results suggest that increased serum FGF-23 may contribute to reductions in 24,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ concentrationsin CKD dogs, and play a role in mineral and bone disorders and potentially, to CKD progression. We failed to identify an association between FGF-23 and 25$(OH)D_3$ concentrations in CKD dogs (Spearman rho = -0.2414, $p= 0.2559$). Lower 25$(OH)D_3$ and 24,25$(OH)_2D_3$ concentrations were previously observed in dogs with advanced CKD (stages 3 and 4), and low serum 24,25$(OH)_2D_3$ levels were similar as reported in mice and human patients with CKD. A high 25$(OH)D_3$ to 24,25$(OH)_2D_3$ ratio observed in our study failed to confirm FGF23-mediated catabolism of vitamin D metabolites assessed by 24,25$(OH)_2D_3$. The reason for 25$(OH)D_3$ concentrations that did not differ between control and CKD dogs, as previously reported, was not investigated in this study. Further studies with a larger sample size are needed for the better understanding of the role of FGF-23-mediated catabolism of vitamin D in dogs with CKD.

NU32

Electrocardiographic Parameters, P and QT Dispersions in Dogs with Chronic Kidney Disease Undergoing Intermittent Hemodialysis

Angélica Alfonso [1], Silvano Salgueiro Geraldes[1], Andre N. V. Le Sueur[2], Priscylla T. C. Guimarães-Okamoto[2], Alessandra Melchert[2], Maria Lucia Gomes Lourenço[1]

[1]São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Botucatu, Sao Paulo, Brazil, [2]School of Veterinary Medicine and Animal Science, São Paulo State University - UNESP Botucatu, Botucatu, Sao Paulo, Brazil

Intermittent hemodialysis (IHD) is used in dogs with chronic kidney disease (CKD) to reduce chronic progressive azotemia, hyperkalemia, and fluid overload. Monitoring the cardiovascular system is important during this treatment to recognize repercussions in the heart. In medicine, dispersions of P wave and QT interval are important mortality risk indicators and can be easily obtained by the difference between their maximum and minimum values in the 10 electrocardiographic leads. The measurement of P wave and QT interval dispersion is related to the risk of developing atrial and ventricular fibrillation, respectively. This study aims at describing P and QT dispersions, as well electrocardiographic changes observed in dogs with CKD stage IV undergoing IHD. The animals were classified considering serum creatinine (> 5mg/dL), urinary protein/creatinine ratio (> 0.5) and

systolic blood pressure (> 160mmHg). Ten dogs weighing 15-30 kg were included in the study and electrocardiographic examinations were performed before and after the first three sessions of IHD. The results were analyzed using ANOVA with Friedman's test, considering a significance level of 5%. There were significant increases in values for P maximum (p = 0.011), QT maximum (p = 0.002), QT minimum (p = 0.023), however, the changes in P and QT dispersions were not significant (p> 0.05). There were increases in PR intervals (p = 0.019), QT intervals (p = 0.034) and a decrease in the amplitude of the R wave (p = 0.002). After the second session, three dogs (30%) developed sinus arrest and another three (30%) developed supraventricular arrhythmias. The results obtained suggest that employing IHD in dogs may cause electrocardiographic changes such as arrhythmias, interferences in atrioventricular conduction, as well as in the ventricular depolarization and repolarization process. These findings highlight the importance of electrophysiological monitoring in animals submitted to this therapeutic modality.

NU33

Dietary Sodium Increases Calcium Excretion and Induced Calcium Oxalate Precipitation in Cats

Jennifer M. MacLeay[1], *Heidi Schiefelbein*[1], *Stephen Davidson*[2]
[1]*Hill's Pet Nutrition, Inc., Topeka, KS, USA,* [2]*2004, Topeka, KS, USA*

Dietary intake of sodium chloride greater than 6g/day (>1.2g sodium/1000 kcal when 2000 kcal/day is consumed), is associated with increased calcium excretion in humans and is not recommended for those with a history of calcium oxalate urolithiasis according to the Consensus Conference for the Metabolic Diagnosis and Medical Prevention of Calcium Nephrolithiasis and its Systemic Manifestations- Rome, March 26–28, 2015[1] and the NIDDK[2]. In cats, feeding a food with a sodium content of approximately 1.4% (3.5g sodium/1000 kcal) has been reported to increase urinary calcium while concomitantly increasing water intake and consequently urine volume when compared to a food containing approximately 0.4% (1g sodium/1000 kcal). When foods with similar Ca, P and Mg restriction but relatively high (3.5g/1000 kcal) and low (1g/1000 kcal) sodium content were compared, it was observed that relative supersaturation (RSS) for calcium oxalate was similar and calcium excretion was significantly greater for the high sodium food.[3] This suggests that a benefit ascribed to lower urinary saturation due to increased urinary water is offset by increased urine calcium content. Long term studies have not been performed in cats comparing RSS for calcium oxalate and recurrence rates for uroliths; however a correlation does exist in humans. Another surrogate for calcium oxalate risk, the Bonn Risk Index as described by Laube et al, has been proposed as a better predictor of future calcium oxalate risk than relative super saturation.[4,5] This study examines low and high sodium feline dry foods by using the calcium oxalate titration (COT) test first described by Davidson[6], which is similar to the human Bonn Risk Index test. In the COT test, centrifuged, unaltered, body temperature urine is titrated with a sodium oxalate solution and monitored at 585nm until precipitation occurs. The COT value is determined by dividing the urine [Ca^{2+}] by the resultant amount of oxalate added at the point of precipitation. Therefore the COT test measures urine stability or capacity as it is influenced by the composition of urine, reflecting the interactions of inhibitors and promoters of crystal formation.

A group of 12 healthy adult cats were fed two commercially available foods[a,b] (1g sodium/1000 kcal and 3.5g sodium /1000 kcal), in series, for 2 weeks each and 24 hour urine samples were collected at the end of each period and evaluated. The COT test was performed and Equil 2 software was used to calculate RSS from urine concentrations of NH_4^+, Ca^{+2}, Cl^-, Citrate, Mg^{+2}, oxalate, PO_4^{-3}, K^+, Na^+, SO_4^{-2} and urine pH. SAS™ version 9.2 was used for all analyses. A repeated measures regression model was used with the value of the analyte as the dependent variable and food as the independent variable. Least-square means and pair-wise differences were calculated for diet comparisons for each analyte. Two-sided tests were performed. An alpha of 0.05 was used to determine statistical significance.

Urine pH (p = 0.4), RSS for struvite (p = 0.6) and RSS for calcium oxalate (p = 0.3) were not significantly different between foods. Urine from cats fed the low sodium food had a significantly (p < 0.01) greater urine specific gravity ((mean±SEM) 1.054 ± 0.001 vs 1.038 ± 0.002), phosphorus (2776 ± 94 ppm vs 1370 ± 102 ppm) and citrate concentration (2993 ± 538 umol/L vs 1154 ± 589 umol/L) and significantly lower calcium (45 ± 7 ppm vs 80 ± 8 ppm) and COT (28 ± 30 vs 202 ± 33) compared to the high sodium food. Compared to the high sodium food, the low sodium food improved urinary crystallization capacity as measured by the COT test by 86% and lowered urine calcium concentration by 44%. With respect to calcium oxalate crystal formation, factors associated with increased urine stability, include urine citrate and urine phosphorus and these were significantly increased by 159% and 103% respectively.

Feline urine is typically metastable for calcium oxalate and complexation is affected by many factors. Increased urine water may help reduce saturation of crystal components but may also dilute inhibitors of crystal formation. This data supports that the dynamic nature of calcium oxalate precipitation testing provides additional information on the capacity of urine to resist induced crystallization which is not reflected in the traditional super saturation calculation. Elevated sodium content of foods influenced calcium excretion and appeared to lower urine resistance to induced crystal formation. Further study of the relationship between RSS and COT and long term risk for crystal and stone formation is warranted.

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NU34

Continuous Flow Cystoscopy: The Next Step

Timothy C. McCarthy
VetMIST(Veterinary Minimally Invasive Surgery Training), Beaverton, OR, USA

Continuous flow cystoscopy facilitates preforming transurethral cystoscopy for complex prolonged interventional procedures.Transurethral cystoscopy has traditionally been performed using a single

cannula system that allows fluid flow in only one direction at a time. These one way flow cystoscopes have worked well in small animal practice for over 30 years and still work well for diagnostic and simple corrective procedures. This system does not work well for more advanced complex transurethral interventional procedures, especially those using lasers or radio-frequency. With only one way flow the visual field is quickly obscured with debris, blood, and smoke. Repeated fluid exchanges are required to maintain adequate image quality. Discontinuing the procedure to drain and refill the bladder is time consuming, frustrating, and increases the risk of bladder trauma by over-distensionContinuous flow cannulas are available for cystoscopes that allow ingress and egress of fluid to occur simultaneously maintaining a constant bladder distension with a clear visual field. These systems are standard instrumentation for human cystoscopy. Continuous flow cannulas are designed with two concentric cannulas rather than the traditional single cannula. The inner cannula is for inflow and delivers a continuous flow of fluid directly in front of the telescope lense to maintain a visual field. The outer cannula with a separate isolated lumen is for simultaneous egress. Each cannula has a separate luer connector with a stop cock. Continuous flow maintains a clear visual field decreasing operative times, decreasing frustration level, and decreasing the risk of bladder trauma due to over-distension. Continuous flow cannulas are available for the 4.0mm, 30 degree, cystoscope for use in large dogs and has a 23 French diameter outer cannula with a 7.5 French working channel. A system for smaller patients requires purchase of an additional telescope and cannula system. This is a 2.0mm, 30 degree, telescope with a 12.5 French diameter outer cannula with a 17cm working length and a 5 French working channel. Unfortunately a continuous flow cannula is not available for the 2.7mm MPRT.

NU35

Prevalence and Risk Factors of Feline Lower Urinary Tract Disease in Chiang Mai, Thailand

Kakanang Piyarungsri[1], *Sahatchai Tangtrongsup*[2], *Phathamaporn Lekklar*[3], *Atiratt Kittinuntasilp*[4], *Niyada Thitaram*[2]
[1]*Department of Companion Animal and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiangmai, Chiang Mai, Thailand,* [2]*Department of Companion Animal and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Chiang Mai, Thailand,* [3]*Faculty of Veterinary Medicine, Chiang Mai University, Chaiang mai, Chiang Mai, Thailand,* [4]*Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Chiang Mai, Thailand*

Feline lower urinary tract disease (FLUTD) is a common problem in cats. The objectives of the current study were to determine the prevalence, clinical signs, causes and risk factors for FLUTD. The medical records of 3486 cats visiting Chiang Mai University Small Animal Veterinary Teaching Hospital between November 2016 to October 2017 were reviewed. Ninety-three cats with FLUTD were identified. A case–control study was performed to determine risk factors for FLUTD in 74 FLUTD cats and randomly selected 74 clinically normal cats. For each animal, potential risk data obtained from medical records and cat owners'; interviews were analyzed for associations with FLUTD. A multivariable logistic regression analysis was performed to estimate odds ratios and to adjust for expected confounding factors. The prevalence of FLUTD in cats in Chiang Mai was 2.7% (95%CI: 2.1 - 3.2). The most common clinical signs identified were urethral obstruction (56.9%), hematuria (23.6%), stranguria (16.7%), and pollakiruria (15.3%), respectively. The most common diagnoses were feline idiopathic cystitis (FIC) (57%), followed by urolithiasis (struvite) (19%), and urinary tract infection (UTI) (12%), respectively. The multivariate logistic regression analysis indicated that FLUTD was more likely to be diagnosed in castrated male cats. In conclusion, FIC and urolithiasis are the most common diagnoses in cat with FLUTD and castration of male cats may be the important risk factor for FLUTD.

NU36

Retrospective Evaluation of the Relationship Between Symmetric Dimethylarginine, Creatinine and Body Weight in Hyperthyroid Cats - Sponsored By IDEXX

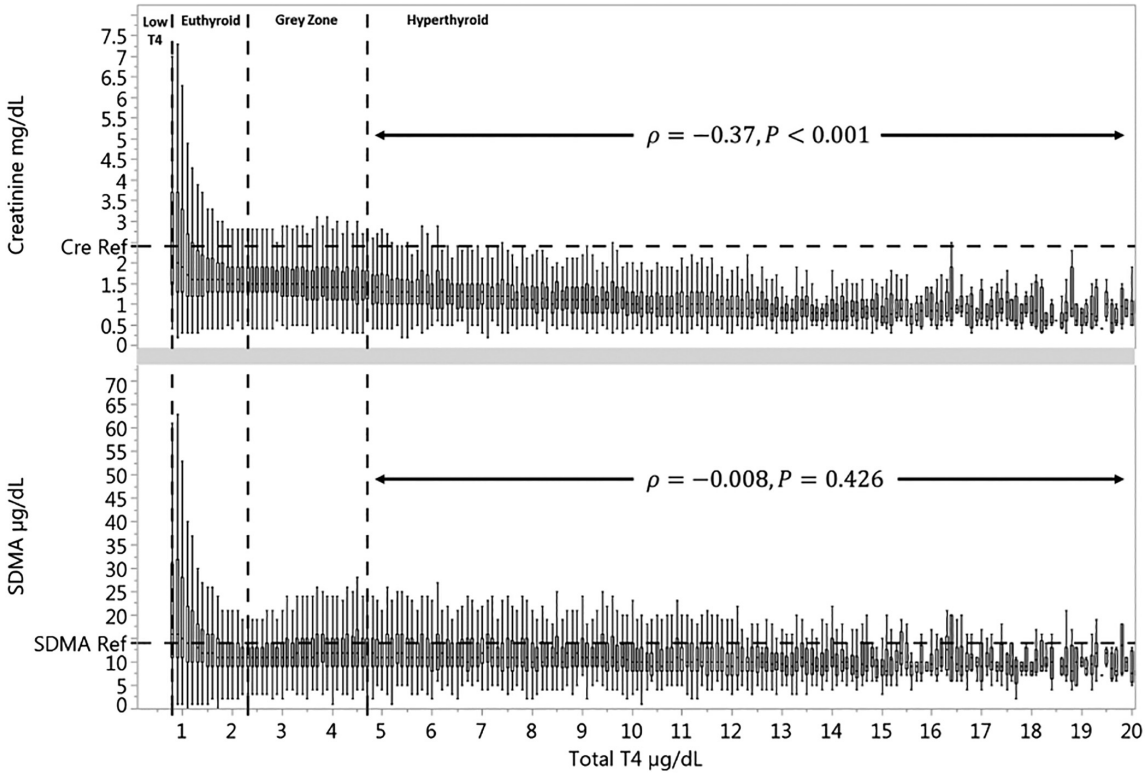
Jessica M. Quimby[1], *Jane E. Robertson*[2], *Donald A. Szlosek*[2], *Jennifer S. Ogeer*[3], *Celeste A. Clements*[2], *Donald J. McCrann*[2]
[1]*The Ohio State University, Columbus, OH, USA,* [2]*IDEXX Laboratories, Inc., Westbrook, ME, USA,* [3]*IDEXX Laboratories, Inc., Markham, ON, Canada*

Hyperthyroidism can mask chronic kidney disease (CKD) because of hyperfiltration and muscle loss. Symmetric dimethylarginine (SDMA) has been shown to increase earlier than creatinine in cats with CKD, and, unlike creatinine, SDMA is not impacted by lean muscle mass. For these reasons, it has been postulated that SDMA could be a more sensitive indicator of renal function in hyperthyroid cats. The aim of this study was to describe the relationship between SDMA, creatinine, body weight and T4 to evaluate SDMA as a potential renal biomarker in hyperthyroid cats.

Cats were retrospectively identified from the US IDEXX Reference Laboratories database where T4, SDMA and creatinine were measured on the same sample. SDMA and creatinine were compared to T4 concentrations for the entire study population of cats. A Spearman correlation test was conducted for cats with T4 > 4.7 μg/dL. A hyperthyroid treated group was identified (T4 ≤ 4.7 μg/dL and decreased by a minimum of 2.5 μg/dL) that had body weight and laboratory results available from more than one visit. Hyperthyroid cats and a 10 to 1 comparative control group of age-matched euthyroid cats were used to evaluate body weight, creatinine, SDMA and T4 pretreatment and at 1-30, 31-60, 61-90, 91-120 days post-treatment.

Of 113,535 cats that met the criteria, 14,861 had a T4 < 0.8 μg/dL, 41,032 were euthyroid (0.8 μg/dL ≥ T4 < 2.3 μg/dL), 20,211 were in the grey zone (2.3 μg/dL ≥ T4 ≤ 4.7 μg/dL) and 37,431 were hyperthyroid (T4 > 4.7μg/dL). Boxplots illustrate the relationship between T4 and creatinine and T4 and SDMA concentrations. A negative relationship was found between creatinine and T4 (ρ = -0.37, P < 0.001). Although there was also a slight negative relationship between SDMA and T4, it was not statistically significant (ρ = -0.008, P < 0.426).

The mean body weight of hyperthyroid treated cats (n = 1,281) at pretreatment and at all post-treatment time points was significantly less than control cats (n = 12,810) (all P values < 0.01). Hyperthyroid cats had a significantly lower mean weight pretreatment, and at 1-30 days and 31-60 days versus 90-120 days (all P values ≤ 0.03). Pretreatment creatinine was significantly decreased in comparison to all



post-treatment time points (all P values < 0.001). Creatinine was significantly increased at 91-120 days in comparison to all other time points (all P values ≤ 0.007). The mean creatinine of hyperthyroid cats was significantly less than in control cats pre-treatment and up to the 61-90 day post-treatment time period (all P values < 0.01). Pre-treatment SDMA was significantly decreased in comparison to all post-treatment time points (all P values < 0.001). However, after the increase in SDMA at 1-30 days, there was no significant difference in mean SDMA concentrations throughout the remainder of the treatment periods. In addition, compared to the control cats, the mean SDMA of hyperthyroid cats was only significantly lower pre-treatment (P < 0.001) but not at any post-treatment time point.

Creatinine significantly decreased with increasing concentrations of T4, whereas SDMA did not. Both SDMA and creatinine concentrations increased during the immediate post-treatment period. During treatment creatinine continued to increase as cats gained weight. In contrast, SDMA remained stable during treatment and was comparable to age-matched control cats. Therefore, SDMA may be a more reliable biomarker of renal function than creatinine in hyperthyroid cats before and during treatment.

NU37

Evaluation of an Herbal Compound Used to Manage Lower Urinary Tract Disease in Healthy Dogs

Ashley Shaw[1], *Cook English*[1], *Joe Bartges*[1], *Sherry Cox*[2]
[1]*The University of Georgia, Athens, GA, USA,* [2]*The University of Tennessee, Knoxville, TN, USA*

Lower urinary tract disease occurs commonly in dogs. While conventional therapy involves modified diet and pharmacological intervention, traditional Chinese and Western herbs have been recommended as alternative and complementary treatments. The purpose of this study was to evaluate efficacy of an herbal supplement. We hypothesized that the herbal supplement would be associated with increased urine volume and decreased urine saturation for calcium oxalate and struvite when compared with placebo.

Fourteen healthy client-owned dogs were evaluated using a randomized placebo cross-over study in a pairwise fashion, each dog receiving treatment every 12 hours for a two-week period. An approximate 12-hour voided urine sample was collected at the end of each treatment period. Samples were analyzed for pH, sodium, potassium, chloride, calcium, phosphorous, magnesium, citrate, oxalate, ammonia, and creatinine. Relative supersaturation for calcium oxalate and struvite was estimated using an iterative computer program (EQUIL 93b, University of Florida, Gainesville, FL). Data were assessed for normal distribution using Shapiro-Wilk. Paired t-test (normal distribution) or Wilcoxon signed rank (non-normal distribution) were used for statistical analysis. Data were analyzed using 2-tailed statistical testing except for relative supersaturation and urine volume where 1-tailed testing was used. A *p*-value < 0.05 was considered significant.

Data from 10 dogs were used due to incomplete urine collection from 4 dogs. There were 7 females and 3 males including 4 Labrador mixes, 1 Havanese, 1 Pit bull, 1 Great Dane mix, and 3 mixed unknown breeds. Urine saturation for calcium oxalate was significantly lower when dogs received supplement compared with placebo (supplement = 2.30 +/- 1.76, placebo = 3.50 +/- 1.61; p= 0.047). There were no differences in other analytes, body weight, or urine volume.

There are several limitations of this study. The study was of short duration and included a small number of healthy, non-urolith forming

dogs. Twelve hour urine samples were collected by owners at home, which may have introduced error in urine volume collected. Additionally, urine was collected overnight and dogs did not drink although they had access resulting in collection of low volumes of urine.

There was a significant decrease in calcium oxalate supersaturation even with these limitations suggesting determination of relative supersaturation is a better determinant of risk of urolith formation than electrolyte & mineral concentrations or excretions. The herbal supplement may be beneficial for managing calcium oxalate uroliths in dogs.

NU38

Platelet Aggregometry and Measurement of Clopidogrel Metabolite Concentrations in Dogs with Protein-Losing Nephropathy

Sarah Shropshire, Tyler Johnson, Christine Olver
Colorado State University, Fort Collins, CO, USA

Dogs with protein-losing nephropathy (PLN) are commonly prescribed drugs for thromboprophylaxis but there is no standardized method to measure response to these medications. Additionally, it is unknown if clopidogrel metabolite concentrations differ between PLN dogs and dogs without renal disease. The purpose of this study was to assess response to aspirin or clopidogrel in dogs with PLN using whole blood impedance platelet aggregometry (PA) and to compare clopidogrel metabolite concentrations between healthy and PLN dogs.

PA using two agonists (adenosine diphosphate; ADP and arachidonic acid; ASPI) and a saline control was performed in six healthy client-owned dogs at baseline and 1 week following clopidogrel administration to establish cut-off values to identify responders (*R*) or non-responders (*NR*). A decrease of ≥ 60% for ADP at 1 or 3 hours post clopidogrel administration defined a *R*. PA was performed in PLN dogs at baseline and 1, 6, and 12 weeks following clopidogrel or aspirin administration. Drug concentrations and PA were measured at 1 and 3 hours post clopidogrel administration (at 1 week or 6 weeks) in both healthy and PLN dogs. Fourteen dogs were enrolled; ten PLN dogs received clopidogrel alone, three received aspirin alone, and one dog was started on aspirin but was switched to clopidogrel. Repeated measures (RM) analysis (one-way ANOVA) was performed and subsequent time points (1, 6, and 12 weeks) were compared to baseline aggregometry in PLN dogs and RM analysis (two-way ANOVA) was performed to evaluate differences in drug concentrations between healthy and PLN dogs.

In PLN dogs receiving clopidogrel; aggregometry differed from baseline at all time points for ADP but no differences were observed for ASPI at any time point. Most dogs were defined as clopidogrel *R* at one or both time points except one dog who showed no response via ADP or ASPI. In PLN dogs receiving aspirin, there were no differences from baseline at any time point for either ADP or ASPI. There were no differences in drug concentrations at either time point between healthy and PLN dogs.

PA may represent an objective method to evaluate response to clopidogrel or aspirin therapy in PLN dogs and PLN dogs appear to metabolize clopidogrel similarly to healthy dogs.

NU39

Alterations in Oral and Fecal Microbiomes in Dogs with Chronic Kidney Disease

Chan-Joo Sung[1], Joo-Hyun Song[1], Bona Ho[1], Ji-Houn Kang[1], Wan-Kyu Lee[2], Taesoo Kwon[3], Ji-Soo Kim[1], Jinhee Lee[1], Minji Lee[4], Hakhyun Kim[1], Haemin Lee[1], Minwoo Kang[1], Byeong-Teck Kang[1], Mhan-Pyo Yang[1]
[1]Veterinary Medical Center, College of Veterinary Medicine, Chungbuk National University, Cheongju, Ch'ungch'ong-bukto, Republic of Korea, [2]College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju, Ch'ungch'ong-bukto, Republic of Korea, [3]Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea, [4]Veterinary Medical Center, College of Veterinary Medicine, Chungbuk National University, cheongju, Ch'ungch'ong-bukto, Republic of Korea

Chronic kidney disease (CKD) in dogs is a common clinical entity characterized by a marked decrease in renal function. In humans, there are several reports that uremia caused by renal failure affects the oral and fecal microbiomes, but there is a lack of information on the oral and fecal microbiomes in dogs with CKD. Therefore, the objective of the present study was to examine the changes in the oral and fecal microbiomes in CKD dogs.

Eleven dogs with CKD presenting with azotemia over 3 months and 9 healthy dogs were included in this case-controlled study. The oral swabs and fecal samples were analyzed by whole genome sequencing using an Illumina MiSeq platform.

The oral microbiome of the CKD dogs showed changes in composition. The isolation frequency of *Proteobacteria* in CKD dogs was decreased, and conversely, that of *Bacteroides* and *Firmicutes* was increased. The predominant species in the oral microbiome of healthy dogs were those of the genera *Neisseria* and *Bacteroides*, whereas those in CKD dogs were *Bacteroides* and *Porphyromonas* species. In fecal samples, lower bacterial species richness was observed in CKD dogs compared to healthy dogs. The proportions of *Fusobacteria*, *Firmicutes*, *Proteobacteria* species were increased in the fecal microbiome of the CKD dogs, but the proportion of *Bacteriodetes* was decreased.

Our results suggest that chronic azotemia might trigger the altered composition of oral and fecal microbiomes in dogs with CKD. Large population-based studies are warranted to determine how these changes affect the manifestation of gastrointestinal complications associated with CKD.

NU40

Serum Lipoprotein Profiles in Cats with Chronic Kidney Disease

Jean-Sébastien Palerme[1], Tomomi Minamoto[2], Punyamanee Yamkate[3], Jean-Sébastien Palerme[1], Jennifer Scaccianoce[1], Jan S. Suchodolski[2], Joerg M. Steiner[4]
[1]Iowa State University College of Veterinary Medicine, Ames, IA, USA, [2]Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, [3]Texas A&M University, College Station, TX, USA, [4]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, College Station, TX, USA

Dyslipidemia affects up to 85% of humans with chronic kidney disease (CKD) and has been associated with increased mortality and progression of CKD in addition to being a risk factor for CKD development. Altered lipoprotein profiles have been documented in dogs with CKD but this has not yet been investigated in cats. The purpose of this

study was to compare serum lipoprotein profiles between cats with CKD and healthy controls. We hypothesized that cats with CKD would have higher total low-density lipoprotein (LDL) and lower total high-density lipoprotein (HDL) when compared with healthy cats.

Client-owned healthy cats and cats with naturally-occurring CKD were prospectively enrolled in the study. Healthy cats had a urine specific gravity of 1.035 or greater and a serum creatinine < 1.6 mg/dl or a normal S-dimethylarginine (SDMA). Cats with CKD had a compatible clinical history, serum creatinine > 1.6 mg/dl and a urine specific gravity < 1.035. After a 12-hour fast, serum was collected for analysis of lipoprotein profiles using a continuous lipoprotein density profiling (CLPDP) technique. Serum cholesterol and triglycerides were also measured using a commercial chemistry analyzer. Area under the curves for triglyceride-rich lipoproteins (TRL), total LDL and total HDL were calculated for statistical analysis. Data were analyzed using a Wilcoxon rank sum test with significance set at $P < 0.05$.

Thirteen cats with CKD and six healthy controls met inclusion criteria. Based on creatinine values at the time of study enrollment, 11/13 cats with CKD were classified as International Renal Interest Society (IRIS) stage 2 and 2/13 cats were classified as IRIS stage 3. Triglyceride rich lipoproteins, total LDL and total HDL were not significantly different between cats with CKD and healthy controls ($P = 0.187$, $P = 0.218$, $P = 0.065$, respectively). Serum cholesterol concentrations and serum triglyceride concentrations were within reference range for all cats and were not significantly different between groups ($P = 0.484$ and $P = 0.080$, respectively).

This study suggests that dyslipidemia may not occur in cats with CKD and implies a possible difference in the pathophysiology of CKD in cats relative to that of dogs and humans. Further studies with a larger sample size and cats with more advanced CKD are needed to confirm our findings.

NU41

Urine is Not Sterile: Evidence of a Urinary Microbiome in Healthy Adult Cats - Sponsored By Purina

Brittany M. Vester Boler[1], Berenice C. Bastien[1], Gail L. Czarnecki[1], Janet R. Jackson[2]
[1]Nestle Purina Research, St. Louis, MO, USA, [2]Nestle Purina, St. Louis, MO, USA

Urine has traditionally been regarded as a sterile fluid. Culture independent techniques have allowed for further exploration of various body sites, including the bladder. Researchers have shown that a urinary microbiome exists in other species, including healthy dogs. This study aimed to characterize the urinary microbiome of healthy adult cats. Urine was collected via cystocentesis from 15 healthy adult domestic shorthair cats showing no signs of urinary tract infection. Cats used in this study included 9 neutered males and 6 spayed females with an average age of 8.3 years, and an average body weight of 4.8 kg. Bacterial genomic DNA was extracted using methods specifically for low-biomass samples. The V4 region was amplified by PCR and sequenced using the Illumina MiSeq platform and compared to the SILVA Database. A rarefaction cutoff of 460 reads per sample was used and 202 OTUs were detected. The major phyla (relative abundance) in the urine samples included Proteobacteria (49.7 %), Firmicutes (32.8 %), Actinobacteria (8.9 %), Bacteroidetes (4.1 %), and Cyanobacteria (1.3 %). Identified genera were highly variable among cats. *Staphylococcus* spp. was the only genus present in all 18 samples. Other genera present in over half of cats included *Enterobacter* spp. (n = 13), *Acinetobacter* spp. (n = 13), *Streptococcus* spp. (n = 12), *Shigella* spp. (n = 12), and *Acinetobacter* spp. (n = 10). Relative abundance of these genera varied widely among cats. Similar to reports in other species, cats do have a urinary microbiome even with no signs of a clinical infection; however, what constitutes a healthy urinary microbiome is currently unknown.

NU42

Daily Oral Chondroitin Sulfate Administration Increases Urinary Chondroitin Sulfate Concentrations in Dogs

MICHAEL Wood
University of Wisconsin-Madison, Madison, WI, USA

In healthy individuals urinary chondroitin sulfate helps prevent uropathogenic bacterial adherence to the bladder wall. In people exogenous chondroitin sulfate/hyaluronic acid administered intravesicularly reduced yearly urinary tract infection recurrence 10 fold. In dogs similar bladder instillation therapy is impractical requiring regular catheterization and extended bladder dwell times. Alternatively chondroitin sulfate is a common veterinary dietary supplement. The described study aimed to determine whether daily oral chondroitin sulfate administration increased canine urinary chondroitin sulfate concentrations (uCS).

This study enrolled 8 client owned dogs. Urine samples collected for 5 consecutive days (days 1-5) established baseline uCS. Dogs then received 20-30mg/kg twice daily of chondroitin sulfate for 8 days (days 8-15) with urine samples collected on days 8-12 and 15. uCS was quantified in μg/ml using high performance liquid chromatography tandem mass spectrometry. Variable urine output, and hence uCS dilution, was accounted for by dividing measured uCS by urine creatinine (uCS:uCrea). Treatment and baseline uCS:uCrea were compared to calculate the fold change in urinary chondroitin sulfate excretion after supplementation.

Oral chondroitin sulfate supplementation increased mean uCS:uCrea 1.81x ($p = 0.02$). No difference in uCS was observed when comparing the first (day 8) and last (day 15) supplementation days ($p = 0.08$).

These data indicate that oral chondroitin sulfate supplementation increases uCS. The increase occurs within 24h and is not additive such that intravesicular instillation concentrations are not achieved. A therapeutic benefit of persistent low concentration chondroitin sulfate supplementation in preventing canine recurrent urinary tract infections is unknown and an on-going research focus.

NU43

Assessment of Indicators for Disease Progression in Feline Autosomal Dominant Polycystic Kidney Disease

Yoshihiko Yu[1], Kate L. Shumway[2], Marie E. Edwards[3], Timothy L. Kline[3], Reuben M. Buckley[2], Jodi S. Matheson[2], Leslie A. Lyons[2]
[1]Nippon Veterinary and Life Science University/College of Veterinary Medicine, University of Missouri, Columbia, Musashinoshi, Tokyo, Japan, [2]College of Veterinary Medicine, University of Missouri, Columbia, Columbia, MO, USA, [3]Mayo Clinic College of Medicine, Rochester, MN, USA

Disease progression variability of feline autosomal dominant polycystic kidney disease (ADPKD) is known, but predictions of end-stage renal disease (ESRD) are not feasible. Additionally, no biomarkers are

available to evaluate the efficacy of drug treatments. In humans, computed tomography (CT) and magnetic resonance imaging (MRI) are used to follow ADPKD progression by evaluating total kidney volume (TKV). The purpose of this study was to examine the efficacy of biomarkers and imaging modalities to evaluate disease progression in feline ADPKD.

Eleven ADPKD cats with the c.10063C>A *PKD1* mutation were imaged using ultrasonography, CT, and MRI. CBC, serum chemistry, urianalysis, symmetric dimethylarginine levels, and glomerular filtration rate were also measured.

All ADPKD cats were clinically normal except for the presence of multiple, bilateral renal cysts. TKV measured by CT correlated with estimates using ultrasonography and MRI. TKV had no correlation with age. Fractional cyst volume (FCV) (0.01 – 0.28 ml) were obtained from CT and had a moderate correlation with age (15.12 – 97.08 months), with a Spearman's rank correlation coefficient of 0.627, and $p = 0.044$. However, FCV per age showed outliers.

Renal cysts are known to increase over time in cats with ADPKD, however, this data indicates cysts progress at different rates as determined by CT-based FCV per age and cats do not have significant increases with age in TKV. Variable FCV per age supports disease progression is individual and suggests modifiers likely influence progression. FCV can evaluate disease progression of feline ADPKD and characterize the therapeutic efficacy of drugs for ADPKD.

NU44

Creatinine and GFR Measurements using Jaffe and Enzyme-Based Methods in Cats - Sponsored By Purina

Brian Zanghi[1], *Patricia Turpin*[2], *Rachel Anderson*[2], *Brittany M. Vester Boler*[2]
[1]*Nestle Purina PetCare, St. Louis, MO, USA*, [2]*Nestle Purina Research, St. Louis, MO, USA*

One component of assessing chronic kidney disease in cats is creatinine (Cr) concentration in blood and urine measured using an automated system based on the Jaffé reaction or enzyme-based assay. Evidence from other species suggests Cr is over-estimated with the Jaffé reaction, but it is not clear if this assumption is accurate for cats. This study compared these 2 automated assay methods, with a -0.3 mg/dL correction as part of the Jaffé methodology, to measure Cr concentration in serum (SCr) and urine (UCr). Cr data was used with voided urine volume (Uvol) to calculate endogenous creatinine glomerular filtration rate (GFR) and compared to serum symmetric dimethylarginine (SDMA) concentrations. Forty-eight-hr Uvol from healthy, domestic shorthair cats ($N = 45$ [23 males/22 females]; 7.8 yrs ± 2.0 SD) occurred while individually housed with inert litter and finished with blood sample collection for SCr and SDMA. GFR was determined using both Cr assay methods. Mean Jaffé reaction SCr, UCr, and GFR are overestimated ($P = 0.002$), UCr ($P = 0.04$) with the overestimation becoming greater with increasing Cr concentration. The overall mean ± SE (min-max range) of SCr, UCr, and GFR based on the enzyme method was 1.6 ± 0.2 mg/dL (1.1-2.1), 323 ± 59 mg/dL (184-457), and 1.10 ± 0.37 mL/min/kg BW (0.46-1.86). UCr and GFR did not differ with age or sex, whereas SCr differed with age ($P = 0.04$), but not sex ($P = 0.74$), as older cats had lower SCr (1.49 ± 0.06; 8-14 yrs) versus middle-aged cats (1.64 ± 0.04; 4-7.5 yrs). In addition, Uvol (range: 3.4-18.4 mL/kg/d) and USG (range: 1.028-1.070 g/mL) did not differ with age or sex, whereas SDMA (range: 6-21 ug/dL) differed with age ($P = 0.032$), but not sex ($P = 0.13$), with lower SDMA in older (12.1 ± 0.7 SE) versus middle-aged cats(14.5 ± 0.8 SE). Therefore, as part of the framework to successfully manage CKD in cats over time, selecting diagnostic laboratories that measure Cr using the enzyme method appears to improve accuracy by eliminating the detection of non-Cr compounds. This is particularly important as these data demonstrate that Cr is increasingly overestimated with the Jaffe method as Cr concentrations increase.

NM01

Prospective Evaluation of a Novel Esophagostomy Feeding Tube Placement Device in Dogs

JD Foster
Friendship Hospital for Animals, Washington, DC, USA

This study evaluated the efficacy and safety of a novel technique for placing esophagostomy feeding tubes (e-tubes) in client owned dogs who were unable to voluntarily consume adequate calories and nutrients.

E-tube placement was performed under general anesthesia. The distal end of the e-tube was advanced to the lower esophageal sphincter. A tunneler device was advanced into the esophagus until the tip was palpated dorsolaterally from the jugular groove. Soft tissues were dissected, and the tip was advanced through the skin. The proximal end of the e-tube was connected to the distal part of the tunneler device. The tunneler was pulled caudally, bringing the proximal end of the e-tube outside the patient. Correct placement of the e-tube was confirmed, and the e-tube was secured to the skin. Statistical analysis was performed to compare time of e-tube placement against patient body weight and e-tube size.

30 dogs were enrolled, with a median weight of 19.7kgs (range 2.75-35.5 kgs). Median placement time was 262.5 seconds (range 78-635 seconds). Placement time was significantly ($p = 0.045$), but weakly ($r^2 = 0.14$) associated with patient body weight. There was no difference in placement time between different sizes of e-tubes ($p = 0.33$). Median duration of e-tube placement was 21 days. The e-tube was removed in one patient due to infection, and minor complications were observed in 3 other patients.

This study confirms the ease, speed, and safety of a novel methodology of e-tube placement. Further analysis is needed to compare adverse effects and placement time of different e-tube placement methods.

NM02

Metabolic and Immunological Effects of Intermittent Fasting in Healthy Dogs Fed a High Fat Diet

Yuet-Ming Leung[1], *Nick J. Cave*[1], *Axel Heiser*[2], *Patrick Edwards*[3], *Timothy Wester*[1]
[1]*School of Veterinary Science - Massey University, Palmerston North, Manawatu-Wanganui, New Zealand*, [2]*AgResearch, Palmerston North, Manawatu-Wanganui, New Zealand*, [3]*Institute of Fundamental Sciences at Massey University, Palmerston North, Manawatu-Wanganui, New Zealand*

Intermittent fasting (IF) has numerous benefits in some species, including increased insulin sensitivity, and improved neuronal repair

following spinal injury from an increase in ketones. The aim of this study was to examine the immunological, metabolic and hormonal effects associated with IF in dogs.

Ten healthy dogs underwent three, 1-week isocaloric feeding regimens in a Latin square design: daily low-fat feeding (20% kcal ME; DLF), IF on a low-fat diet (IFLF), and IF on a high-fat (70% kcal ME) diet (IFHF). Body weight, intake, activity, lymphocyte proliferation, macrophage/neutrophil phagocytosis and respiratory burst, along with fasting blood glucose, beta-hydroxybutyrate (BHOB), leptin, ghrelin, and insulin were measured. NMR spectroscopy was used to identify changes in the fasted plasma metabolome.

Dogs on the IFLF lost weight [mean (±SD) (-1.7 ± 1.5%)] from a lower voluntary food intake. Dogs were more active at night when fed daily than when fed intermittently. Mean fasting BHOB concentrations were highest during the IFHD phase (0.061 ± 0.023mM) and lowest during the DLD phase (0.018 ±0.004mM). Mean fasting plasma glucose was greatest when fed daily, and lowest during the IFHD phase. Intermittent and daily feeding separated on metabolomics analysis. Hormone concentrations did not differ, nor were there biologically significant differences in immune assays between dietary phases.

Unlike humans and rodents, short term fasting in dogs does not produce detrimental immunological effects. The increase in plasma BHOB with IF on a high fat diet highlights its potential as a beneficial feeding regime for dogs with spinal disease.

NM03

Effects of Continuous and Intermittent Caloric Restriction Regimens on Body Fat Loss in Obese Dogs

Yuanlong Pan[1], Ms. Julie Fish[2], Ms. Xuemei Si[1], Hui Xu[3], Sandeep Bhatnagar[3], Isabelle Mougeot[2]
[1]Nestle Purina Research, St Louis, MO, USA, [2]CanCog Technologies Inc., Toronto, ON, Canada, [3]Nestle Purina PTC, St Louis, MO, USA

Obesity has become the most common health problem and can reduce longevity, increase risk of many chronic diseases, and adversely affect quality of life in dogs. Continuous caloric restriction (CCR) regimens have been applied for obesity treatment by creating a daily negative energy balance. However, CCR leads to decreased energy expenditure, which makes it difficult to continue to lose excess body fat and to maintain body weight after weight loss. The objective of this study was to compare the effect of CCR to an intermittent calorie restriction (ICR) regimen on body fat loss, and post-weight loss maintenance energy requirement (MERs) in obese dogs.

Thirty naturally obese dogs with BCS of 9 were randomized into two groups based on MERs, body weight, and % body fat. Dogs in the CCR group were fed 75% of their baseline MERs for 6 months, while the dogs in the ICR group were fed with the repeats of 75% baseline MERs for one week, and then 100% baseline MERs for one week for 6 months. Then, the caloric restriction in both groups was adjusted to 65% of baseline MERs for 2 additional months. ICR and CCR led to similar % body fat at the end of the study (35.40%± 1.38 vs 33.89% ± 1.52, $p > 0.05$). Dogs in the CCR group significantly ($p = 0.0005$) lowered their post-weight loss MERs by 17.92% compared with their baseline MERs. However, dogs in the ICR group numerically increased their post-weight loss MERs by 3.43% ($p = 0.08$).

NM04

Effect of Different Carbohydrate Sources on Glycemic Index and Satiety-Related Gut Hormones in Dogs

Alexandra Rankovic[1], Jennifer Adolphe[2], Anna-Kate Shoveller[3], Dan Ramdath[4], Adronie Verbrugghe[5]
[1]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Petcurean Pet Nutrition, Chilliwack, BC, Canada, [3]Department of Animal Biosciences University of Guelph, Guelph, ON, Canada, [4]Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, ON, Canada, [5]University of Guelph Ontario Veterinary College, Guelph, ON, Canada

Obesity and diabetes are leading nutrition-related disorders among pets in North America and have significant impacts on animal morbidity and mortality. The glycemic index (GI), a measure of post-prandial blood glucose response, has been shown in humans to significantly impact glucose control, insulin sensitivity, weight management, and chronic disease risk.

The purpose of this research was to determine the GI of commercially available pet foods containing different carbohydrate sources; in addition, the effects on postprandial glycemic and insulinemic response and hormones related to satiety were also investigated.

Four commercial extruded diets containing different carbohydrate sources were tested. The diets were classified based on the type of carbohydrate sources they contained: traditional grains (corn, wheat), whole grains (oats, rye, barley), grain-free (peas, lentils, chickpeas) or vegan (no animal ingredients). Each diet was tested once in eleven healthy adult Siberian Huskies and the control (50% glucose solution) was tested twice per dog in a randomized cross-over design. Each meal and control provided 25g of available carbohydrate. Pre- and postprandial blood samples at 15, 30, 45, 60, 90, and 120 minutes were collected to measure whole blood glucose concentrations using a handheld blood glucose monitor. Serum insulin, ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide 1 (GLP-1), leptin, total peptide tyrosine-tyrosine (PYY), and pancreatic polypeptide (PP) concentrations were also analysed at these time points using a magnetic bead multiplex assay.

Glycemic index was calculated as the post-prandial incremental area under the curve (iAUC) for glucose of each test diet, divided by the iAUC of the control. Glycemic indices were compared using two-way ANOVA. Concentrations of glucose, insulin and satiety hormones over time were compared between treatments using repeated-measures ANOVA. This study was approved by the University of Guelph Animal Care Committee (AUP#3650).

The effect of diet on GI was insignificant ($p = 0.589$), although numerical differences were observed. The diet containing traditional grains presented the highest GI of 76.9 ± 18.3, while the grain-free diet presented the lowest GI of 50.5 ± 13.1. The whole grain diet and vegan diet had GI values of 60.8 ± 7.4 and 71.4 ± 17.3, respectively. No significant effects of diet were observed for postprandial glycemic response, insulinemic response or on any of the satiety hormones measured ($p > 0.05$).

The lack of observed differences could be related to the gelatinization of starch during extrusion processing, resulting in a high content of rapidly digestible starch. These results suggest that different carbohydrate sources in commercial extruded diets may not have a significant

effect on GI or satiety in dogs. Further research investigating the appropriateness of using the GI methodology in humans is necessary due to the high variability in postprandial glucose response observed in this study, and the unknown health benefits of low glycaemic foods in dogs.

NM05

Differences in Postprandial Metabolites related to Lipid Metabolism between Lean and Overweight Labrador Retriever Dogs

Josefin Söder, Katja Höglund, Johan Dicksved, Ragnvi Hagman, Johnny Östman, Ali Moazzami, Sara Wernersson
Swedish University of Agricultural Sciences, Uppsala, Uppsala Lan, Sweden

Obesity in pet dogs is an increasing problem associated with morbidity, shortened life span and poor life quality. As overweight dogs exhibit postprandial hyperlipidemia it is of interest to identify specific metabolite changes. This study aimed to investigate postprandial metabolites related to lipid metabolism in a feed-challenge test and to identify alterations related to overweight in dogs. Twenty-eight healthy male Labrador Retriever dogs were included, 12 of which were classified as lean (body condition score (BCS) 4-5 on a 9-point scale) and 16 as overweight (BCS 6-8). After overnight fasting, plasma samples were collected and dogs were fed a high-fat meal followed by postprandial plasma sample collection hourly for 4 hours. High-resolution liquid chromatography–mass spectrometry (LC-ToF-MS) was used, and selected metabolites were evaluated based on relative signal areas in mixed model repeated measures analysis. In all dogs, propionylcarnitine and stearoylcarnitine increased in response to food intake ($P \leq 0.005$) while the metabolite C18:1 decreased ($P = 0.0003$), resembling findings in humans. The overall taurine, carnitine and acetylcarnitine responses were lower in overweight compared to lean dogs ($P \leq 0.017$). Notably, in lean dogs the acetylcarnitine signal area was higher than in overweight dogs at fasting ($P = 0.001$) and was decreased one hour postprandially ($P < 0.0001$), while in overweight dogs there was no response to feeding. In conclusion, the lower signal areas of taurine and carnitine in overweight dogs suggest the possibility of deficiencies in this group. Moreover, the acetylcarnitine pattern in overweight dogs could indicate decreased fatty acid oxidation at fasting and metabolic inflexibility to food intake.

NM06

Nutrient-Enriched Water Enhances total Water Intake and Hydration in Dogs

Brian Zanghi[1], Cari Gardner[2]
[1]Nestle Purina PetCare, St. Louis, MO, USA, [2]Nestle Purina Research, St. Louis, MO, USA

Water intake and hydration were evaluated in healthy dogs offered a nutrient-enriched water (NW) supplement while fed a maintenance dry food to maintain BW with ad libitum tap water (TW) in a bucket. Baseline (day -7) urine specific gravity (USG) was analyzed in Beagles ($N = 30$; 2-11 years) and 16 were selected with ≥ 1.015 USG, then equally divided into 2 groups balanced for USG. Groups received either TW or NW (containing whey protein, poultry flavoring, and glycerol) in a bowl for 56 days. Dose for each dog was 0.5:1 water-to-calorie ratio (mL:kcal ME/d) from days 1-49 and 2:1 mL:kcal ME/d for days 50-56 based on baseline calorie intake. Food calorie and total liquid intake (TLI; g/d; sum of NW or TW in a bowl and bucket water) recorded daily and used to calculate weekly mean intake amounts. USG was measured on days -7, 14, 42, 56. Linear mixed effects models were performed with main effects of treatment, time, and time-by-treatment interaction. Calorie intake was not different ($P > 0.49$). A time-by-treatment interaction was significant ($P = 0.07$) versus baseline. A significant ($P < 0.01$) on days 42 (1.018 g/mL) and 56 (1.014 g/mL) versus baseline (1.026 g/mL). This study indicates that all dogs offered the NW supplement, while having free access to TW, increased their TLI and produced a more dilute urine, which suggests an improvement in overall hydration.

NM07

Pea and Barley as a Starch Source Impact on Hyperlipidemia of Diabetic Dogs

Marcio Antonio Brunetto[1], Daniela Pedrosa Machado[2], Juliana Toloi Jeremias[3], Mariana Ramos Queiroz[2], Cristiana Ferreira Fonseca Pontieri[3], Marcio Antonio. Brunetto[4]
[1]School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, Sao Paulo, Brazil, [2]School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, São Paulo, Sao Paulo, Brazil, [3]Grandfood Industria e Comercio LTDA, Dourado, Sao Paulo, Brazil, [4]School of Veterinary Medicine and Animal Science - University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil

Diabetes mellitus is a chronic disorder that results in hyperglycemia and hyperlipidaemia by insulin deficiency, sometimes leading to fatal

(NM07)

Table 1 – Mean of plasmatic tryglicerides and cholesterol concentration of 12 diabetic dogs after two months feeding each diet.

Variables	Triglycerides			Cholesterol		
(mg/dL)	Ba	PB	Co	Ba	PB	Co
T0	192.4 (238.8)^{a}	83.8 (43.9)^{b}	194.8 (283.3)^{a,b}	334.6 (113.3)	318.1 (96.7)	358.8 (119.6)
T2	282.1 (262.8)	205.9 (144.4)	328.4 (340.2)	316.4 (100.1)^{a,b}	288.7 (85.2)^{a}	350.2 (113.9)^{b}
T4	285.9 (254.7)^{a,b}	188.3 (175.9)^{a}	389.5 (392.9)^{b}	312.5 (106.0)^{a}	297.2 (101.4)^{a}	362.7 (127.1)^{b}
T6	187.6 (167.5)	113.4 (125.3)	301.2 (381.4)	319.9 (106.3)^{a,b}	299.5 (102.5)^{a}	351.2 (112.3)^{b}
T8	109.9 (76.7)	75.8 (46.6)	211.6 (306.6)	307.6 (123.8)^{a,b}	302.1 (91.6)^{a}	351.0 (117.1)^{b}
T10	81.5 (51.0)	67.5 (30.2)	152.7 (195.5)	297.6 (113.9)^{a}	296.7 (93.0)^{a}	345.9 (115.3)^{b}
Mean	189.9 (161.9)	120.6 (79.1)	263.0 (309.9)	314.8 (108.6)^{a,b}	303.0 (96.3)^{a}	353.3 (116.6)^{b}
Minimum	76.4 (51.7)	63.7 (30.7)	141.9 (198.8)	282.7 (104.4)^{a}	284.5 (85.2)^{a}	334.2 (108.5)^{b}
Maximum	316.8 (260.4)	221.7 (180.5)	397.8 (386.3)	341.4 (116.5)^{a,b}	323.9 (102.7)^{a}	376.2 (122.6)^{b}
AUC*	2004.7	1349.8	2809.0	3145.2^{a}	2856.0^{a}	3534.8^{b}

Different superscript letters means statistically difference in line between diets ($p<0.05$). AUC = area under the curve (mg/dL/min). Ba = basal diet; PB = pea and barley diet; Co = Corn diet

complications. In this study we evaluated the effect of pea and barley as only starch source on lipid profile of diabetic dogs.

Twelve diabetic dogs received, randomly and by a double blind way, three diets differenced by fat level and starch source: Ba (mix of rice, pea and barley; 6.7 g of fat/1000kJ), PB (pea and barley; 9.6 g) and Co (corn; 9.8 g). A plasmatic cholesterol and triglycerides curve was performed, every 2 hours for 10 hours beginning immediately before the morning meal and insulin injection, after 60 days of each diet treatment. Statistical tests were performed and p Pea and barley-based diet caused lesser plasmatic lipid concentration, mainly cholesterol, comparably to corn diet (table 1). PB diet results the same lipid value that Ba diet, allowing decrease of lipids levels with higher fat content diet. Probably it happened due to better glycemic control of diabetic dog receiving slow assimilation starch, and to phenolics compounds presents in pea and barley ingredients. Then, we believe that pea and barley could be interesting starch source to hyperlipidemic diabetic dogs.

NM08

Changes in Pet Feeding Practices Over the Past Decade

Sarah AS Dodd[1], *Nick J. Cave*[2], *Jennifer Adolphe*[3], *Anna-Kate Shoveller*[4], *Adronie Verbrugghe*[5]

[1]*University of Guelph, Bradford, ON, Canada*, [2]*School of Veterinary Science - Massey University, Palmerston North, Manawatu-Wanganui, New Zealand*, [3]*Petcurean Pet Nutrition, Chilliwack, BC, Canada*, [4]*Department of Animal Biosciences University of Guelph, Guelph, ON, Canada*, [5]*University of Guelph Ontario Veterinary College, Guelph, ON, Canada*

Research investigating nutritional sufficiency and deficiency have been prominent in animal nutrition and veterinary literature for the past few decades, resulting in development of complete and balanced commercial pet foods. Regardless, trends in companion animal nutrition often shadow trends in human nutrition, and a perception exists among veterinarians that many pet owners now prefer 'natural'; and 'holistic'; diets over processed foods. The purpose of the study was to determine what and how dog and cat owners feed their pets and compare to a study published ten years ago (Laflamme et al. 2008). Both studies involved pet owner surveys: Laflamme and colleagues'; 2008 questionnaire was administered by students over the phone to randomly selected telephone directories, while this present questionnaire was administered online and was distributed to pet owners on social media. The survey was promoted to general pet owning and pet appreciation groups and not to veterinary, nutrition or other special interest groups, and was open for sharing to reach a wide audience and acquire a sample representative of the general population of pets and pet owners. The study was approved by the research ethics boards of participating institutions (REB #17-08-029). Descriptive data were reported as percentages, while significant differences between the two studies were determined using Chi Square analysis, with significance set at $P < 0.05$. Information from Australia and the United States only were included for analysis. The results of both surveys are summarised in Table 1 below.

Where data were comparable, significant differences in feeding practices over time included: increase in feeding homemade and raw diets, decrease in daily feeding of treats, and decrease in feeding a commercial diet as the main food source in both dogs and cats; while an increase in exclusive feeding of homemade diets was also detected in cats only. In essence, the trend appears to be a shift in pet nutrition away from the complete and balanced diets currently recommended by most veterinarians, in favour of home-prepared 'human'; foods and raw meat diets. These changing practices may predispose the nations'; pets to dietary imbalances, nutritionally-responsive diseases, and infectious diseases. Given this information, clinicians in both general and specialty practice must be aware of the risks associated with homemade and raw diets, obtain comprehensive dietary histories from their clients, and be prepared to diagnose and treat associated diseases.

Reference:

Laflamme DP, Abood SK, Fascetti AJ, et al. Pet feeding practices of dog and cat owners in the United States and Australia. *J Am Vet Med Assoc* 2009;5:687-694.

NM09

Correlation between Dietary Macronutrients and Gut Microbiota Bacterial Groups

Hannah L. Klein[1], *Rachel Pilla*[2], *Jan S. Suchodolski*[3]

[1]*Gastrointestinal Laboratory, Texas A&M University, College Station, TX, Bryan, TX, USA*, [2]*Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX., College Station, TX, USA*, [3]*Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA*

The purpose of this study was to correlate the percentage of protein, fat, and crude fiber in commercial dry dog food diets to major bacterial groups and canine microbiota dysbiosis index.

(NM08) Table 1: Feeding practices of dog and cat owners

	Never		Daily		Inclusive		Exclusive	
Variable	**2008**	**2018**	**2008**	**2018**	**2008**	**2018**	**2008**	**2018**
Dogs								
Commercial†	<10%	18.6%	-	65.3%	93.2%[a]	81.4%[b]	>60%[a]	14.4%[b]
Homemade	>80%[a]	18.2%[b]	1%[a]	34.0%[b]	<20%[a]	81.8%[b]	<5%	10.9%
Raw	83.8%[a]	34.4%[b]	0%[a]	32.5%[b]	16.2%[a]	65.6%[b]	-	13.7%
Scraps	74.6%[a]	19.6%[b]	20.0%	10.4%	25.4%[a]	80.4%[b]	-	0.8%
Treats	36.4%[a]	14.5%[b]	41.2%[a]	26.6%[b]	63.6%[a]	85.5%[b]		
Cats								
Commercial†	<5%	10.0%	-	78.9%	98.8%[a]	90.0%[b]	>80%[a]	33.4%[b]
Homemade	>80%[a]	39.4%[b]	0.2%[a]	15.6%[b]	<15%[a]	60.6%[b]	0%[a]	7.0%[b]
Raw	90.4%[a]	45.2%[b]	0.2%[a]	20.6%[b]	9.6%[a]	54.8%[b]	-	12%
Scraps	85.6%[a]	48.7%[b]	5.3%	3.3%	14.4%[a]	51.3%[b]	-	0.3%
Treats	61.2%[a]	35.5%[b]	21.4%[a]	8.7%[b]	38.8%[a]	64.5%[b]		

Significant differences between years are denoted by superscript. In the present study, data was collected for 1,486 dogs and 757 cats. In 2008 study, data was collected for 621 dogs and 449 cats.

† Commercial category includes conventional cooked kibble or wet diets

Fecal samples were collected from healthy dogs (n= 73). Owners completed a survey with information about health status and dietary history. Nutrient content was obtained from online product guides, if the information was not provided, the pet food companies were contacted directly. The dysbiosis index (DI) was evaluated by combining the quantitative polymerase chain reaction values for *E. coli*, *Streptococcus*, *Turicibacter*, *Faecalibacterium*, *Fusobacterium*, *Blautia*, and *Clostridium hiranonis*. Statistical significance was set to p< 0.05. Correlations between abundance of bacterial taxa and the macronutrients were performed using Spearman';s correlations. Significance was set at p< 0.05.

Results indicated there was statistical significance between an increase in crude fiber in the diet and a decrease in *Fusobacterium* (r= - 0.255; p= 0.0330). However, there was no correlation between any bacterial group and protein or fat and no other correlations with the other bacterial groups and changes in fiber content in the commercial dry food diets. When correlating protein to the bacterial groups there were no significant changes in *E. coli* (p= 0.8793), *Faecalibacterium* (p= 0.3733), *Fusobacterium* (p= 0.1588), or the Dysbiosis Index (p= 0.3747). When correlating fat to the bacterial groups there were no significant changes in *E. coli* (p= 0.7026), *Faecalibacterium* (p= 0.4963), *Fusobacterium* (p= 0.0675), or the Dysbiosis Index (p= 0.8907). And finally when correlating crude fiber to the bacterial groups there were no significant changes in *E. coli* (p= 0.7137), *Faecalibacterium* (p= 0.7946)or the Dysbiosis Index (p= 0.3243).

In conclusion, a significant correlation was found only between crude fiber and *Fusobacterium*. Results suggest that the macronutrient content in commercial diets has only modest impact on the abundance of major bacterial taxa in the canine microbiota.

NM10

Feline Plasma and Urine Lipidome: A Novel Source of Biomarker Candidates for Feline Medicine

Sol Rivera, Sandy Chen, Nicolas Villarino
Washington State University College of Veterinary Medicine, Pullman, WA, USA

The study of lipids in biological fluids has led to substantial advances in diagnosis and therapeutics of human obesity, cancer, and kidney diseases. Considering that most of these conditions also afflict cats, we hypothesize that lipids could also serve as biomarkers for studying and diagnosing diseases in cats. However, the study of lipids in feline plasma has been limited to a few number of lipid species, mainly, fatty acids and triacylglycerols, whereas it is yet unknown if the feline urine contains any lipids. Considering this gap of knowledge, the objective of this study was to characterize the lipid composition in plasma and urine samples collected from clinically healthy young-adult cats (n=8).

Plasma samples were obtained from clinically healthy young-adult (< 2 years-old) intact female cats by collecting 1 mL of blood into tubes containing citrate and then centrifuged at 1,800 x g for 8 min. Urine samples were collected by ultrasound-guided cystocentesis and immediately centrifuged at 1,000 x g for 8 min. Every cat was given a baseline complete history, physical examination, and laboratory evaluation. All had normal complete blood count, serum chemistry, and urinalysis. Lipids were analyzed using liquid chromatography/quadrupole time-of-flight mass spectrometry.

In total, 212 lipids were characterized in feline plasma and urine samples. The origin of these metabolites was mainly from food and endogenous

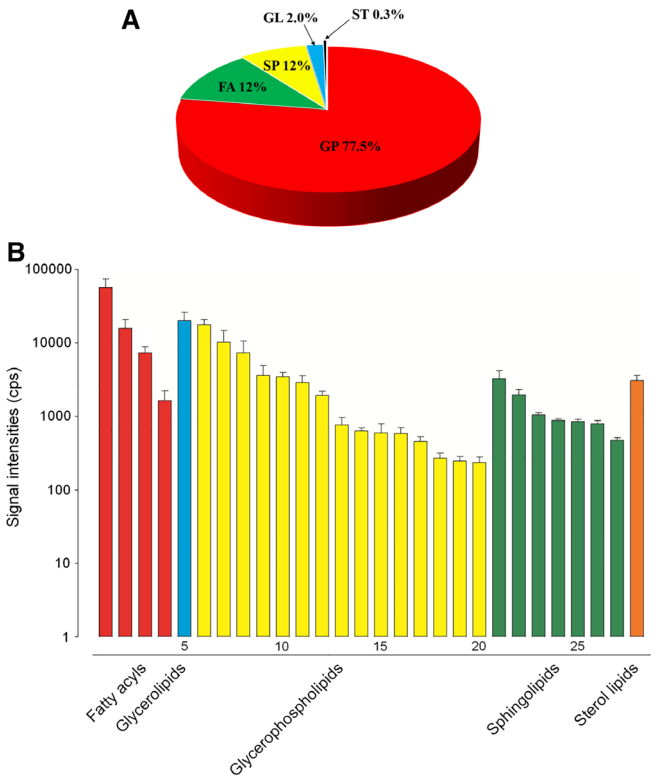


FIGURE 1 A) Percentages of each category of lipids found in feline plasma. Fatty acyls, FA; glycerolipids, GL; glycerophospholipids, GP; sphingolipids, SP; and sterol lipids, ST. B) Signal intensities (mean ± SEM) for the known lipids detected in feline urine

compounds. In plasma, glycerophospholipids showed the higher number of chemical species detected (n=101) followed by sphingolipids (n=35), glycerolipids (n=23), fatty acyls (n=22) and sterol lipids (n=3). In urine, the highest structure diversity was observed for polar lipids, namely, glycerophospholipids and sphingolipids, with 15 and 7 chemical species identified within each category, respectively. Figure 1 shows the relative abundance of each lipid category based on signal intensity found in the feline plasma and urine samples. Of note, 150 out of 212 lipids identified in this study have not been reported previously in cats.

This is the first study describing the lipid composition in plasma and urine of healthy cats comprehensively. Many of the lipids found in this study have been used as biomarkers in human medicine for different pathologies including linoleic and arachidonic acids for kidney diseases; SM(38:1) and SM(42:1) for insulin resistance and nonalcoholic fatty liver disease; LPC(16:0), LPC(18:0), PC(34:1), PC(34:2), PC(36:4) and cholesterol for hepatocellular carcinoma, among others. Thus, the novel information generated in this study could lead to the discovery of novel biomarker candidates for detecting and studying feline diseases and individualized treatments for cats.

NM11

Protein Carbonyl Content as an Indicator of AGEs Formation in Diabetic Dogs

Sol Rivera, Nicolas Villarino
Washington State University College of Veterinary Medicine, Pullman, WA, USA

Glycation is a non-enzymatic reaction between the carbonyl group of reducing sugars and a free amino group in the protein. During this

process, plasma proteins form a variety of heterogeneous structures known as advanced glycation end products (AGEs). These compounds have been associated with several pathologies including obesity, diabetes, cirrhosis, cardiovascular disease, renal failure, and neurological disorders.

In contrast to human medicine, little attention has been given to AGEs in veterinary medicine. Of note, fructosamine is a biomarker used for monitoring blood sugar concentrations, but it only provides information about early glycation products. Conversely, carbonyl groups are formed during both the early and late stages of protein glycation.

Considering the fact that canine albumin contains similar glycosylatable amino acids (lysine and arginine) to those in humans, we hypothesize that abnormally high levels of glucose in a dog';s plasma leads to the formation of AGEs during the process of protein glycation. In order to address this hypothesis, plasma from healthy dogs was enriched with glucose levels similar to those reported in diabetic patients with good and poor glycemic control. Glucose-enriched and control samples were incubated for 40 days at 37°C.

The high levels of glucose glycated plasma proteins and led to the formation of AGEs as determined by 2,4-dinitrophenylhydrazine assay. The carbonyl content in plasma increased ($P < 0.05$) in a glucose concentration-dependent manner ($P < 0.05$, Figures 1 and 2) and was positively associated with the concentration of fructosamine ($P < 0.05$). The fructosamine levels obtained in this study were comparable to those found in dogs suffering from diabetes (> 340 µmol/L plasma fructosamine levels).

This is the first study reporting the content of protein carbonyls in canine plasma. These results demonstrate that protein carbonyls are formed in higher amount in dog';s plasma with glucose levels similar to diabetic patients compared with the plasma control. Although, these results were obtained using an ex vivo system, it provides new information about the occurrence of AGEs during glycation of canine plasma proteins. Certainly, future studies should be performed using samples from diabetic patients in order to expand our knowledge about the formation and clinical impact of these compounds in dogs.

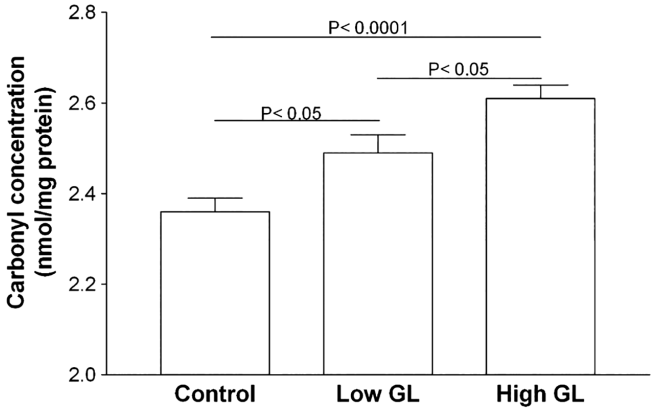


FIGURE 1 Changes in carbonyl content in glycated canine proteins that were incubated for 4 weeks with different amounts of glucose. Values are reported as mean ± SE. Low GL, low glucose concentration and, High GL, high glucose concentration

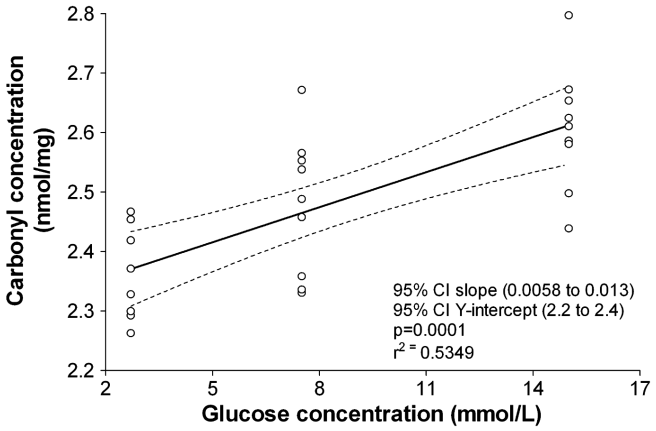


FIGURE 2 Correlation between carbonyl and glucose concentrations in canine plasma

The study of the formation of protein carbonyls, due to the glycation of proteins, could represent a new tool to elucidate the metabolic pathways that lead to the formation of AGEs and consequently aid in the development of new therapeutic strategies for the treatment of diabetic patients in dogs.

OT01

The Relationship Between Pet-Owner Satisfaction and Loyalty: the Mediating Role of Communication

Bryan R. Brown
Merck Animal Health, Virginia Beach, VA, USA

Loyalty is one of the greatest intangible assets that any organization can possess, and improving client loyalty is a primary marketing goal that can have a significant financial impact on any business. This quantitative study examined the mediating role of communication on the relationship between satisfaction and loyalty (attitudinal and behavioral) in veterinary clinics, along with the moderating roles of trust, commitment, perceived value, and relational characteristics. Responses collected from 351 pet-owners through social media were analyzed using descriptive and inferential statistics. The results show that attitudinal loyalty (AL) has a strong positive relationship with communication at multiple points in a veterinary clinic whereas the relationship with behavioral loyalty was not as clear. Additional findings suggest that AL, which is influenced by trust in the veterinarian, communication from staff members and commitment, has a strong positive relationship with behavioral intentions, increases the number of products and services that a pet-owner consumes at his or her primary veterinary clinic, and attenuates the role of cost in receiving veterinary care. The youngest and oldest cohorts in the study exhibited the lowest levels of AL, while pet-owners who see the same veterinarian at each visit have higher AL than those who do not. A set of recommendations is made for each communication point within a veterinary clinic to improve AL. These findings can help veterinary clinic owners and managers in developing and implementing relationship strategies that improve pet-owner loyalty.

OT02

The Effect of Client Complaints on Small Animal Veterinary Internists

Alyssa Bryce[1], Tracey Rossi[1], Colleen Tansey[2], Lisa A. Murphy[3], Reid Nakamura[4], Ruth Murphy[5]
[1]Southern California Veterinary Specialty Hospital, Irvine, CA, USA, [2]Inland Valley Veterinary Specialists and Emergency "Center", Upland, CA, USA, [3]Veterinary Speciality "Center" of Delaware, Newcastle, DE, USA, [4]Veterinary Emergency and Speciality "Center", Thousand Oaks, CA, USA, [5]Beaumont Hospital, Dublin, Ireland, Galway, Galway, Ireland

Multiple studies have found veterinarians to be at a higher risk for suicide compared to the general population. Risk factors that have been associated with psychological distress in veterinarians include being a practice associate, practicing for less than 20 years and practicing shelter medicine. For human physicians, patient complaints lead to increased risk of depression, anxiety and suicide. Secondary effects of these complaints on physicians include practicing medicine more defensively, avoiding high risk procedures or patients and performing unnecessary diagnostics. Veterinary medicine, due to the need for owners to use their own disposable income, leads to different potential sources of conflict between veterinarians and clients,

The purpose of this study was to investigate the effect of client complaints on small animal veterinary internist's welfare, their job satisfaction, as well as the way they practice veterinary medicine. A web-based anonymous questionnaire was made available through the American College Veterinary Internal Medicine sub-specialty Small Animal Internal Medicine E-mail List Serve between January 1st and March 31st 2017.

92 surveys were obtained and available for review. 64% of respondents had received a client complaint within the preceding 6 months with the cost of care the most common reason. The majority of respondents (96%) worried to varying degrees about a client complaint being made against them. More concerning, almost 35% reported being verbally assaulted by a client within the preceding six months and 27% reported being threatened with litigation. A majority reported that they have changed the way they practice veterinary medicine to avoid a complaint being made against them and 43% said they had considered changing their career because of client complaints.

The study confirmed that client complaints are a common source of distress for veterinary internists. The high percentage of conflicts being due to cost of care illustrates a central problem in veterinary medicine that is an issue most veterinarians cannot control. Veterinarians need to consider how client education on cost of care can be achieved. There also needs to be a way or forum that allows veterinarians to openly discuss client complaints and how to deal with them in a supportive environment. As veterinary medicine becomes more advanced and we offer more expensive treatments and procedures, the issue of client complaints and communication will likely continue to grow.

OT03

Strategies to Improve Case Outcome When Referral is Not Affordable

Alyssa Sullivant[1], Andrew Mackin[2]
[1]Mississippi State University, Starkville, MS, USA, [2]Mississippi State University, Mississippi State, MS, USA

Referral to an internist is often in the best interest of a patient, particularly if the case involves a chronic, refractory, or undiagnosed condition. Unfortunately, many pet owners cannot afford the cost of referral. In these instances, the general practitioner is challenged with treating these complicated cases on a limited budget. Owners may even elect euthanasia due to cost of care. General practitioners may seek guidance from an internist in order to discuss diagnostic and treatment options for patients that need referral care but whose owners cannot afford it. Such consultations are frequently limited to a single phone conversation or email, and outcome is therefore unknown by the consulting internist. Additional strategies to improve options and outcome in these financially challenging cases may be beneficial to both general practitioners and patients. Furthermore, increasing the awareness of and preparedness of veterinary students for these challenges may improve their abilities to approach cases more efficiently when owners are on a limited budget.

A descriptive study was performed to determine the usefulness of on-site consultations with a boarded internist, case follow-up with an internist, and written diagnostic and therapeutic protocols for selected internal medicine conditions. Fourth-year veterinary students participated in weekly on-site consultations and development of protocols. A total of 35 veterinarians and 15 small animal veterinary practices participated in the study. Each practice was visited three times over the course of one year, and 133 total consults were conducted. A collection of 38 protocols for different internal medicine conditions was given to each practitioner.

Data was collected from pre- and post- participation surveys of fourth-year veterinary students and participating veterinarians. Prior to participation, 90% of veterinary students did not feel confident choosing the best diagnostic and therapeutic approach to internal medicine cases when owners were on a budget and could not afford referral. 100% of students felt that training in this area should be part of their education. After participation in the on-site consults and protocol development, 63% of students felt confident choosing the best approach to these cases, and 59% were "highly likely" to use the protocols in general practice.

Lack of a diagnosis, uncertainty about the most appropriate diagnostics, and the need for a special procedure were the three most common reasons general practitioners recommended referral. Over 50% of participating veterinarians noted that only 25% of dog owners agreed to referral, and 94% of veterinarians noted that expense was the primary reason owners declined referral. Only 28% of veterinarians were "always comfortable" with these cases and 72% were "sometimes comfortable." 83% of veterinarians expressed the desire for lower-cost options, such as treatment protocols, when owners cannot afford referral. After completion of the project, 83% of veterinarians noted that both the on-site consultations and protocols were "highly beneficial." The areas of greatest improvement were their comfort level with treating referral-level cases in general practice, case outcome, and patient care. 100% of veterinarians believed that patient quality of life improved, and 100% wanted to continue participating in on-site consultations.

In summary, on-site internal medicine consultations and cost-effective diagnostic and treatment protocols for various internal medicine conditions benefitted both veterinary students and general practitioners.

The cost of veterinary care, especially referral medicine, is not affordable for many owners. Veterinary students should be exposed to these challenges and should be trained in the most cost-effective approach to these cases. Similarly, general practitioners may be able to more successfully and efficiently diagnose and treat challenging internal medicine cases with the proposed strategies when owners decline referral.

OT04

An Investigation into Multi-Organ Fibrosis in the Cavalier King Charles Spaniel

James H. Warland[1], *Stephen Greenhalgh*[2], *Will Howat*[3], *Margaret Carter*[4], *Tania Ledger*[4], *Clare Rusbridge*[5], *Fernando Constantino-Casas*[6], *Penny Watson*[6]
[1]*University of Cambridge, Cambridge, England, United Kingdom,* [2]*Royal (Dick) School of Veterinary Studies, Midlothian, Scotland, United Kingdom,* [3]*AstraZeneca, Cambridge, England, United Kingdom,* [4]*Cavalier Matters, Croydon, England, United Kingdom,* [5]*University of Surrey, Guildford, England, United Kingdom,* [6]*Department of Veterinary Medicine, Cambridge, Cambridge, England, United Kingdom*

Studies show Cavalier King Charles Spaniels (CKCS) have a high prevalence of chronic, fibrosing pancreatitis; fibrotic renal lesions; and CNS perivascular fibrosis in syringomyelia.

We hypothesised that CKCS, irrespective of disease status, would have increased fibrosis in multiple organs compared with other breeds.

Post-mortem samples of CKCS were collected prospectively though a donation scheme available to owners and veterinarians. Collection was irrespective of disease state or antemortem diagnosis. Clinical information was obtained where possible. Liver, pancreas, kidney and lung were stored as formalin-fixed paraffin-imbedded samples. Tissues were matched by age and histopathologic diagnosis to tissues from non-CKCS breed dogs. Sirius red staining, highlighting fibrosis, was performed and slides digitized for objective analysis. Software analysed for red staining and scored this as a proportion of the tissue volume. Those analysing were blinded to breed and pathologic diagnosis.

Forty CKCS (liver n=38; pancreas n=36; kidney n=37; lung n=35) & 31 non-CKCS (liver n=14; pancreas n=9; kidney n=11; lung n=12) were included, comprising 22 breeds. There was no significant difference between organ fibrosis scores in CKCS and controls. However, in CKCS, moderate but significant correlations were identified between liver fibrosis and pancreas or lung fibrosis; additionally, significant correlation was found between lung and renal fibrosis. Insufficient cases with multiple organs precluded analysis in non-CKCS.

In conclusion, CKCS didn';t show increased fibrosis compared with matched controls, but fibrosis was correlated across organs. Further studies are indicated to assess multiple organs in other dog breeds to confirm the significance of this finding.

OT05

Hereditary Deafness by BEAR Testing in Purebred Angora Cats

Hasan Albasan[1], *Seyda Tike-Avci*[2], *Nevra Keskin*[3], *Sebahattin Cureoglu*[4], *Cemil Mutlu*[5]
[1]*Department of Otolaryngology-Head and Neck Surgery, University of Minnesota, Minneapolis, Minnesota, USA / Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ankara, Ankara, Turkey, Diskapi, Ankara, Turkey,* [2]*Tarım ve Kırsal Kalkınmayı Destekleme Kurumu, SELCUKLU, Konya, Turkey,* [3]*Department of Internal Medicine, Faculty of Veterinary Medicine, Ankara University, Diskapi, Ankara, Turkey,* [4]*Department of Otolaryngology- Head and Neck Surgery, University of Minnesota, Minneapolis, MN, USA,* [5]*Department of ENT, Kayseri Research and Teaching Hospital, Health Science University, Kocasinan, Kayseri, Turkey*

Absence or abnormality of melanocytes in the stria vascularis in white-coated cats with *White (W)* gene leading degeneration in cochlea more pronounced in the organ of Corti and cochlear hair cells results in hereditary deafness which can be diagnosed by behavioral and/or Brainstem Auditory Evoked Response (BAER) tests.[1,2]

In the present study, auditory function was assessed in purebred Angora cats with BAER testing with sedation. Auditory thresholds were determined by applying stimuli between 54-120 dB SPL. Moreover, stimuli of ≥55 dB SPL was considered as deaf while stimuli of ≤54 was considered as hearing. A total of 9 Angora cats (6 males and 3 females) were included in the study. Mean age was 22.8±52.4 months-old (range:1.5-156). Of 9 Angora cats, 7 (77.7%) were unilateral deaf, and 2 (22.3%) were hearing. Of 7 unilateral deaf cats, 4 were males (3 right and 1 "left"-sided deaf) and 3 were females (1 right and 2 "left"-sided deaf). Of 4 unilateral deaf male cats, 2 were blue, 1 was blue-amber, and 1 was amber eye colors. Of 3 unilateral deaf female cats, 1 was blue, 1 was blue-amber, and 1 was amber eye colors. Remaining 2 cats with hearing were males with blue eye colors.

Results of present study revealed that Angora cats have partial but not bilateral hereditary deafness. There is only one report available indicating 1 of 11 Angora cats had unilateral deafness while no bilateral deafness with BAER testing.[3] On the other hand, another study performed by behavioral testing in Angora cats reported 11% bilateral deafness.[4]

As a conclusion, further epidemiologic studies should be performed in larger populations to determine the prevalence of uni-and/or bilateral hereditary deafness along with gender or eye color association.

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OT06

Computed Tomographic Appearance of Abdominal Lymph Nodes in Healthy Cats

Michael Perlini, Andrew Bugbee, Scott Secrest
University of Georgia College of Veterinary Medicine, Athens, GA, USA

Computed tomography (CT) is commonly used in veterinary medicine and plays an important role in disease identification and cancer staging. Differentiation of normal versus disease affected abdominal lymph nodes on CT is important not only for clinical and therapeutic

decision making, but also for cancer staging and prognostication. The purpose of this study was to describe the presence, number, size, shape, attenuation and enhancement pattern of abdominal lymph nodes on CT in healthy cats and assess for correlations to patient characteristics.

Sixteen healthy cats without clinical or biochemical evidence of disease underwent sedated pre-and post-contrast CT exams of the entire abdomen. The number, size, shape, attenuation, enhancement pattern and presence of intranodal fat of the following lymph "center"s were evaluated by two readers, including a board-certified radiologist: aortic lumbar, renal, hepatic, splenic, gastric, pancreaticoduodenal, jejunal, ileocecal, colic, caudal mesenteric, medial iliac, sacral and inguinal. Assessments were compared to patient characteristics with Pearson's correlation and student t-tests used for the statistical analysis.

A total of 525 abdominal lymph nodes were readily identified on CT with caudal mesenteric, colic, hepatic, inguinal, and pancreaticoduodenal lymph nodes identified in 16/16 cats. Body weight and sex were not associated with differences in the overall number of lymph nodes identified (p =0.3845 and 0.8565, respectively). Lymph node size and shape varied between lymph "center"s with nearly all lymph nodes homogenously contrast enhancing (515/525). Significant negative correlations were identified between the length (p =0.0166) and width (p =0.0387) of abdominal lymph nodes as well as age and the number of sacral lymph nodes (p =0.0493). Intranodal fat was present in 18/525 lymph nodes.

CT readily permitted identification and characterization of feline abdominal lymph nodes. This study provides subjective and objective data on the CT characteristics of abdominal lymph nodes in 16 healthy cats. The size and shape of abdominal lymph nodes varied depending on the lymph "center" evaluated with younger cats having larger abdominal lymph nodes and a greater number of sacral lymph nodes. Otherwise the vast majority of abdominal lymph nodes should be soft tissue attenuating with homogenous contrast enhancement and only occasional intranodal fat. This information should be considered when trying to identify diseased lymph nodes on CT and for cancer staging purposes.

OT07

Intravenous Fluid Prescribing Rates for Dogs at a Veterinary Teaching Hospital

Michael J. Verschoor-Kirss[1], *Elizabeth Rozanski*[2], *Shelby Karp*[3], *Jonathan Babyak*[2]
[1]*Foster Hospital for Small Animals at Cummings Veterinary Medical "Center", Westborough, MA, USA,* [2]*Tufts University, North Grafton, MA, USA,* [3]*Washington University at St. Louis, N. Grafton, MA, USA*

While published veterinary guidelines for fluid therapy emphasize an individualized approach, studies evaluating daily changes in fluid rates are limited. The aim of this observational prospective study was to describe changes in fluid therapy in all hospitalized dogs over a one-month period at a veterinary teaching hospital.

All hospitalized dogs treated with intravenous fluids during the study period were eligible for inclusion. The initial fluid therapy (rate [ml/kg/d] and fluid type) and any in-hospital adjustments in fluid rate were recorded until discharge or death as well as the managing service (ECC, Surgery or ACVIM based specialty). Data normality was assessed using the Shapiro-Wilk test and analyzed with a t-test with a $p < 0.05$ considered significant.

Two hundred and eighty-three dogs were included, receiving 862 dog-days of fluid therapy, with a median duration of 3 days (range 1-20 days). The mean starting fluid rate was 83±4 ml/kg/d and the ending fluid rate was 68±3 ml/kg/d ($p < 0.001$). Throughout hospitalization, almost all dogs (99%) received crystalloid fluids, of which lactated Ringer';s solution was the most common (93% of dogs). Three dogs were treated with hydroxyethyl starches and no dogs received maintenance type fluids. Fluid rates were adjusted on average once per hospitalization, although 140 (49%) dogs had no fluid rate changes. Dogs managed by surgeons had lower average starting fluid rates (71±5 ml/kg/d) than those managed by ACVIM -based specialties and criticalists (86±4 ml/kg/d, $p < 0.001$) but other aspects of fluid therapy were the same ($p > 0.05$).

Fluid therapy is approached similarly throughout this veterinary teaching hospital. Although daily fluid rates decreased during hospitalization, daily fluid rate changes were infrequent.

OT08

The Effects of 3% Hydroxyethyl Starch-Hypertonic Saline on Resuscitation of Dogs in Controlled Hemorrhagic Shock

Woosun Kim[1], *Junwoo Bae*[2], *Hyunwoo Kim*[3], *Hayoung Yang*[4], *Soomin Ahn*[4], *Jinho Park*[5], *Dong-In Jung*[6], *Do-Hyeon Yu*[6]
[1]*College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea/May Animal Medical Center, Jeonju-si, Cholla-bukto, Republic of Korea,* [2]*College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea/May Animal Medical Center, Jeonju, Cholla-bukto, Republic of Korea,* [3]*College of Veterinary Medicine, Chonnam National University, Gwangju 54976, South Korea, Gwangju, Cholla-namdo, Republic of Korea,* [4]*College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju, Kyongsang-namdo, Republic of Korea,* [5]*College of Veterinary Medicine, Chonbuk National University, Jeonju 54596, Republic of Korea, Iksan-si, Cholla-bukto, Republic of Korea,* [6]*College of Veterinary Medicine, Gyeongsnag National University, Jinju 52828, South Korea, Jinju-si, Kyongsang-namdo, Republic of Korea*

The adequate fluid resuscitation in hemorrhagic shock is essential to expand volume and improve global and regional oxygen delivery thus the proper fluid selection is important. Hypertonic saline with colloids was newly proposed for clinical use but its effect and optimal ratio were still in question. This study introduces 3% hydroxyethyl starch-hypertonic saline (3% HHES) and compares to four conventional fluids (lactated Ringer';s solution (LRS), 6% hydroxyethyl starch (6% HES), 7% hypertonic saline (7% HS), and 7% hydroxyethyl starch-hypertonic saline (7% HHES) in dogs with experimental hemorrhage.

Twelve healthy dogs were repeatedly used after full recovery at least twice. Dogs were bled to remain mean arterial blood pressure at 40 to 50 mmHg over 60 minutes and were resuscitated as in 5 groups: LRS (n=5); 6% HES (130:40) (n=5); 7% HS (n=5); 3% HHES (n=5) and 7% HHES (n=5). Intravascular volume expansion using Evans blue dye, cardiac output, stroke volume, preload (stroke volume variation, pulse pressure variation, central venous pressure) for hemodynamic indices and systemic oxygenation (oxygen extraction ratio, central venous oxygen saturation), lactate, base excess, electrolytes, and pH were monitored at baseline, after 60 minutes of hemorrhage, and 15, 30, 60, and 120 minutes after fluid resuscitation. Acid-base status was

examined thoroughly with the Stewart's method and the Fencl's semi-quantitative method. Along with monitoring, TNF-α and IL-6, and nitric oxide in plasma were measured.

This study evaluated the effects of 3% HHES in controlled hemorrhagic shock dog model compared to four conventional fluids in three aspects: volume expansion, systemic and tissue perfusion response, and acid-base status. In the aspect of volume expansion, LRS, 6% HES, and 3% HHES resuscitated significantly larger volume than 7% HS and 7% HHES but LRS and 6% HES were considered as over-expansion of intravascular volume ($p < 0.05$). Hemodynamic data coincides with volumetric results. Three percent HHES as well as other four fluids provided sufficient systemic oxygenation and perfusion yet the further examination should be conducted in hemorrhagic shock model based on oxygen debt. Infusion of 3% HHES was beneficial to the acidotic state of hemorrhagic shock without dilutional acidosis owing to transient hypernatremia and hyperchloremia.

In conclusion, the data suggest that 3% HHES support sufficient volume expansion without overloading, return of systemic oxygenation indices and lactate to control level, and the superior resuscitation benefits on acid-base status and inflammatory stability after reperfusion injury than conventional resuscitation fluids. Thus, 3% HHES could be the best alternative solution that compensate dosage disadvantages of conventional crystalloid or colloid administration.

OT09

Urinary F2-Isoprostanes: A Comparison of Two Methods for Measurement in Cats

Andrew Woolcock, Ashley Leisering, Pierre Deshuillers, Janet Roque-Torres, George Moore
Purdue University College of Veterinary Medicine, West Lafayette, IN, USA

Oxidative stress is primarily assessed through the activity of antioxidant enzymes, the concentration of endogenous antioxidants, and byproducts of oxidative damage. F2-isoprostanes, a byproduct of lipid peroxidation, were determined to be the best marker of oxidative injury in a rodent model of oxidative stress, and are stable and easily measured in urine. In people, the gold standard for measurement of urinary F2-isoprostanes is gas chromatography-mass spectrometry (GC-MS), but in animals the majority of studies have utilized enzyme-linked immunosorbent assay (ELISA) for measurement of F2-isoprostanes. A previous study compared these two methods in small populations of dogs, cats, horses, and cows. Poor agreement between these methods was identified in dogs, horses, and cows. However, fair agreement between these methods was identified in cats. The objective of this study was to evaluate the agreement between GC-MS and ELISA for measurement of urinary F2-isoprostanes in a large population of cats including a range from healthy to clinically ill cats.

Skeletally mature cats were recruited. Health status in each cat was determined by a physical examination, complete blood count (CBC), serum chemistry profile, thyroid hormone (T4) and urinalysis. Cats were defined as systemically ill if evidence of illness was identified on physical examination (persistent fever, injected mucous membranes) and/or evidence of inflammation and/or organ system dysfunction. Urine was collected non-invasively (free catch) in all cats unless otherwise indicated due to the disease process in the systemically ill cats. Urinary F2-isoprostanes were measured by GC-MS and ELISA in all urine samples.

Fifty cats were enrolled in the study. Twenty-five cats were determined to be healthy, while the other 25 were determined to be systemically ill. All urine samples had detectable levels of F2-isoprostanes. A significant negative correlation was identified between the two methods ($\rho = 0.364$, P=0.009). No significant correlation was identified when the healthy and systemically ill cats were compared in sub-group analysis. Passing and Bablok regression showed poor agreement between the two methods. This comparison demonstrated a non-linear relationship and proportional bias. The concentration of urinary F2-isoprostanes as measured by ELISA was significantly lower in systemically ill cats when compared to healthy cats (P=0.002). No significant difference was identified when urinary F2-isoprostanes were measured by GC-MS (P=0.068).

These results indicate that GC-MS is the only method recommended for the assessment of urinary F2-isoprostanes in cats. The expected increase in urinary F2-isoprostanes in systemically ill cats was not identified, so caution is still warranted in interpreting this test regardless of method. Future research should use GC-MS to identify normal reference ranges and variability of urinary F2-isoprostanes in cats.

OT10

Canine Mesenchymal Stem Cells Pre-Treated with TNF-α/IFN-γ Enhance Anti-Inflammatory Effects by Up-Regulating the COX-2/PGE2 Pathway

Woo-Jin Song, Qiang Li, Jin-Ok Ahn, Min-Ok Ryu, Ju-Hyun An, Aryung Nam, Hwa-Young Youn
Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Mesenchymal stem cells (MSCs) have been used in studies on treatment of various diseases, and their application to immune-mediated diseases has garnered interest. Various methods for enhancing the immunomodulation effect of human MSCs have been used; however, similar approaches for canine MSCs are relatively unexplored. Accordingly, we evaluated immunomodulatory effects and mechanisms in canine MSCs treated with TNF-α and IFN-γ.

Canine MSCs were stimulated with TNF-α and IFN-γ for 24 hours to produce conditioned media (CM). Lipopolysaccharide (LPS)-stimulated RAW 264.7 cells were co-cultured with the MSCs for 48 h in CM. Expression of RNA was assessed by quantitative reverse transcription PCR (qRT-PCR), and protein levels were assessed by western blot.

Expression of inducible nitric oxide synthase (iNOS), IL-6 and IL-1β was significantly (one-way ANOVA) decreased in LPS-stimulated RAW 264.7 cells co-cultured with naïve canine MSCs compared to that in LPS-stimulated RAW 264.7 cells alone. Furthermore, anti-inflammatory effects of TNF-α- and IFN-γ-primed canine MSCs were significantly increased compared with those of naïve canine MSCs. Expression of cyclooxygenase 2 (COX-2) and prostaglandin E_2 (PGE_2) were likewise significantly increased in primed canine MSCs. The level of iNOS protein in LPS-stimulated RAW 264.7 cells co-cultured with

the primed canine MSCs was decreased, but it increased when the cells were treated with NS-398(PGE_2 inhibitor).

In conclusion, compared with naïve canine MSCs, cells primed with TNF-α and IFN-γ cause a greater reduction in release of anti-inflammatory cytokines from LPS-stimulated RAW 264.7 cells; the mechanism is upregulation of the COX-2/PGE_2 pathway.

P01

Localization and Quantification of Cannabinoid Receptors in Canine Tissue

Matthew E. Miller, Kamoltip Thungrat, Jey W. Koehler, Dawn M. Boothe
Auburn University College of Veterinary Medicine, Auburn, AL, USA

The endocannabinoid system (ECS) is comprised of endogenous signaling molecules known as endocannabinoids, and the G-protein-coupled receptors to which they bind. In recent decades, research has found relationships between the ECS and memory, nociception, inflammation, appetite, metabolism, and more.

In the interest of providing a framework for the eventual development of safe and effective cannabinoid therapies in veterinary species, this paper sought to characterize the two predominant cannabinoid receptors—CB1R and CB2R—by quantification and localization.

Tissue samples were acquired from living adult dogs that presented to the AU-SATH Surgery Department for procedures related to the tissues obtained. Following collection, the tissue samples were placed in either RNA*later*® or formalin. RNA*later*® samples were stored until processing. Formalin samples were submitted to histopathology for processing. In all, 35 tissue samples were collected in RNA*later*®, and 12 were collected in formalin.

RNA was extracted from tissues stored in RNA*later*®, converted to cDNA, and quantified using quantitative PCR (qPCR). The data were reported as ratios of *CB1R* or *CB2R* gene expression to the constitutively-expressed housekeeping gene *B2M*.

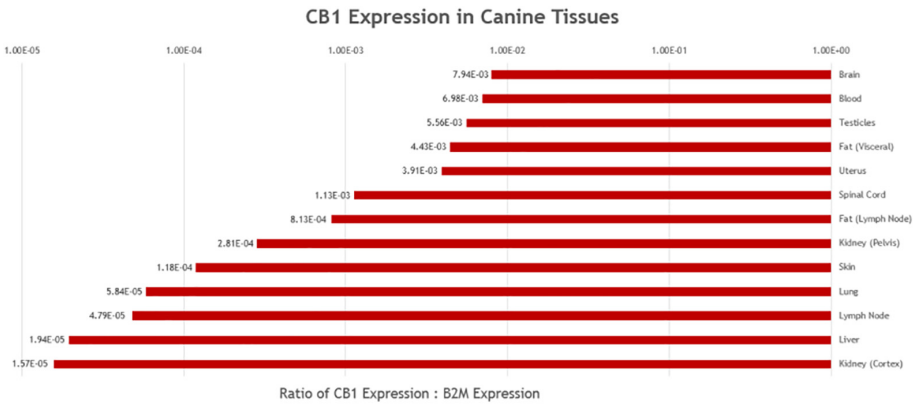
Immunohistochemistry was run using previously-described antibodies, and selective staining was verified by Western blot.

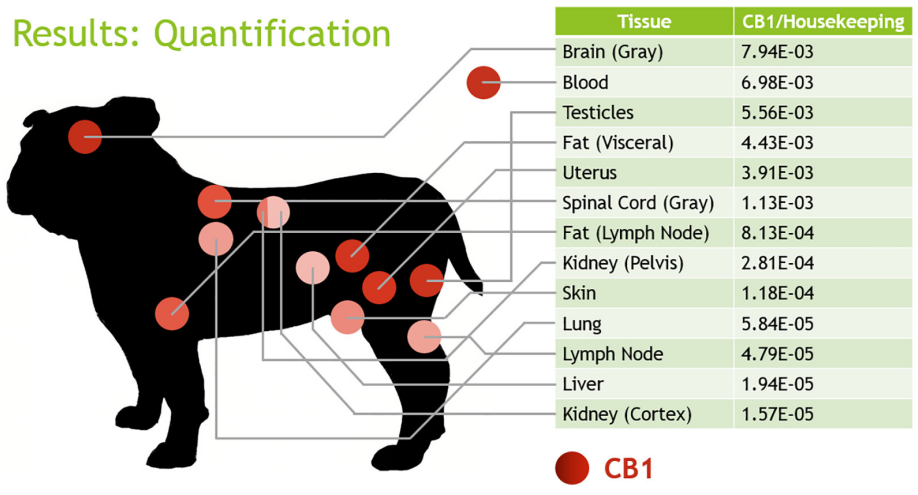
Previously undescribed findings in the immunohistochemistry were limited; CB2 receptors stained most darkly in the endothelial cell membranes of most tissues, with less-significant staining scattered throughout the parenchyma, localized microscopically to the cell membranes.

Quantification showed high expression of *CB1R* gene in the blood, brain, testicles, ovary and uterus, but low expression in kidney, lung, liver and lymph node. Expression values for the *CB2R* gene have been somewhat limited but show high expression in blood and lymph nodes.

While many findings from previous studies were confirmed, such as the high concentration of CB1R in gray matter and the high concentration of CB2R in blood and lymph nodes, the study also found unexpectedly high quantities of CB2R in both the male and female gonads. Additionally, unexpectedly low levels of CB2R expression were found in the lung and the liver compared to human and mouse models.

These results invite future investigation into the reproductive applications of cannabinoids, as well as possible upregulation following exogenous exposure to explain the relatively low expression of *CB1R* in





Tissue	CB1/Housekeeping
Brain (Gray)	7.94E-03
Blood	6.98E-03
Testicles	5.56E-03
Fat (Visceral)	4.43E-03
Uterus	3.91E-03
Spinal Cord (Gray)	1.13E-03
Fat (Lymph Node)	8.13E-04
Kidney (Pelvis)	2.81E-04
Skin	1.18E-04
Lung	5.84E-05
Lymph Node	4.79E-05
Liver	1.94E-05
Kidney (Cortex)	1.57E-05

liver and lung tissues. Quantification of specific regions of the canine brain may also prove useful in the development of pharmaceutical cannabinoids.

P02

Effect of Glutathione on Itraconazole-Induced Cytotoxicity in Canine Hepatocytes

Jennifer M. Reinhart
Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA

Itraconazole and other azole antifungal medications are essential in the treatment of deep mycoses, but the development of hepatotoxicity may limit their clinical utility. Anecdotally, glutathione precursors (e.g. S-adenosylmethionine) may ameliorate hepatotoxicity induced by itraconazole in dogs. The purpose of this study was to determine the effect of glutathione on itraconazole-induced cytotoxicity in an *in vitro* cell model. Canine hepatocytes in suspension (1.0 x 10^5 cells/ml) were treated with combinations of itraconazole (0, 2, 10, 50 μM) and reduced glutathione (0, 10, 100, 1000 μM). Cytotoxicity was determined at 0.5, 3, and 6 hours of incubation using a commercial lactate dehydrogenase assay, which measures leakage of intracellular enzyme from dead cells. Differences in % cytotoxicity were assessed by ANOVA. Without glutathione, % cytotoxicity increased over time at all concentrations of itraconazole ($p = 0.0001 – 0.0060$). Similarly, % cytotoxicity was increased at higher concentrations of itraconazole compared to lower concentrations at all time points ($p = 0.0001 – 0.0078$). At 6 hours, glutathione demonstrated a dose-dependent reduction in % cytotoxicity in cells treated with 2 μM itraconazole ($p = 0.0333$), but this effect was not seen at earlier time points (0.5 and 3 hours) or higher concentrations of the drug (10 and 50 μM). These findings suggest that glutathione may decrease low-dose, itraconazole-induced cytotoxicity in canine hepatocytes. Prospective clinical studies are needed to determine whether glutathione precursors are useful in the treatment or prevention of itraconazole-induced hepatotoxicity in dogs. Additionally, these results establish dose- and time-dependent cytotoxicity of itraconazole in canine hepatocytes *in vitro*, which may be a useful model for studying the pathogenesis of this adverse drug reaction.

P03

Pharmacodynamics of Mycophenolate Mofetil after Multi-Dose Oral Administration in 10 Healthy Cats

Jennifer E. Slovak, Julianne Hwang, Sol Rivera, Nicolas Villarino
Washington State University College of Veterinary Medicine, Pullman, WA, USA

The immunosuppressant Mycophenolate mofetil (MMF) has recently gained popularity in veterinary medicine. MMF is a pro-drug for the active moiety mycophenolic acid (MPA), a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH inhibition, ultimately leads to suppressed lymphocyte proliferation.

The goal in this study was to evaluate the pharmacodynamics (PD) of MPA after 1 week of varying oral doses of MMF in 10 healthy cats. The PD of MPA was evaluated following oral administration of MMF 10mg/kg PO BID (n=3), 15mg/kg PO BID (n=3), and 15mg/kg PO TID (n=4) for up to 1 week in 10 cats. Blood samples were taken prior to the first administration of MMF, 24 hours into treatment, at day 7 and 12 hours after the last oral dose of MMF.

Isolation of peripheral blood mononuclear cells (PBMC) was performed using standard methods after each blood draw. All samples were cryopreserved and later thawed for subsequent flow cytometry and analyzed in 1 batch. Percentage of positively stained cells for $CD4^+$ and $CD8^+$ antibody were determined by flow cytometry.

Total isolated PBMC numbers were variable in all cats tested at premedication, after initiation of oral MPA on day 1, day 7, and after 12 hours of the last dose of oral MMF. There was minimal to no change in the average $CD4^+$ and $CD8^+$ lymphocyte counts in the 10mg/kg BID and 15mg/kg BID groups. There was a mild reduction in $CD4^+$ but not $CD8^+$ lymphocytes in cats treated with 15mg/kg TID of MMF. Overall, the $CD4^+$:$CD8^+$ ratios were nearly the same in the 10mg/kg BID, 15mg/kg BID, and 15mg/kg TID treatment groups.

This study describes the PD of multi-day oral administration of MMF in 10 healthy cats. There was little to no change in the $CD4^+$ lymphocyte counts in the 2 BID groups, and a mild reduction in the 15mg/kg TID group. The $CD8^+$ lymphocytes and the $CD4^+$:$CD8^+$ ratios, showed little to no change for all 3 groups. The results of this study did not show an appreciable reduction in T cell suppression. This may be due a short treatment period or due to the presence of un-activated lymphocytes as the cats were healthy. Future studies will evaluate the PD of MPA in clinically affected feline patients receiving MMF.

P04

Genetic Polymorphisms in CYP3A12 and Clinical Outcomes of Vinblastine Chemotherapy in Dogs with Mastocytoma

Kamoltip Thungrat[1], Chenphop Sawangmake[2]
[1]Auburn University College of Veterinary Medicine, Auburn, AL, USA, [2]Chulalongkorn University, Bangkok, Krung Thep, Thailand

Canine mast cell tumors (MCT) are the most common malignant skin cancers in dogs. A combination of anticancer drugs including vinblastine, prednisone, and tyrosine kinase inhibitors (toceranib and masitinib) along with surgery and/or radiation therapy is commonly used to treat MCT. Chemotherapy can result in unpredictable clinical outcomes in both responses to therapy and drug toxicity. A patient';s variability of genetic polymorphism CYP3A12 enzyme might affect drug metabolism and clinical outcomes. Many single nucleotide polymorphisms (SNPs) at the human CYP3A locus have been characterized. Recent studies have highlighted the potential for CYP3A variation in susceptibility to several common phenotypes, including cancer. The objectives of this study were to analyze the sequencing of the canine *CYP3A12* gene (NCBI accession NP_001003340) from thirteen client-owned dogs with MCT (Grade II-III) and to investigate the patterns of sequence variation that related to clinical response to therapy. Fisher'; s exact test was used to assess the correlation between SNPs and clinical response. All 13 patients receiving vinblastine showed signs of toxicity, including vomiting, anorexia, and thrombocytopenia. Three sets of point mutations were found in 6 patients with silent ($T^{12574}C$, $n = 1$), missense ($T^{12564}G$, $n = 2$) with a change in the amino acid (Ile169Leu), and frameshift mutations (Asp153Glufs, $n = 3$) in the coding sequences. Three of 6 patients that showed missense ($n = 1$) and

frameshift mutations (n = 2) presented with recurrent MCT after vinblastine chemotherapy. This could represent a further step toward predicted the clinical response of anticancer chemotherapy.

P05

Therapeutic Drug Monitoring and Population Analysis of Cyclosporine in Dogs with Immune-Mediated Diseases

Kamoltip Thungrat
Auburn University College of Veterinary Medicine, Auburn, AL, USA

Cyclosporin (CsA) is one of the most effective and commonly used immunosuppressants. Variable clinical efficacy can be seen between patients due to significant variation in drug disposition. The process of adjusting drug doses based on CsA therapeutic drug monitoring (TDM) facilitates successful patient management. The objectives of this study was to determine the population TDM parameters of serum CsA, and to compare disposition and clinical control in canine patients. CsA TDM information was collected from the Clinical Pharmacology Laboratory database between October 2012 and September 2017. All statistical analyses were computed using SAS (version 9.3., SAS institute, Cary, NC). Disposition, plasma drug concentration, and serum half-life [$t_{1/2}$]) of CsA and individual clinical response were compared between patients with controlled clinical signs (n=127, 55%) versus those with uncontrolled disease (n=105, 45%). The most common diseases being treated were encephalitis (n = 84), immune-mediated hemolytic anemia or thrombocytopenia (n = 73), inflammatory bowel disease (IBD, n = 59), and other (n = 16, e.g. atopic dermatitis, anal furunculosis, uveitis). CsA peak levels for controlled (1119 ± 704 ng/ml) versus uncontrolled patients (994.84 ± 606 ng/ml) failed to demonstrate a statistical difference (P = 0.15). The $t_{1/2}$ of controlled patients (20 ± 40 hrs) was significantly longer than uncontrolled patients (11 ± 20 hrs) (P = 0.02). Significant variability was found in both peak CsA levels and $t_{1/2}$. Co-treatment with prednisolone, leflunomide, and ketoconazole, and diseases also increased variability. Variability in CsA disposition supports the need for TDM to establish patient therapeutic range.

P06

Is SDMA a Wonder Biomarker for Detecting Kidney Disease in Cats?

Nicolas F. Villarino[1], Liam Broughton[1], Pablo Piñeyro[2], Suarez Martin[1]
[1]College of Veterinary Medicine, WSU, Pullman, WA, USA, [2]College of Veterinary medicine, Iowa State University, Ames, IA, USA

The IDEXX-SDMA™ test is a non-invasive test being marketed for diagnosing and monitoring chronic kidney disease in cats and dogs. The objective of this study was to determine if the serum concentration SDMA and creatinine increase in cats with meloxicam-induced kidney damage.

Female cats (n=12) were allocated to 2 experimental groups: control group (n=6) and meloxicam group (n=6). Cats in the control and meloxicam groups were treated with saline and meloxicam, respectively. Serum SDMA IDEXX™ and creatinine concentrations were assessed before and after the administration of the treatments. Histopathology was performed on kidneys from all cats.

In the meloxicam group, severe tubular changes were observed in 5 out of 6 cats. Before starting the administration of the treatments, all cats had comparable serum concentrations of creatinine (≤1.6 mg/dL) and SDMA (≤14mg/dL). Unexpectedly, SDMA concentrations rose above the normal reference range only in 3 out of the 5 cats with meloxicam-induced kidney damage. The time required for SDMA and creatinine to surpass the reference concentration was similar for each cat.

This study is the first one reporting the changes in the serum concentration of SDMA in healthy cats that develop NSAID-kidney damage. Results of this study suggest that; **(i)** the serum concentration of SDMA did not detect kidney tubular damage induced by the repeated administration of meloxicam earlier than creatinine in cats and **(ii)** clinicians may be unable to detect extensive kidney tubular damage in some cats by using SDMA and creatinine, at least until glomerulus are severely affected.

P07

Pharmacokinetics of Oral Fluconazole in a Clinical Population of Dogs and Cats

Kate KuKanich[1], Butch KuKanich[1], Zhoumeng Lin[1], Dean Andrew Hanzlicek[2], Amy Rankin[1], Jean-Sebastien Palerme[3], Audrey Cook[4], Jonathan Bach[5]
[1]Kansas State University, Manhattan, KS, USA, [2]Oklahoma State University, Stillwter, OK, USA, [3]Iowa State Univesity, Ames, IA, USA, [4]Texas A&M University, College Station, TX, USA, [5]University of Wisconsin, Madison, WI, USA

Fluconazole is commonly prescribed for dogs and cats, yet pharmacokinetic data are limited. This study determined fluconazole pharmacokinetics in client-owned dogs and cats with clinical disease.

This was a multi-institutional study (n=5 university hospitals). Patients prescribed oral fluconazole for confirmed or suspected fungal disease were enrolled. Dosing protocols were determined by treating clinicians. One to three serum samples were collected at opportunistic times per fluconazole dose interval; samples from multiple visits were encouraged. Samples were stored frozen and analyzed with a validated liquid chromatography mass spectrometry method.

Forty dogs with a mean (range) body weight 24.7 (2.75-58.2) kg and 37 cats with body weight 3.88 (2.38-6.70) kg were enrolled. Most patients provided 1-2 samples (range 1-12), yielding 109 canine and 110 feline samples. Diagnoses included histoplasmosis (46), blastomycosis (7), fungal keratitis, cryptococcosis (4), coccidioidomycosis (4), aspergillosis (1), protothecosis (1), and cutaneous fungal (1). Six dogs and two cats were treated for suspected fungal disease pending diagnostic results.

The serum concentrations (dose normalized) varied widely and are directly proportional to the oral clearance (Cl/F). The Cl/F of fluconazole in cats was 0.615 (0.138-2.24) mL/min/kg compared to a mean 0.48-0.9 mL/min/kg in research cats. Renal or hepatic disease had no apparent effect on Cl/F or serum concentrations, but Cl/F progressively increased over 5 months in a cat with hypertrophic cardiomyopathy. The Cl/F in dogs was 0.650 (0.161-2.24) mL/min/kg, with 6 dogs with hepatic and/or renal disease respectively having similar Cl/F. Widely variable plasma concentrations may affect clinical efficacy and adverse effects.

R01

Blood Cultures as Minimally Invasive Surrogates in Diagnosis of Canine Bacterial Pneumonia: A Pilot Study

Aida I. Vientós-Plotts[1], Carol Reinero[2]
[1]University Of Missouri College of Veterinary Medicine, Columbia, MO, USA, [2]College of Veterinary Medicine University of Missouri, Columbia, MO, USA

Despite risk and expense, cytology and culture of bronchoalveolar lavage fluid (BALF) are essential for correct identification and treatment of bacterial pneumonia. Blood cultures could be a minimally-invasive alternative to BALF culture. The study objective was to determine agreement between cultivable bacteria in BALF and blood in canine bacterial pneumonia.

Six client-owned dogs with bacterial pneumonia having BALF with septic suppurative inflammation and/or positive aerobic/anaerobic cultures were prospectively enrolled. Blood cultures were obtained within 2 hours of BALF collection. Exclusion criteria included antibiotics (prior 14 days) and weight < 8kg (ensuring adequate blood volume collection for culture).

Clinical signs included fever (n=6), cough (5), tachypnea (3), regurgitation (2), ptyalism (1), hematemesis (1), and hemoptysis (1). On CBC, peripheral neutrophilia (2) and neutropenia (1) were noted; bands or neutrophil toxicity were common (5). On BALF cytology, degenerate neutrophils (4/6) and intracellular bacteria (3/6) were observed. Secondary bacterial pneumonia (4) was associated with multiple (3/4) or single (1/4) organisms on BALF culture: *Escherichia coli* (2), *Pseudomonas putida* (2), *Klebsiella pneumoniae* (1), *Streptococcus canis* (1), *Stenotrophomonas maltophilia* (1), *Achromobacter xylosoxidans* (1), *Pseudomonas oleovorans* (1). Primary/community-acquired pneumonia (2) had single organisms identified in culture (*Pasturella canis* and *Haemophilus haemoglobinophilus*). Only 1/6 blood cultures were positive with both blood and BALF having *E. coli*, *Klebsiella pneumonia* and *Streptococcus canis*. Three dogs were euthanized; three dogs recovered completely.

Blood cultures appear insensitive as surrogates for BALF cultures in canine bacterial pneumonia. Future analysis of banked BALF and blood for microbiome analysis in these dogs may prove more rewarding.

R02

Development of Novel Thoracic Radiographic Measurements for Discrimination of Feline Mediastinal Masses versus Pleural Effusion

Kaitlyn Belanger[1], Laura Nafe[1], Ryan Baumwart[1], Mackenzie Hallman[1], Mark Payton[1], Carol Reinero[2]
[1]Oklahoma State University, Stillwater, OK, USA, [2]College of Veterinary Medicine University of Missouri, Columbia, MO, USA

Discriminating feline mediastinal masses from pleural effusion on thoracic radiography may be challenging. The study objective was to determine if the carina is displaced caudally and dorsally in cats with a mediastinal mass (mass) compared to cardiogenic pleural effusion (CPE), non-cardiogenic pleural effusion (NCPE), and no thoracic disease (normal). Medical records from 2007-2017 at the OSU VMTH and the MU VHC were reviewed. Advanced imaging or necropsy was required for inclusion into the mass, CPE, and NCPE groups. Three evaluators (radiologist, cardiologist, and 4th year veterinary student) blinded to diagnosis obtained measurements on a lateral radiograph: number of intercostal spaces from the first rib to carina (ICS), distance from caudal aspect of manubrium to carina (MC), standardized vertebral score by drawing MC from the fourth thoracic vertebrae and counting numbers of included vertebral bodies (VS), and distance from first rib to carina (RC). A two factor ANOVA was used; data were reported as mean±SEM. Seventy-eight cats were included. The mass group had increased ICS, MC, VS, and RC compared to other groups. Compared to normal, cats with CPE and NCPE had increased ICS, and CPE cats had increased MC and RC.

Group	ICS	MC (cm)	VS	RC (cm)
Mass (n=19)	6.0 ±0.15[a]	8.9±0.17[a]	7.4±0.16[a]	7.4±0.16[a]
CPE (n=20)	5.4±0.07[b]	8.0±0.09[b]	6.5±0.08[b]	6.5±0.1[b]
NCPE (n=14)	5.2±0.07[b]	7.7±0.16[bc]	6.3±0.1[b]	6.1±0.15[bc]
Normal (n=25)	4.8±0.05[c]	7.3±0.1[c]	6.2±0.06[b]	5.9±0.09[c]

Data with different superscripts are significantly different from each other ($P < 0.0001$).

These radiographic measurements may aid clinicians in differentiating between mediastinal masses and pleural effusion in cats.

R03

Comparison of Two Aerosolized Gentamicin Protocols for Bordetella Bronchiseptica Lower Airway Infection in Dogs

Morgane Canonne[1], Elodie Roels[2], Frederic Billen[2], Ghita Benchekroun[3], Cécile Clercx[2]
[1]Ecole National Vétérinaire de Maisons Alfort, France, Maisons Alfort, Ile-de-France, France, [2]NA, Liège, Liege, Belgium, [3]NA, Maisons Alfort, Ile-de-France, France

Despite the widespread use of vaccines, *Bordetella bronchiseptica* (*Bb*) infection is still commonly diagnosed in dogs with lower airway infection. As fatal pneumonia or chronic presentation may be observed, efficient treatment is required. Aerosolized gentamicin was historically described to maximize local drug delivery with minimal systemic absorption but clinical response to standardized protocols has not been reported.

The objective of this study was to compare clinical response to two different protocols of aerosolized gentamicin in dogs with confirmed respiratory *Bb* infection.

Diagnosis of *Bb* infection was based on compatible clinical signs and positive bacterial culture or PCR on bronchoalveolar lavage fluid. Dogs were recruited over a 5 years'; period (2012-2017). In all dogs, gentamicin was aerosolized for [3]10 minutes twice daily for [3]3 weeks, using a face mask and various types of ultrasonic nebulizers. Either a dose of 4 mg/kg of gentamicin diluted with 1-4 parts of saline was used (protocol 1, n=13) or a fixed amount of undiluted 5% gentamicin (protocol 2, n=24). At first recheck after 3 to _4 weeks, treatment was either stopped or pursued in 3-week increments until clinical cure. Clinical cure was defined as resolution of clinical signs (absence of cough) and radiographical lesions improvement/resolution. Proportion of dogs clinically cured at first recheck and median total duration of treatment were compared between treatment protocols; additionally, the effect of some factors (age, breed, co-infections with other bacteria including *M. cynos*, presence of alveolar lesions, previous steroid therapy and severity of neutrophilia of the lavage) on cure and treatment duration was analysed (Chi-square and Mann-Whitney tests).

Table 1: Proportion of dogs with clinical cure at 3-4 weeks and median duration of treatment depending of type of protocol, signalement, co-infection, radiographical lesions, previous steroid therapy and cytological parameters of the lavage.

	Cure at 3-4 weeks	Median duration
Protocol 1, n = 13	3/13[a]	6[b]
Protocol 2, n = 24	13/24[a]	4[b]
Less than 1 year old, n = 31	13/31	5
More than 1 year old, n = 6	3/6	4.5
Less than 6 month old, n = 27	10/27	5
More than 6 month old, n = 10	6/10	4
Co-infection with other bacteria, n = 5	3/5	4
No co-infection with other bacteria n, = 32	13/32	5
Co-infection with *M. cynos*, n = 16	7/16	5
No co-infection with *M. cynos*, n = 8	3/8	5
Alveolar lesions, n = 20	8/20	5
No alveolar lesions, n = 17	8/17	5
Brachycephalic* dogs, n = 13	4/13	5,5
Non brachycephalic dogs, n = 24	12/24	4,5
Previous steroid therapy, n = 7	5/7	4
No previous steroid therapy, n = 30	14/30	5
TCC > 1000, n = 20	8/20	5
TCC < 1000, n = 12	7/12	4
% neutrophils > 50%, n = 23	11/23	4,5
% neutrophils < 50%, n = 9	4/9	5

[a]: proportion tending to be different (p = 0.06, Chi-square test)

[b]: median duration significantly different (p = 0.03, Mann-Whitney test)

* Brachycephalic breeds: Boxer, French and English Bulldogs, Shar Pei

TCC: total cell count of the bronchoalveolar lavage
% neutrophils: proportion of neutrophils of the bronchoalveolar lavage

Thirty-seven dogs were recruited (18 males, 19 females). Median age was 6 months [5weeks–7years] and median BW was 6.8 kg. Brachycephalic breeds, Yorkshires and Chihuahuas were over-represented (20/37 dogs (54%)) and 35/37 dogs (95%) had previously been treated with oral antimicrobial therapy with poor or no response. Twenty dogs (54%) had alveolar pattern on thoracic radiographs suggestive of bronchopneumonia. There was no difference in demographic and clinical data between dogs included in both protocols. Treatment was well tolerated in all dogs and no undesirable effect was observed. Clinical cure at first recheck tended to be more frequently observed in dogs treated with protocol 2 (13/24 [54%] dogs) compared with protocol 1 (3/13 [23%] dogs) (p=0.06). The median duration of treatment was shorter with protocol 2 (4 weeks, range 3-9) compared with protocol 1 (6 weeks, range 3-8) (p=0.03). None of the clinical or pathological parameter studied was associated with clinical response.

In conclusion, while being more time-consuming than oral antimicrobial therapy, aerosolized gentamicin appears safe and promising for treating dogs with *Bb* infection, particularly for cases refractory to oral antimicrobial therapy. Protocol 2 using undiluted 5% solution for [3]10 minutes, that allows delivery of gentamycin in amounts proportional to the individual minute volume, could offer shorter duration of treatment.

R04

Aerodigestive Disorders Identified in Dogs Evaluated for Cough Using Videofluoroscopic Swallow Studies

Megan Grobman[1], Teresa Lever[2], Carol Reinero[3]
[1]University of Missouri, Columbia, MO, USA, [2]University of Missouri Department of Otolaryngology-Head and Neck Surgery, University of Missouri School of Medicine, COLUMBIA, MO, USA, [3]College of Veterinary Medicine University of Missouri, Columbia, MO, USA

Aerodigestive diseases may occur without obvious esophageal/gastrointestinal signs. Though well characterized in humans, these are under-diagnosed in dogs due to poor clinical recognition and diagnostic limitations. The study objective was to evaluate dogs presenting for the primary clinical complaint of cough by videofluoroscopic swallow study (VFSS).

Dogs with a primary complaint of cough, thoracic radiographs, and a VFSS presenting to the University of Missouri between 8/2015-12/2017 were included retrospectively. Exclusion criteria included cough of cardiac origin or esophageal/gastrointestinal signs (regurgitation, vomiting, belching) within the preceding 6 months. Abnormalities on VFSS were categorized as gastroesophageal reflux (GER), hiatal hernia, dysmotility and aspiration.

Thirty-two cases met inclusion criteria. Median(IQR) age was 6(18.1) years with no identified breed or sex predisposition. Median(IQR)

duration of cough was 4(7.25) months. Thoracic radiographs were unremarkable in 10/32 dogs, with evidence of aspiration pneumonia in 4/32. Abnormalities on VFSS were detected in 30/32 dogs. Abnormalities included GER in 13/30 dogs with large volume GER in 5/6 dogs later diagnosed with laryngeal paralysis and 3/3 dogs diagnosed via VFSS with sliding hiatal hernia. Pharyngeal and/or esophageal hypomotility were each found in 7/30 dogs. Megaesophagus was noted in 4/30 dogs. Macroaspiration was identified in 4/30 dogs. Final respiratory diagnoses for dogs with abnormal VFSS included chronic bronchitis (9), laryngeal paralysis (6), and laryngeal polyp (1); in 14 dogs, the primary cause of cough was not of respiratory origin.

Canine aerodigestive disorders can occur without obvious signs of esophageal/gastrointestinal disease. VFSS is a useful adjunctive diagnostic in dogs with cough.

R05

Tracheal Stent Following Tracheal Rings for Management of Tracheal Collapse 9 Cases: (2010-2017)

Nathan Squire[1], Elizabeth Rozanski[2], Michael Goodrow[1], Robert McCarthy[1]
[1]Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA, [2]Tufts University, North Grafton, MA, USA

Tracheal collapse is a devastating disease primarily affecting small breed dogs. When medical management fails, surgical therapy with cervical tracheal rings and/or tracheal stents may be used for palliation of clinical signs. Tracheal rings may be preferred in cases of cervical or thoracic inlet collapse in order to prevent stent-associated complications such as granulation tissues, persistent cough or stent fracture. However, as tracheal collapse is considered progressive, dogs that initially improved following tracheal ring placement may have recurrent intra-thoracic airway obstruction that requires an intrathoracic stent for palliation. The purpose of this study is to evaluate the characteristics and outcome of dogs that received tracheal stents following earlier placement of tracheal rings.

The electronic medical record was searched for dogs that underwent placement tracheal rings. The records were retrieved, and records of dogs that subsequently underwent placement of a tracheal stent were further evaluated for breed, age at placement of rings and duration between rings and stent were identified.

Forty-seven dogs underwent tracheal ring placement during the study period, including 36 Yorkshire terriers and 11 Non- Yorkshire terriers. Two dogs had both rings and stents placed during a single hospitalization prior to discharge. One dog was euthanized after ring placement following the development of suspected ARDS. Forty-four dogs were discharged following isolated tracheal ring placement. Nine Yorkshire terriers (19%) subsequently underwent tracheal stent placement a median of 3.6 years (range 1.3 to 5.8 years) following initial placement of tracheal rings. Dogs that underwent both tracheal rings and stenting were significantly (p = 0.03) younger than dogs that did not ultimately have both procedures at the time of the original ring placement (5.6 ±1.3 years; 7.6±2.6 years). One dog that underwent both procedures had a seizure and died at home two days following discharge. Two dogs subsequently were euthanized due to progressive airway obstruction from stent-associated granulation tissue, and one dog died due to pneumonia.

Tracheal stenting may be performed after earlier ring placement in dogs with progressive airway disease. Younger age at initial ring placement as well as being a Yorkshire terrier may increase the likelihood undergoing subsequent tracheal stenting.

R06

Epiglottic Retroversion: Concurrent Diseases, Management, and Outcome in 13 Cases (2012-2017)

A. Taylor, Elizabeth Rozanski, J. Gladden
Tufts University, North Grafton, MA, USA

Epiglottic retroversion (ER) is the displacement of the epiglottis into the lumen of the larynx resulting in inspiratory airflow limitation and/or distress. It is unclear how often ER is a primary disease in dogs versus a condition that develops secondarily to other upper airway obstructive diseases. The goal of this study was to describe the concurrent diseases, management, and outcome in dogs with ER.

The electronic medical records system was searched for "epiglottic retroversion," "epiglottic entrapment" and "epiglotto-pexy." Diagnosis was made upon sedated oral examination and tracheoscopy, with or without doxapram administration. The records were reviewed and data was collected for descriptive analysis.

Thirteen dogs met the inclusion criteria. The following breeds were represented: Yorkshire terrier (n=5), and one each of chihuahua, havanese, miniature schnauzer, Parson Russell terrier, pomeranian, pug, shih tzu, and toy poodle. Seven dogs were males (6 neutered) and six were females (5 spayed). The mean age was 9.3 ± 2.4 years, and the mean weight was 4.6 ± 2.8 kg.

Two dogs had ER with no evidence of concurrent upper airway disease. The remaining eleven dogs had a combination of concurrent tracheal collapse, elongated soft palate, and laryngeal paralysis with concurrent elongated soft palate being the most common (n=6).

Treatment included an epiglotto-pexy performed in 12 of 13 dogs, and a epiglottectomy was performed in one dog. Four dogs had a staphylectomy, and one dog underwent an arytenoid lateralization procedure. All dogs recovered and were discharged.

Epiglottic retroversion may contribute to respiratory distress and is commonly is associated with other airway diseases. Additionally, epiglotto-pexy in dogs with ER may help reduce clinical signs attributable to ER.

R07

Comparison of Propofol and Alfaxalone Induction Anesthesia for Evaluation of Laryngeal Function in Healthy Dogs

Po-ching (Lydia) Pan[1], Christine Savidge[2], Pierre Amsellem[2], Stephanie Hamilton[2]
[1]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PI, Canada, [2]Atlantic Veterinary College, University of Prince Edward Island, CHARLOTTETOWN, PI, Canada

Objectives of this study were to compare effects of alfaxalone and propofol on laryngeal function and evaluate laryngeal function with a numerical, objective scoring system via computerized software.

Ten healthy beagle dogs were randomly assigned to receive either propofol or alfaxalone in this crossover prospective study. Propofol was administered at 5 mg/kg/min intravenously and alfaxalone was administered at 2 mg/kg/min intravenously until the dogs'; mouths could be opened for videolaryngoscopy. Two weeks later, dogs were evaluated using the other anesthetic agent. Videos were analyzed by GlotAnTools and Image J software.

Values of the area of the rima glottidis (A) and major length axis (L)(Figure 1) were processed at 0.02 second increments from the final 30 seconds of recording. A normalized measure was computed as A/L, representing the "elongatedness" of the rima glottidis. Small (large) values correspond to thin (thick) shapes along the length axis. The breath with the greatest laryngeal movement was identified. The difference between maximal and minimal values in each breathing cycle was calculated.

Shape values ranged between 9.6 and 138 (pixel lengths) and the differences between maximal and minimal values ranged between 2.6 and 61 (pixel lengths). The final statistical model showed a significant drug effect (p=0.016); the variability in shape for alfaxalone was about 40% (39.5%, 95% CI: 19.6%-79.6%) of that for propofol.

Propofol proved superior for evaluation of laryngeal function in healthy beagles. Software programs provided objective numbers for comparison.

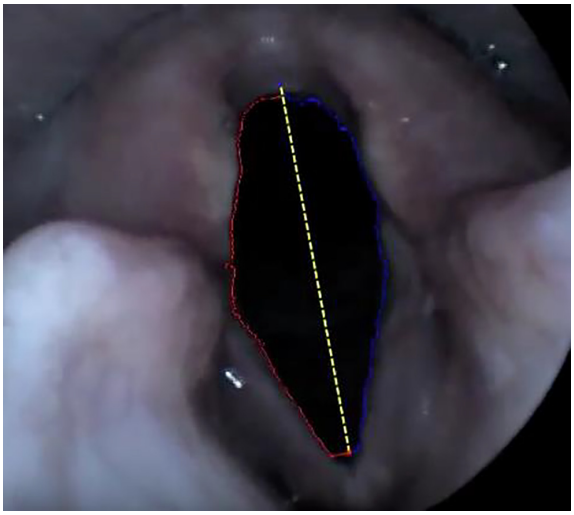


FIGURE 1 Photo exported through GlotAnTool. The glottal gap area (A) was traced around the arytenoid cartilages and the vocal cords as shown by the red and blue line. The long axis of the glottal gap (L) was measured from the center of the dorsal connection between the arytenoid cartilages to a central point at the base of the vocal cords by Image J as shown by the yellow line