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ABSTRACT

2021 ACVIM Forum Research Abstract Program

2021 ACVIM Forum Research Abstract Program
June 9 – September 31, 2021
On Demand for 3 Months
Index of Abstracts

Time	#	Presenting Author	Abstract Title
LIVE ORAL PRESENTATIONS—WEDNESDAY, JUNE 9			
CARDIOLOGY			
1:30 PM	C01	Justin Allen	Percutaneous left atrial decompression in the cat: A pilot study
1:45 PM	C02	Sonya Wesselowski	Pre-procedural femoral vessel ultrasound in dogs with patent ductus arteriosus: Diameter, image quality and outcome
2:00 PM	C03	Samantha Gomart	Pre-operative use of atenolol in dogs with pulmonic stenosis undergoing interventional procedures (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)
2:15 PM	C04	I-Jung Chi	Optimizing fluoroscopic projections for canine pulmonary valve intervention as determined by electrocardiogram-gated cardiac computed tomography
LIVE ORAL PRESENTATIONS - THURSDAY, JUNE 10			
CARDIOLOGY			
11:30 AM	C05	Deepmala Agarwal	High-sensitivity and point-of-care cardiac troponin I in apparently healthy boxers with and without ventricular arrhythmia
11:45 AM	C06	Kentaro Kurogochi	New bipolar electrocardiographic lead configurations for specific evaluation of atrial depolarization in dogs

[Correction addded on 24 Aug 2021, after the first online publication: Author list have been updated for the abstracts EN09,GI07,GI09,GI11,GI12,GI14,HP05 and title of the abstract GI13 has been updated.]

[Correction added on 20 October 2021, after first online publication: The author list has been updated for abstracts C01, C03, C04, C05, C06, C07, C14, C15, C17, C18, C26, C27, C29, C30, C33, C36, C38, C39, C40, C42, C47, E01, E03, E04, E07, E08, E12, E14, E16 E20 E21 E28 E29 E35 E37 E38 E39 E40 E44 E45 E46 E47 E48 E49 E50 E53 E54 E55 E56 E58 E60 F01 F02 F08 F09 N02 N08 N09 N12 N13 N15 N17 N23 O02 O07 O15 O19 GI03 GI04 GI10 GI13 GI15 HM07 HM08 HP02 HP03 ID05 ID06 ID14 IM01 IM04 NM01 NM05 NM06 NU03 NU05 NU09 NU13 NU18 NU19 NU20 NU21 NU22 OT03 OT04 OT05 P01 P03 P04 P06 and R03. The title of abstract E137 has been updated.]

12:00 PM	C07	Lance Visser	Regurgitant fraction in dogs with mitral regurgitation: Method comparison, effects of pimobendan, and reproducibility
12:15 PM	C08	Eric Owens	Comparison of echocardiographic measurements and cardiac biomarkers in healthy dogs eating non-traditional or traditional diets (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)

ONCOLOGY

2:30 PM	O01	Lucas Rodrigues	Canine cancer mutations homologous to human hotspots: precision medicine opportunity
2:45 PM	O02	Andi Flory	Characterization of tumor-derived genomic alterations and heterogeneity in dogs with cancer by noninvasive liquid biopsy
3:00 PM	O03	Margaret Musser	L-CHOP in combination with monoclonal antibody (AT-005, Tactress) for naïve canine intermediate/high-grade, peripheral t-cell lymphoma
3:15 PM	O04	Nanna Jacobsen	Procoagulant platelet microparticles associate with regional metastatic cancer in dogs

LIVE ORAL PRESENTATIONS—FRIDAY, JUNE 11

EQUINE

11:30 AM	E07	Hannah Kinsella	The insulin-modified frequently sampled intravenous glucose tolerance test in healthy neonatal foals and horses (ACVIM Resident Research Award eligible)
11:45 AM	E31	Kallie Hobbs	Syndecan-1 as a biomarker of endothelial glycocalx shedding in adult horses with sepsis (ACVIM Resident Research Award eligible)
12:00 PM	E41	Kimberly Young	Comparison of cerebrospinal fluid between three collection sites in adult equids with neurologic disease (ACVIM Resident Research Award eligible)
12:15 PM	E48	Sofia Bedford	Survival proportions and risk factors for non-survival in hospitalized foals from Ontario, Canada (ACVIM Resident Research Award eligible)

LIVE ORAL PRESENTATIONS—SATURDAY, JUNE 12

EQUINE

11:30 AM	E01	JoAnn Slack	Cardiac arrhythmias in 75 horses competing in the cross-country phase of equine eventing
11:45 AM	E53	Estelle Manguin	Inhaled corticosteroids influence pulmonary microbiome in severe equine asthma
12:00 PM	E30	Julie Potier	Investigation of serum markers of hepatic fibrosis in horses
12:15 PM	E49	Melissa Mercer	Pharmacokinetics and antipyretic efficacy of acetaminophen in adult horses with experimentally induced endotoxemia

ON DEMAND ORAL PRESENTATIONS

#	Presenting Author	Abstract Title
CARDIOLOGY		
C09	Hillary Hammond	The renin-angiotensin-system fingerprint® in healthy dogs and dogs with subclinical myxomatous mitral valve disease (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)
C10	Ashley Walker	Ambulatory electrocardiography, heart rate variability, and pharmacologic stress testing in cats with subclinical hypertrophic cardiomyopathy (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)
C11	Keaton Morgan	Long-term outcomes following transmembrane stent placement for cor triatriatum dexter in 6 dogs (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)
NEUROLOGY		
N01	Caitlin Doran	Drug-drug interaction between cannabidiol and phenobarbital in healthy dogs (ACVIM Resident Research Award eligible)

N02	Caitlin Doran	Handheld raman spectroscopy for intraoperative differentiation of normal brain tissue from neoplasia in canine patients (ACVIM Resident Research Award eligible)
N03	Dylan Djani	A retrospective evaluation of the efficacy of zonisamide in controlling seizures in 59 cats (ACVIM Resident Research Award eligible)
N05	Christian Woelfel	Cervical locked facet injuries in the dog: neurologic signs, advanced imaging findings, treatment, and outcomes (ACVIM Resident Research Award eligible)

ONCOLOGY

O05	Emi Sasaki	Canine hepatobiliary neuroendocrine neoplasia: An immunohistochemical and proteomic study

SMALL ANIMAL INTERNAL MEDICINE—GASTROENTEROLOGY

GI01	Naina Dinesh	Evaluation of serum zonulin in canine chronic enteropathies (ACVIM Resident Research Award eligible)
GI02	Melody Chen	DECiSION study: does endoscopy change clinicians' supportive interventions or not? (ACVIM Resident Research Award eligible)

SMALL ANIMAL INTERNAL MEDICINE—HEMATOLOGY

HM01	Avin Arjoonsingh	Novel cold storage method using platelet additive solution to prolong storage of canine platelet concentrates (ACVIM Resident Research Award eligible)
HM02	Alyssa Cannavino	Characterization of post-transfusional anti-FEA 1 alloantibodies in transfusion-naive FEA 1 negative cats (ACVIM Resident Research Award eligible)
HM03	Paige Hafner	Anticoagulant effects of rivaroxaban, prednisone, alone and in combination, in healthy dogs (ACVIM Resident Research Award eligible)
HM05	Emilie Véran	Validation of the use of bedside agglutination card for dal blood typing in dogs (ACVIM Resident Research Award eligible)

SMALL ANIMAL INTERNAL MEDICINE—IMMUNOLOGY

IM01	Petra Cerna	Antiviral immune responses and in vitro suppression of FIPV replication by novel liposome-TLR immune therapeutic (ACVIM Resident Research Award eligible)

SMALL ANIMAL INTERNAL MEDICINE—NEPHROLOGY/UROLOGY

NU01	Emmelyn Hsieh	Diagnostic yield of cystoscopy compared to ultrasonography for evaluating lower urinary tract disorders in dogs (ACVIM Resident Research Award eligible)
NU02	Melissa Milligan	Prophylactic use of tetrasodium EDTA in 95 subcutaneous ureteral bypass devices in 66 cats (ACVIM Resident Research Award eligible)
NU03	Kenneth Siu	Urine bacterial culture growth and association with clinical findings in cats with acute kidney injury (ACVIM Resident Research Award eligible)
NU04	Whitney Vickery	Reliability of Crit-Line® monitors in predicting hematocrit and change in blood volume during canine hemodialysis (ACVIM Resident Research Award eligible)

SMALL ANIMAL INTERNAL MEDICINE—PHARMACOLOGY

P01	Marianne Pan	Influence of feeding on IL-2 expression and peak blood concentration in dogs administered oral cyclosporine (ACVIM Resident Research Award eligible)

EQUINE

E08	Brianna Clark	Evaluation of an HMGA2 variant contribution to height and insulin in a population of ponies (ACVIM Resident Research Award eligible)
E09	Katheryn Johnson	Cortisol and adrenocorticotropin (ACTH) response to corticotropin-releasing-hormone (CRH) stimulation tests in healthy and hospitalized foals (ACVIM Resident Research Award eligible)
E10	Hannah Kinsella	Comparison of proxies of insulin sensitivity and insulin secretion between foals and adult horses (ACVIM Resident Research Award eligible)
E26	Céline Loubière	Immune cell population in the duodenal mucosal in asthmatic horses (ACVIM Resident Research Award eligible)
E42	Kallie Hobbs	Magnetic resonance imaging of the normal equine pituitary gland (ACVIM Resident Research Award eligible)

E45	Caitlin Malik	The comparison of two glucose monitoring systems for use in horses (ACVIM Resident Research Award eligible)
E60	Roxane Westerfeld	Effects of soaked hay on lung function and inflammation in horses with severe asthma (ACVIM Resident Research Award eligible)

FOOD ANIMAL

F03	Cileah Kretsch	Agglutination and hemolytic crossmatches to determine transfusion reaction differences between large and small breed goats (ACVIM Resident Research Award eligible)
F08	Camilo Jaramillo	Serum haptoglobin concentrations in sick calves (ACVIM Resident Research Award eligible)

EPOSTER PRESENTATIONS

#	Presenting Author	Abstract Title
CARDIOLOGY		
C12	Maiken Bach	Syringomyelia and myxomatous mitral valve disease in Cavalier King Charles spaniels
C13	Brian Barnett	Differentiating preclinical myxomatous mitral valve disease stages using electrocardiography in Cavalier King Charles spaniels
C14	Gianninne Bedoya Nader	Interaction of transforming growth factor-beta and serotonin induced mouse valvulopathy model: A pilot study
C15	Bruna Del Nero	Vasovagal tonus index in boxers with and without arrhythmogenic right ventricular cardiomyopathy (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)
C16	Erin Duble	Pulmonary arterial end-diastolic forward flow measurement in dogs with pulmonary hypertension and pulmonic stenosis
C17	Emily Gavic	Chagas disease in 12 dogs translocated from Texas
C18	Catherine Georges	Evaluation of platelet-dependent thrombin generation in healthy cats on antithrombotic therapy with rivaroxaban and clopidogrel
C19	Tung Hsueh	Resolution of congestive heart failure in dogs with myxomatous mitral valve disease
C20	Marta Karn	Retrospective study of nutritional cardiomyopathy in dogs
C21	Yoon-Mi Kim	Concentration of plasma galectin-3 in dogs with myxomatous mitral valve disease
C22	Dong Won Kim	Circulating cell free DNA concentration in canine myxomatous mitral valve disease according to ACVIM stage
C23	Dohee Lee	Contribution of chronic kidney disease to the progression of myxomatous mitral valve disease in dogs
C24	Man-Cham Lam	Orthostatic hypotension induced by rapid altitude raise in geriatric toy-breed dogs
C25	Rebekah Mack	Increased N-terminal pro-B-type natriuretic peptide is associated with increasing concentrations of renal biomarkers over time
C26	Ciara McGrath	Prevalence of pacemaker-lead-induced thrombosis in dogs diagnosed on echocardiography following transvenous cardiac pacing
C27	Anna McManamey	Population pharmacokinetics of oral pimobendan and its metabolite in dogs with myxomatous mitral valve degeneration (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)
C28	Tomoya Morita	Interventricular inflow time difference assessed by echocardiography in dogs with myxomatous mitral valve disease
C29	Lynne Nelson	Comparison of a wireless patch ambulatory ECG monitor to standard Holter monitor in six dogs
C30	Yasuyuki Nii	Hypoglycemia after cardiac surgery is disassociated with insulin and glucagon in small breed dogs
C31	Dmitrii Oleynikov	Levels of plasma microRNA and ribonuclease activity in cats with primary or secondary myocardial hypertrophy
C32	Lisbeth Høier Olsen	Long-term effects of mandatory breeding restrictions against mitral valve disease in Cavalier King Charles spaniels
C33	Tatsuyuki Osuga	Prognostic value of left ventricular-arterial coupling estimated using echocardiography in canine myxomatous mitral valve disease
C34	David Pelio	Evaluation of owner medication adherence for management of cardiovascular disease in small animal practice
C36	Ashley Saunders	Prospective cardiac evaluation in 50 asymptomatic dogs naturally-infected with *Trypanosoma cruzi*

C37	Laurel Gardner	Computed tomography in dogs with decompensated and compensated right heart disease compared to normal dogs
C38	Jessica Gentile-Solomon	Frequency of arrhythmias detected in patients using a computer aided electrocardiogram
C39	Ronald Li	Effects of single versus dual-agent antithrombotic therapy on feline platelet function
C40	Samantha Fousse	Whole genome sequencing identifies a large structural variant associated with pulmonary valve stenosis in bulldogs
C41	Caitlin Stoner	Prospective evaluation of complications associated with transesophageal echocardiography in dogs with congenital heart disease (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)
C42	Caitlin Stoner	Prospective evaluation of probes for transeosphageal echocardiography in small dogs: imaging capabilities, image quality, usability (ACVIM Resident Research Award eligible & Cardiology Research Abstract Award eligible)
C43	Kazuki Takamura	Presence of previous left atrial rupture scar in dogs undergoing mitral valve repair surgery
C44	Selena Tavener	Profiling of circulating miRNAs in canines with heart murmur and associated valvular cardiovascular disorder
C45	Lauren Wiley	The efficacy of isosorbide dinitrate in the treatment of congestive heart failure: A pilot study
C46	Yunosuke Yuchi	Influence of heart rate on right ventricular function in healthy dogs
C47	Ryohei Suzuki	Myocardial function assessed by two-dimensional speckle-tracking echocardiography in cats with cardiomyopathy and congestive heart failure

NEUROLOGY

N06	Anika Biercher	Using deep learning to detect spinal cord diseases on thoracolumbar magnetic resonance images of dogs
N07	Gilad Fefer	Relationship between hearing and cognitive function in senior and geriatric canines
N08	Sarah Foth	Canine idiopathic vestibular syndrome: unilateral decrease in inner ear signal in fluid-attenuated inversion recovery sequences
N09	Robert Hildebrandt	The effect of medium-chain triglycerides on brain metabolism and neurotransmitter concentration
N10	Molly Kelley	Sexual dimorphism of relative corpus callosum size in dogs as measured via magnetic resonance imaging
N11	Shintaro Kimura	Novel chemical chaperon inhibitors for aggregation of mutant canine superoxide dismutase 1 protein
N12	Yoonhoi Koo	Evaluation of 18F-fluorodeoxyglucose uptake of beagle dogs in different durations of isoflurane anesthesia
N13	Melissa Lewis	Calprotectin and canine pancreatic lipase activity in dogs treated surgically for thoracolumbar intervertebral disc herniation
N14	Christopher Mariani	Adapting laser interstitial thermal therapy for treatment of intracranial lesions in dogs
N15	Rebecca McBride	Developing a predictive model for spinal shock in dogs with acute spinal cord injury (ACVIM Resident Research Award eligible)
N16	Eiji Naito	MRI visualization of the volume changes in dorsal root ganglia in dogs with degenerative myelopathy
N17	Jasmin Nessler	Canine meningoencephalitis of unknown origin: The search for infectious Agents via next-generation sequencing
N18	Rowena Packer	Evaluating the impact of canine epilepsy on their caregivers
N19	Rell Parker	Prevalence of and risk factors for early postoperative seizures in dogs following intracranial surgery
N20	Aryana Razmara	Canine central nervous system metastatic melanoma: A retrospective analysis and comparative review
N21	Ashley Riffe	Region of interest compared to semi-automated diffusion tensor imaging metrics
N22	Joana Tabanez	Evaluation of a novel dorsal cemented technique for atlantoaxial stabilization in 12 dogs
N23	Taesik Yun	Neurofilament light chain as a biomarker of meningoencephalitis of unknown etiology in dogs

ONCOLOGY

O06	Julie Allen	Cancer anorexia cachexia syndrome and associated biomarkers in dogs with lymphoma
O07	Yeon Chae	Characteristic and comparison of physiologic FDG uptake in cats and dogs with positron emission tomography
O08	Lauren Holtvoigt	Veterinarian attitudes toward blood-based 'liquid biopsy' testing for cancer detection in dogs
O09	Matthew Cook	A retrospective analysis of 12 dogs with surface osteosarcoma (Early Career Clinical Oncology Research Award eligible)

O10	Migyeong Geum	Investigation of the mechanism of impaired skin barrier function in dogs with malignant tumors
O11	Lynn Griffin	The prognostic significance of metabolic tumor volumes F18-FDG PET/CT in dogs with appendicular osteosarcoma
O12	Chad Johannes	CCNU in combination with monoclonal antibody (AT-005, tactress) for naïve canine intermediate/high-grade, peripheral t-cell lymphoma
O13	Nai Kay Karen Koo	Expression and prognostic value of receptor c-met in canine malignant melanoma
O14	Mark Mamula	Vaccine-induced ErB (EGFR/HER2)-specific immunity in spontaneous canine cancer
O15	Andrea Montano Hernandez	Single-agent procarbazine chemotherapy for naïve multicentric B cell lymphoma in dogs
O16	Mo Morsey	An anti-canine PD-1 monoclonal antibody for immunotherapy of cancer in dogs
O17	Margaret Musser	mRNA expression of the prostaglandin receptor EP4 in canine lymphoma
O18	Chelsea Tripp	Gold nanoparticles & photothermal ablation as a novel approach for treating canine soft tissue sarcomas
O19	Hiroki Yamazaki	Molecular design of hypoxia-targeting therapy for intestinal T-cell lymphoma in dogs

SMALL ANIMAL INTERNAL MEDICINE—ENDOCRINOLOGY

EN01	Joana Aguiar	RNA-sequencing as a novel hypothesis generating tool to unravel the pathogenesis of feline hyperthyroidism (ESVE Award Winner)
EN02	Arnon Gal	Blood-to-saliva glucose time lag in sedated dogs
EN03	Pamela Galati	The use of desmopressin acetate to reduce polyuria and polydipsia associated with prednisolone administration
EN04	Chen Gilor	Safety and tolerability of OKV-119: A novel exenatide long-term drug-delivery-system in cats
EN05	Guido Linari	Evaluation of glycemic variability in cats with diabetes mellitus and prediction of diabetic remission
EN06	Bérénice Lutz	Outcome of radioiodine therapy for feline hyperthyroidism: Fixed dosage versus individualized dosage
EN07	Allison O'Kell	Targeted metabolomic analysis in canine diabetes
EN08	Marta Dinis	Clinical and laboratory findings in dogs with low basal cortisol levels: A retrospective case-control study
EN09	Joseph Cyrus Parambeth	Impact of six minutes of physical activity on baseline cortisol concentrations in clinically healthy dogs
EN10	Kristina Pascutti	The effect of capromorelin on glycemic control in healthy dogs (ACVIM Resident Research Award eligible)
EN11	Ashley Wood	Determination of serum TSH using novel, feline-optimized TSH immunoassay: new diagnostic test for hyperthyroid cats?
EN12	Cynthia Ward	Assessment of once daily dosing with ProZinc® insulin in diabetic beagle dogs

SMALL ANIMAL INTERNAL MEDICINE—GASTROENTEROLOGY

GI03	Allison Collier	Investigating fecal microbial transplant in dogs with inflammatory bowel disease: A pilot study
GI04	Alice Defarges	Metoclopramide effect on capsule endoscopy evaluation of the gastrointestinal tract in 17 healthy dogs
GI05	Kenjiro Fukushima	Effect of a commercially available synbiotic on mycophenolate mofetil associated diarrhea
GI06	Jodie Green	Incidence of relapse of inflammatory protein-losing enteropathy in dogs and associated risk factors
GI07	Patricia Eri Ishii	Detection of entero-invasive *Escherichia coli* in dogs with granulomatous colitis using immunohistochemistry
GI08	Aarti Kathrani	The effect of a hydrolyzed diet on the fecal microbiota of cats with chronic enteropathy
GI09	Mohammad Khattab	Effect of different storage conditions of fecal samples on some diagnostic markers of canine dysbiosis
GI10	Dohee Lee	Clinical signs, histopathology and serum high-mobility group box-1 concentrations in dogs with inflammatory bowel disease
GI11	Evangelia Stavroulaki	The effects of amoxicillin/clavulanic acid or doxycycline on the fecal microbiota in young cats
GI12	Chi-Hsuan Sung	Profiling of the fecal microbiome in cats with chronic enteropathies using quantitative PCR
GI13	Fabio Teixeira	Diabetic dogs had higher gall bladder volume than healthy dogs
GI14	Naila Telles	Comparison of gastrointestinal pH and transit times in the fasted state between cats and dogs

GI15	Emily Jachec	Clinical utility of fecal scoring and evaluation of daily fecal scoring fluctuations in healthy dogs

SMALL ANIMAL INTERNAL MEDICINE—HEMATOLOGY

HM06	Brian Barnett	A pilot study of sample tube temperature changes in three simulated 24-hour shipping conditions
HM07	Cindy Corrales M	Production and characterization of an anti-DAL murine monoclonal antibody for blood typing in dogs
HM08	Melanie Dickinson	Pharmacodynamic monitoring of clopidogrel therapy in dogs with thrombosis or risk for thrombosis (ACVIM Resident Research Award eligible)
HM09	Austin Viall	Characterization of a flow cytometric assay for anti-canine erythrocyte IgG utilizing novel standardized assay controls
HM10	Justin Hildebrand	Prevalence, severity, and concurrent clinical characteristics of microcytosis in cats
HM11	Pak Kan Tang	Comparison of i-STAT® point-of-care blood gas parameters between non-anticoagulated and heparinized whole blood in cats

SMALL ANIMAL INTERNAL MEDICINE—HEPATOLOGY

HP01	Maxime Derre	Ultrasonographic characteristics of the portal venous system of 37 healthy unsedated student-owned cats: Prospective study
HP02	Rommaneeya Leela-Arporn	Morphometric characteristics of liver masses in dogs: A promising tool for predicting malignancy
HP03	Elizabeth Schooley	Using big data to compare single versus paired bile acid testing in dogs
HP04	Rachael Sirois	Evaluation of outcomes using various treatments for extrahepatic biliary obstructions in dogs and cats: 2012-2019
HP05	Adrian Tinoco Najera	Evaluation of serum MicroRNAs 15b, 181a and 150 as biomarkers for chronic hepatitis in dogs
HP06	Jessica Villm	Gallbladder motility in dogs with hyperlipidemia

SMALL ANIMAL INTERNAL MEDICINE—IMMUNOLOGY

IM02	Paul Hess	Feline leukocyte antigen class I global survey via SMRT sequencing reveals prevalent alleles and supergroups
IM03	Yun-Fan (Eva) Kao	Cytokine dysregulation and effect of corticosteroids during treatment in cats with acute cytauxzoonosis
IM04	Alison Manchester	Differential modulation of innate immune response by primary and secondary bile acids in dogs

SMALL ANIMAL INTERNAL MEDICINE—INFECTIOUS DISEASE

ID01	Nida Chornarm	Anti-erythrocyte and anti-platelet antibodies in dogs co-infected with *Babesia gibsoni* and candidatus *Mycoplasma haematoparvum*
ID02	Jonathan Dear	Disseminated *Rasamsonia argillacea* complex infections in 8 dogs
ID03	Geghani Galustanian	Computed tomographic findings of dogs and cats with cryptococcal mycotic rhinitis
ID04	Kate KuKanich	Susceptibility of imipenem-resistant *Pseudomonas aeruginosa* isolated from canine otitis
ID05	Kathrin Langner	Clinical accuracy of cryptococcal antigen lateral flow assay for diagnosis of canine and feline cryptococcosis
ID06	Michael Lappin	Effect of a novel liposome-TLR immune therapeutic on ocular FHV-1 infections in experimentally inoculated cats
ID07	Rodolfo Oliveira Leal	Current trends on antileishmanial treatment: A questionnaire-based survey among general practitioners from Portugal
ID08	Dan O'Neill	New information on skin fold dermatitis in dogs under primary veterinary care the UK
ID09	Krystle Reagan	Outcome and prognostic factors in canine infective endocarditis: 113 cases (2005-2020)
ID10	Rafael Ricardo Santisteban-Arenas	Prevalence of *Dirofilaria immitis* and *Ehrlichia canis* infection in dogs of San Andres Island, Colombia
ID11	Ri Scarborough	Urinary tract infections in dogs and cats: how can we optimize empirical antibiotic therapy?
ID12	Kira Schmitt	Hand hygiene compliance and antimicrobial-resistant microorganisms on the hands of veterinary healthcare worker
ID13	Ben Walker	Effect of discussion of antimicrobial guidelines on prescribing habits in a small animal first-opinion practice

ID14	Alexis McLaine	Prevalence of SARS-Cov-2 in domestic cats presenting to a teaching hospital during the COVID-19 pandemic

SMALL ANIMAL INTERNAL MEDICINE—NEPHROLOGY/UROLOGY

NU05	Emily Coffey	Characterization of the urinary microbiome in miniature schnauzers with calcium oxalate urolithiasis
NU06	David Conway	Effect of prazosin on rate of recurrent urethral obstruction in cats: preliminary results
NU07	Linda Fleeman	Is glucosuria in dogs fed jerky treats associated with excessive intake of soluble phosphorus?
NU08	JD Foster	Centrifugal mononuclear cell collection in dogs: Safety and efficacy of treatment
NU09	Courtney Gallant	Complications and outcome in female dogs with ectopic ureter treated by laser ablation or surgery
NU10	Federica Cagnasso	A retrospective evaluation of neutrophil and platelet ratios in cats with obstructive uropathy
NU11	Rita Hanel	Evaluation of the automated FIRSTract rapid urine culture to detect canine and feline bacteriuria
NU12	Sarah Adrianowycz	Canine urine ammonia-to-creatinine ratio reference interval
NU13	Alexis Hoelmer	Nephroureterolithiasis in dog breeds predisposed to calcium oxalate urolithiasis
NU14	Huiyeon Ko	Cystatin C and neutrophil gelatinase-associated lipocalin as early biomarkers for chronic kidney disease in dogs
NU15	Jack Lawson	Isolation of urinary extracellular vesicles from healthy cats and cats with chronic kidney disease
NU16	Julie Lecavalier	Use of telmisartan to address proteinuria in dogs in various clinical contexts: A retrospective study
NU17	Jennifer Macleay	An updated relative supersaturation program, EQUIL-HL21, compared to EQUIL93 applied to healthy dogs and cats
NU18	Lia McCoy	Urine and renal cultures from geriatric cats with and without chronic kidney disease
NU19	Rene Paschall	Assessment of capillary rarefaction in cats with and without CKD
NU20	Stacie Summers	Effect of dietary protein concentration on serum concentrations of gut-derived uremic toxins in healthy cats
NU21	Craig Sutter	Evaluation of a rapid immunoassay for detection of UTI in dogs
NU22	Taesik Yun	Progression of chronic kidney disease in dogs with concurrent myxomatous mitral valve disease

SMALL ANIMAL INTERNAL MEDICINE—NUTRITION / METABOLISM

NM01	Hailey Davis	Effect of *Bifidobacterium longum* 999 supplementation on stress associated findings in cats with FHV-1 infection
NM02	Chelsea Iennarella-Servantez	Effects of Macronutrient Composition on Adipose Deposition and Body Weight in Healthy Dogs Fed Westernized-Diet
NM03	Ram Jinka	Fortetropin Safety and Tolerability in Cats
NM04	Selena Lane	Evaluation of Blood Thiamine Concentration in Hospitalized Dogs with and without Critical Illness
NM05	Johnny Li	Gut Dysbiosis and Its Association with Microbiota-Derived Metabolites in Myxomatous Mitral Valve Disease in Dogs
NM06	Sarah Lorbach	Ghrelin Sample Storage and Stability in Healthy Cats (ACVIM Resident Research Award eligible)

SMALL ANIMAL INTERNAL MEDICINE—OTHER

OT01	James Barton	Comparison between non-contact handheld cutaneous infrared thermometer and standard rectal thermometer in dogs and cats
OT02	Hatice Çolakoğlu	Short-term effects of early-age ovariohysterectomy in cats: A preliminary report
OT03	Kohtaro Hayashi	Evaluation of the diagnostic utility of serum amyloid A in 206 diseased cats
OT04	Kellyn McNulty	Developing an end-of-life survey to capture cause of death and reasons for euthanasia in dogs
OT05	Kellyn McNulty	Development and reliability of a survey instrument used to create a canine multimorbidity index
OT06	Sonya Wesselowski	Prevalence of burnout, depression and excessive daytime sleepiness in academic veterinary intern and resident trainees

SMALL ANIMAL INTERNAL MEDICINE—PHARMACOLOGY

P02	Sarah Ezell	Genetic analysis of the cannabinoid receptor: 1 gene in three beagle dogs

P03	Henrique Ellrich	Comparison of pharmacodynamic effects of different modified cyclosporine formulations in dogs on comparable oral doses
P04	Andrzej Ogrodny	Effect of inhaled albuterol on whole blood potassium concentrations in dogs
P05	Jennifer Reinhart	Cytochrome P450 reaction phenotyping of itraconazole hydroxylation in dogs
P06	Rafael Ricardo Santisteban-Arenas	Preoperative administration of cannabidiol (CBD) in healthy dogs undergoing elective surgery in Colombia: 16 cases

SMALL ANIMAL INTERNAL MEDICINE—RESPIRATORY

R01	Loren Easterwood	Comparing nasal sampling techniques for culture and mycoplasma polymerase chain reaction in canine nasal disease (ACVIM Resident Research Award eligible)
R02	Elizabeth Luciani	Identification and prevalence of aerodigestive disease in dogs with hiatal hernia
R03	Nevra Keskin Yılmaz	Distribution of allergens in cats with feline asthma: Clinical experience in Ankara, Turkey
R04	Mark Nagel	Development and evaluation of a novel respiratory airway model of a cat
R05	Craig Sutter	Patient-specific three dimensional-printed nasopharyngeal stents in dogs

EQUINE

E02	Lisa De Lange	First successful applications of closed loop stimulation pacemakers with remote monitoring in two syncopal miniature donkeys (ECEIM Award Winner)
E03	Todd Holbrook	Renin-angiotensin-aldosterone system profiling in horses before and after exercise
E04	Rikke Buhl	Atrial fibrillatory rate as predictor of recurrence of atrial fibrillation in horses
E05	Sian durward-Akhurst	Utility of a modified wearable ECG patch for 7-day monitoring in horses
E06	Ludovic Tanquerel	Repeatability of heart rate variability measurements during standardized treadmill exercise test in thoroughbred horses
E11	Nicholas Bamford	Comparison of basal adrenocorticotropic hormone concentrations among different equine breeds
E12	François-René Bertin	The effect of transferring equine plasma into silicate-containing tubes to improve short-term ACTH stability
E13	Sarah Vaughn	Effects of oral RRR-A-tocopherol on plasma oxidative stress and endocrine markers in healthy horses
E14	Erin Pinnell	Lamellar ribosomal protein S6 activation in three forms of experimentally induced equine laminitis
E15	Caroline Burglass	Adrenocorticotropin concentrations vary with assay used in equids with and without pituitary pars intermedia dysfunction (ACVIM Resident Research Award eligible)
E16	Aimee Colbath	Lumbar vertebral bone density is decreased in aged horses with pituitary pars intermedia dysfunction
E17	Harold Schott	Steroid hormone profiles of horses with pituitary pars intermedia dysfunction
E18	Mathijs Theelen	Temporal changes in the fecal microbiota and its functional capacity of ponies developing insulin dysregulation
E19	Kathryn Timko	Effect of AMPK agonists on incretin hormone secretion in horses with experimentally-induced insulin dysregulation
E20	Kathryn Timko	Effect of AMPK agonists on serum lipid and adipokine concentrations in horses receiving dexamethasone
E21	Kathryn Timko	Incretin hormone response to an oral sugar test in horses with dexamethasone-induced insulin dysregulation
E22	Rosemary Bayless	Investigation of plasma cell-free DNA (cfDNA) as a novel biomarker in equine colic patients
E23	Clémence Loublier	Microbial composition and viability in equine feces after processing for transplantation
E24	Cosette Ayoub	Machine learning for the analysis of the fecal microbiota in horses with colitis
E25	Elizabeth Graham-Williams	Use of equine omega complete for prevention of gastric ulcers and supplementation of vitamin E
E27	Kate McGovern	Prophylactic treatment of equine laparotomy incisions with manuka honey reduces the incidence of incisional infection
E28	Luiza Zakia	Equine enterocolitis: A retrospective post-mortem study (2007-2019) (ACVIM Resident Research Award eligible)
E29	Megan Palmisano	Effect of plasma administration on serum amyloid a in neonatal foals

E32	Rosemary Bayless	Treatment with withaferin A inhibits respiratory burst, adhesion, and chemotaxis by equine neutrophils
E33	Ashton Miller	EHV-specific immune responses to an EHV 1 & 4 vaccine in horses
E34	Sophie Sage	First single-cell gene expression analysis of equine bronchoalveolar cells
E35	Luis Arroyo	Isolation of a novel species of neorickettsia that causes potomac horse fever
E36	Kimberly Martin	Do veterinarians want increased reporting of equine strangles?
E37	Veridiana Nadruz	Efficacy of disinfection of endoscope contaminated by *Streptococcus equi* subspecies equi
E38	Nicola Pusterla	Effect of bi-weekly administration of diclazuril on antibody kinetics to *Sarcocystis neurona* in healthy horses
E39	Laszlo Hunyadi	A prospective study of serum amyloid a in relation to plasma administration in neonatal foals
E40	Thomas Ternisien	Minimally invasive removal of obstructive ureteral stones by lithotripsy in horses: 3 cases
E43	Gemma Cock	Opioid-sparing sedation for atlantoaxial cerebrospinal fluid collection in standing horses
E44	Rebeca Scalco	Sensorineural auditory loss associated by intravenous administration of gentamicin in healthy adult horses
E46	Jose Gonzalez Carballo	Type of metabolic acidosis and its association with survival in critically ill horses
E47	Erin Pinnell	Effects of vaccination with a commercially available xenogenic DNA vaccine in 15 horses with melanoma
E50	Daniela Luethy	Pharmacokinetics of oral and intravenous metoprolol tartrate in clinically healthy horses
E51	Emily Hess	Pharmacokinetics of thiamine hydrochloride (Vitamin B1) in horses after administration of three single intravenous doses
E52	Katherine Wilson	Potassium penicillin and gentamicin pharmacokinetics in conscious and anesthetized horses
E54	Séléna de Wasseige	Oscillometry bronchodilator response does not differentiate horses with severe asthma in remission and healthy controls
E55	Florence Dupuis-Dowd	Bronchial smooth muscle remodeling in mild and moderate equine asthma
E56	Sarah Reuss	Evaluation of the safety of ciclesonide delivered via SoftMist™ inhaler in horses
E57	Allison Thriffiley	Effect of treatment with excede and azithromycin on culture of transtracheal washes from healthy foals
E58	Clare Ryan	Effect of treatment with excede and azithromycin on the respiratory microbiota of healthy foals
E59	Clare Ryan	Equine asthma management: survey of horse owners demonstrates interest in mobile phone app
FOOD ANIMAL		
F01	Yoko Nakamae	Gastrointestinal foreign bodies in pet pigs: 17 cases
F02	Jessie Ziegler	Comparison of transfaunate collected from two different rumen compartments in a healthy fistulated donor cow
F04	Joe Smith	Comparison of a point-of-care hematocrit assay and an automated microcentrifuge for cattle and sheep
F05	Meera Heller	Novel caprine coronavirus: ELISA development and serologic survey of exposed herds in Northern California
F06	Ingrid Lorenz	A high plane of nutrition is a protective factor against calf diarrhea on dairy farms
F07	Joe Smith	Clinical findings of gastrointestinal parasitism in camels presenting to a Veterinary Teaching Hospital
F09	Katherine Wilson	Lack of Oral Absorption of Grapiprant in adult alpacas

C01

Percutaneous left atrial decompression in the cat: A pilot study

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Congestive heart failure (CHF) and arterial thromboembolism (ATE) are common in cats with severe cardiomyopathy. Left atrial decompression (LAD) involves creating an iatrogenic atrial septal defect (iASD) via percutaneous jugular catheterization, which reduces left atrial hypertension. It may also reduce left atrial stasis by providing an additional outlet into the lower-pressure right atrial chamber. We hypothesized that LAD would result in reduced left atrial pressure with concurrent reduction in left atrial stasis in cats with chronic CHF and left atrial stasis, with or without historical ATE.

Cats with cardiomyopathy and chronic left CHF and/or historical ATE were recruited for evaluation in this pilot study. Four cats with chronic CHF were recruited: one cat with hypertrophic cardiomyopathy

(HCM), two with HCM and ATE, and another with restrictive cardiomyopathy and ATE.

Left atrial pressure was reduced in all cats following LAD. None of the cats developed signs of left heart failure (pleural effusion or pulmonary edema) requiring hospitalization during short-term follow-up. Reductions in diuretic doses were achieved in all patients, though escalation of diuretics over time were necessary in 2/4. There were improvements in left atrial function in all cats, with increases in left atrial shortening fraction and left auricular velocity in all patients. Spontaneous contrast was markedly diminished or eliminated in all patients postoperatively, suggesting a reduction in the degree of left auricular stasis.

Left atrial decompression results in rapid reduction of left atrial pressure in cats with CHF, and may reduce left atrial stasis in cats at risk for ATE.

C02

Pre-procedural femoral vessel ultrasound in dogs with patent ductus arteriosus: Diameter, image quality and outcome

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Patent ductus arteriosus (PDA) in dogs is often treated via minimally invasive transvascular occlusion using right femoral artery (RFA) access. This study compared ultrasound-derived diameter and image quality score of the RFA and right femoral vein (RFV) in dogs with PDA using a linear ultrasound probe (L-P) and a phased-array transthoracic echocardiography probe (TTE-P). Outcome of cases undergoing RFA catheterization was assessed.

Forty-five client-owned dogs with PDA were prospectively enrolled. Ultrasound-measured RFA and RFV diameters were obtained on images acquired with both ultrasound probes pre-operatively and compared using Bland-Altman analysis. Image quality of the RFA and RFV were scored on L-P and TTE-P images.

Comparison of RFA and RFV diameter measured on L-P versus TTE-P images revealed: [Mean difference (limits of agreement): RFA=0.009 mm (-0.78-0.79mm), RFV=0.523mm(-1.75-2.79mm)]. Image quality scores were significantly higher for L-P than TTE-P ($p < 0.0001$). In 5 dogs, measurable images were unattainable with TTE-P. Dogs of similar body weight had variable RFA diameter. Twenty-seven dogs had RFA catheterization. In 21/27 dogs, RFA diameter was larger than the external diameter of the introducer used for catheterization and in 6/27 it was smaller.

Pre-procedural ultrasound of the RFA in dogs with PDA is useful given variable RFA diameter relative to body weight. Despite poorer image quality, RFA diameter from TTE-P images was negligibly different from L-P images on average, suggesting TTE-Ps are suitable for pre-procedural planning in most dogs. Vasospasm, hypotension or differences in the location of ultrasound measurement versus catheterization might produce variation in pre-procedural versus intraoperative RFA size.

C03

Pre-operative use of atenolol in dogs with pulmonic stenosis undergoing interventional procedures

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Ventricular arrhythmias can be triggered during right heart catheterization, potentially resulting in hypotension, ischemic damage or even patient death. This study aimed to evaluate if pre-procedure atenolol treatment reduces ventricular arrhythmias, anesthetist interventions or shortens procedure time.

This single center, prospective, randomized, open-label study enrolled dogs with pulmonic stenosis prior to balloon valvuloplasty or transpulmonic stent implantation. Dogs were randomized to treatment with atenolol or no treatment pre-operatively for a minimum of 10 days. Variables recorded included an intra-operative ECG, total procedure time and administration of anti-arrhythmic treatment, vasopressors, chronotropes or fluid boluses by the attending anesthetist. Statistical analysis included Fisher's exact test, Chi-squared test and Mann–Whitney U test where appropriate.

Fifteen dogs were enrolled in each group. Dogs receiving atenolol had lower mean heart rates during the procedure (atenolol 100 ± 11bpm vs untreated 115 ± 19bpm, $p=0.01$). There were no significant differences between the atenolol and untreated groups in the frequency of ventricular ectopic complexes (535 [6-5296] vs 553 [79-2863], $p=0.90$), ventricular couplets (46 [0-481] vs 29 [3-121] $p=0.58$), ventricular triplets (20 [0-265] vs. 16 [1-82] $p=0.67$), ventricular tachycardia (8 [0-224] vs. 8 [1-118] $p=0.99$), proportion exhibiting R-on-T phenomenon (11/15 vs. 14/15, $p=0.33$), proportion receiving intra-operative lidocaine (1/15 vs. 3/15, $p=0.60$), vasopressors/chronotropes (11/15 vs. 5/15, $p=0.06$) or fluid boluses (12/15 vs. 7/15, $p=0.12$). The procedure time was similar (atenolol 41min [23-68] vs. untreated 35min [18-98], $p=0.91$).

In conclusion, no benefits of pre-operative atenolol treatment were identified in dogs undergoing pulmonic balloon valvuloplasty or stenting.

C04

Optimizing fluoroscopic projections for canine pulmonary valve intervention as determined by electrocardiogram-gated cardiac computed tomography

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Optimal fluoroscopic projections (OFP) for valvar interventions should be orthogonal to the transverse plane or *en face* projection (EFP) to minimize foreshortening artifacts and visualize anatomic features of the valve and surrounding structures important for performance of the intervention. Using ECG-gated cardiac computed tomography (CCT), we sought to characterize the OFP for canine pulmonary valve

(PV) interventions and explore the difference in projection angles between normal dogs and dogs with PV stenosis (PS).

CCTs were obtained from 6 normal dogs and 12 dogs with PS. The EFP of the PV was planimetered using dedicated software and a curve generated predicting all projections orthogonal to the EFP (S-curve), which was plotted on a coordinate grid with the degrees of left/right anterior oblique rotation (LAO/RAO) and cranial/caudal (CRA/CAU) angulation. By inspecting all projections along the S-curve, the OFP was determined by maximal separation of the intermediate and left pulmonary cusps from central positioning of the right cusp. C-arm coordinates of each dog's OFP were collected as spherical data and analyzed under the assumption of Fisher distribution (alpha=0.05); mean and 95% confidence limits of each OFP were calculated.

Mean OFP was not different (P=0.33) between normal (LAO 92±8 / CAU 18±8) and PS (LAO 83±9/CAU 22±8) dogs. Pre-procedural CCT is useful for determining the OFP of the PV in dogs and suggests a lateral projection with moderate caudal angulation is optimal. This approach likely has application for most structural heart interventions requiring fluoroscopic guidance.

C05

High-sensitivity and point-of-care cardiac troponin I in apparently healthy boxers with and without ventricular arrhythmia

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The utility of a high-sensitivity cardiac troponin-I (hs-cTnI) assay for the detection of ventricular arrhythmias in the Boxer dog population has not yet been reported. The objective of this study was to evaluate and compare the diagnostic value of an hs-cTnI assay and a conventional point-of-care cTnI (POC cTnI) assay in the identification of premature ventricular complexes (PVCs) in apparently healthy Boxers.

Prospective study. 167 client-owned Boxers were screened. Thirty purebred, apparently healthy Boxers >1 year old, not receiving cardiac medications were enrolled, based on pre-defined inclusion and exclusion criteria. The frequency of PVCs/24h was assessed via 24-hour Holter. Circulating cTnI concentrations [cTnI] were measured by the two assays. Twenty-four dogs had PVCs (median, 10 PVCs/24h; range, 1–767). The [cTnI] medians were 140.5 ng/L (hs-cTnI) and 60 ng/L (POC cTnI). Circulating [hs-cTnI] were significantly higher than the POC [cTnI] ($P < 0.0001$). A significant modest positive correlation existed between [hs-cTnI] and the frequency of PVCs/24h ($r = 0.53$, $P = 0.002$). At [hs-cTnI] cutoff of 128.5 ng/L, the sensitivity and specificity to identify Boxers with >100 PVCs/24 h were 100% and 59%, respectively. No significant correlation existed between POC [cTnI] and PVCs/24h ($r = 0.30$, $P = 0.10$). At POC [cTnI] cutoff of >55 ng/L, the sensitivity and specificity to identify Boxers with >100 PVCs/24 h were 75% and 41%, respectively. Hs-cTnI and POC cTnI assays cannot be used interchangeably. The hs-cTnI assay may be more sensitive and specific than the POC cTnI to detect PVCs in apparently healthy Boxers.

C06

New bipolar electrocardiographic lead configurations for specific evaluation of atrial depolarization in dogs

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The Lewis Lead, described by Thomas Lewis, is an electrocardiograph configuration to detect atrial activity and its relationship to ventricular activity. In recent years, the configuration has been used to recognize P waves during wide QRS tachycardia. However, no equivalent configuration has been proposed in the field of veterinary medicine. We explored new bipolar lead configurations to specifically evaluate atrial depolarization and investigated their usefulness by comparing them with conventional leads. We hypothesized that a lead depicting both high absolute value of the P wave (|P|), and high ratio of the P wave to the QRS complex (|P|/|QRS|) might be a useful configuration.

The electrocardiograph was recorded using bipolar limb leads (I, II, III, aVL, aVF, and aVR) and unipolar precordial leads (1st-R, C2, C3, C4, C5, C6, M1, M2, M5, M6, CV6LL, and V10) in six Beagle dogs in the standing position. The new bipolar leads were attached in the following configuration: the right forelimb electrode was attached to M6, and the left forelimb electrode or the left hindlimb electrode was sequentially applied to C2, C3, C5, M1, M2, and M5. We named them M6C2, M6C3, M6C5, M6M1, M6M2, and M6M5, respectively. Statistical significance was set at $p < 0.05$.

The waveforms obtained using the new bipolar leads, especially M6M1, showed significantly higher values of |P|/|QRS| and |P| than those obtained from most conventional leads.

Our findings suggest that M6M1 may be a potent lead configuration to indicate P waves without amplifying the QRS waves, compared to conventional configurations.

C07

Regurgitant fraction in dogs with mitral regurgitation: Method comparison, effects of pimobendan, and reproducibility

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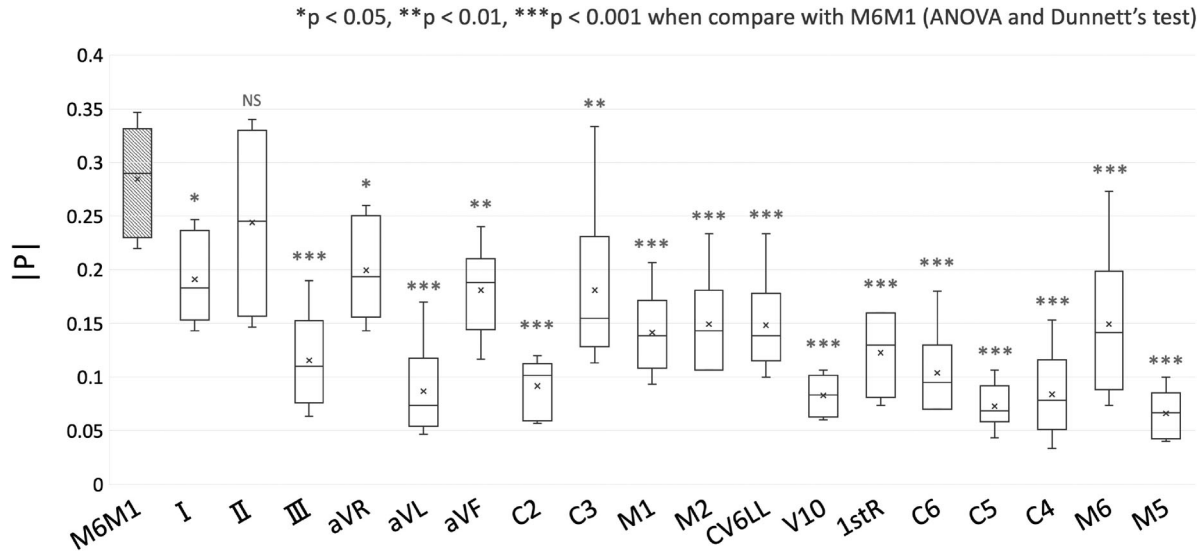
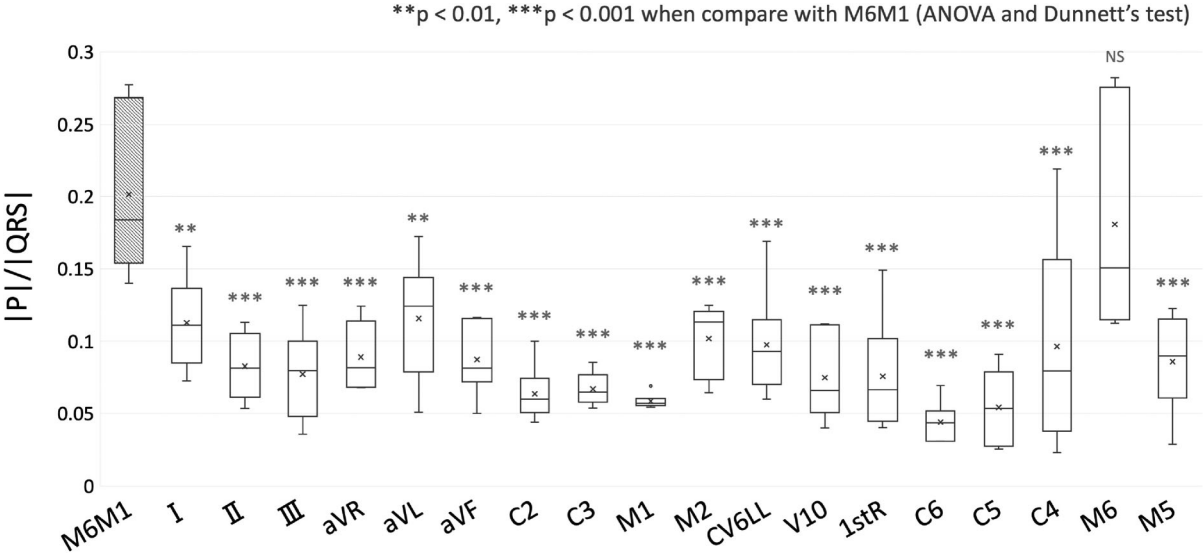
We sought to determine echocardiographic regurgitant fraction (RF) using Simpson's method of disks (RF_SMOD) to estimate total stroke volume (TSV) in a large number of dogs with myxomatous mitral valve disease (MMVD) and compare it to RF using M-mode to estimate TSV (RF_M-mode). We also sought to evaluate the effect of pimobendan on RF and the reproducibility of RF.





Eighty-one dogs with MMVD were prospectively enrolled and underwent an echocardiographic examination. Ten dogs underwent a second echocardiographic examination after receiving pimobendan 0.3 mg/kg PO q12h for 7-10 days. Nine dogs underwent six echocardiographic examinations by two operators on three nonconsecutive days within one week for reproducibility analysis. The relationship between left atrial size (LA/Ao) and RF_SMOD was determined using regression analysis. Agreement between RF_SMOD and RF_M-mode was evaluated using Bland-Altman's method. Wilcoxon's test was used to compare RF before and after pimobendan. Reproducibility was quantified with reproducibility coefficients (RC).

Dogs with stage B1 (n=30), B2 (n=38), and heart failure (n=13) were enrolled. RF_SMOD exhibited a curvilinear relationship with LA/Ao (R^2=0.60; P< 0.001). RF_SMOD versus RF_M-mode revealed a mean (SD) bias of -8.0 (12.0)% with significant proportional bias. Pimobendan caused a significant (P< 0.001) decrease in RF_SMOD and RF_M-mode (median IQR percent changes of -25.8 [-36.8, -15.6] and -37.6 [-47.2, -33.2]%, respectively). Between-day intraoperator and between-operator RCs for RF_SMOD were 19.1% and 24.6%, respectively, and for RF_M-mode were 24.8% and 28.0%, respectively.

RF_SMOD might aid the echocardiographic assessment of MMVD and should be considered for multifactorial approaches to determine disease severity.

C08

Comparison of echocardiographic measurements and cardiac biomarkers in healthy dogs eating non-traditional or traditional diets

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There has been a recent association between dogs eating non-traditional diets and the development of a dilated cardiomyopathy phenotype. The purpose of this study was to compare echocardiographic measurements

and cardiac biomarkers between healthy dogs eating non-traditional versus traditional diets.

Healthy dogs at least three years of age were enrolled in a prospective, breed- and age-matched, cross-sectional study. Dogs were divided into groups based on diet ingredients: traditional (grain-containing diets with no potatoes or pulses in the top 10 ingredients) or non-traditional (grain-free diets or those with potatoes or pulses in the top 10 ingredients). Dogs underwent three-dimensional (3D), two-dimensional (2D), and Doppler echocardiographic examinations and analysis of N-terminal B-type natriuretic peptide (NT-proBNP), cardiac troponin I, and whole blood and plasma taurine concentrations. Data were compared between the non-traditional and traditional diet groups.

There was no significant difference in age, breed, or weight between the traditional (n = 23) and non-traditional (n = 23) diet groups. Mean 3D left ventricular end-diastolic ($p = 0.001$) and end-systolic ($p < 0.001$) volumes as well as 2D left-ventricular end-diastolic ($p = 0.04$) and end-systolic ($p = 0.002$) volumes (all indexed to body weight) were significantly higher in dogs eating non-traditional diets compared to those eating traditional diets. Mean 3D ejection fraction ($p < 0.001$), global longitudinal strain ($p < 0.001$), and 2D ejection fraction ($p < 0.001$) were significantly lower in dogs eating non-traditional diets compared to those eating traditional diets. No statistical differences were observed between groups for 2D fractional shortening, normalized left ventricular internal dimensions at end-diastole or end-systole, NT-proBNP, cardiac troponin I, or taurine concentrations.

Healthy dogs eating non-traditional diets had significantly reduced left ventricular systolic performance compared to dogs eating traditional diets, albeit within reference ranges for most variables. Further studies are required to elucidate possible associations between non-traditional diets and cardiac function.

C09

The renin-angiotensin-system fingerprint® in healthy dogs and dogs with subclinical myxomatous mitral valve disease

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The renin-angiotensin system (RAS) Fingerprint® utilizes high performance liquid chromatography and mass spectrometry to create a comprehensive profile of circulating RAS. The primary study aim was to compare the circulating RAS Fingerprint® and aldosterone concentrations between normal dogs (N) and dogs with subclinical (American College of Veterinary Internal Medicine stage B1 and B2) myxomatous mitral valve disease (MMVD). No dogs were receiving cardiac medications. Seventy-eight dogs (37 apparently healthy, 27 stage B1, and 14 stage B2) were included. Surrogates for angiotensin-converting enzyme 2 (ACE2): (angiotensin [Ang]1-5/AngII), renin: (AngI+AngII), and angiotensin-converting enzyme (ACE): (AngII/AngI) activities and peptide concentrations of AngII, Ang1-7, and aldosterone were compared between the three groups using the Kruskal-Wallis test with Dunn's post-test. Median (IQR) ACE2 activity surrogates for N, B1, and B2 were 0.76 (0.55-1.2), 0.83 (0.54-1.3), and 1.4 (0.86-1.9), respectively and differed significantly (P = 0.02). No significant differences were found for AngII, Ang1-7, and aldosterone concentrations or the surrogates for renin and ACE activities. Angiotensin-converting enzyme 2 is an endogenous counter-regulator of the RAS and could be useful as a marker for disease severity or as an indicator of prognosis in canine MMVD.

C10

Ambulatory electrocardiography, heart rate variability, and pharmacologic stress testing in cats with subclinical hypertrophic cardiomyopathy

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The utility of ambulatory electrocardiography (AECG) to evaluate cats with subclinical hypertrophic cardiomyopathy (HCM) for arrhythmias and heart rate variability (HRV) is not well defined but may provide important information regarding risk stratification.

This prospective study used AECG monitoring to evaluate ectopy and HRV in cats with subclinical HCM compared to healthy controls and is the first to implement a pharmacologic cardiac stress test. Twenty-three purpose-bred, Maine coon cross cats (16 HCM, 7 control) underwent 48-hours of continuous AECG. Terbutaline (0.2 - 0.3 mg/kg) was administered orally at 24 and 36 hours. Heart rate, ectopy frequency and complexity, and HRV parameters, including standard deviation of normal R-R intervals (SDNN) were compared pre-terbutaline and post-terbutaline as well as across disease status, genotype and sex.

Frequency of ventricular ectopic beats was significantly associated with genotype (A31P vs wildtype; p=0.013) and maximal left ventricular wall thickness (p=0.039) across all cats. Seven HCM cats and zero healthy cats exhibited sinus arrhythmia. Mean heart rate was significantly higher post-terbutaline ($p < 0.0001$). HCM cats had significantly greater HRV compared to controls (SDNN: p=0.0006). Male cats had significantly higher HRV (SDNN: p=0.0001) and lower mean heart rates (p=0.0001). HRV decreased post-terbutaline (SDNN: p=0.0008) and changes in HRV observed between sexes were attenuated by terbutaline.

Administration of oral terbutaline is an effective, feasible, cardiac stress test in cats with subclinical HCM. Significant differences in heart rate and HRV were found between sex. Cats positive for the A31P MYBPC3 mutation and those with greater left ventricular hypertrophy were more likely to have ventricular ectopy.

C11

Long-term outcomes following transmembrane stent placement for Cor Triatriatum Dexter in 6 dogs

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Report the long-term outcomes following transmembrane stent placement as therapy for Cor Triatriatum Dexter (CTD).

Retrospective case series including 6 dogs with CTD treated with stent placement. Follow up information was obtained including the persistence of presenting clinical signs, additional therapies required, and survival analysis.

Median follow up time was 24 months (range 15-76 months). Of the 6 dogs included, 3 dogs (50%) had immediate resolution of clinical signs without the need for medical therapy. These dogs had no concurrent congenital heart defects. The three dogs with persistent clinical signs had varying degrees of tricuspid valve dysplasia (TVD), two of which also had a right-to-left shunting patent foramen ovale. One of these 3 dogs died 23 months post-stent placement during attempted open-heart repair of the TVD and foramen ovale due to severe exercise intolerance. Another is alive 15 months post-operatively stable on medical therapy for right sided congestive heart failure secondary to TVD. The final dog demonstrated improved but persistent mild exercise intolerance up to 76 months post-operatively associated with mild TVD and a concurrent patent foramen ovale. Interestingly, two dogs had failed previous balloon dilation procedures prior to stent placement, after which 1 dog had immediate resolution of clinical signs and the other is the aforementioned dog with persistent mild exercise intolerance.

Stent treatment for CTD is a viable long-term treatment option with improvement or resolution of clinical signs. In the presence of concurrent congenital heart disease, long-term medical management may be necessary with a corresponding impact to prognosis.

C12

Syringomyelia and myxomatous mitral valve disease in cavalier King Charles Spaniels

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[1]Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Hovedstaden, Denmark, [2]Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark, [3]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark, [4]Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [5]Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark

Cavalier King Charles spaniels (CKCS) are genetically predisposed to both syringomyelia (SM) and myxomatous mitral valve disease (MMVD). This study investigated if a possible association between the occurrence of the two diseases exists.

This combined retrospective and prospective study running 2007-2015 included 55 CKCS: Forty-one dogs with syrinx identified with MRI, defined by a hypointense area in T1 > 2mm in width, and 14 controls (> 5 years of age) with no syrinx on MRI. Of dogs with SM, 19 dogs were asymptomatic while 22 were symptomatic. Echocardiography (> 4 years of age) were performed in all dogs. A possible association between syringomyelia and MMVD disease severity (left ventricle internal diameter in diastole (LVIDD), LVIDD normalized to bodyweight (LVIDDN) and left atrium to aortic ration (LA:Ao)) was tested using multivariable linear regression analysis adjusting for sex and age.

There was no significant difference in LVIDD, LVIDDN and LA:Ao (median [IQR]) between CKCS with SM (28.20 [27.0–30.80], 1.50 [1.40–1.60], 1.20 [1.10–1.40]) and CKCS without SM (28.90 [27.15–36.75], 1.55 [1.43–1.83], 1.30 [1.20–1.80]). Yet, LVIDD, LVIDDN and LA:Ao were smaller in CKCS with symptomatic SM (27.20 [25.85–28.80], 1.45 [1.30–1.50] and 1.20 [1.10–1.28]) compared to CKCS with asymptomatic SM (30.80 [28.40–38.40] $p < 0.001$, 1.60 [1.50–1.90] $p < 0.001$, 1.40 [1.20–1.75] $p = 0.004$).

We found no overall association between MMVD and SM. Dogs affected with symptomatic SM had less severe MMVD compared to dogs with asymptomatic SM indicating it is important to be aware of both diseases when breeding CKCS.

C13

Differentiating preclinical myxomatous mitral valve disease stages using electrocardiography in Cavalier King Charles spaniels

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Echocardiography is the recommended test to establish a diagnosis of Stage B2 myxomatous mitral valve disease (MMVD). However, echocardiography is not always available. The diagnostic utility of electrocardiography (ECG) and heart rate variability (HRV) variables to differentiate Stage A and Stage B1 from Stage B2, as defined by the 2019 ACVIM MMVD consensus statement has not been evaluated in Cavalier King Charles Spaniels (CKCS).

The goal of this study was to evaluate diagnostic utility of a variety of ECG-derived variables alone and in combination to differentiate Stage A and Stage B1 from Stage B2 MMVD in CKCS.

Two-hundred and twenty-seven apparently healthy CKCS that were not receiving any cardiac medications were prospectively recruited. Dogs were staged with auscultation and echocardiography: Stage A (N = 14; 6.2%), Stage B1 (N = 169; 74.4%), Stage B2 (N = 44; 19.4%). As part of

the study, a 30-second resting right lateral six-lead ECG was performed and analyzed off-line using IDEXX CardioPet. Measured variables included; P wave duration (ms), P wave amplitude (mV), PR interval (ms), R wave peak time (ms), QRS duration (ms), R wave amplitude (mV), QT interval (ms). Twenty sequential RR intervals were used to calculate average heart rate (HR) and vasovagal tonus index (VVTI) to assess HRV. Additionally, QRS+P duration (ms), a novel ECG variable, was calculated.

Receiver operating characteristic (ROC) curves were constructed for each variable to determine their discriminatory utility. The area under the curve (AUC) was ≤ 0.6 for P and R amplitude, PR and QT interval, R wave peak time, HR and VVTI, demonstrating poor discrimination. The AUC for P and QRS duration was 0.742 and 0.759 respectively, demonstrating fair discrimination. The AUC for QRS+P duration was 0.812 (standard error = 0.037; 95% confidence interval = 0.750 - 0.884; $p < 0.0001$), demonstrating good discrimination. A cut-off of 79 ms for QRS+P duration had good specificity (85%) and fair sensitivity (59%) for identification of Stage B2, suggesting it may be useful to rule-out Stage B2 in CKCS with preclinical MMVD with a false negative rate of 10%. However, the false positive rate remains high at 51%. Subsequent decision tree analysis using P (≤ 40 ms) and QRS (≤ 39 ms) in series reduced the false negative rate to 6% but the false positive rate remained high at 42%.

These data suggest that P and QRS durations combined as P+QRS or in series may represent a good screening test to rule-out Stage B2 MMVD in CKCS with preclinical MMVD with a negative predictive value 90-94%.

C14

Interaction of transforming growth factor-beta and serotonin induced mouse valvulopathy model: A pilot study

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Serotonin and Transforming Growth Factor-beta (TGF-β) signaling have been proposed to play an important role in the development of myxomatous mitral valve degeneration (MMVD) but the interaction between the pathways remains unknown. Antagonism of the TGF-β type I receptor (ALK5) induces valvular lesions in rats, which are similar to MMVD. Administration of high levels of serotonin to rats results in valve lesions similar to carcinoid heart disease and administration of serotonergic drugs. We propose that TGF-β signaling functions in an overseer role, regulating multiple other tissue pathways, including serotonin in which impaired TGF-β function results in dysregulated tissue growth. We hypothesize that the morphologic valvular changes induced by ALK5 antagonism will be reduced or prevented by the administration of the serotonin antagonist cyproheptadine. Twenty-two mice were used for this study; 5 negative controls (Group A), 10 received the ALK5 antagonist AZ12799734 (Group B), and 7 received cyproheptadine and AZ12799734 (Group C). After seven days of AZ12799734, the hearts were collected for histologic analysis. Valve morphology was evaluated using a semi-quantitative scoring system, fibrosis was assessed using Masson's Trichrome, and IHC was used to quantify alpha-smooth muscle actin (SMA). Administration of AZ12799734 induced morphologic changes in all mice and were notably different from the control group. While there was a significant expansion in the spongiosa and an increase in interstitial cells in Group B compared to control ($p=0.005$), this was not true for Group C ($p=0.072$); total valve score was also not significantly different between Groups A and B ($p=0.064$), Groups A and C ($p=0.078$), or Groups B and C ($p=1.00$). There was no significant difference in fibrosis or SMA between any groups.

The administration of the ALK5 antagonist AZ12799734 to mice resulted in morphologic changes to the valves although this pilot study was underpowered. The administration of the serotonin antagonist cyproheptadine did not prevent these changes. We conclude that 1) serotonin is not a key element in this valvulopathy model 2) alterations in the TGF-β pathway likely play a key role in MMVD, and 3) these results further add to the complexity of this important disease process.

C15

Vasovagal tonus index in boxers with and without arrhythmogenic right ventricular cardiomyopathy

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Heart rate variability (HRV) is a short-term indicator of autonomic nervous system (ANS) status. Vasovagal tonus index (VVTI) is a simple method of HRV analysis that has not been evaluated in canine ARVC. The objective of this study was to compare VVTI between Boxers with and without ARVC, and between ARVC Boxers with and without sotalol treatment. We hypothesized that VVTI would be lower in Boxer dogs with ARVC compared to normal Boxers, but that sotalol would increase their VVTI reflecting less pronounced sympathetic tone predominance. Medical records from January 2011 through December 2019 were reviewed to identify client-owned Boxers with a 24-hour Holter monitor, without evidence of systolic dysfunction, congestive heart failure, or other significant diseases (n=60). Dogs were categorized with ARVC (≥100 VPCs/24h or fewer with complexity; ARVC-S if receiving sotalol [n=10] or ARVC-NS if not receiving sotalol [n=24]) or normal (< 100 VPCs/24h, no complexity [n=26]). The VVTI measurements from the maximum heart rate (HHR_{max}) hour and the minimum heart rate (HHR_{min}) hour were calculated using the following formula: VVTI = ln[VAR(R1–R20)], and VAs were quantified. The VVTI at HHR_{max} was significantly lower than at HHR_{min} ($p< 0.0001$). The VVTI did not differ significantly among groups and was not significantly correlated to the frequency of VAs. In Boxers, ARVC with and without sotalol treatment is not characterized by ANS imbalance detectable by short-term evaluation using VVTI. Other methods of HRV assessment are warranted to more fully evaluate the role of the ANS in Boxers with ARVC.

C16

Pulmonary arterial end-diastolic forward flow measurement in dogs with pulmonary hypertension and pulmonic stenosis

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Main pulmonary artery end-diastolic forward flow (EDFF) has been studied in humans with repaired tetralogy of Fallot as a potential indicator of restrictive right ventricular physiology (r-RVP). We sought to evaluate EDFF in dogs with two other conditions characterized by r-RVP – pulmonic stenosis (PS) and precapillary pulmonary hypertension (PH). We proposed that individuals in these disease groups have increased EDFF magnitude compared to dogs with structurally and functionally normal hearts, and that increasing EDFF maximum velocity in these patients is associated with more severe right ventricular (RV) hypertrophy. Retrospective analysis of echocardiographic studies of 50 PS patients, 50 PH patients, and 12 (6 age and weight matched individuals for each disease group) normal dogs evaluated by the University of Tennessee Veterinary Medical Center Cardiology service between

Table 1. Mann-Whitney U nonparametric test assessing the differences between maximum EDFF velocity in the pulmonary hypertension and pulmonic stenosis disease groups and the maximum EDFF velocity in their respective age and weight-matched normal groups

Pulmonary Hypertension Patients vs. Age and Weight Matched Normal Dogs	Maximum EDFF velocity (cm/s)		PH Patients	Normal Dogs
		N	50	6
		Median	31.57	21.86
		Standard deviation	22.33	7.11
		Mann-Whitney U	65.00	
		Exact significance (2*1-tailed significance)	0.001	
Pulmonic Stenosis Patients vs. Age and Weight Matched Normal Dogs	Maximum EDFF velocity (cm/s)		PS patients	Normal Dogs
		N	50	6
		Median	67.18	25.40
		Standard deviation	26.61	18.86
		Mann-Whitney U	30.00	
		Exact significance (2*1-tailed significance)	0.001	

Abbreviations: EDFF – end-diastolic forward flow; PH – pulmonary hypertension, PS – pulmonic stenosis

Table 2. Spearman's rho nonparametric correlations between maximum EDFF velocity and right heart structural measurements, and between maximum EDFF velocity and functional indicators of disease severity.

Pulmonary Hypertension Patients			iRVIDd 2D LA4C (cm)	iRVFWd 2D RPSAX (cm)	iRVFWd M mode (cm)	iRAAs 2D LA4C	TR (m/s)
	Maximum EDFF velocity (cm/s)	Correlation coefficient	0.017	-0.300*	-0.152	0.049	0.079
		2-tailed significance	0.909	0.034	0.355	0.733	0.590
		N	50	50	39	50	49
Pulmonic Stenosis Patients			iRVIDd 2D LA4C (cm)	iRVFWd 2D RPSAX (cm)	iRVFWd M mode (cm)	iRAAs 2D LA4C	PV PPG mmHg)
	Maximum EDFF velocity (cm/s)	Correlation coefficient	0.015	0.121	0.297*	0.134	0.406**
		2-tailed significance	0.918	0.404	0.047	0.370	0.004
		N	50	50	45	47	49

*Correlation significant at 0.05 level (2-tailed)

** Correlation significant at 0.01 level (2-tailed)

Abbreviations: EDFF – end-diastolic forward flow; iRVIDd 2D LA4C – right ventricular internal diameter at end-diastole measured on a two-dimensional left apical four chamber view and indexed to body weight ($RVIDd/BW_{kg}^{0.33}$); iRVFWd 2D RPSAX – right ventricular free wall thickness at end-diastole measured on a two-dimensional right parasternal long axis view and indexed to body weight ($RVFWd/BW_{kg}^{0.25}$); iRVFWd M mode - right ventricular free wall thickness at end-diastole measured from an M mode image and indexed to body weight ($RVFWd/BW_{kg}^{0.25}$); iRAAs 2D LA4C – right atrial area at end-systole measured on a two-dimensional left apical four chamber view and indexed to body weight ($RAAs/BW_{kg}^{0.71}$); TR – tricuspid regurgitation measured with spectral doppler centered on the tricuspid valve (indicator of pulmonary hypertension clinical significance); PV PPG – peak pressure gradient across the pulmonic valve measured with spectral doppler centered on the right ventricular outflow tract (indicator of pulmonic stenosis severity).

2007-2020 addressed these two hypotheses. EDFF was retrospectively measured according to the methods described in Kutty et al., 2018, and right heart structural measurements were retrospectively obtained according to the methods described in Gentile-Solomon et al., 2016. We found that maximum EDFF velocity was significantly higher in individuals with PH or PS compared to age and weight matched normal dogs (Table 1). In addition, we found that maximum EDFF velocity was significantly positively correlated with end-diastolic RV free wall thickness indexed to body weight (correlation coefficient = 0.297, $p < 0.05$) and with pulmonic valve peak pressure gradient (correlation coefficient = 0.406, $p < 0.005$) in PS patients only (Table 2). Our findings support our first hypothesis, as maximum EDFF velocities were significantly greater in our two disease groups compared to their respective age and weight matched normal groups, suggesting that EDFF of the velocities observed in our disease groups is abnormal and potentially correlated with their disease states. Our second hypothesis was partially supported, as there was an association between EDFF and RV hypertrophy in PS patients, but not in PH patients. Interobserver agreement was also evaluated, comparing the primary observer's retrospective EDFF and right heart structural measurements (ED) to those of a board-certified veterinary cardiologist (LK) – no statistically significant differences were found for any of the measurements assessed.

C17

Chagas disease in 12 dogs translocated from Texas

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Canine Chagas disease is caused by *Trypanosoma cruzi*. Infections can remain asymptomatic or result in acute or chronic disease most often due to myocardial injury. Increasingly, Chagas disease is diagnosed in non-endemic regions of the United States.

This retrospective case series describes the history, clinical presentation and outcome in 12 client-owned dogs diagnosed with Chagas disease in the northern U.S. (CO, NC, MI, NJ, NY, OH, WA, WI) that were translocated from Texas. Diagnosis was confirmed by indirect immunofluorescence antibody titer ≥1:20 (n=9), quantitative PCR (n=1), or post-mortem histopathology (n=2). Time spent in Texas was < 1 year in 7/12 dogs, while 5/12 spanned 2-8 years. 9/12 dogs spent < 1 year in the northern U.S. prior to diagnosis; 3/12 spent >3 years. Presenting complaints included respiratory signs (4/12), collapse (3/12), abdominal distension (3/12), arrhythmia (3/12), exercise intolerance (1/12), lethargy (1/12), gastrointestinal signs (1/12). Dogs also presented for wellness exam (1/12) and because a littermate tested positive (1/12). At diagnosis, median age was 2.6 years (range, 0.2-8.8); median weight was 18.5kg (range, 4.5-44.0). Examination findings included arrhythmia (8/12) and effusion (ascites 4, pleural 4, both 1). Electrocardiographic abnormalities included ventricular arrhythmias (9/11), supraventricular arrhythmias (3/11) and third-degree atrioventricular block (2/11). Echocardiography revealed left ventricular systolic dysfunction (9/10), ventricular dilation (left 7/10; right 6/10; biventricular 4/10), and atrial dilation (right 5/10, left 3/10). At the time of reporting, outcomes included alive (n=4, survival time following diagnosis ranging 60-269 days), sudden death (n=5), euthanized for heart disease (n=2), and lost to follow-up (n=1).

These cases highlight the variable clinical signs and diagnostic findings in dogs diagnosed with Chagas disease. Further, Chagas disease should be considered when dogs originating from endemic regions present with arrhythmia, systolic dysfunction or dilated cardiomyopathy phenotype, and reinforces the value of a comprehensive medical and travel history and physical examination.

C18

Evaluation of platelet-dependent thrombin generation in healthy cats on antithrombotic therapy with rivaroxaban and clopidogrel

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Cats with hypertrophic cardiomyopathy (HCM) are at risk of cardiogenic arterial thromboembolism (CATE), an often-fatal disease sequela. Studies have investigated the use of single-agent antithrombotic therapies for their role in the treatment of CATE and prevention of recurrence. Dual-agent therapy, using multiple antithrombotic therapies, is superior to single-agent therapy in the treatment and prevention of thromboembolic disease in people. Despite this, little is known about the combined effect of rivaroxaban (RVX) and clopidogrel on platelet function and the potential use of this dual-agent therapy in preventing complications of feline HCM. We aimed to compare the safety and efficacy of dual-agent treatment with RVX and clopidogrel with single-agent therapy with RVX. We hypothesized that dual-agent therapy would safely reduce platelet-dependent thrombin generation (TG) more effectively than single-agent treatment.

TG on platelet-rich plasma was compared in nine healthy cats before and after 7 days of RVX (2.5 mg PO q24h), or dual treatment with RVX and clopidogrel (18.75 mg PO q24h). TG over 1 hour was measured by fluorogenic assay.

Dual therapy modulated platelet-dependent thrombin generation potential [Median (IQR) AUC 3.55 e + 10 (2.05 e + 10, 6.45 e + 10)

and 2.35 e + 10 (2.1 e + 10, 4.75 e + 10) for RVX and dual therapy respectively; p = 0.012]. All treatments significantly affected peak thrombin generation and thrombin kinetics. No side effects were noted in any of the cats throughout the study.

Based upon improved modulation of TG, combined RVX and clopidogrel therapy in cats may represent a superior antithrombotic treatment strategy compared to clopidogrel alone. Further studies are necessary to investigate clinical outcomes and additional measures of antithrombotic effects.

C19

Resolution of congestive heart failure in dogs with myxomatous mitral valve disease

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Myxomatous mitral valve disease (MMVD) is one of the causes of congestive heart failure (CHF) in small-breed dogs. Based on our clinical observations, few dogs with CHF secondary to MMVD are stable to the extent that the dose of diuretic can be lowered, tapered, or stopped. Therefore, this study aimed to investigate the differences between dogs with MMVD that showed resolution of CHF and those that died due to cardiac causes.

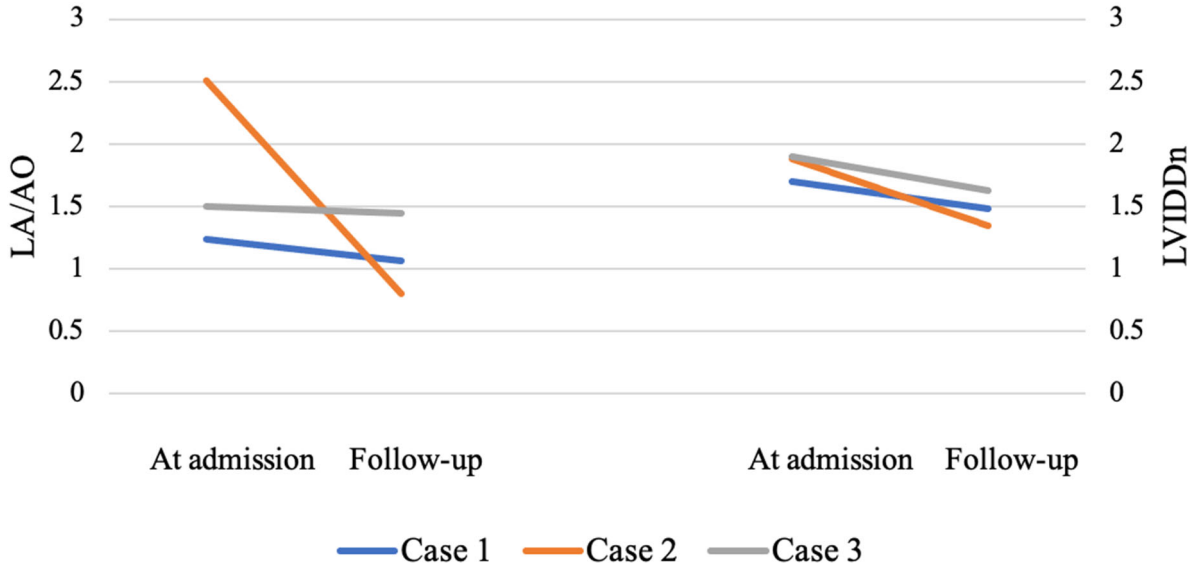
Electronic medical records of dogs with a new diagnosis of MMVD from October 2012 to December 2020 were retrospectively reviewed. Dogs that were diagnosed with pulmonary edema at admission, and for whom the dose of diuretic could be lowered (furosemide not exceeding 3 mg/kg/day), tapered, or stopped for at least one year without recurrent pulmonary edema were included. Of the 1,223 MMVD cases, 8 (8/1223, 0.65%) met the inclusion criteria and were allocated to the resolved group (5 Maltese, 2 toy poodles, and 1 schnauzer). Breed- and sex-matched controls, which were also diagnosed with CHF secondary to MMVD and ultimately died of cardiac reasons, were allocated to the progressive group. This was a

Table 1. Baseline comparisons between resolved group and progressive group.

	Resolved group	Progressive group	*P* value
Breed			
(Maltese/toy poodle/schnauzer)	5/2/1	5/2/1	1.00
Sex (M/MN/F/FN)	3/1/1/3	3/1/1/3	1.00
Age (years)	8 (6-17)	9.5 (7-13)	.336
Body weight (kg)	3.4 (2.4-6.9)	3.1 (1.4-5.2)	.645
VHS	11.5 (10.9-13.4)	12.0 (10.6-14.9)	.721
VLAS	2.9 (2.5-3.2)	3.2 (2.6-4.0)	.328
LVIDSn	0.83 (0.61-1.03)	1.18 (0.49-1.30)	.105
LVIDDn	1.95 (1.76-2.03)	2.30 (1.68-2.64)	.234
FS (%)	55.1 (46.6-66.9)	51.1 (45.5-70.6)	.328
Mitral E/A	1.85 (1.48-2.55)	1.61 (0.87-3.65)	.442
Mitral E/E'	11.7 (8.96-34.5)	15.4 (11.1-22.1)	.645
LADn	20.9 (19.4-26.7)	26.3 (19.0-35.0)	.259
LA/AO- 2D	2.03 (1.52-2.68)	1.93 (1.64-3.02)	.535
LA/AO- M-mode	1.79 (1.36-2.51)	1.90 (1.39-2.71)	.878
LA ejection fraction (%)			
Reservoir	0.50 (0.37-0.59)	0.45 (0.30-0.59)	.366
Conduit	0.34 (0.25-0.45)	0.29 (0.10-0.46)	.295
Contractile	0.27 (0.09-0.31)	0.23 (0.05-0.48)	.805
Flailed mitral valve (Y/N)	4/4	3/5	1.00
PH (Y/N)	2/6	6/2	.132

Values are shown as median and range.
E/A, the ratio of early and late diastolic inflow velocity; E/E', the ratio of early diastolic inflow velocity and myocardium velocity; F, female; FN, neutered female; FS, fractional shortening; LA/AO, the ratio of left atrium to aorta dimension; LADn, normalized left atrial dimension; LVIDDn, normalized left ventricular internal dimension in diastole; LVIDSn, normalized left ventricular internal dimension in systole; M, male; MN, neutered male; PH, pulmonary hypertension; VHS, vertebral heart size; VLAS, vertebral left atrial size,

Figure 1. Decreased left atrium and left ventricle size in 3 resolved cases without identifiable systemic disease.



retrospective case-control study. Breed, sex, age, body weight, vertebral heart size, vertebral left atrial size, normalized left ventricular internal dimension in systole and diastole, left ventricular fractional shortening, mitral E/A, mitral E/E′, normalized left atrial dimension, left atrium to aorta ratio (2D and M-mode), variables representing left atrial functions, and the presence of a flailed mitral valve and pulmonary hypertension were compared between the two groups.

All variables showed no statistically significant difference between the two groups. In the resolved group, more than half of the patients (5/8, 62.5%) had other systemic diseases that required tapering of diuretic therapy; two were diagnosed with Cushing's disease and one each had proteinuria, chronic kidney disease, and urinary obstruction. Of the remaining three dogs, without evidence of other systemic diseases, two were still under follow-up, and the dose of diuretics was unchanged for 1079 and 1035 days. Diuretic therapy had been stopped in the third dog 779 days after the first admission. None of them showed evidence of pulmonary hypertension at the first admission, and all of them showed decreased left atrium and left ventricle size upon re-evaluation by echocardiography (ranging from 255 to 910 days after the first admission).

For dogs with MMVD and CHF, the occurrence of other systemic diseases that could alter the hydration status may lead to the resolution of CHF signs, followed by the modification of diuretic therapy. This emphasizes the importance of regular follow-up in dogs with MMVD and CHF. For unknown reasons, few cases without other identifiable systemic disease can be stable for a long time. The reason why only very few dogs with MMVD and CHF need lowering or cessation of diuretic therapy warrants further study.

C20

Retrospective study of nutritional cardiomyopathy in dogs

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The purpose of this study was to evaluate cases of presumed nutritional cardiomyopathy and to note specific treatment recommendations that led to the improvement of cardiac structure and function.

This was a single-center, retrospective analysis of canine patients with a diagnosis of dilated cardiomyopathy (DCM), left ventricular dilation, and/or systolic dysfunction. Age at diagnosis, normalized left ventricular internal dimension in diastole (LVIDdN), fractional shortening (FS), left ventricular ejection fraction (LVEF) via Modified Simpson method (when applicable), improvement or lack thereof (as noted by the primary cardiologist), diet, and medical treatment recommendations were analyzed in 51 cases of presumed nutritional cardiomyopathy.

Visit data from 5/4/2017 to 11/27/2020 were reviewed. The age of canines with diagnosis of nutritional cardiomyopathy ranged from 17 months to 14 years old (mean= 6.05 years, median= 7 years). Improvement, as noted by a decrease in LVIDdN, increase in FS and/or LVEF was seen in 48 out of 51 dogs (94%) between 3 months and 1 year and 7 months after diagnosis. Fractional shortening significantly improved over time with a mean increase of 8% (95% confidence interval: 6-9%). Normalized LVIDd was significantly different over time with a median decrease of 0.22 (95% confidence interval: 0.16-0.3). Sixteen of improved patients were switched to a diet that meets the WSAVA guidelines: Royal Canin Veterinary Diet (33.3%),

13 to a Purina diet (27.1%), 10 to Hill's/Science Diet (20.8%), 1 to Iams (2.1%); the rest of the patients were either switched to a non-WSAVA diet or no data was available (10.4% and 6.3%, respectively). Taurine was prescribed to 49 patients in the analysis (96%) and pimobendan was used in 30 dogs (58.8%). Ten patients were presented in congestive heart failure (19.6%) and treated with pimobendan, furosemide, and an ACE-inhibitor (enalapril or benazepril). Only 1 patient from that pool did not improve due to presumed concurrent idiopathic DCM.

In conclusion, the majority of dogs who were presented for evaluation improved with at least a diet change. This data solidifies the potential for improvement with a diet change and taurine supplementation, as well as an improvement in function with proper congestive heart failure treatment.

C21

Concentration of plasma galectin-3 in dogs with myxomatous mitral valve disease

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The aims of the study were to investigate if plasma galectin-3 concentrations were different in normal dogs versus dogs with myxomatous mitral valve disease (MMVD) and to explore the potential association of other cardiac biomarkers, inflammatory cytokines, echocardiographic estimates, and dog characteristics on galectin-3.

The study prospectively recruited 10 healthy dogs and 30 dogs with MMVD. Dogs were divided into control and MMVD groups based on the presence of MMVD, which was verified by echocardiography. Dogs with MMVD were further categorized according to the severity of MMVD (stage A, B1, B2, and C). Canine-specific enzyme-linked immunosorbent assays were used to analyze plasma galectin-3, serum N-terminal pro-brain natriuretic peptide (NT-proBNP), and cardiac troponin I (cTnI). Inflammatory cytokines were measured using real-time polymerase chain reaction (PCR). The nonparametric Mann-Whitney U-test and Kruskal-Wallis test were used to compare the indices between the control and MMVD groups and the severity of MMVD. Multiple regression analyses were used to investigate the potential associations between galectin-3, conventional cardiac biomarkers (NT-proBNP and cTnI), inflammatory cytokines (tumor necrosis factor-α, interleukin-1β (IL-1β), IL-2, IL-8, and IL-33), and dog characteristics (age, clinical sign scores, duration of clinical signs, body weight, heart rate, respiratory rate, body temperature, and systolic blood pressure). Logistic regression was used to develop models for the detection of MMVD.

Significantly higher concentrations of plasma galectin-3 were observed in dogs with MMVD (2.94 [interquartile range 1.61 – 5.20] ng/mL), compared to concentrations in the control group (1.56 [0.69 – 1.84] ng/mL, $P = 0.009$). Additionally, dogs with MMVD stage C showed significantly higher plasma galectin-3 levels (3.484 [2.03 – 5.58] ng/mL) than those in the control group (1.56 [0.69 – 1.84] ng/mL, $P = 0.002$). Plasma galectin-3 levels increased significantly with increasing heart rate and body weight ($R^2 = 0.273$, $P = 0.003$). However, other cardiac biomarkers, inflammatory cytokines, and echocardiographic parameters were not significantly associated with galectin-3 expression. In logistic regression analysis, galectin-3 and age were considered suitable for the detection of MMVD (predictive accuracy = 90.0%, $P < 0.05$).

In conclusion, concentrations of plasma galectin-3 were higher in dogs with MMVD compared to healthy dogs, especially in dogs with higher MMVD severity. In addition, the results suggest that galectin-3 is sufficiently useful to screen for MMVD and is a novel cardiac biomarker in dogs with MMVD. Further research is required to investigate the changes in plasma galectin-3 levels during long-term follow-up and its role in determining the prognosis of dogs with MMVD.

C22

Circulating cell free DNA concentration in canine Myxomatous mitral valve disease according to ACVIM stage

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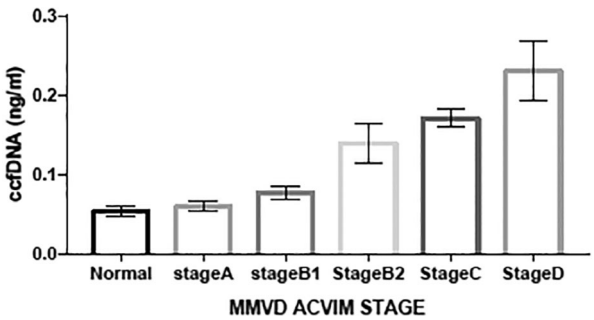
The circulating cell free DNA (ccfDNA) is released by apoptosis or cell necrosis and flows along the bloodstream. The concentrations of ccfDNA have been found to increase not only in tumors, but also in myocardial infarction, trauma, sepsis in human medicine. Dogs with myxomatous mitral valve disease (MMVD) causes mitral regurgitation and can proceed with left heart failure (LHF). Since ccfDNA leaks due to cell apoptosis and cell necrosis even in myocardial infarction, experiments were conducted on dogs with LHF caused by MMVD which can make myocardial cell apoptosis ultimately. The hypothesis of the experiment was that concentration of ccfDNA would be higher in dogs with LHF than normal dogs. The 40 dogs with LHF and 14 normal dogs were diagnosed and graded through thoracic radiography and echocardiography, and blood tests to exclude any other diseases. The results of study show that ccfDNA has increased with the stage overall. Especially, ccfDNA has increased dramatically between stage B1 and stage B2 ($P < 0.01$) which has strong recommendation for initiation of Pimobendan. The result of this study may suggest that apoptosis or necrosis occurs in LHF due to MMVD and that the level of ccfDNA increases due to the active modulation of the heart from stage B2.



C23

Contribution of chronic kidney disease to the progression of Myxomatous mitral valve disease in dogs

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In veterinary medicine, the understanding of the interaction between renal and cardiovascular system is increasing, but it has not yet been completely elucidated. Therefore, in this study, we aimed to investigate the cardiorenal system in dogs by evaluating chronic kidney disease (CKD) as a risk factor for the progression of myxomatous mitral valve disease (MMVD).

The medical records of 63 dogs diagnosed with MMVD stage B1, B2, or C, according to the guidelines of the American College of Veterinary Internal Medicine, were retrospectively reviewed. The progression of MMVD was examined 6 months after the first diagnosis, and the mortality rate was recorded. The indicators for the progression of MMVD and mortality rate were compared between the dogs with only MMVD (MMVD group) and the dogs with both CKD and MMVD (concurrent group).

In MMVD stages B2 and C, change in vertebral heart score was significantly greater in the concurrent group than in the MMVD group ($P < 0.01$ and $P = 0.02$, respectively). In all stages of MMVD, the concurrent group showed a greater change in left ventricular end-diastolic diameter normalized for body weight than the MMVD group ($P < 0.05$). However, no significant differences were found in the progression of murmur grade and left atrium/aorta ratio between the two groups ($P > 0.05$). The mortality of the concurrent group was significantly higher than that of the MMVD group ($P < 0.01$).

These results provide an insight into the relationship between CKD and MMVD, and suggest the potential role of CKD as a risk factor for the progression of canine MMVD.

C24

Orthostatic hypotension induced by rapid altitude raise in geriatric toy-breed dogs

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Orthostatic hypotension (OH), defined as systolic blood pressure (SBP) decrease of ≥20 mmHg, is frequently observed in elderly people who feel fainting on rapid rising. An old Maltese Terrier presented for syncope while being brought up quickly from the ground by her owner. The simulated manipulation reduced the dog's SBP, and this raised a question of whether OH could be induced by rapid altitude raise (RAR), which is a common daily situation in the lives of toy breeds. The purpose of this study was to prospectively investigate the SBP change by RAR test in geriatric toy-breed dogs.

Twenty-five client-owned dogs >8 years old with a body height of < 50 cm were included. Dogs were excluded if critically ill, uncooperative, or having baseline SBP < 100 mmHg. Baseline SBP was measured by Doppler sphygmomanometry, and measurements were immediately repeated during the subsequent 180 seconds after the dog was quickly lifted in a 2-leg standing posture up from the ground to a height of approximately 120–130 cm.

The average SBP after RAR test decreased by ≥20 (defined as OH) and 10–20 mmHg (suspected OH) relative to the baseline SBP in 8% (−29.6 ± 6.2 mmHg) and 28% (−15.0 ± 2.9 mmHg) of the dogs, respectively. None of these dogs experienced syncope or weakness. Age, gender, severity of heart disease, and current antihypertensive medication did not statistically affect the SBP drop.

In conclusion, OH can occur after RAR in geriatric toy-breed dogs, but it is uncommon to induce clinical signs.

C25

Increased N-terminal pro-B-type natriuretic peptide is associated with increasing concentrations of renal biomarkers over time

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Interplay between the cardiovascular and renal systems is increasingly recognized in both health and disease. The nature of this cardiorenal axis in disease in dogs and cats is still incompletely characterized and there is a need for more information about biomarkers to identify patients at risk for disease in the interlinked organ systems. The n-terminal pro-B-type natriuretic peptide (NT-proBNP) is a biomarker for identifying cardiomyocyte and wall stretch, heart failure, and hypertrophic cardiomyopathy in small-breed dogs and cats. Symmetric dimethylarginine (SDMA) and creatinine (Cr) are biomarkers of loss of renal excretory function. There have been conflicting reports about whether there is a relationship between these biomarkers in cardiorenal disease. We hypothesized that there is a relationship between increased NT-proBNP (>150 pmol/dL for cats and >900 pmol/dL for dogs) and trends in SDMA and Cr over time. Using a large retrospective dataset of dogs and cats with at least 3 SDMA and Cr concentrations over 24 months, we evaluated trends in SDMA and Cr for three groups: 1) patients with an increased NT-proBNP concentration (2,627 dogs and 6,171 cats); 2) patients with a NT-proBNP within the reference interval (3,905 dogs and 15,012 cats); and 3) patients without a NT-proBNP (90,487 dogs and 103,659 cats). For animals with NT-proBNP measurements the 24-month study period started with that measurement. Dogs were only included if they were breeds classified as "small" or "toy" breeds by the American Kennel Club and not Italian Greyhounds or Cavalier King Charles Spaniels. Animals were weighted by

boosted logistic regression models to balance patient characteristics between the three groups. Increased SDMA and Cr were. SDMA was more commonly increased than Cr: increased SDMA (>14mg/dL) was found in < 25% of dogs and approximately 33% of cats. Dogs with increased NT-proBNP had significantly higher mean SDMA and Cr at time of NT-proBNP testing ($p < 0.001$) than did dogs in comparison groups. Additionally, dogs and cats with increased NT-proBNP had greater increases in mean SDMA and Cr concentrations over the 24-month trending period (all $p < 0.001$) than comparison groups. These data indicate there is a relationship between increased NT-proBNP and SDMA/Cr in this large population and that patients with increased NT-proBNP may be at higher risk of declining renal excretory function. While NT-proBNP can be increased in patients with decreased renal excretory function, most patients were not azotemic when NT-proBNP was measured. Further study into the relationship between these markers and into the ability of these markers to predict or identify patients at risk for cardiorenal disease is needed.

C26

Prevalence of pacemaker-lead-induced thrombosis in dogs diagnosed on echocardiography following transvenous cardiac pacing

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Pacemaker implantation is the treatment of choice for patients with clinically relevant bradyarrhythmias. Pacemaker-lead-induced thrombosis (PLIT) occurs in 30-45% of human patients after implantation of transvenous pacemakers, with serious thrombo-embolic complications reported in 0.6-3.5%. The prevalence of PLIT in veterinary patients has not been documented.

This was a retrospective multicenter study. Objectives were to describe the prevalence, treatment and outcome in dogs diagnosed with PLIT.

606 dogs at 7 centers had a pacemaker implanted between 2012 and 2019. 528/606 (87.1%) dogs had a transvenous pacemaker placed, with 260/528 (49.2%) having at least one follow-up echocardiogram. 27/260 (10.4%) patients were diagnosed with PLIT at 175 (6-1853) days post implantation. Patients with PLIT were more likely to have proteinuria at the time of pacemaker implantation (25.9% with PLIT vs 9.4% without PLIT, $p = 0.010$). 10/12 (83.3%) patients in which urine protein:creatinine ratio was measured at the point of thrombus diagnosis were proteinuric. 22/27 (81.5%) patients were treated with anti-thrombotics. Follow-up echocardiography was performed in 15/27 (55.6%) patients and the thrombus resolved in 9/15 (60.0%) patients. 10/27 (37.0%) had complications due to the thrombus. 15/606 (2.5%) dogs had a suspected pulmonary thromboembolism, of which 7/15 (46.7%) had PLIT identified on echocardiography. Patients with PLIT had a shorter median survival time from implantation compared to those without PLIT (677 (9-1988) days vs 912 (0-2661) days, $p = 0.021$)

PLIT is a common complication of transvenous lead implantation, is potentially associated with proteinuria, causes complications in a high proportion of cases and reduces overall survival times.

C27

Population pharmacokinetics of oral Pimobendan and its metabolite in dogs with myxomatous mitral valve degeneration

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Pimobendan is an important therapy in canine myxomatous mitral valve degeneration (MMVD). The goal of this study was to determine the pharmacokinetics of oral pimobendan and its active metabolite O-desmethyl-pimobendan (ODMP) in a population of dogs with naturally occurring MMVD of various stages.

Fifty-four client-owned dogs with MMVD ACVIM Stage B2, C, or D on steady state pimobendan were prospectively enrolled. Blood samples were collected at predetermined times using a sparse-sampling protocol at intervals from zero minutes to 12 hours post-pimobendan administration. Plasma pimobendan and ODMP concentrations were determined via high-pressure liquid chromatography and fluorescence detection. A population pharmacokinetic approach and nonlinear mixed effects modeling were used to analyze the data.

The half-life (T½) of elimination was approximately 1, and 1.4 hours for pimobendan and the metabolite, respectively. The T½ was highly variable for pimobendan with a coefficient of variation of 59.1% compared to 11.9% for the metabolite. The peak concentration was 32.7 and 29.3 ng/mL, for pimobendan and the metabolite, respectively. The area-under-the-curve and peak concentration were similar for pimobendan and the metabolite with ratios of 1.1 and 0.9, respectively.

Although the concentrations and pharmacokinetic parameters were highly variable among dogs, the stage of MMVD (B2, C, D) was not a significant source of variability in the parameters. Additional covariates will be explored to determine if they contribute to the source of variability which may lead to a better understanding of optimal pimobendan dosing in the management of canine MMVD.

C28

Interventricular inflow time difference assessed by echocardiography in dogs with myxomatous mitral valve disease

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In healthy humans, tricuspid valve opening (TO) precedes mitral valve opening (MO). The interventricular inflow time difference (IVID) is a time interval between MO and TO, which reflects both LV and RV hemodynamics and the recent human study has revealed that the precedence of MO (MOP) was associated with adverse outcome in heart failure patients. There is limited research in veterinary medicine on IVID in dogs. The purpose of this prospective cohort study was to investigate the characteristics of IVID and MOP in dogs with myxomatous mitral valve disease (MMVD).

Forty-seven dogs without heart disease and ninety dogs with MMVD were prospectively enrolled. Transmitral and transtricuspid flow were simultaneously recorded using dual pulsed-wave Doppler, and IVID was calculated by subtracting TO from MO. Dogs were divided into 2 groups (TOP or MOP) based on the precedence of TO or MO. Multivariate analysis was used to determine an independent predictor of the MOP group.

Almost all dogs (98%) without heart disease were subdivided into TOP group. Dogs in the MOP group had a higher transmitral early diastolic inflow velocity (E-wave), impaired RV Tei index, a longer TO, and a shorter MO. Multivariable analysis showed E-wave (odds ratio, 1.69; 95% confidence interval, 1.27-2.43) and RV Tei index (odds ratio, 1.75; 95% confidence interval, 1.17-2.81) were independent predictors of the MOP group.

Impaired LV diastolic function and RV dysfunction were associated with MOP group due to delayed TO and earlier MO in dogs with MMVD.

C29

Comparison of a wireless patch ambulatory ECG monitor to standard holter monitor in six dogs

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Wireless ambulatory 24-hour ECG monitoring devices (patches) are increasing in availability and are attractive for veterinary use. Advantages include ease of application, light in weight and the small footprint increases the range of patients on which it can be applied. The purpose of this study was to compare the Carnation Ambulatory Monitor (CAM, Bardy Dx) to the standard Holter monitor (Forest Medical, LLC) recording for accuracy in analyzing abnormal ECG rhythms in dogs. Seven dogs were identified with arrhythmias in which a 24-hour monitoring device was recommended. The CAM device was applied simultaneously with the Holter monitoring device. The CAM device was analyzed by a company technician experienced in reading canine recordings. The Holter monitor was analyzed by an experienced veterinary technician. One dog was eliminated from comparison due to extensive artifact encountered by the CAM device. In the remaining six dogs, the agreement between the CAM and the Holter recording for arrhythmia detection was overall poor. The mean agreement between the 2 recordings for ventricular arrhythmias was 38 % (range 6-98 %) and for supraventricular arrhythmias was 36 % (range 1-96 %). Common inaccuracies that occurred with the CAM recording were: sinus tachycardia recorded as supraventricular tachycardia, normal sinus arrhythmia recorded as atrial premature contractions, lack of detection of ventricular bigeminal rhythms, and ventricular ectopy recorded as supraventricular ectopy. The CAM tended to record more artifact with high heart rates; the dog that was eliminated from study was a patient with atrial fibrillation with an average heart rate > 200 bpm. The wireless CAM patch monitor was not considered an accurate tool for the assessment of arrhythmias in dogs given its lack of agreement with the standard Holter monitor and increased artifact recording.

C30

Hypoglycemia after cardiac surgery is disassociated with insulin and glucagon in small breed dogs

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Hyperglycemia due to stress reactions commonly occurs after cardiac surgery in human medicine. Conversely, we occasionally encounter cases of hypoglycemia after cardiac surgery with cardiopulmonary bypass. The purpose of this study was to investigate the incidence, predictors, and relationship between serum insulin and plasma glucagon of hypoglycemia in dogs undergoing mitral valve repair.

This prospective study included 109 dogs that underwent mitral valve repair. For the predictors, multivariate analysis was performed using variables such as age, weight, sex, body condition score, muscle condition score, cardiopulmonary time, cardiac arrest time, blood transfusion, and opioid use. Blood samples were acquired four times: before and during surgery and 6–8 and 16–20 hours after surgery. Serum insulin concentrations were measured using a chemiluminescent immunoassay method. Plasma glucagon concentrations were measured using a radio-immunoassay method.

The hypoglycemia incidence was 14.7% (16/109), and hypoglycemia significantly occurred in small dogs (median, 2.50 [range: 1.88–5.82] kg body weight; odds ratio = 0.334, 95% confidence interval 0.165–0.675, P = 0.002). There were significant differences between dogs with hypoglycemia (median, insulin; 0.27 [interquartile range: 0.25–0.32] ng/mL, glucagon; 587 [417–822] pg/mL) and normal dogs (median, insulin; 0.47 [0.35–0.67] ng/mL, glucagon; 363 [305–488] pg/mL) (P < 0.001 and P < 0.003, respectively).

Our findings suggest that low body weight is a useful predictor of postoperative hypoglycemia. Unexpectedly, low insulin and high glucagon concentrations were observed in dogs with hypoglycemia. Cardiopulmonary bypass and/or mitral surgery may affect glucose metabolism in dogs with low body weight.

C31

Levels of plasma microRNA and ribonuclease activity in cats with primary or secondary myocardial hypertrophy

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Hypertrophic cardiomyopathy is the most common heart disease in cats. It has specific phenotypical markers, such as concentric hypertrophy of the left ventricle, dynamic obstruction of the left ventricle outflow tract, left atrial enlargement, diastolic dysfunction, myocardial fibrosis. Like in humans it is genetically determined. But there are, like in human medicine, several diseases that can mimic this phenotype: hyperthyroidism, arterial hypertension, the chronic renal disease that can share the same phenotypical markers of myocardial hypertrophy. At a certain stage, it could be a problem in the differentiation of primary and secondary hypertrophy. In this study, we analyze the clinical utility of muscle-associated miR1, miR133, and miR208 and fibrosis-associated miR21 and miR223 as circulating biomarkers of myocardial hypertrophy.

This study included 46 cats, divided into 3 groups: control healthy cats without markers of myocardial hypertrophy (HC, n=20); cats with diagnosed hypertrophic cardiomyopathy (HCM, n=11); cats with the markers of myocardial hypertrophy secondary to chronic renal disease (CKD, n=15). Diagnostic criteria included: clinical signs, arterial pressure measurements, echocardiography, electrocardiography, clinical and biochemical blood analyses, serum Troponin I evaluation. Plasma samples we obtained for miRNA analysis and ribonuclease activity evaluation. Levels of circulating in plasma miR1, miR21, miR133, miR208, and miR223 were analyzed by RT-qPCR. Ribonuclease activity in plasma was evaluated by analysis of the degradation of spiked-in exogenous RNA.

Data analysis

The nonparametric Mann-Whitney test was used to compare the quantification cycle (Cq) between two groups. Two-sided P values less than 0.05 were considered statistically significant. GraphPad Prism 8.00 (GraphPad Software Inc., La Jolla, CA, USA) was used to perform statistical analysis and visualizations. Differences between groups were deemed statistically significant if p-value < 0.05. The relative levels of gene expression (RQ) in each sample were calculated as RQ = 2^(Cq max – Cq sample).

In the CKD group, a significant decrease of miR1 level and an increase of miR223 level (P < 0.01, for both) in comparison to healthy cats were observed. Levels of miR1, miR21, miR133, miR208, miR223 remain unchanged between groups of primary and secondary myocardial hypertrophy. Ribonuclease activity of plasma in cats with CKD was more than 10 times higher (P < 0.01) compared to both healthy cats and cats with HCM. There was no difference in plasma ribonuclease activity between HCM and healthy cats.

Investigated muscle- and fibrosis-associated miRs cannot be used as plasma biomarkers to differentiate primary and secondary myocardial hypertrophy. CKD was shown to be associated with increased plasma ribonuclease activity. However, changes in plasma ribonuclease activity are unlikely to affect circulating plasma miRs levels, since mature miRs are believed to be protected from degradation by protein complexes.

C32

Long-term effects of mandatory breeding restrictions against mitral valve disease in Cavalier King Charles Spaniels

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Effects of breeding restrictions against myxomatous mitral valve disease (MMVD) in Cavalier King Charles Spaniels (CKCS) in Denmark have already been reported after 8-10 years of breeding using a mandatory programme based on auscultation and echocardiography, and open access to cardiac findings. The aim of the present study was to evaluate the effects after additional 8-9 years of breeding, and to evaluate the effects in a parallel Danish CKCS population where findings were only accessible for the individual dog owner. Cardiac certificates were reviewed from 2460 CKCS examined from 2002-2019 (open programme) and 848 CKCS examined from 2007-2019 (closed programme). In the open programme, odds of having mitral regurgitation (MR)-murmur were significantly reduced in dogs examined in 2018-2019 (n = 226) compared with dogs examined in 2010-2011 (n = 327) (odds ratio (OR) 0.40 (0.18-0.84 (95% confidence interval); $P = 0.019$)). Odds of having moderate to severe mitral valve prolapse (MVP > 1) did not differ between the periods. Comparing dogs examined in 2018-2019 with dogs examined in 2002-2003 (n = 215), odds for having MR-murmur (OR 0.21 (0.09-0.42); $P =< 0.0001$) and MVP > 1 (OR 0.64 (0.42-0.97); $P = 0.037$) were both significantly reduced. In the closed programme, odds were reduced for having MVP > 1 (OR 0.56 (0.31-0.99); $P = 0.049$) in 2017-2019 (n = 122) compared to 2007-2009 (n = 135), no difference in odds was found regarding MR-murmur ($P = 0.36$). In conclusion, mandatory breeding restrictions already running for 8-10 years against MMVD in CKCS had additional effect after 8-9 years of further breeding. Open access to cardiac results may improve effects of mandatory breeding restrictions.

C33

Prognostic value of left ventricular-arterial coupling estimated using echocardiography in canine myxomatous mitral valve disease

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The interaction between left ventricle (LV) and systemic arterial system, left ventricular-arterial coupling (VAC), has been evaluated based on the effective arterial elastance (Ea) to LV end-systolic elastance

(Ees) ratio (Ea/Ees). The Ea is an index of total arterial load of LV, while Ees is an index of LV systolic function. A recent study found that inappropriate VAC based on increased Ea/Ees estimated using echocardiography is associated with advanced disease severity in dogs with myxomatous mitral valve disease (MMVD). This prospective cohort study investigated the prognostic value of VAC assessed by echocardiographic estimation of Ea/Ees in dogs with MMVD.

Eighty-nine dogs with MMVD underwent baseline echocardiographic examinations. The Ea was echocardiographically estimated using the formula: mean blood pressure/(forward stroke volume/body weight). The Ees was echocardiographically estimated using the formula: mean blood pressure/(LV end-systolic volume/body weight). The Ea/Ees was then calculated.

The Ea, Ees, and Ea/Ees were predictors of cardiac-related death in univariate Cox proportional hazard analysis. Multivariate Cox proportional hazard analysis showed that Ea/Ees (hazard ratio [HR], 1.46 per 0.1-unit increase; 95% confidence interval [CI], 1.25-1.71), body weight (HR, 0.69 per 1-kg increase; 95% CI, 0.54-0.88), peak systolic mitral annular velocity (HR, 1.21 per 1-cm/s increase; 95% CI, 1.03-1.43), and the peak early diastolic transmitral velocity to peak early diastolic mitral annular velocity ratio (HR, 1.15 per 1-unit increase; 95% CI, 1.02-1.30) were independent predictors of cardiac-related death among echocardiographic indices.

The assessment of VAC based on echocardiographically-estimated Ea/Ees provides useful prognostic information in dogs with MMVD.

C34

Evaluation of owner medication adherence for management of cardiovascular disease in small animal practice

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A prospective questionnaire-based study was used to evaluate owners' medication adherence for management of cardiovascular disease in the small animal specialty setting. Owners completed a written survey evaluating medication adherence, and difficulties encountered for medication adherence. Owners were free to decline participation in the study. One hundred questionnaires were completed during a 3-month period at a small animal specialty hospital.

Fifty-seven respondents (57%) reported treating their pet for over a year for cardiovascular disease and myxomatous mitral valve disease was the most common diagnosis (73%). Thirty-one respondents felt they could consistently administer at most 3 medications daily and 50 respondents reported twice daily dosing was the highest frequency of medications they could consistently administer. Twenty-four respondents (24%) reported their pet required daily medications for at least one other condition besides cardiac disease. Twenty-one

Table 1: Characteristics of Cardiac Medications Administered

Where do you get your pets medications? (Select all that applies)	
From Investigators' Hospital	47 (47%)
From my local veterinarian	29 (29%
From an online pharmacy	44 (44%)
From a local human pharmacy	29 (29%
From a compounding Pharmacy	14 (14%)
How many different places do you get your pet's medications?	
One	45 (45%)
Two	43 (43%)
Three	9 (9%)
Four	3 (3%)
Five or More	0 (0%)
How many people consistently medicate your pet?	
One	54 (54%)
Two	44 (44%)
Three	0 (0%)
Four or More	2 (2%)
What heart medications is your pet currently on? (Choose all that Apply)	
Furosemide or Torsemide	61 (61%)
Pimobendan	88 (88%)
Ace-inhibitor (enalapril or benazepril)	42 (42%)
Spironolactone	15 (15%)
Potassium gluconate	2 (2%)
Atenolol or sotalol or other beta-blocker	8 (8%)
Sildenafil	13 (13 %)
Taurine	1 (1%)
Mexiletine	1 (1%)
Diltiazem	1 (1%)
Which heart medication do you feel is the hardest to administer correctly	
Furosemide or Torsemide	9 (9%)
Pimobendan	30 (30%)
Ace-inhibitor (enalapril or benazepril)	1 (1%)
Potassium gluconate	1 (1%)
Atenolol or Sotalol or other beta-blocker	0 (0%)
Spironolactone	0 (0%)
Diltiazem	2 (2%)
Sildenafil	1 (1%)
All Medications	2 (2%)
None	54 (54%)
What is the primary reason the medication listed above is the most difficult to administer consistently to your pet?	
Number of Pills	5 (5%)
Frequency of pills	3 (3 %)
Size of pills	19 (19%)

(Continued)

Difficulty breaking up the medications	4 (4%)
Taste of medication	12 (12%)
None	1. (54%)
Pet Hates process	1 (1%)
Pet Will Not Take pills	2 (2%)
How do you administer the medications to your pet? (Select all that apply)	
Wrapped in a treat	56 (56%)
Hidden in their food	41 (41%)
Manually by hand	22 (22%)
Manually by use of a device	3 (3%)
Other	10 (10%)
Crushed	5 (5%)
Gel Capsule	4 (4%)
Syringe	1 (1%)
How many different methods administering the medication did owners report?	
One	71 (71%)
Two	24 (24)
Three	5 (5%)
Four	0 (0%)
Are you able to administer the medication to yourself or do you required a second person to give the medications to your pet?	
I am able to medicate my pet by myself	100 (100%)
I require a second person to administer the medications	0 (0%)
Have you even been injured attempting to medicate your pet?	
Yes	5 (5%)
No	95 (95%)
If you have been injured attempting to medication your pet, did it require formal medical attention (i.e., seeing your doctor, urgent care, emergency care, etc.)	
Yes	0 (0%)
No	5 (5%)
Not applicable (I have not been injured by my pet)	95 (95%)
How many different heart medications is your pet currently receiving per day?	
One	24 (24%)
Two	32 (32%)
Three	27 (27%)
Four	13 (13%)
Five or More	4 (4%)
How many different medications do you feel you can consistently administer to your pet daily?	
One	7 (7%)
Two	7 (7%)
Three	31 (31%)

(Continues)

(Continued)

Four	14 (14%)
Five or More	41 (41%)
What is the highest frequency of medications you feel you can consistently administer to your pet daily?	
Once daily	3 (3%)
Twice daily	50 (50%)
Three times a daily	27 (27%)
Four times a day or more	20 (20%)

Table 2: Issues with Medication Adherence

Is your pet currently being chronically medicated for any other disease that requires at least once a day dosing of medications?	
Yes	24 (24%)
No	76 (76%)
How many additional chronic conditions (besides heart disease) does your pet have that requires chronic daily medications	
None	76 (71%)
One	23 (28%)
Two	1 (1%)
Three	0 (0%)
Four or more	0 (0%)
What percentage of medications dose your pet miss per week?	
I never miss any medications	79 (79%)
< 10 % of medications	16 (16%)
10-20% of medications	4 (4%)
20-30 % of medications	1 (1%)
> 30% of medications	0 (0%)
Has missing medications negatively affected your pet?	
Yes	7 (7%)
No	14 (14%)
Not applicable (never missed medications)	79 (79%)
Have missing medications resulted in your pet needing to be re-examined by your veterinarian or cardiologist sooner than was expected/planned?	
Yes	2 (2%)
No	19 (19%)
Not applicable (never missed medications)	79 (79%)
Have missing medications ever resulted in hospitalization	
Yes	1 (1%)
No	20 (20%)
Not applicable (never missed medications)	79 (79%)
Have you ever overdosed your pet?	
Yes	8 (8%)
No	92 (92%)

(Continues)

(Continued)

Did over-dosing your pet require re-examination by your veterinarian or cardiologist sooner than was expected?	
Yes	0 (0%)
No	8 (8%)
Not applicable (never over-dosed my pet)	92 (92%)
Did over-dosing your pet require hospitalization by your veterinarian or cardiologist sooner than was expected?	
Yes	0 (0%)
No	8 (8%)
Not applicable (never-overdosed my pet)	92 (92%)
Do you have at least one other pet at home that requires chronic medical management (i.e., medications being administered daily for at least 1 month or longer – does NOT need to be heart disease)?	
Yes	24 (24%)
No	76 (76%)
Have you researched your pet's medications to get more information regarding the efficacy, safety, etc.?	
Yes	71 (71%)
No	29 (29%)
How did you research your pet's medications? Multiple answers were allowed	
Online search engine	66 (66%)
Facebook or other Social Media	4 (4%)
Veterinary School Websites	14 (14%)
Relevant Veterinary Literature	16 (16%)
Package Insert of the medication	19 (19%)
Other	4 (4%)
• Other veterinarian	3 (3%)
• RVT	1 (1%)
• Other Website	1 (1%)
I have not researched	29 (29%)
Have you ever modified your pet's medications on your own based on your research without informing your veterinarian?	
Yes	4 (4%)
No	96 (96%)

respondents (21%) reported at least occasionally missing medications although only 5% reported that it was more than 10% of medications. Medications adherence was high in this study population with 79% reported never missing medications. Almost one third (31%) of owners felt they could at most administer 3 medications per day and approximately one quarter of patients (24%) were treated for at least one chronic condition besides cardiac disease requiring daily medications. Clinicians should be aware of these factors when determining optimal treatment protocols for management of cardiovascular disease in dogs and cats.

C36

Prospective cardiac evaluation in 50 asymptomatic dogs naturally-infected with *Trypanosoma cruzi*

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The protozoal parasite, *Trypanosoma cruzi*, causes myocarditis and cardiac morbidity in dogs. The objective of this study was to perform cardiac evaluations in naturally-infected, asymptomatic dogs.

Fifty client-owned dogs with prior positive *T. cruzi* serology were prospectively enrolled. Diagnostics at enrollment included tests for *T. cruzi* (immunofluorescent antibody (IFA), 2 rapid tests (InBios, StatPak), PCR), SNAP 4DX (IDEXX), cardiac troponin I (cTnI), ECG (5-minute 6-lead, Holter), and comprehensive echocardiogram. Descriptive statistics were performed; normally distributed data are reported as mean±SD.

Dogs were 5.2±3.4 years-old and weighed 21.9±9.4kg. Median IFA titer was 1:640 (range, 1:20-1:40,960). Six dogs with prior positive IFA were IFA negative (5 were positive on 2 rapid tests, 1 was positive on 1 rapid test). 14/49 had positive blood PCR results, suggesting acute or re-infection. 2/50 were positive for other infectious diseases (*Dirofilaria immitis*, *Ehrlichia spp*). Median cTnI was 0.12ng/ml (range, 0.007-5.63). The most common abnormalities on 5-minute ECG included ventricular arrhythmias (n=6, modified Lown score 1), first-degree atrioventricular block (n=5), bundle branch block (n=4); on Holter included ventricular arrhythmias (n=43; modified Lown score 1 in 26, >1 in 17), supraventricular arrhythmias (n=20), second-degree atrioventricular block (n=11), sinus arrest >4 seconds (n=8). Left and right ventricular systolic and diastolic dysfunction and chamber enlargement were identified in a subset of dogs. Select echocardiographic variables: left ventricular internal dimension in diastole and systole normalized to body weight (1.58±0.19 and 1.07±0.19, respectively), fractional shortening (28.3±6.7%), ejection fraction (62.4 ±11.4%), mitral E/A (1.18±0.27), left atrium to aorta ratio short axis (1.31±0.18), tricuspid annular plane systolic excursion indexed to body weight (4.55±0.85mm/kg$^{0.297}$), tricuspid E/A (1.27±0.30). All dogs had an abnormality detected on at least 1 test (ECG, echocardiography, cTnI).

Abnormal cardiac test results were common and variable in this asymptomatic group of dogs with *T. cruzi* infection. Longitudinal, preventative and treatment studies are needed.

C37

Computed tomography in dogs with decompensated and compensated right heart disease compared to normal dogs

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The computed tomographic (CT) findings of right heart disease (RHD) in dogs are poorly characterized. CT studies of 27 dogs were analyzed: 12 with right-sided congestive heart failure (R-CHF), 10 with compensated RHD, and 5 normals. The cross-sectional area (CSA) and effective diameter of the caudal (CaVC) and cranial (CrVC) caval veins plus 3 major hepatic veins (HV) were measured, as was right atrial volume (RAVol). Linear measurements were normalized to height of the 4th thoracic vertebral body (T4), CSA was normalized to body surface area, and RAvol was normalized to body weight.

Ratios of CaVC diameter to T4 height at a point equidistant between the cavoatrial junction and first HV were 1.5, 1.3, and 1.0 in R-CHF, compensated RHD, and normal dogs respectively. The CSA of the CaVC and HVs were larger in dogs with R-CHF compared to normal and to dogs with compensated RHD (all $P < 0.02$). Neither the CrVC diameter to T4 ratio nor its CSA were different between groups, when measured at an equidistant point between cavoatrial junction and costocervical vein. Average RAvol was significantly different between groups ($P = 0.0002$) with R-CHF median 9.2 ml/kg, compensated RHD 2.2 ml/kg, and normal dogs 1.1 ml/kg. Multiple qualitative findings of congestive hepatopathy were noted in all R-CHF dogs, 4 of 10 dogs with compensated RHD, but none of the normal dogs. This study demonstrates the utility of CT to detect and quantify changes in the right atrium, caval veins, HVs, and hepatic parenchyma in canine RHD.

C38

Frequency of arrhythmias detected in patients using a computer aided electrocardiogram

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Arrhythmias may increase morbidity and mortality in both dog and cat patients. This study aimed to describe the occurrence of arrhythmias in dog and cat patients from general practice settings. A total of 57,950 dog and 10,638 cat electrocardiograms were reviewed by board certified cardiologists. Exclusion criteria included treatment with antiarrhythmic drugs, atropine, sedation (including alpha-2 agonists), missing weight, sex or age. Each breed had to have at least 30 cases to be included in the study. A total of 53,708 dog electrocardiograms were included from 108 different dog breeds and 9,440 electrocardiograms were included from 16 different cat breeds. Two Firth's logistic regression models (one for each species) was performed with arrhythmia (present or absent) as the dependent variable and breed, age, weight (for dogs), and sex as independent variables. Due to the high number of comparisons, the false discovery rate (FDR) method was used to adjust p-values for multiplicity. The overall proportion of arrhythmias was 3.27% (1758/53,708) for dogs and 2.64% (249/9440) for cats. Senior dogs had higher odds of having an arrhythmia compared to adult dogs (aged 8 to 11 years, OR: 1.93, 95% CI: 1.67 - 2.22, $P < 0.001$). Both juvenile (aged < 2 years, OR: 0.80, 95% CI: 0.66 - 0.98, $P < 0.027$) and geriatric (aged 11+ years, OR: 0.79, 95% CI: 0.70 - 0.89, $P < 0.001$) dogs had lower odds of having an arrhythmia compared to adult dogs. Geriatric (aged 14+ years, OR: 1.75, 95% CI: 1.13 - 2.74, $P < 0.012$) and senior (aged 9 to 14 years, OR: 1.85, 95% CI: 1.23 - 2.84, $P < 0.003$) cats had higher odds of having an arrhythmia compared to adult cats. For dog breeds, Whippets (OR: 5.31, 95% CI: 2.07 - 12.34, $P < 0.039$), King Charles Spaniels (OR: 4.37, 95% CI: 1.80 – 9.72, $P < 0.045$), Cavalier King Charles Spaniel (OR: 2.58, 95% CI: 1.41 - 4.72, $P < 0.045$), American Cocker Spaniels (OR: 3.46, 95% CI: 2.08 - 5.95, $P < 0.001$), and Boxers (OR: 2.93, 95% CI: 1.85 - 4.85, $P < 0.001$) had the highest odds of having an arrhythmia. For cat breeds the Ragdoll (OR: 4.19, 95% CI: 1.76 - 8.69, $P < 0.036$) had the highest odds of having an arrhythmia. The top three arrhythmias identified were ventricular premature complexes (Dogs: 1.38% (740/53708); Cats: 1.63%, (154/9440)), supraventricular premature complexes (Dogs: 0.71% (380/53708); Cats: 0.35% (33/9440)) and first-degree AV block (Dogs: 0.63% (337/53708); Cats: 0.06% (6/9440)). These findings demonstrated the value of including electrocardiograms in screening protocols for dog and cat patients, especially at-risk age brackets and breeds, to create suitable anesthetic protocols and guide further diagnostics and treatment.

C39

Effects of single versus dual-agent antithrombotic therapy on feline platelet function

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Cardiogenic arterial thromboembolism (CATE) is a devastating outcome in cats with hypertrophic cardiomyopathy. The use of dual-agent therapy with clopidogrel and rivaroxaban may provide superior protection against thromboembolism or further delay the recurrence of CATE when compared to either drug alone. This study aimed to evaluate the safety and efficacy of dual-agent treatment with rivaroxaban and clopidogrel (R+C) in comparison to either rivaroxaban or clopidogrel alone. We hypothesized that combined clopidogrel and rivaroxaban treatment would further reduce platelet function when compared to either rivaroxaban or clopidogrel in cats. Platelet function by P-selectin expression using flow cytometry in the presence or absence of adenosine diphosphate (ADP) and thrombin and ADP-induced platelet aggregometry was assessed in 9 healthy cats before and after each treatment. Suppression of P-selectin expression was calculated as fold change (FC).

Adverse effects were not noted in any cats throughout the study period. Rivaroxaban alone lowered P-selectin in resting platelets ($FC = -0.53 \pm 0.31$) compared to clopidogrel (-0.19 ± 0.18, $p=0.0060$) and R+C (-0.066 ± 0.098, $p=0.0003$) but augmented platelet responsiveness to ADP ($FC=0.088 \pm 0.13$). R+C treatment increased ADP

response compared to clopidogrel alone (-0.25 ± 0.17 vs -0.48 ± 0.18, p=0.006). All 3 treatments modulated thrombin-mediated P-selectin expression (p=0.95). Rivaroxaban did not inhibit ADP-induced platelet aggregation (AUC: 100.7 ± 44.46 vs 120.3 ± 24.36, p=0.28) while R+C did not further inhibit aggregation compared to clopidogrel alone (p > 0.99).

Rivaroxaban indirectly inhibits platelet activation in resting platelets but potentiates platelet response to ADP. Addition of clopidogrel to rivaroxaban may represent a superior antithrombotic therapy compared to rivaroxaban alone.

C40

Whole genome sequencing identifies a large structural variant associated with pulmonary valve stenosis in bulldogs

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Pulmonary valve stenosis (PS) is a common congenital heart disorder in dogs. Bulldogs have a high predisposition and documented PS heritability. The purpose of this study was to identify a genetic mutation(s) associated with PS in Bulldogs.

All Bulldogs were client-owned and had previously received a full echocardiographic examination by a board-certified cardiologist. High quality DNA on six unaffected Bulldogs (normal pulmonary outflow tract velocity and morphology) and twelve severely affected Bulldogs (pulmonary outflow tract velocity > 4.5m/s) were submitted for whole genome sequencing at 30x coverage. An established bioinformatic pipeline generated variant call files. Variants were scored based on segregation with phenotype, localization in a previously identified genome wide association region, mode of inheritance, and predicted effect on protein function. The top variants were submitted for MassArray analysis to analyze segregation in a larger cohort of phenotyped Bulldogs (60 cases and 53 controls). Variants found to be statistically significant were reviewed for expression in the heart and biological function. One million base pairs flanking each statistically significant variant were analyzed manually using the Integrated Genome Viewer to identify structural variants if present.

A large 24kb structural variant that segregated cases and controls was identified on chromosome 1. This variant disrupts ZNF446, a transcription factor involved in embryonic heart development. This finding should prompt further functional research into the possible role of ZNF446 and heart development in Bulldogs. It may also lead to the development of a genetic test to reduce the prevalence of this disease in Bulldogs.

C41

Prospective evaluation of complications associated with transesophageal echocardiography in dogs with congenital heart disease

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Transesophageal echocardiography (TEE) is an imaging modality in canine cardiology. Complications with TEE occur at a low rate in humans but have not been prospectively evaluated in dogs. The objective of this study was to prospectively evaluate the safety of TEE and report complications.

Forty client-owned dogs with confirmed congenital heart disease were enrolled. Thirty dogs weighing ≥ 4kg had TEE performed with a 6VT-D probe (GE) and 10 dogs < 4kg had TEE performed with two probes in sequence; a 10T-D microprobe (GE) followed by an intracardiac echocardiography catheter-based probe. Direct visualization of the oropharyngeal region and esophagus by endoscopy was performed before and immediately after each TEE study to score gross changes to esophageal mucosa. The times for probe within the esophagus, active imaging, and imaging in three-dimensions were recorded. Following anesthetic recovery, dogs were monitored for signs of nausea until the morning after TEE.

Esophageal mucosal abnormalities were present in 4 dogs at baseline and in 8 following TEE (4 unchanged from baseline; 4 with mild post-TEE hyperemia involving < 25% of lower esophageal sphincter circumference. Median (range) times for TEE were: 27 minutes (10-141) for the probe within the esophagus, 24.5 minutes (10-102) active imaging, 2.5 minutes (0-8) three-dimensional imaging. The 6VT-D probe obstructed fluoroscopic views in 3/30 dogs. Four dogs had evidence of nausea documented post-procedure.

Complications encountered were mild and included esophageal mucosal hyperemia (4/40), post-procedural nausea (4/40) and probe interference (3/30). An awareness of imaging time, probe manipulation and patient monitoring are recommended when using TEE.

C42

Prospective evaluation of probes for transesophageal echocardiography in small dogs: Imaging capabilities, image quality, usability

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Patient size is a limiting factor for transesophageal echocardiography (TEE) probe placement and image acquisition for interventional management of canine heart disease. The objective of this study was to evaluate the GE 10T-D microprobe and intracardiac echocardiography (ICE) catheter-based probe for TEE in small dogs to describe and compare imaging capabilities, ease of use, and image quality.

Ten client-owned dogs of various breeds weighing < 4kg with confirmed congenital heart disease were prospectively enrolled. Each dog

had TEE performed with both probes in alternating order. The ease of probe placement, ability to acquire specific diagnostic images including region of interest, and image quality were recorded for each probe.

Median weight was 2.4kg (range, 1.0-3.2). Congenital abnormalities included patent ductus arteriosus (n=9) and pulmonic stenosis (n=1). The 10T-D microprobe has anteflexion and retroflexion capabilities but lacks lateral motion and was not difficult (easy, n=8) to mildly difficult (achievable with manipulation, n=2) to place. A complete imaging study (8/10) and images of the defect of interest (9/10) was possible. Diagnostic image quality was scored good (3/10) to excellent (7/10). The ICE probe was difficult to place without external support, was difficult to operate, and was rarely able to acquire a full sequence of standardized diagnostic images.

While the ICE catheter-based probe was not useful as a TEE probe, the 10T-D microprobe was able to provide good to excellent image quality in the majority of dogs < 4kg; however, the lack of lateral motion of the probe head can diminish its utility in some dogs.

C43

Presence of previous left atrial rupture scar in dogs undergoing mitral valve repair surgery

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In myxomatous mitral valve disease (MMVD), left atrial rupture (LAR) is rarely reported. LAR scar was macroscopically examined during mitral valve repair (MVR) and dogs with an evidence of LAR were reviewed. The objective of this study is to evaluate the prognosis, and pre-operative clinical signs and echocardiographic findings.

We retrospectively reviewed dogs with MVR performed between September 2014 to December 2020. Data collected from the medical records included signalment, clinical history, radiographic and echocardiographic findings and long-term outcome. The surgical findings were also recorded.

The 1309 dogs underwent MVR during the investigation period. 16 of them showed presence of a previous LAR scar (Incidence rate 1.22%). Preoperative syncope was observed in nine dogs. Severe left atrial enlargement and increase in left atrial pressure were identified in all dogs. The median left atrial / aortic ratio and E wave were 2.43 and 1.32 (m/s). Pericardiac effusion was identified in the 13 dogs before MVR. 93.7% survival rate was observed at two year after MVR. One dog died from perioperative complication. LAR is uncommon, accounting for only 1.22% of all cases of MVR performed to our center within the time-period studied.

The elevated intra-atrial pressure and severe atrial enlargement is thought to have contributed to the rupture. LAR should be considered in dogs with MMVD if they are presented with fainting, syncope, collapse with an evidence of pericardial effusion. Furthermore, MVR is an effective treatment for dogs with previous rupture of the left atrium.

C44

Profiling of circulating miRNAs in canines with heart murmur and associated valvular cardiovascular disorder

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Circulating microRNAs (miRNAs) are increasingly recognized as important biomarkers of cardiovascular dysfunction including dilated cardiomyopathy and valvular heart disease. One of the characteristics of mitral valve disorder (MVD) in dogs is heart murmur. While the underlying causes of MVD are not clear, pathological changes in MVD include alterations in the extracellular matrix (ECM) and collagen, possibly as a result of the activation of matrix metalloproteinases (MMPs), leading to subsequent myocardial re-modeling. Inflammatory cytokines are also hypothesized to influence progression of the valve lesion contributing to valvular regurgitation. We assessed the circulating levels of miRNAs using Qiagen's miScript miRNA PCR array Dog Cardiovascular Disease, in the blood of dogs collected at the end-of-life. Data were analyzed using the DDCt method. In this retrospective study, five dogs had been clinically diagnosed as having at least grade 3 heart murmur when alive (2 dogs had grade 5/6, 2 had 4/6 and 1 had 3/6 heart murmur) and most were diagnosed as having MVD by a veterinarian. RNA was extracted from blood samples collected at the end-of-life from dogs with heart murmurs (n=5; 12.8-14.9 yr) and control dogs (n=6; 13.3-14.6 yr) with no known history of cardiovascular problems. There was a significant up-regulation in the levels of miRNAs *cfa*-miR-222 and *cfa*-miR-365 in dogs with heart disorders when compared to controls ($p < 0.05$). miR-222 has been demonstrated to inhibit TIMP-2 and TIMP-3, natural inhibitors of matrix metalloproteinases (MMPs), enabling MMPs to be up-regulated and act on ECM proteins. MMP-2, a target of TMP-2, plays an important role in the progression of MVD. In addition, miR-365 also negatively regulates TIMP-1 and TIMP-3, and TIMP3 reduction leads to poor cardiac remodeling. Other miRNAs that were also upregulated (> 2-fold increase) when compared to controls include miR-7, miR-18a, miR-19a, miR-21, miR-23a, miR-26a, miR-27a, miR-27b, miR-29a, miR-29b, miR-29c, miR-30e, miR-99a, miR-218, miR-223, and miR-499 (NS vs Con). In contrast, *cfa*-miR-494 was significantly down-regulated in dogs with cardiovascular problems when compared to controls ($p < 0.05$). Decreased levels of miR-494 can increase cytokine expression and inflammation, as reported in a macrophage cell line. In addition, c*fa*-miR-143 was also downregulated compared to controls (NS) and targets of miR-143 include tropomyosin-4 (TPM-4), a structural protein, and angiotensin-converting enzyme (ACE), a major regulator of contraction, and thus, a decreased level of mir-143 may contribute to heart dysfunction. Taken together, these results suggest that circulating miRNAs may serve as clinical biomarkers in dogs with cardiovascular valve disorders as well as in assessing its progression, and possibly as targets for nutritional intervention.

C45

The efficacy of isosorbide dinitrate in the treatment of congestive heart failure: A pilot study

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Preload reducers (i.e., nitroprusside) have been used to treat dogs with degenerative mitral valve disease (DMVD) in congestive heart failure (CHF) with anecdotal success. Now that nitroprusside is cost prohibitive in most cases, alternative preload reducers warrant exploration. Isosorbide dinitrate is a preload reducer for which pharmaco-kinetic and -dynamic research has been performed in dogs. The purpose of this study is to determine if the use of the preload reducer, isosorbide dinitrate, will improve heart failure more rapidly without significantly lowering blood pressure in patients with CHF in a prospective, randomized, double-blinded, placebo-controlled clinical trial. DMVD dogs in their first episode of CHF and requiring hospitalization were enrolled. Study dogs were randomized into a placebo group (n = 10) or a preload reducer group (n = 7) in which patients received isosorbide dinitrate 5-7 mg/kg PO q12. The outcome parameters time in oxygen and hospital, indirect blood pressure, fraction of inspired oxygen (FiO2), and total furosemide administered were measured at 0, 6, 12, and 24 hours of hospitalization. Creatinine and BUN were measured at admission and discharge (defined by time animals were weaned out of an oxygen cage). Data were statistically evaluated using standard paired t-tests at the time points (significance set at P < 0.05). No statistical difference in baseline characteristics was seen between the groups. There was no statistical difference in furosemide administration at presentation, or at the time points of 6, 12, 24 or mg/kg/day at time of discharge between the placebo and preload-reducing groups. No statistical difference was found in respiratory rate, blood pressure, BUN, creatinine or time to discharge between placebo and preload-reducing groups. While the blood pressure and respiratory rates initially decreased in the preload-reducing group compared to the placebo group, statistical significance was not met. Isosorbide dinitrate did not significantly decrease blood pressure throughout hospitalization. The use of isosorbide did not more rapidly improve heart failure morbidity in DMVD patients with first time CHF.

C46

Influence of heart rate on right ventricular function in healthy dogs

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Heart rate (HR) is one of the main factors which might influence right ventricular (RV) function. We aimed to evaluate the influence of HR on RV function assessed by right heart catheterization and echocardiography.

Eight healthy anesthetized beagles were enrolled in this study. Right heart catheterization and echocardiography were performed at each right atrial pacing rate (baseline, 120, 140, 160, 180 bpm). Maximum and minimum RV pressure, volume and first derivative of RV pressure (dP/dt) were measured using RV pressure-volume loop. End-systolic elastance (Ees) was calculated using single beat method. Normalized tricuspid annular plane systolic excursion (TAPSEn), normalized RV fractional area change (RV FACn), and myocardial systolic velocity of lateral tricuspid annulus (RV s') were measured for RV systolic function. Only RV free wall and RV global (6seg) strain and strain rate (RV-SrL) were measured using two-dimensional speckle tracking echocardiography.

There was no significant change in RV pressure, minimum dP/dt, RVs', RV strain, and RV-SrL_{3seg} among each HR. With the increase of HR, RV volume and TAPSEn decreased (all were $P < 0.01$), and maximum dP/dt and Ees increased (both were $P < 0.05$). Multiple regression analysis showed that RV-SrL_{6seg}, RV FACn, and HR were significantly associated with Ees (all were $P < 0.05$).

Results of RV volume may reflect the decreased venous return due to increased HR. However, RV contractility evaluated by Ees and maximum dP/dt was increased reflecting the force-frequency relationship. The RV-SrL_{6seg} and RV FACn might be useful tools to evaluate RV contractility.

C47

Myocardial function assessed by two-dimensional speckle-tracking echocardiography in cats with cardiomyopathy and congestive heart failure

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Congestive heart failure (CHF) is life threatening condition in cats with cardiomyopathy. However, no known studies have evaluated the involvement of myocardial dysfunction in cats with CHF. In this study, we aimed to evaluate the relationship between the existence of CHF and myocardial function assessed by two-dimensional speckle-tracking echocardiography (2D-STE).

Sixteen client owned healthy cats and thirty-two cats with cardiomyopathy were enrolled in this study. Cats were classified into three group: normal, cardiomyopathy without CHF (CM), and cardiomyopathy with CHF and/or atrial thromboembolism (CHF/ATE). Left ventricular (LV) longitudinal and circumferential strain (SL and SC, respectively), and right ventricular (RV) SL were measured using 2D-STE. Logistic regression analysis was performed to assess the relationship between the existence of CHF and echocardiographic variables including 2D-STE.

Results comparing between Normal and CM versus CHF/ATE showed that increased left atrial-to-aortic diameter ratio and decreased LV apical SC were significantly associated with the existence of CHF (odds ratio [95% confidence interval]: 1.40 [1.16–1.78] and 1.59

[1.06–2.36], respectively). Results comparing between CM versus CHF/ATE showed that increased end-diastolic RV internal dimension and decreased RV SL were significantly associated with the existence of CHF (odds ratio [95% confidence interval]: 1.07 [1.00–1.13] and 1.34 [1.07–1.68], respectively).

Left atrial enlargement and depressed LV apical myocardial function might be useful tools to predict the progression to CHF in cats. Furthermore, the RV enlargement and dysfunction might lead to CHF onset in asymptomatic cats with cardiomyopathy.

E01

Cardiac arrhythmias in 75 horses competing in the cross-country phase of equine eventing

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Sudden death during the cross-country (XC) phase of eventing has raised interest in the frequency and types of exercise-associated cardiac arrhythmias in these equine athletes. A prospective, observational study with convenience sampling was undertaken at events across the United States. Continuous electrocardiographic recordings were obtained from 75 horses during and immediately after XC exercise. For statistical purposes, horses were grouped according to level into Division 1 (beginner novice, novice and training), Division 2 (preliminary and CIC1*) and Division 3 (Intermediate, advanced, CIC/CCI 2*, CIC/CCI 3*). Arrhythmias were grouped according to presence (Y/N) and complexity (couplets, triplets, salvos and tachyarrhythmias or combinations). Multivariate logistic regression was applied to determine risk factors for various arrhythmia groupings ($p \leq 0.05$). A total of 42 horses (56%) had at least one arrhythmic event and 5 horses (7%) had complex arrhythmic events (salvos or paroxysmal tachyarrhythmias) during XC. Horses competing in Division 3 had increased odds (OR = 17.5, $p = 0.0001$) of having an arrhythmia during XC compared to Division 1. Increasing time with heart rate (HR) > 199 was predictive of complex arrhythmias ($p=0.0432$). Age ($p = 0.0037$) and maximal HR ($p = 0.0045$) were predictive of triplets. The presence of arrhythmia at rest was predictive of recovery period arrhythmias ($p = 0.0016$). The presence of arrhythmias during exercise was not predictive of performance. In summary, simple and complex arrhythmias occur in apparently healthy eventers during XC competition. Division, age, maximal HR and amount of time HR > 199 bpm are predictive of various arrhythmia groupings.

E02

First successful applications of closed loop stimulation pacemakers with remote monitoring in two syncopal miniature donkeys (ECEIM Award Winner)

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Advanced second or third-degree atrioventricular (AV) block can be treated by pacemaker implantation. Pacemaker rate-adaptability has typically been obtained by a built-in accelerometer. Closed-loop (CLS) stimulation is a new rate adaptive technology which is based on myocardial impedance changes due to altered sympathetic tone, and achieves a rate-adaptation closer to physiological needs. Automatic remote monitoring allows daily wireless based exchange of pacemaker functional parameters between the patient and an online server, which automatically sends warning messages to the clinician in case of suboptimal pacemaker function. Both CLS and remote monitoring have not yet been investigated in veterinary medicine so far.

In two miniature donkeys with symptomatic AV block, a rate-adaptive single chamber pacemaker with accelerometer, CLS and remote monitoring functionality (Eluna 8 SR-T, Biotronik) was implanted. A bipolar steroid eluting screw-in lead in the right ventricular apex was connected to the pacemaker. After full recovery, rate-adaptivity was assessed. In contrast to periods where no physical activity was present, during low-level exercise, increases in heart rate could be obtained with both the accelerometer and CLS function. During periods of stress, without any physical activity, only the CLS function produced physiological heart rates. With a receiver nearby the donkey (< 4m distance), successful wireless remote monitoring was obtained in both cases with exchange of data to the clinician.

CLS functionality successfully allowed to achieve physiologically-paced heart rate adaptation in relation to actual needs. It also resulted in rate response without physical motion. Remote monitoring allowed automatic reporting of pacemaker function which facilitate follow-up.

E03

Renin-angiotensin-aldosterone system profiling in horses before and after exercise

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The renin-angiotensin-aldosterone system (RAAS) regulates blood pressure, sodium, and fluid balance. Angiotensin-converting enzyme

(ACE) cleaves angiotensin I to angiotensin II to cause vasoconstriction, sodium retention, and inflammation. Its homologue, ACE2, regulates the counter-regulatory RAAS pathway by degrading angiotensin II to angiotensin 1-7, which causes vasodilation, natiuresis, and anti-inflammation. The RAAS is implicated in pulmonary disease and ACE2 protects against lung injury in people. Evaluation of the effect of exercise on the RAAS in horses is necessary before investigating whether dysregulation occurs in diseases such as exercise-induced pulmonary hemorrhage. We hypothesized that exercise would activate the RAAS and ACE2 would be detectable in horses.

The RAAS was evaluated in serum samples from 25 healthy horses before and immediately after exercise using equilibrium analysis (4 Thoroughbreds, treadmill-exercise [TB-TM]; 10 Arabians, 50-mile endurance ride; 5 Thoroughbreds, 1 mile race [TB-R]; 6 Quarter Horses-race 250-500 yards [QH-R]). Exercise increased angiotensins I, II, 1-5, 1-7, aldosterone, renin activity, and ACE2 ($P < .001$ all) and decreased aldosterone:angiotensin II ($P = 0.002$). The concentration of ACE2 ($P < 0.001$) but not neutral endopeptidase ($P = 0.5$) increased after exercise indicating that ACE2 mediated the conversion of angiotensin II to angiotensin I-7. TB-R had higher post-exercise angiotensin II, renin activity, and % change ACE2 than TB-TM (adjusted $P = 0.03$). TB-R and QH-R had higher post-exercise angiotensin 1-5 than TB-TM (adjusted $P < 0.03$).

Exercise activates the RAAS in healthy horses, with prominent ACE2-mediated conversion of angiotensin II to angiotensin 1-7 and greater activation in TB-R compared to TB-TM.

E04

Atrial fibrillatory rate as predictor of recurrence of atrial fibrillation in horses

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Recurrence rate of atrial fibrillation (AF) in horses after cardioversion to sinus rhythm (SR) is high. Atrial fibrillatory rate (AFR) derived from surface ECG is considered a biomarker for electrical remodeling and could potentially be used for prediction of AF recurrence after cardioversion. This retrospective multicenter study evaluated if AFR could predict AF recurrence in horses.

Horses with persistent AF admitted for cardioversion with either medical treatment (quinidine) or transvenous electrical cardioversion (TVEC) were included and AFR was calculated from the surface ECGs. Cox regression and Kaplan-Meier survival curves analyses was performed to assess the relationship between AFR and the risk of AF recurrence.

Of the 204 horses included, 74 received quinidine and 130 received TVEC treatment. The median AFR was higher in the 10 horses receiving quinidine that did not cardiovert to SR, compared to the successfully quinidine-cardioverted horses (383 [min-max 353-422, range 89] vs 351 [min-max 176-469, range 294] fibrillations per minute (fpm), $p < 0.01$). Within the first 180 days, 12 % of the quinidine and 34 % of the TVEC horses had AF recurrence. For the horses successfully cardioverted with TVEC, AFR above 370 fpm was significantly associated with AF recurrence (Hazard Ratio = 5.4, 95 % CI 2.3-12.2, $p < 0.001$).

In conclusion, high AFR was associated with failure of quinidine cardioversion as well as AF recurrence after successful TVEC. As a non-invasive marker retrieved from surface ECG, AFR can be clinically useful in predicting the probability of maintaining SR after electrical cardioversion and identification of non-responders subjected to quinidine treatment.

E05

Utility of a modified wearable ECG patch for 7-day monitoring in horses

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Idiopathic arrhythmias are an important cause of sudden death and diminished exercise performance in horses. The frequency of these arrhythmias in sedentary horses is not known. Available ECG methods to detect arrhythmias are not suitable for long-term (>24 hour) monitoring and are impractical for large studies or routine clinical use in horses.

The FDA-approved Cardea SOLO enables non-invasive ECG monitoring in people for up to 7 days. We modified the device's wearable adhesive patch ECG monitor to record a single ECG lead with one electrode behind the right elbow and the second on the left behind the proximal scapula to allow convenient ECG monitoring of horses. After 7 days the ECG recordings were analyzed by proprietary computer software. For the first and last 24 hours, a Televet was placed to allow comparison between ECG traces.

The modified SOLO was well-tolerated by all 15 sedentary mixed breed horses (14 mares, 1 stallion). During an average ±SD 6.5±1.1 days of monitoring, the mean ±SD heart rate was 40±4 beats/minute. Brief episodes of bradycardia and second-degree atrioventricular block were common in all subjects. Two horses had one or more episodes of paroxysmal supraventricular tachycardia and one horse had 3 episodes of brief non-sustained ventricular tachycardia (fastest rate 141 bpm for 10.6 seconds). Atrial fibrillation was not observed. Overall, the SOLO recordings correlated well with those obtained with Televet.

These results suggest that the modified Cardea SOLO monitor is an accurate and practical long-term ECG recording method for detecting paroxysmal arrhythmias in horses.

E06

Repeatability of heart rate variability measurements during standardized treadmill exercise test in thoroughbred horses

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Heart Rate Variability (HRV) is increasingly used in equine sport medicine to detect arrhythmias during exercise. Although cut-offs of HRV parameters have been calculated between normal HRV and abnormal arrhythmias, repeatability of HRV has not been evaluated.

The objective was to evaluate if HRV parameters measurements (mean RR intervals, mean heart rate, standard deviation of RR intervals (SDRR), triangular index (TI), triangular index triangular interpolation of RR intervals (TIRR), square root of mean squared differences between successive RR intervals (RMSSD), %RR deviation) are repeatable during Standardized Treadmill Exercise Test (STET).

Five healthy Thoroughbred mares with normal ECG at rest (mean age 5,2 yo [3-9], mean weigh 467 kg [406-516]) were included. All mares had grade IV left recurrent laryngeal neuropathy, and were acclimated to the treadmill. STET were performed two times (3 days apart), as follows: 4 min walk (2 m/s), 2 min trot (4 m/s), 2 min canter (8 m/s), 1 min gallop (11 m/s), 2 min trot (4 m/s), 4 min walk (2 m/s). Exercise ECG was recorded by a modified base-apex lead configuration with Televet® recording system. RR intervals were visually checked and analyzed with Kubios® and Graphpad®. Bland-Altman analysis was performed to evaluate repeatability of HRV parameters at each speed and repeatability was considered acceptable if standard deviation of bias was within limits of agreement.

All horses were able to perform the tests. No horses showed exercise intolerance or arrhythmias. For all parameters measured, repeatability was acceptable at all speeds, and HRV was the smallest during the trot pace recovery. Mean and standard deviation of RR intervals, RMSSD and % RR deviation are represented in figure 1 at all speeds. A

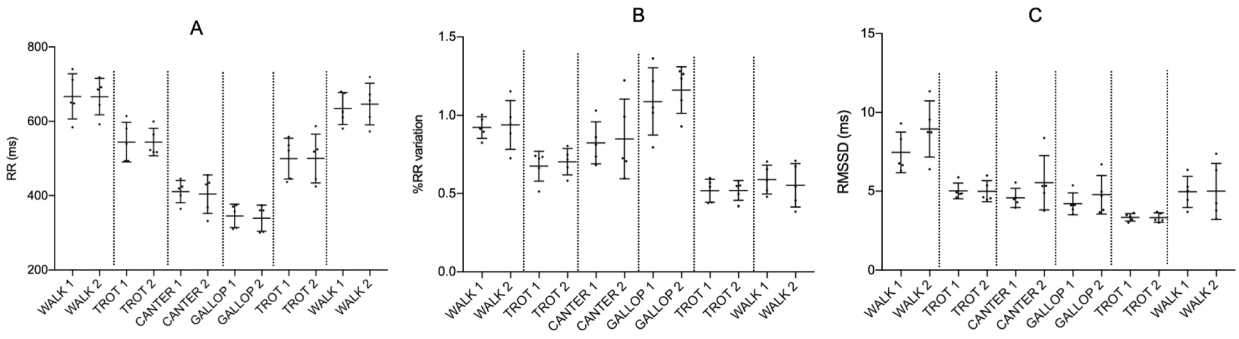

Figure 1 : A : RR intervals means (ms) at each pace (mean and SD) ; B : % RR deviation means (%) at each pace (mean and SD) ; C : RMSSD means (ms) at each pace (mean and SD).

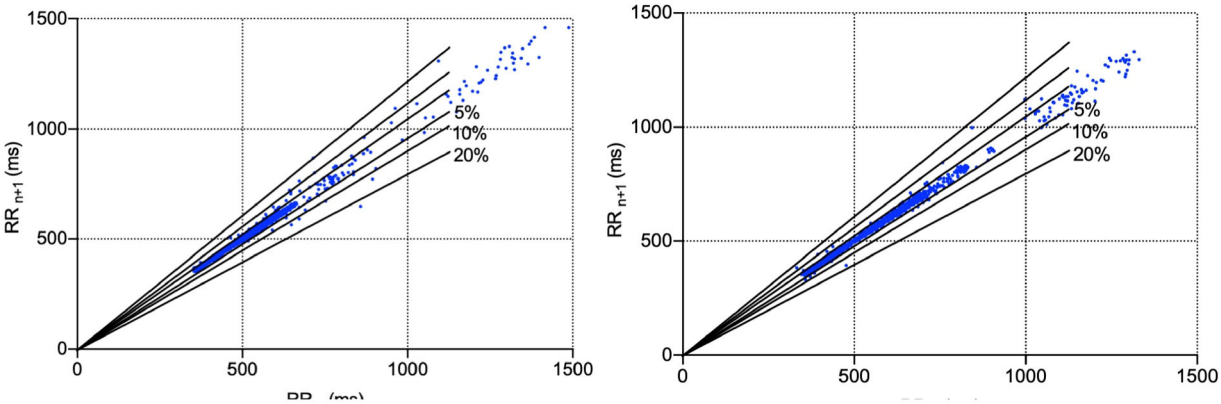

Figure 2 : Pointcarré plots of RR intervals (ms) of the same mare during the 2 exercise tests. This mare had the highest HRV parameters of all horses included.

pointcarré plot shows HRV of the mare with the highest values of HRV during the 2 STET.

HRV parameters measurements appear to be repeatable during standard exercise treadmill. Main limitations: limited number of horses.

E07

The insulin-modified frequently sampled intravenous glucose tolerance test in healthy neonatal foals and horses

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The equine neonate is considered insulin resistant due to delayed maturation of the energy endocrine system. Few studies have investigated insulin sensitivity in newborn foals using dynamic testing methods. The objective of this study was to assess insulin sensitivity by comparing the insulin-modified frequently sampled intravenous glucose tolerance test (I-FSIGTT) between neonatal foals and adult horses. The I-FSIGTT was performed on healthy neonatal foals (n = 12), 24-60 hours of age, and adults (n = 8), 3-14 years of age, using dextrose (300 mg/kg/IV) and insulin (0.02 IU/kg/IV). Insulin sensitivity (SI), acute insulin response to glucose (AIRg), glucose effectiveness (Sg), and disposition index (DI) were calculated using minimal model analysis. Nonparametric statistical methods were used for comparison. SI (median, interquartile range [IQR]) was significantly higher in foals (18.3 $L{\cdot}min^{-1}{\cdot}mU^{-1}$ [13.4-28.4]) compared to horses (0.9 $L{\cdot}min^{-1}{\cdot}mU^{-1}$ [0.5-1.1]); ($p < 0.0001$). DI (median, IQR) was significantly higher in foals (1.2×10^4 [0.8-1.4]) compared to horses (0.04×10^4 [0.02-0.07]); ($p < 0.0001$). AIRg and Sg were not significantly different between foals and horses. While the acute insulin response to glucose (AIRg) and ability of glucose to mediate its own disposal (Sg) were not different between foals and horses, insulin had a higher capacity to promote glucose disposal and inhibit endogenous glucose production (SI) in foals versus adults. These results suggest that healthy neonatal foals may not be insulin resistant, but insulin sensitive, and this could be an adaptation to conserve energy in the transition to extrauterine life.

E08

evaluation of an hmga2 variant contribution to height and insulin in a population of ponies

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Ponies are at increased risk for insulin dysregulation (ID). A missense variant (G >A) in the *HMGA2* gene was previously identified as being correlated with height and insulin concentration in American Welsh ponies. However, the pleiotropic effect of this variant in other pony populations has not been investigated. Ponies of various breeds were evaluated for presence of the *HMGA2* variant and its correlation with height and insulin concentrations. Ponies with suspected pituitary pars intermedia dysfunction were excluded from the study, resulting in 238 ponies with a mean age of 15 years and representing 8 breeds including 120 (50.4%) Shetland, 66 (27.7%) Welsh, and 26 (10.9%) Australian ponies. DNA was isolated and genotyping assay performed for the HMGA2 variant. Baseline serum insulin was measured utilizing an immunochemiluminescent assay. To account for censored data, Tobit regression was performed including the covariates age and sex. Pearson's correlations coefficients were calculated between insulin concentration, height, and the additive effect of the A allele. The A allele frequency was 62% with 112 homozygotes A/A and 71 heterozygotes. There were statistically significant correlations between genotype and height (-0.35; $p = 2.71e\text{-}08$), height and insulin concentration (-0.14; $p = 0.03$), and genotype and insulin concentration (0.15; $p = 0.02$). Thus, the A allele was correlated with both height and insulin concentrations and its presence was not limited to Welsh ponies. A better understanding of genetic contributions to ID will help make informed decisions on how to reduce the risk of ID in individual ponies.

E09

Cortisol and adrenocorticotropin (ACTH) response to corticotropin-releasing-hormone (CRH) stimulation tests in healthy and hospitalized foals

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CRH stimulation tests can be used to assess the hypothalamic-pituitary-adrenocortical axis (HPAA) function in neonatal foals. Information on CRH stimulation tests in foals is lacking.

The purpose was to evaluate the response of cortisol and ACTH to different doses of CRH in foals. We hypothesized that, cortisol and ACTH will increase in response to exogenous CRH in healthy and hospitalized foals, and there will be a dose dependent effect.

CRH (0.1, 0.3, and 1μg/kg) was administered intravenously to healthy and hospitalized foals < 7 days of age. Blood samples were collected before and 15, 30, 60, and 90 minutes after administration of CRH for determination of plasma ACTH and cortisol concentrations.

In healthy foals, cortisol concentrations increased at 15 minutes from baseline with 0.1 and 0.3μg/kg and at 30 and 60 minutes from baseline with 1μg/kg CRH ($P < 0.05$). The delta cortisol (0-30min, 0-60min) and ACTH (0-30min, 0-60min) was higher for the 1μg/kg compared with the 0.1μg/kg CRH ($P < 0.05$). In the hospitalized foals, cortisol and ACTH concentrations increased at 15 minutes from baseline with the 0.1μg/kg of CRH and ACTH concentrations increased from baseline at 30 minutes with 1μg/kg ($P < 0.05$). The delta ACTH 0-15min was higher for the 1μg/kg compared to the 0.1μg/kg

(P < 0.05). There was no difference in cortisol and ACTH response to CRH between healthy and hospitalized foals.

The results suggested, cortisol and ACTH increased in response to administration of all 3 doses of CRH, and 1μg/kg dose of CRH is optimal for HPAA assessment in healthy and hospitalized foals.

E10

Comparison of proxies of insulin sensitivity and insulin secretion between foals and adult horses

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In the postnatal period, neonatal foals are considered to be insulin resistant secondary to elevated concentrations of catecholamines and cortisol. Insulin resistance may also be a consequence of prematurity or critical illness. Proxy measurements of insulin sensitivity and insulin secretory response have been used in horses to assess insulin sensitivity and dysregulation. The goal of this study was to compare proxies between healthy newborn foals and horses in order to investigate insulin resistance in newborn foals. Proxies were calculated from fasted blood glucose and insulin concentrations in healthy neonatal foals (n = 12), 24-60 hours of age, and horses (n = 8), 3-14 years of age. Glucose concentrations (median, interquartile range [IQR]) were significantly higher in foals (165.5 mg/dL [159.8-184.0]) compared to horses (103.5 mg/dL [97.3-109.0]); p < 0.0001. The modified insulin to glucose ratio (MIRG; [median, IQR]) was significantly lower in foals (1.72 $mIU_{insulin}^2/10{\cdot}mg_{glucose}$ [1.43-2.68]) compared to horses (3.91 $mIU_{insulin}^2/10{\cdot}mg_{glucose}$ [2.57-7.89]); p = 0.009. Fasting insulin concentrations, the quantitative insulin sensitivity check index (QUICKI), the homeostatic model assessment of insulin resistance (HOMA-IR), and the reciprocal square root of insulin (RISQI) were not different between foals and horses. Our results show that neonatal foals have similar insulin sensitivity to horses, but decreased insulin secretory ability relative to elevations in blood glucose, based on low MIRG values. These results suggest that neonatal foals may be more insulin sensitive in the first days of life to compensate for reduced insulin secretory ability. These results contradict previous conclusions on insulin sensitivity in newborn foals.

E11

Comparison of basal adrenocorticotropic hormone concentrations among different equine breeds

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Recent evidence has suggested differences in basal ACTH concentrations between different equine breeds, which has implications for the diagnosis of pituitary *pars intermedia* dysfunction (PPID). The aim of this cross-sectional study was to compare basal ACTH concentrations among a population of horses and ponies.

Three breed groups were studied: Thoroughbred horses (n = 127), Shetland ponies (n = 131), and ponies of non-Shetland breeds (n = 141). Inclusion criteria comprised: aged ≥ 8 years; no current illness, lameness, or medications; no clinical signs consistent with PPID; and no historical administration of pergolide mesylate. Paired blood samples were collected, within 2 weeks of the autumn equinox and within 3 weeks of the spring equinox. Plasma ACTH concentrations were measured using an immunochemiluminescent assay (Immulite 1000). Groups were compared using the Kruskal-Wallis test with Dunn's *post hoc* test.

Autumnal ACTH concentrations were higher in Shetland ponies (median [IQR] pg/mL; 114 [75-207]) compared with Thoroughbreds (46 [35-65]; p < 0.001) and non-Shetland ponies (72 [43-119]; p < 0.001), and higher in non-Shetland ponies compared with Thoroughbreds (p < 0.001). Spring ACTH concentrations were ≤ 40 pg/mL for all animals, with higher ACTH concentrations in Shetland ponies (24 [18-28]) compared with Thoroughbreds (20 [17-24]; p < 0.001), while non-Shetland ponies were not significantly different to other breed groups (22 [17-26]; p ≥ 0.13). These findings, in animals with no clinical signs of PPID, indicate that breed should be considered when interpreting ACTH results, and that breed-specific reference intervals and clinical cut-offs could be warranted.

E12

The effect of transferring equine plasma into silicate-containing tubes to improve short-term ACTH stability

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Pituitary pars intermedia dysfunction (PPID) is a common endocrinopathy affecting approximately 20% of horses ≥ 15 years. PPID is confirmed by increased plasma ACTH concentrations; however, ACTH has a poor stability due to proteolytic degradation and possible interaction with blood cells. It is advised to collect blood into EDTA tubes and transfer plasma into cryovials after centrifugation for transportation to laboratories. However, in clinical practice cryovials are rarely available and plasma is transported in silicate-containing tubes. Some laboratories have questioned if this would affect ACTH concentration as the effect of transferring plasma into silicate-containing tubes has not been evaluated. Whole blood was collected into EDTA tubes from 16 horses ranging from 12 to 17 years of age (9 PPID-positive with endogenous plasma ACTH concentration > 35 pg/mL and 7 PPD-negative). After centrifugation, plasma was aliquoted to either cryovials or silicate-containing tubes. Samples were stored at 4°C and 20°C. ACTH concentrations were measured using a chemiluminescent assay at 1, 24 and 48 hours after sample collection. There was a significant effect of time on plasma ACTH concentrations in PPID-positive horses at 4°C (P = 0.02) and 20°C (P < 0.0001), and in both groups at 20°C (P ≤ 0.0001) with decreasing plasma ACTH concentrations overtime. However, no significant effect of

transferring plasma into a silicate-containing tube was detected in either groups. The use of silicate-containing tubes does not alter ACTH stability. In all cases, it is recommended to keep plasma samples at 4°C to be analyzed within 24 hours.

E13

Effects of oral RRR-A-tocopherol on plasma oxidative stress and endocrine markers in healthy horses

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Equine Pituitary Pars Intermedia Dysfunction (PPID) is a common, progressive, neurodegenerative disorder resulting from oxidative damage to the hypothalamus. Anti-oxidant therapy with RRR-a-tocopherol (vitamin E) might slow PPID progression if it can reduce systemic oxidative stress. The objective of this study was to assess the effect of two RRR-a-tocopherol formulations on plasma oxidative stress and endocrine markers in healthy horses. We hypothesized that oral administration of RRR-a-tocopherol alters basal plasma oxidative stress and endocrine markers in healthy horses, and the degree of this effect varies between formulations. Ten healthy horses received 5000 IU (~10 IU/kg) of RRR-a-tocopherol acetate powder, water-dispersible liquid, or tap water orally once daily for 21 days in a randomized crossover design. Horses underwent a ≥ 28-day washout between treatments. Blood was collected on before and on days 0, 3, 7, 14 and 21 of treatment for measurement of plasma reactive oxygen metabolites (dROMs), antioxidant capacity (PAC), cortisol, ACTH, and leptin via photometric and immunoassays respectively. Data were analyzed with repeated measures ANOVA, Friedman Test, paired t-test, Wilcoxon signed rank test as appropriate ($P < 0.05$). Endocrine parameters did not differ significantly between days 1 and 21 in any of the three treatment groups. There was also no effect of RRR-a-tocopherol treatment on dROMS or PAC. This RRR-a-tocopherol dosing regimen did not alter oxidative stress or endocrine parameters in healthy horses, but further study is needed to assess if treatment could have an effect on animals with endocrine disease or increased oxidative stress

E14

Lamellar ribosomal protein s6 activation in three forms of experimentally induced equine laminitis

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A comprehensive research effort has revealed signaling pathways contributing to lamellar stretching, failure, and laminitis in experimental models. However, pathophysiological mechanisms common in all forms of laminitis have remained elusive. Ribosomal protein S6 (RPS6) is activated downstream of insulin-like growth factor-1 receptor and is active within the lamellae during laminitis. The objectives of this study were 1) determine the concentration of phosphorylated-RPS6 (pRPS6 S240-244 and S235-236) following experimentally-induced endocrinopathic (euglycemic-hyperinsulinemic clamp model [EHC]), sepsis-associated (carbohydrate overload [CHO]), and supporting-limb laminitis models (SLL), and 2) evaluate the effect of distal limb cryotherapy (CRYO) on pRPS6 concentrations. Healthy adult light-breed horses were used for three different laminitis models (CHO n=6, control n=6), (EHC n=8, control n=8), (SLL n=7, control n=6). Additionally, CRYO was applied to one forelimb while the other forelimb remained at ambient temperature (AMB) in CHO and EHC models. Digital lamellar tissue was collected following humane euthanasia and the concentrations of p-RPS6, total RPS6, and β-actin were determined using western immunoblotting. Lamellar [p-RPS6] was significantly increased in all models of equine laminitis at the onset of clinical signs (CHO p =0.0006, 0.05; EHC p=0.0002, < 0.0001; SLL p=0.0005, 0.0017 [235-236 and 240-244, respectively]). Cryotherapy significantly attenuated RPS6 activation in the CHO and EHC models (p=< 0.0001 and p=0.0078). RPS6 appears to play a role in the pathophysiology of all three forms of laminitis and is attenuated by a practice known to prevent disease in high-risk individuals. Evaluation of pharmacological inhibition of RPS6 for laminitis prophylaxis and treatment is warranted.

E15

Adrenocorticotropin concentrations vary with assay used in equids with and without pituitary pars intermedia dysfunction

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Plasma adrenocorticotropin (ACTH) concentration is measured to support a diagnosis of pituitary pars intermedia dysfunction (PPID). However, baseline ACTH and ACTH after IV administration of thyrotropin releasing hormone (TRH) vary with season and assay used. To assess performance of two ACTH assays (Scantibodies® immunoradiometric assay [S] and Immulite® 2000 immunoassay [I]) for diagnosis of PPID, ACTH was measured before and 10 minutes after administration of TRH in three groups of horses: 1) 6 young horses (6 ± 3 years, no hypertrichosis and a pituitary gland [PG] histology score of 1-3/5); 2) 7 aged horses (28 ± 6 years, no hypertrichosis and a PG histology score of 2-3/5); and 3) 6 PPID-affected equids (29 ± 6 years [one pony], all with hypertrichosis and a pituitary gland [PG] histology score 5/5). All equids underwent TRH stimulation in non-fall months with necropsy examination within 7 days. Baseline ACTH was elevated (>35 pg/mL) with S in 1/6, 1/7, and 5/6 and with I in 2/6,

2/7, and 5/6 of young, aged, and PPID-affected equids, respectively. After TRH administration, ACTH was elevated (>110 pg/mL) with S in 0/6, 0/7, and 4/6 and with I in 4/6, 4/7, and 6/6 of young, aged, and PPID-affected equids, respectively. Mean increases in ACTH after TRH administration were 4.0-, 7.2-, and 4.6-fold greater with I compared to S in young, aged, and PPID-affected equids, respectively. In conclusion, plasma ACTH (or immunoreactive ACTH) concentration before and after TRH administration varies substantially with different assays and can affect diagnostic interpretation.

E16

Lumbar vertebral bone density is decreased in aged horses with pituitary pars intermedia dysfunction

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Osteoporosis, most marked in the spine, was recognized by Harvey Cushing in association with basophil adenomas of the pituitary gland (PG) in 1932. Similarly, osteopenia is a recognized complication of canine hyperadrenocorticism, with a 25% loss of bone mineral density (BMD) reported in trabecular bone of thoracic and lumbar vertebrae. Pathologic fractures have also been reported in horses with pituitary pars intermedia dysfunction (PPID), suggesting that PPID may be also be associated with loss of BMD. We hypothesized that BMD of lumbar vertebrae would be lower in PPID-affected equids, as compared to healthy aged horses. Following euthanasia, the lumbar spine was removed from 7 aged (29 ± 6 years) horses with hypertrichosis and thyrotropin (TRH) stimulation test results supportive of PPID ($PPID^+$, PG histology scores of 4-5/5) and 6 aged horses (26 ± 5 years) without hypertrichosis and non-supportive TRH stimulation test results ($PPID^-$, PG histology score 2-4). Computed tomographic (CT) scans were performed and images were imported into MIMICS 21.0 (Materialise, Leuven, Belgium) and density (Hounsfield Units [HU]) of regions of interest of mid-vertebral bodies of L3, L4, and L5 were measured in a sagittal plane. Data were analyzed by a two factor ANOVA. BMD of the lumbar spine was lower ($P < 0.01$) in $PPID^+$ horses (391 ± 18 HU) than in $PPID^-$ horses (477 ± 19 HU). In conclusion, these data support a loss of BMD in horses with PPID that could make PPID-affected horses at increased risk of fracture, especially of non-weight-bearing bones.

E17

Steroid hormone profiles of horses with pituitary pars intermedia dysfunction

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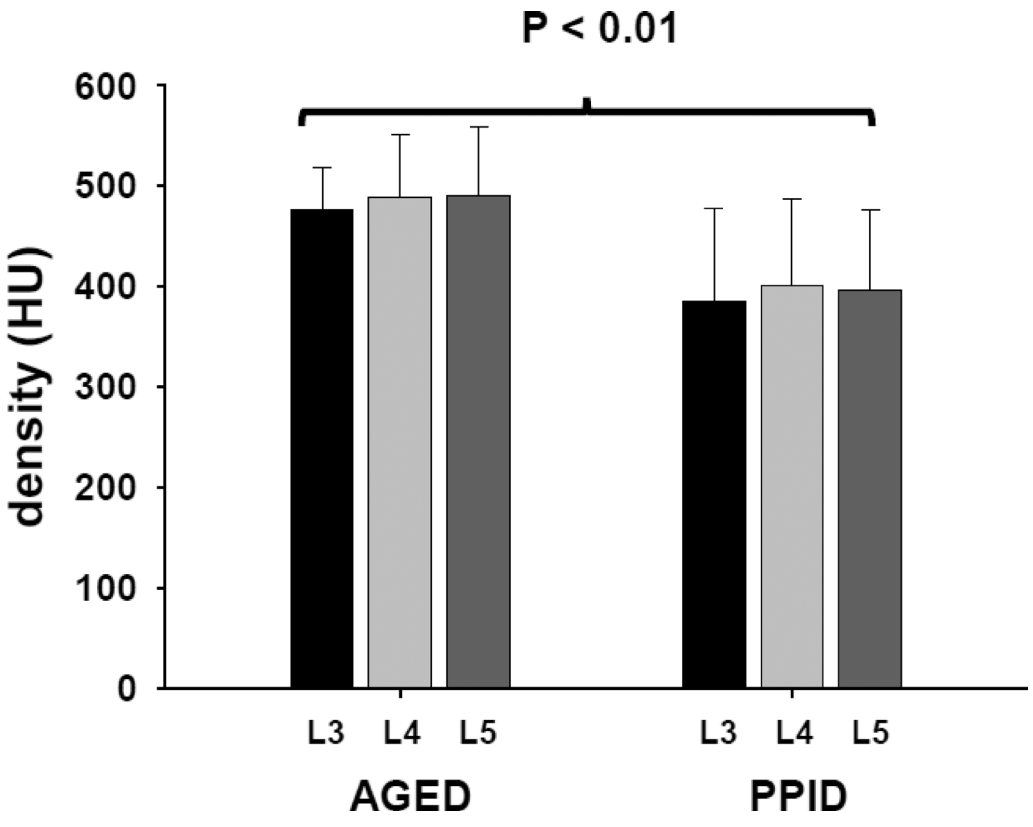
Equids with pituitary pars intermedia dysfunction (PPID) can have a docile attitude and other neurological (cerebral) deficits. Altered neurosteroid hormones contribute to behavioral abnormalities accompanying equine



neonatal encephalopathy. Consequently, we speculated that altered neurosteroid hormones could contribute to neurological deficits accompanying PPID. Thirty-one steroid hormone concentrations were measured by liquid chromatography-mass spectroscopy in jugular venous blood collected from 10 horses with PPID (pituitary gland [PG] histology scores 4-5/5) and 9 normal horses (PG histology scores 1-2/5). Steroid hormone concentrations were analyzed with non-paired t-tests and Mann-Whitney U tests. Six steroid hormones were different ($P < 0.05$) between PPID and control horses. Plasma cortisol and pregnenolone concentrations were lower while plasma 20-hydroxyprogesterone, cortexone, androstanediol, and androstenediol concentrations were higher in PPID, as compared to normal horses. A lower cortisol concentration in PPID horses, despite increased activity of the hypothalamic-pituitary-adrenal axis, could support more rapid metabolism and excretion of cortisol metabolites and/or altered tissue regulation of cortisol by 11-β-hydroxysteroid dehydrogenase 1 or 2. We had suspected that pregnenolone, a potent neurosteroid, might be increased in PPID-affected horses and could contribute to their docile attitude; however, we found the opposite. Curiously, circulating pregnenolone concentrations are also lower in humans with Parkinson's Disease and there is evidence that pregnenolone might have neuroprotective effects. Reasons for differences in the remaining steroid hormone concentrations remain unknown. In conclusion, PPID is likely a complex disorder with multiple neurohumoral imbalances and these preliminary data provoke interest in expanding mechanisms that could contribute to the clinical syndrome of PPID.

E18

Temporal changes in the fecal microbiota and its functional capacity of ponies developing insulin dysregulation

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This study evaluated changes in the fecal microbiome of ponies receiving a high-energy diet and developing insulin dysregulation.

Nine Shetland ponies received diet A (100% net energy requirement), and nine ponies received diet B (200% net energy requirement) for 37 weeks in two separate study cycles. At week 0, week 9-13, week 18-23, and week 27-29, oral glucose tolerance tests (OGTT) were performed, and fecal samples were collected. Illumina Miseq 16S sequencing was performed on all fecal samples. Shotgun metagenomic sequencing was performed on one sample per pony. Richness, α-diversity, β-diversity, and relative abundance of taxa and bacterial metabolic pathways were compared over time and between diets. PERMANOVA was used for statistical analysis.

Figure 1 *PCoA plot of beta diversity at different time points for control diet A (on the left) and the high-energy diet B (on the right) for both study cycles. A clear temporal effect is visible for ponies on the high-energy diet (B) as the samples shift to the right across the X-axis with increasing study period.*

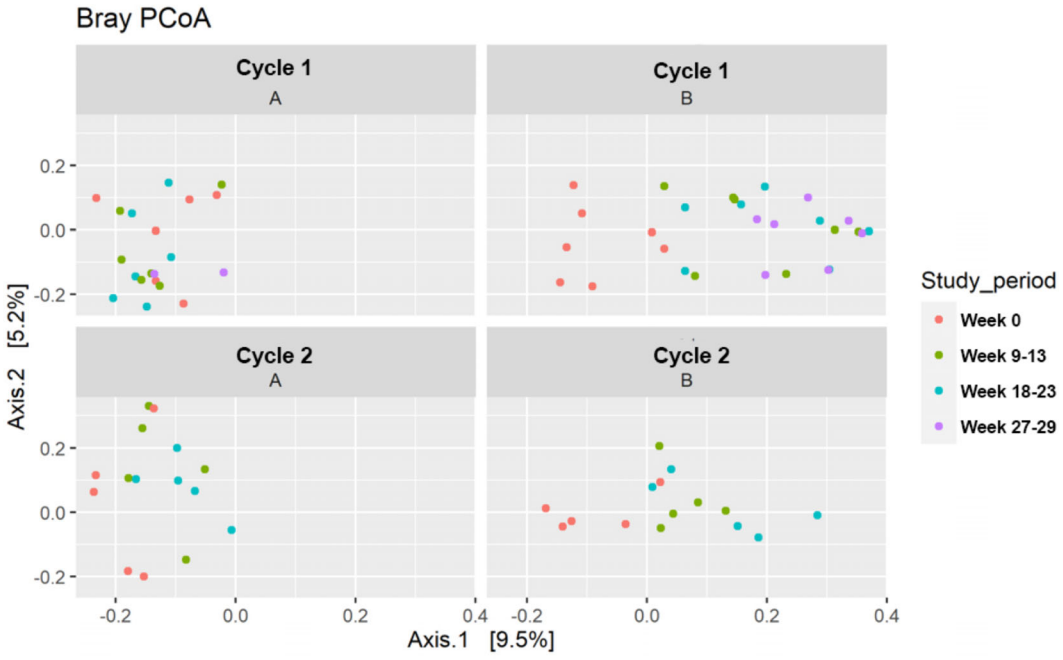
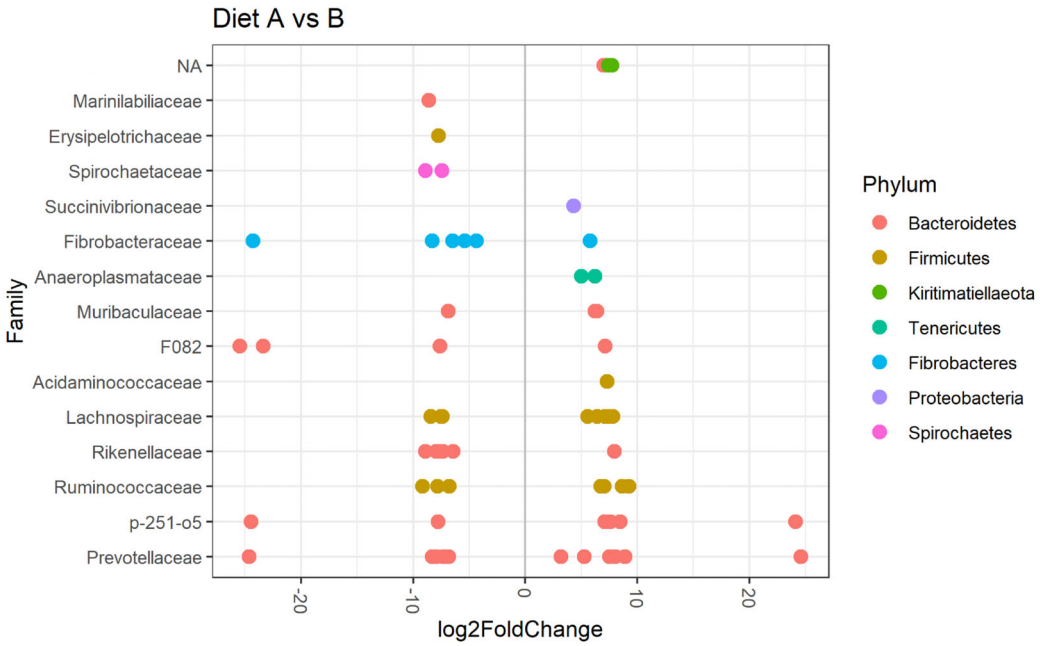


Figure 2 Differentially abundant amplicon sequence variants (ASVs) identified through DESeq2 testing. Each ASV is annotated at the family level and plotted according to their log2 fold change, calculated as the levels in samples collected from ponies on diet B relative to the levels in samples collected from ponies on diet A. Left side of the plot: bacterial families that are proportionally less abundant in fecal samples collected from ponies on the high-energy diet (B). Right side of the plot: bacterial families that are proportionally more abundant in fecal samples collected from ponies on the high-energy diet (B).



Richness and α-diversity were increased at week 9-13 and week 27-29 in ponies on both diets, indicating a seasonal effect. Ponies on diet B showed a significant temporal shift in fecal microbiota composition (cycle 1: R2 9.1; P < 0.001; cycle 2: R2 9.7; P = 0.025), see Figure 1. These ponies also had a higher α-diversity (P = 0.019). Microbiota composition (R2 11.7; P = 0.001) and relative abundance of several bacterial families differed significantly from ponies on diet A (Figure 2). Bacterial metabolic pathways 'PWY-5973 cis-vaccenate biosynthesis' and 'PWY-7663 gondoate biosynthesis (anaerobic)' were significantly more abundant in ponies on diet B (Benjamin-Hochberg q-value < 0.1)

This study demonstrated a clear temporal shift in microbiota composition of ponies on a high-energy diet. Also, two bacterial metabolic pathways involved in fatty acid synthesis were significantly more abundant in these ponies.

E19

Effect of AMPK agonists on incretin hormone secretion in horses with experimentally-induced insulin dysregulation

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Insulin dysregulation (ID) in horses increases the risk of endocrinopathic laminitis. The enteroinsular axis includes the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinoptropic peptide (GIP) and supports insulin secretion after meal feeding. Adenosine 5′-monophosphate activated protein kinase (AMPK), a highly conserved enzyme involved in energy regulation, is a therapeutic target in humans with metabolic syndrome. The objective of this study was to investigate the response of GIP, GLP-1, and active GLP-1 (GLP-1a) to an oral sugar test (OST) before and after the induction of ID and in response to metformin and aspirin administration (both AMPK agonists). Insulin dysregulation was induced in 7 light-breed horses with dexamethasone (0.08 mg/kg PO q24h for 7 days). Then, horses received metformin (30 mg/kg PO q12h) and aspirin (10 mg/kg PO q24h) for 7 days in addition to dexamethasone. An OST was performed at baseline, ID, and ID + combination timepoints, and serum incretin hormones were measured at 0, 30, 60, 90, 120, and 150 minutes. The $AUC_{GIP0\text{-}150}$ and $AUC_{GLP\text{-}1a0\text{-}150}$ were significantly increased at the ID (P = 0.005, 0.05) and ID + combination therapy (P = 0.006, 0.004) timepoints compared to baseline. The $AUC_{GLP0\text{-}150}$ was significantly increased at ID + combination therapy compared to baseline and ID (P = 0.03, 0.03). Incretin hormone secretion is altered following dexamethasone administration. The administration of

metformin and aspirin in combination enhances secretion of GLP-1 and is associated with hyperinsulinemia.

E20

Effect of AMPK agonists on serum lipid and adipokine concentrations in horses receiving dexamethasone

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Insulin dysregulation (ID) is a hallmark component of equine metabolic syndrome (EMS) and has been shown to increase the risk of endocrinopathic laminitis and other metabolic derangements. Dexamethasone, a commonly used therapeutic in equine medicine, can induce insulin dysregulation and alter other metabolic pathways in equids. Adenosine-5'-monophosphate-activated protein kinase (AMPK) is a cellular energy enzyme and important therapeutic target in humans with metabolic syndrome. The objective of this study was to assess plasma lipid fractions (triglycerides, non-esterified fatty acids [NEFA]) and adipokines (adiponectin and leptin) in dexamethasone-induced ID and their response to AMPK agonist administration (metformin [Met], aspirin [ASP], and combination [MET/ASP]). ID was induced in 14 adult light-breed horses with dexamethasone (0.08 mg/kg PO q24h); horses were assigned to groups and received either ASP (10 mg/kg PO q24h; n = 7) or MET (30 mg/kg PO q12h; n = 7) for 7 days. Seven horses then received MET/ASP for 7 days. Serum concentrations of triglycerides, non-esterified fatty acids, leptin, and adiponectin were measured at baseline, ID, ID + monotherapy, and ID + combination timepoints. Serum [triglyceride] and [leptin] were increased following dexamethasone administration (P = 0.02, 0.04). Serum [NEFA] was decreased following dexamethasone administration (P = 0.0002). Serum [adiponectin] was not significantly altered following dexamethasone administration (P = 0.41). AMPK agonist therapy did not significantly affect leptin, adiponectin, NEFA, or triglyceride concentrations. The administration of dexamethasone significantly alters lipid and adipokine concentrations, which may have clinical relevance for horses receiving dexamethasone.

E21

Incretin hormone response to an oral sugar test in horses with dexamethasone-induced insulin dysregulation

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Insulin dysregulation (ID) is a hallmark component of equine metabolic syndrome (EMS) and has been shown to increase the risk of endocrinopathic laminitis and other metabolic derangements. The enteroinsular axis (including the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)) increases insulin secretion after meal feeding and perpetuates hyperinsulinemia in insulin dysregulated animals. The objective of this study was to evaluate secretion of the incretin hormones GIP, GLP-1, and the active form of GLP-1 (GLP-1a) in response to an oral sugar test (OST) before and after induction of ID with dexamethasone. An OST was performed on 7 adult light-breed horses; serum measurements were obtained at 0, 30, 60, 90, 120 and 150 minutes for GIP, GLP-1, and GLP-1a. Then, ID was induced with dexamethasone (0.08 mg/kg PO q24h for 7 days), after which a repeat OST was performed, and incretin hormones were measured. Following the induction of ID, the $AUC_{GIP0\text{-}150}$ and $AUC_{GLP\text{-}1a0\text{-}150}$ were significantly increased (P = 0.002, 0.02 respectively). The $AUC_{GLP\text{-}1\ 0\text{-}150}$ was not statistically different following the induction of ID (P = 0.35). Previous work from our group has shown that treatment with dexamethasone significantly raises the $AUC_{insulin(0\text{-}150)}$ (P = 0.044) during an OST. The incretin response is altered by dexamethasone-induced ID, which further supports its role in the hyperinsulinemia associated with naturally-occurring ID.

E22

Investigation of plasma cell-free DNA (cfDNA) as a novel biomarker in equine colic patients

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Plasma cell-free DNA (cfDNA) is a biomarker of cell death and neutrophil extracellular trap release that is increased in humans and dogs with ischemic gastrointestinal lesions or severe systemic inflammation. We hypothesized that cfDNA in equine plasma would be measurable using a compact fluorometer, be elevated in colic patients vs. healthy horses, and be significantly different among clinically-relevant groupings of colic patients. Our objectives were to 1) measure cfDNA in plasma and plasma-extracted DNA, 2) determine the correlation between paired samples, and 3) compare median cfDNA concentrations between colic patients and healthy horses and among groups of colic patients. Blood was collected from 67 adult colic patients (≥2 years) upon admission to a university hospital and 19 healthy controls. The Qubit™ 4 Fluorometer and dsDNA Broad-Range or dsDNA High-Sensitivity Assay Kits were used to quantify cfDNA in paired plasma and extracted samples, respectively. Colic cases were categorized based on patient data, including lesion type, SIRS score, treatment, and survival to discharge. Cell-free DNA concentrations were compared between paired samples using Spearman correlation, and median values were compared between groups using Mann-Whitney tests. Plasma and extracted cfDNA were not significantly correlated (p = 0.69). Median extracted cfDNA concentrations were significantly higher in colic patients relative to healthy controls (p < 0.0001) and in colic patients with SIRS (score ≥2) compared to non-SIRS cases (p = 0.010). Research to optimize Qubit measurement of cfDNA in equine plasma, as well as determine clinical relevance of cfDNA as a biomarker in horses, is ongoing.

E23

Microbial composition and viability in equine feces after processing for transplantation

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Fecal microbiota transplantation has been used empirically for decades in equine medicine but evidence-based information on this technique is scarce. This *in vitro* study aimed to assess the effect of a common fecal pre-transplantation processing method on microbiota composition and viability of the fecal inoculum.

A fresh equine fecal sample was divided in three 300g aliquots, to be processed identically as follows: feces (T_0) were mixed with 1 L of lukewarm water and chopped using an immersion blender to obtain a mixture (T_1), which was left uncovered during 30 minutes (T_2) and filtered through a sieve to obtain a fecal infusion (T_3). Samples were taken at different steps of the procedure (T_n) and immediately stored at 4°C < 6 hours until processing. Bacterial 16S rDNA amplicon profiling associated to propidium-monoazide treatment was performed in each of the 12 samples to select living bacteria. Total flora was measured using quantitative PCR analysis. Bacterial community structure, composition and total flora were statistically compared (significance $p < 0.05$).

No significant differences in ecological indices or abundance of the major bacterial genera were identified for the different steps of fecal processing. Estimated total flora (mean ± SD) in the final fecal infusion was not significantly lower (10.06 ± 0.07 log gene copies/g) than in the original manure (10.29 ± 0.3 log gene copies/g). Results were similar for all three fecal aliquots.

These results suggest that the processing method described in this study is replicable and the resultant fecal inoculum contains live bacteria and a microbial population similar to the original fecal sample.

E24

Machine learning for the analysis of the fecal microbiota in horses with colitis

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The objective of this study was to profile and compare the fecal microbiota of healthy horses (H; n = 50) and those with colitis (C; n = 58). Horses with colitis were further subdivided into survivors (C-S; n = 29) and non-survivors (C-NS; n = 29); as well as those which developed laminitis (C-L; n = 15) and those which did not (C-NL; n = 43). The fecal microbiota was profiled using next generation sequencing. Alpha- and beta-diversity were measured and compared between groups. Three supervised machine learning algorithms including random forest (RF), support vector machine (SVM), and neural networks (NNW) were used to distinguish H vs, C; C-S vs. C-NS; C-L vs. C-NL. Receiver operator characteristic (ROC) curves were constructed to assess the predictive value of the microbiota profile to differentiate pairs of groups.

Community membership and structure was altered in C compared to H (AMOVA $P < 0.001$). Community membership was different between C-S and C-NS (AMOVA $P < 0.03$, but no differences were observed between C-L and C-NL (AMOVA $P > 0.05$). ROC curve analysis showed that the RF, SVM and NNW models had excellent prediction of colitis (area under the ROC curve = 0.98, 0.96, 0.92, respectively). The RF analysis found *Treponema*, *Fibrobacter* and *Faecalibacterium*, as some of the bacteria with the highest relative importance in H, whereas *Enterococcus* and an unclassified genus of the Proteobacteria class had the highest relative importance in the C group.

Machine learning microbiota models indicated that colitis was strongly associated with microbial variations. This study further reiterate that the gut microbiota undergoes alterations in horses with colitis and this may be an exploitable venue for the treatment and prevention of equine colitis.

E25

Use of equine omega complete for prevention of gastric ulcers and supplementation of vitamin E

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The oral supplement Equine Omega Complete (EOC), containing omega-3 fatty acids and α-tocopherol (vitamin E), may anecdotally reduce gastric ulcer formation in horses. The objectives of this study were to determine if EOC supplementation prevents gastric ulcer formation and increases serum α-tocopherol concentrations in adult horses. Nine Thoroughbred geldings were enrolled, ranging from 5-13 years of age. The study was performed in a prospective randomized block design, repeated in a crossover model. Horses were divided into three groups and administered EOC, omeprazole, or water (control) orally for 28 days. Gastric ulcers were experimentally induced on days 21 through 28 via intermittent feed deprivation, while continuing treatment. Gastroscopies were performed on days 0, 21, and 28 and serum α-tocopherol concentrations were assessed on days 0 and 28. Data were analyzed using a linear mixed model (fixed effects: block, treatment, time), with significance set at $p < 0.05$ and 95% confidence intervals (CI) reported for post-hoc contrasts. Gastric ulcers were induced between days 21 and 28 ($p = 0.0009$, CI -2.89 to -0.93), with a significant effect of treatment ($p < 0.0001$) but not block ($p = 0.09$). Ulcers were significantly induced in the control group ($p = 0.002$, CI 0.41-1.88) but not the omeprazole group. Gastric ulcer grades increased in EOC-treated horses following ulcer induction; increasing from a median score of 0 (0-2) on day 0 to 3 (2-4) on day

28 (p < 0.0001, CI -2.08 to -0.68). Ulcer grades at day 28 were similar in control and EOC groups. Serum α-tocopherol increased in all experimental groups over time (p =0.0005), with no significant difference between treatment groups (p =0.97). Twenty-eight-day supplementation with EOC did not protect against experimental gastric ulcer formation and failed to increase serum α-tocopherol over other treatment groups. The approved omeprazole formulation in horses prevents experimentally induced gastric ulceration.

E26

Immune cell population in the duodenal mucosal in asthmatic horses

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Weight loss of unknown origin is observed in asthmatic horses and could be due to inflammation in the small intestine, which could be secondary to systemic inflammation that is observed in asthmatic horses and similar to some pulmonary diseases in humans. The objectives of this study were to quantify lymphocytes and eosinophils in the mucosa of duodenum in asthmatic horses. These cells are most frequently observed in inflammatory bowel disease. Control (n = 8) and asthmatic (n = 10) horses were studied. Asthmatic horses were evaluated in a symptomatic and asymptomatic status (3 months after environmental changes (n = 4) and fluticasone treatment (n = 6)). Duodenal biopsies were endoscopically (n= 4-6) taken in each horse. Eosinophils were counted on hematoxylin-eosin-phloxin-saffron (HEPS) stained slides. HEPS and immunohistochemistry evaluated T-lymphocytes (CD3) and B-lymphocytes (CD20). The best quality biopsies were used for histomorphometry. The duodenal epithelium of asthmatics and control horses contained exclusively T-lymphocytes (CD3). Symptomatic asthmatic horses, compared to controls, had a significant higher number of T-lymphocytes (CD3) in the epithelium (p = 0.016) and the adjacent lamina propria of villi (p = 0.04) but not in lamina propria adjacent to crypts. The duodenal lamina propria contained B-lymphocytes in asthmatic and control horses without significant difference. Taken together, these results suggest that asthmatic horses have more lymphocytes in the duodenal mucosa indicating a certain degree of inflammation which could be due to a systemic inflammatory effect and/or a local effect of ingested hay allergens in asthmatic horses

E27

Prophylactic treatment of equine laparotomy incisions with manuka honey reduces the incidence of incisional infection

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Manuka honey has anti-bacterial properties and can promote wound healing when applied topically. Surgical site infection (SSI) of laparotomy incisions in horses is a common complication. The purpose of the study was to document if a single application of medical grade Manuka honey to the linea alba prior to skin closure in horses undergoing laparotomy would reduce SSI incidence.

Records of horses undergoing laparotomy from January 2017-January 2020 were evaluated retrospectively. Data recorded included use of honey, and whether and when SSI developed. Owners were contacted for follow-up. Cases were included only if 4 weeks follow-up after surgery was available, unless SSI had already developed. Horses undergoing re-laparotomy in the 4-week period were excluded.

Exploratory laparotomy was performed on 257 occasions on 240 horses. Various subcutaneous and skin closure methods were used. Cases were excluded (113) due to death (6), euthanasia (56), multiple surgeries (33), lack of follow-up (13) or other (5). Manuka honey was used in 72 included cases and not in a further 72 cases, according to surgeon preference. SSI occurred in 13/72 (18%) in the honey group and 23/72 (31.9%) with no honey (p=0.05, Chi squared test). Median time to SSI was 10 days (range 5-23) with honey and 9.5 days (range 3-24) without.

Manuka honey reduces SSI incidence when applied to the linea alba in horses undergoing laparotomy. Limitations include lack of blinding or randomization.

E28

Equine enterocolitis: A retrospective post-mortem study (2007-2019)

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This study aimed to determine etiological agents and histopathological lesions detected on *post-mortem* examination of horses with enterocolitis. Records of horses > 1-year-old presented to the Ontario Veterinary College between 2007 and 2019 that died or were euthanized were reviewed. Horses confirmed with enteritis and/or colitis on *post-mortem* examination were included.

A total of 196 cases fulfilled the inclusion criteria. Overall, 83% (162/196) cases were cultured for Salmonella and 8% (15/196) were positive. *Clostridium perfringens* and *Clostridioides difficile* cultures were performed on 77% (150/196) and 25% (48/196) of the cases, respectively. Toxin detection via ELISA was performed in 31% (*C. perfringens*) and 41% (*C. difficile*) of which 1.5% (3/196) and 5% (10/196) were positive, respectively. Out of the 24% (47/196) cases tested for *Neorickettsia risticii* PCR on feces collected *post-mortem*, 3% (6/196) were positive. The 6% (11/196) cases tested for Coronavirus PCR were negative. A median of 3 (range: 0 to 6) *post-mortem* tests to investigate the etiological agent were performed in each case. Etiological diagnosis was reached on 45% (88/196) of the cases. A total of 3% of the cases presented enteritis (6/196) and typhlitis (5/196) alone, whereas 53% (104/196) were classified as colitis. 12%

(23/196) had enterocolitis, 26% (51/196) were typhlocolitis, and 3% (7/196) were enterotyphlocolitis.

These results highlight the lack of standardization for etiological diagnosis of entero-typhlo-colitis and emphasize the importance to investigate other possible etiological pathogens causing colitis. It also demonstrates that the inflammation is not always limited to the colon.

E29

Effect of plasma administration on serum amyloid a in neonatal foals

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Serum amyloid A (SAA) is a non-specific marker of acute inflammation in equids and has been used to aid in the diagnosis and monitoring of treatment response in sick foals. Anecdotal evidence suggests that plasma transfusions increase plasma SAA concentrations ([SAA]) in healthy neonatal foals. This study aimed to determine whether plasma administration was associated with an increase in plasma [SAA] in healthy foals with adequate transfer of passive immunity (TPI) and foals with failure transfer of immunity (FTPI). This study was conducted using samples from 46 healthy neonatal foals. Foals were divided into 3 groups, adequate TPI (IgG > 800 mg/dL) transfused with hyperimmune plasma for prophylaxis against *R. equi* (*RE* group, n = 26), FTPI (IgG < 800 mg/dL) treated with hyperimmune plasma transfusion (FTPI group, n = 13), and a control group of healthy foals with adequate TPI (Control group, IgG > 800 mg/dL) that were not transfused (n = 7). Blood samples were collected before (t0, age: 6h to 19h of age) and after plasma transfusion (t1, age: 27 to 40h) and in healthy foals at time 0 (t0, age: 10h to 24h) and time 1 (t1, age: 32 to 47h). Plasma [SAA] was determined using the *Stablelab*® EQ-1 Handheld Reader. The foals were monitored throughout the first month of life for any illness or treatment. Steel-Dwass test was used to assess differences among groups or between time points. The age at t0 and t1 was similar in all groups (P > 0.05). Plasma [SAA] at t0 was similar between groups. No differences were detected in [SAA] between t0 and t1 in the control group (P = 0.401), but [SAA] increased significantly from t0 to t1 in both FTPI and *RE* groups (P < 0.001 and 0.048, respectively). None of the foals developed disease during the first 30 days of life. Comparison among the 3 groups of foals showed that plasma administration was associated with an increased plasma [SAA].

E30

Investigation of serum markers of hepatic fibrosis in horses

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Liver disease is encountered commonly in equine practice and is investigated using a combination of historical, clinical, serum

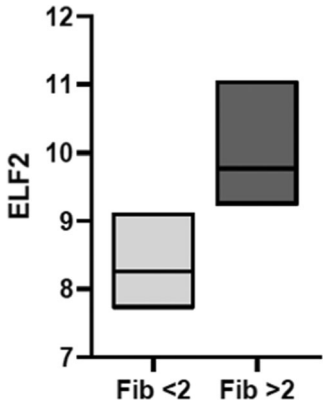

Fig. 1: Distribution of the calculated modified ELF2 score in groups H and C combined categorised with low or high fibrosis scores ($p < 0.001$).

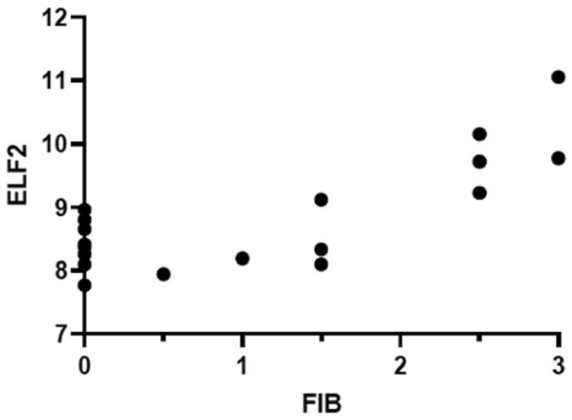

Fig. 2: Comparison of the modified ELF2 scores and the fibrosis scores for all individuals in groups H (n = 10) and C (n = 9) (r = 0.6, $p = 0.006$).

chemistry, ultrasound and histopathological data [1-3]. Collection and examination of liver biopsy specimens is probably the most reliable means of confirming the presence of liver disease as well as guiding therapeutic choices, offering a prognosis for recovery and monitoring progress [2]. Fibrosis is the single most concerning finding in liver biopsy specimens and is a useful prognostic marker [2]. Although historical safety concerns appear to have receded in recent years [4], biopsy is still a relatively underused methodology perhaps due to lack of clinical familiarity and also financial constraints in many cases. Studies in human patients have revealed several proxy markers for hepatic fibrosis measured in blood samples including hyaluronic acid (HA), type IV collagen 7S, platelet count, aspartate aminotransferase (AST)-to-alanine aminotransferase (ALT) ratio, and different scores obtained from algorithms based on these markers and the age, gender, levels of haptoglobin, α_2-macroglobulin, apolipoprotein A1, γ-glutamyl transpeptidase (GGT), bilirubin and/or cholesterol [5-8]. This study aimed to examine the usefulness of a combination test known as the Enhanced Liver Fibrosis (ELF) test based on measurement of 3 serum

analytes comprising HA, amino-terminal propeptide of type III procollagen (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP-1) [9].

Three groups of equids were included in the study. Groups H and D consisted of 10 horses and ponies (group H) and 10 donkeys (group D) that were found to have increased liver-derived serum enzymes and were subject to percutaneous liver biopsy. Group C consisted of 9 horses and ponies that had serum biochemistry testing performed for various clinical concerns and were found to have values for GGT, GLDH, AST and bile acids all to be within the laboratory reference intervals. All serum samples were promptly frozen at -20°C until assay on an ADVIA Centaur analyser (Siemens Healthineers Ltd, Camberley, UK). All samples were analyzed for concentrations of HA, PIIINP and TIMP-1. Measured values were compared between categories of group and also histopathologic fibrosis score [2] using the Mann-Whitney test, and the correlation between variables was tested using Spearman's product-moment test.

Median age was 12 years (range 4-33), breeds included Warmblood, Arab, Cob, Welsh, Connemara, and Shetland, as well as Donkeys. The histopathologic liver fibrosis scores ranged from 0/3 to 3/3 in group D (median 2.5/3) and from 0.5/3 to 3/3 (median 2/3) in group H, and were assumed to be 0/3 in group C. The HA and PIIINP values were obtained in all cases but the TIMP-1 values were undetectable except one donkey. Therefore, ELF scores could not be calculated and a novel ELF2 score was then calculated based on HA and PIIINP only (ELF2 score = 2.278 + 0.851 ln[HA] + 0.751 ln[PIINP]). Values of HA, PIIINP and ELF2 score were not significantly different between groups H and C. However, when all horses and ponies (groups H and C) were considered together and categorized according to histopathologic fibrosis score, HA, PIIINP and ELF2 scores were significantly greater in individuals with a fibrosis score > 2 compared with those with a fibrosis score < 2 (HA $p < 0.001$; PIIINP $p = 0.04$; ELF2 $p < 0.001$) (Fig.1). There were no significant differences observed with the data obtained from the donkeys with greater or lesser fibrosis scores. A significant correlation was found between fibrosis score and HA ($r = 0,54$, $p = 0.02$), PIIINP ($r = 0.46$, $p = 0.05$) and ELF2 score ($r = 0.60$, $p = 0.006$) for horses and ponies in groups C and H combined (Fig 2). No correlation was found in the data from the donkeys.

This is the first study investigating HA, PIIINP and ELF scores as diagnostic markers associated with liver fibrosis in horses, ponies, and donkeys. HA, PIIINP and the modified ELF2 score could be useful for prediction and monitoring of liver fibrosis in horses and ponies with elevated liver enzymes and functional parameters as an alternative to liver biopsy.

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E31

Syndecan-1 as a biomarker of endothelial glycocalx shedding in adult horses with sepsis

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Syndecan-1 (SYND1) is a principal component of the endothelial glycocalyx (EGX). Increasing evidence shows that elevated plasma SYND1 concentration is a sensitive biomarker for endothelial dysfunction in sepsis. Elevated plasma SYND1 concentrations result from pathological dismantling of the EGX during sepsis in other species. Loss of the normal EGX barrier causes increased vascular leakage, tissue edema, activated thrombosis, and microvascular dysfunction, hallmarks of reduced perfusion and organ failure in sepsis. Higher and persisting circulating levels of SYND1 are associated with unfavorable treatment outcomes and death. The purpose of this study was to investigate plasma SYND1 levels in equine patients and to explore the potential value of SYND1 as a predictive marker of vascular damage in septic adult horses. Plasma SYND1 concentrations were determined for horses admitted to the University of Missouri Veterinary Hospital between 2017-2019. Using clinical diagnosis and clinical laboratory data at the time of admission, horses were classified as either systemically healthy (Group 1), affected with nonseptic illness (Group 2), or likely septic (Group 3). Differentiation between septic and nonseptic individuals was further facilitated by determination of a Systemic Inflammatory Response Syndrome score for each horse. Soluble equine SYND1 was measured in frozen heparinized-plasma samples (archived

frozen at admission) using a commercially available species-specific ELISA kit. Statistical analysis entailed ANOVAs and assessment of linear regression (Stata SE 15.1). Results from this study indicate that plasma SYND1 concentrations were higher in septic patients ($p < 0.05$) vs nonseptic counterparts and that plasma SYND1 concentrations may serve as a biomarker for EGX degradation in septic horses.

E32

Treatment with withaferin a inhibits respiratory burst, adhesion, and chemotaxis by equine neutrophils

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Although neutrophils are central to the innate immune response to bacterial pathogens, dysregulation of neutrophil defense mechanisms can exacerbate disease pathology and contribute to morbidity and mortality. Withaferin A (WFA), a steroidal lactone derived from the *Withania somnifera* plant, has well-recognized anti-inflammatory properties, but direct effects of WFA on neutrophil function are unknown. We hypothesized that WFA would inhibit neutrophil effector functions and determined the effect of WFA on equine neutrophil respiratory burst, adhesion, and chemotaxis. Neutrophils isolated from healthy adult horses ($n = 4$-6) were pre-treated with varying WFA concentrations, vehicle control, or media for 30 minutes prior to stimulation. Production of reactive oxygen species in response to stimulation with granulocyte-macrophage colony-stimulating factor (GM-CSF)/lipopolysaccharide (LPS), phorbol 12-myristate 13-acetate (PMA), or insoluble immune complexes was detected via luminol-enhanced chemiluminescence. Calcein-AM-loaded neutrophils were used to determine adhesion to immune complexes immobilized to plastic and PMA- or interleukin-8 (IL-8)-induced adhesion to fetal bovine serum-coated plastic and to assess chemotaxis toward IL-8. Treatments were compared using one-way repeated measures ANOVA with Holm–Sidak multiple comparison testing. WFA significantly ($p < 0.05$) inhibited equine neutrophil respiratory burst, adhesion, and chemotaxis compared to vehicle control for all stimuli evaluated. Trypan blue exclusion and annexin V/propidium iodide data indicate that these concentration-dependent effects are not explained by decreased viability in WFA-treated neutrophils. Our ongoing research into the therapeutic potential of WFA for neutrophil-mediated diseases includes investigation of the molecular mechanism(s) for suppression of neutrophil functions and study of additional means for WFA to modulate neutrophilic inflammation.

E33

EHV-specific immune responses to an EHV 1 & 4 vaccine in horses

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To evaluate humoral and cellular immune responses to an inactivated equine herpesvirus (EHV) 1/4 vaccine, peripheral blood was collected from thirty-five horses, ages 3-26 years, on Days 0 (D0), 21 (D21), 42 (D42), 111 (D111), 201 (D201), and 229 (D229). After randomization based on pre-study EHV titers, Vetera® XP 1/4 (Boehringer Ingelheim) or saline was administered to twenty-eight and seven horses, respectively, on D0, D21, and D201. EHV 1/4 titers were measured, and gene expression after *in vitro* stimulation with Vetera® XP 1/4 vaccine was evaluated via RT-PCR. Data were natural-log transformed and analyzed using a linear mixed model (SAS 9.4) with $P < 0.05$ considered significant.

EHV-1 titers increased after D0 only in vaccinated horses (VH) and were higher than control horses (CH) on D42 and D229. EHV-4 titers shared similar results but did not reach significance.

After *in vitro* stimulation, VH had greater expression of IFNγ and TNFα on D21, IL-1β on D42, and IL-6 and IL-10 on D21 and D42 than CH. VH had greater CD4 expression but did not reach significance. Perforin, granzyme B, CD8, IL-2, and IL-18 expression after stimulation did not differ between groups. VH increased and/or maintained D0 IFNγ, IL-6, IL-1β, and TNFα expression. VH maintained D0 IL-10 expression through D42 before decreasing. CH decreased and/or maintained D0 IL-6, IL-10, IL-1β, and TNFα expression but IFNγ expression increased from D0 for D111-D229.

Vaccination increased EHV titers and influenced several *in vitro* immune responses. Additional research is needed to explore these findings.

E34

First single-cell gene expression analysis of equine bronchoalveolar cells

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The transcriptomic profile and cellular composition of a biological sample can be conjointly determined with single-cell mRNA sequencing (scRNA-seq). We applied scRNA-seq technology to cryopreserved equine bronchoalveolar lavage fluid (BALF) cells as proof of concept.

The BALF from three horses was frozen at -80°C until scRNA-seq analysis with Chromium Single Cell 3′ Solution (10X Genomics). The cDNA libraries underwent paired-end 2x150 bp sequencing. Mapping and quality analyses were performed with Cell Ranger 4.0. Seurat (R package) was used for post quality and clustering analysis.

We profiled 5,078 cells, with a median of 722 genes/cell. Unsupervised graph-based clustering identified 9 distinct cell clusters, grouped into 6 major cell types. Cluster 2 represented monocytes-macrophages based on CD163, APOE, MARCO and CD68 expression. A subpopulation of cluster 2 with highly expressed MHC-II genes (DRA, DRQ) but no CD163 expression was assigned to dendritic cells. Clusters 1, 3, 4, 5 and 7 represented T/NK cells based on GZMA, CCL5, CTSW, CD3E and CD3G expression. Cluster 6, highly expressing TG, CSF3R, TREM1 and RGS2, was labeled as neutrophils. Cluster 9 consisted in two distinct populations. One was identified as B cells based on MS4A1, CD79A, DRB and JCHAIN (immunoglobulin) expression. The other represented mast cells based on LTC4S, HPGDS and GCSAML expression. Cluster 8 (200 cells) represented dead or dying cells, as indicated by a high level of mitochondrial gene transcripts.

The scRNA-seq technology allows identification of major immune cell types in BALF after cryopreservation, thus allowing large-scale single-cell experiments to investigate equine respiratory conditions.

E35

Isolation of a novel species of neorickettsia that causes potomac horse fever

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Neorickettsia risticii is the current known cause of Potomac horse fever (PHF). There is evidence that PHF has been an endemic disease in southern Ontario since 1924. In recent years, equine practitioners have reported horses presenting with clinical signs of PHF that tested PCR negative, however, responded to IV oxytetracycline treatment. In order to investigate these cases, blood samples were collected for cell culture and molecular analysis of *Neorickettsia* species. Here we report the cell culture isolation of a new *Neorickettsia* species found in two locations in eastern Ontario, Canada in 2016 and 2017 from *N. risticii* PCR-negative horses with clinical signs of PHF. Gene sequences of 16S rRNA and the major surface antigen P51 of this new *Neorickettsia* species were distinct from those of all previously characterized *N. risticii* strains and *Neorickettsia* species, except for those from an uncharacterized *Neorickettsia* species culture isolated from a horse with PHF in Finley, Ohio in 1991. The new *Neorickettsia* species, nonetheless, had the characteristic intramolecular repeats within the surface antigen Ssa3, which were found in all sequenced Ssa3 of *N. risticii* strains. Experimental inoculation of two naïve ponies with the new *Neorickettsia* species produced severe and subclinical signs of PHF, respectively, and the bacteria were re-isolated from both of them, fulfilling Koch's postulates. Whole genome sequence analysis of the new *Neorickettsia* species revealed unique features of this bacterium compared with *N. risticii*. We propose to classify this new bacterium as *Neorickettsia findlayensis* sp. nov. This finding will increase clinician awareness, improve laboratory diagnosis and vaccine development, environmental risk assessment of PHF, and enhance our understanding of PHF pathogenesis and *Neorickettsia* spp. biology.

E36

Do veterinarians want increased reporting of equine strangles?

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Although equine strangles is reportable in all states, synchronous reporting of this disease does not occur across the country. States have variable regulations on reporting (actionable, notifiable, and monitored) and no mandatory comprehensive databases exist for tracking prevalence. We hypothesized that veterinarians would prefer increased synchronous reporting of the disease nationally. A questionnaire was disseminated via social media and email to veterinarians across the United States to solicit their opinions on reporting of strangles and factors influencing their opinion. A total of 250 veterinarians participated: 84 participants (34%) believed that strangles should continue to be nationally monitored and that individual states should have jurisdiction over laboratory confirmed positive cases; 58 (23.2%) believed strangles should become nationally monitored with mandatory notification of positive cases to a central forum; 24 participants (10.5%) thought strangles should become notifiable nationally; and 44 (19.2%) thought strangles should become notifiable and actionable. Veterinarians who already reported strangles cases were 87% more likely ($P = 0.054$) to want increased reporting, as did veterinarians who ranked strangles as "very important" or "important" relative to other infectious diseases (OR 3.77, $P = 0.037$). Veterinarians practicing in the southwest ($P = 0.01$) and west ($P = 0.04$) were significantly less likely than northeast practitioners to rank strangles of higher importance. Opinions on equine strangles and desire for increased reporting were varied in the sampled veterinary community.

E37

Efficacy of disinfection of endoscope contaminated by *Streptococcus equi* subspecies equi

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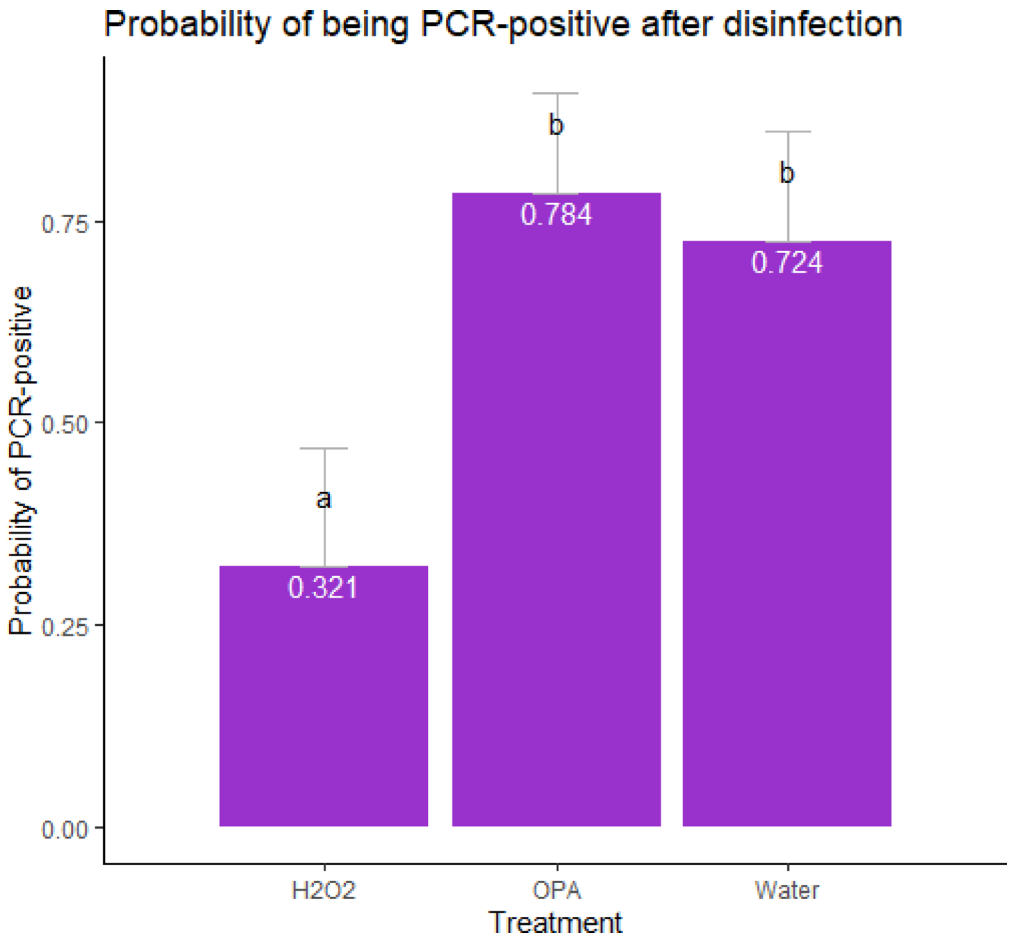
Disinfection of endoscopes is imperative when testing horses for *Streptococcus equi* subspecies *equi* (*S. equi*) to prevent spread or false diagnosis of the disease. The purpose of the study was to evaluate the efficacy of manual high-level disinfection between two commonly used disinfectants, 0.6% *ortho*-phthalaldehyde (OPA) versus 2%



accelerated hydrogen peroxide (H_2O_2) following endoscope contamination with standardized *S. equi* brain heart infusion (BHI) broth. Samples were collected for quantification *S. equi* real-time polymerase chain reaction (qPCR) and culture before and after disinfection. Endoscope disinfection using OPA (12 minutes), H_2O_2 (8 minutes), or water as a control (8 minutes) was performed according to manufacturer guidelines following standard enzymatic cleaning. Endoscopes were contaminated and disinfected 30 times for each group with a randomized block design (15 disinfections performed per day for 6 days). Personnel performing culture and qPCR were blinded to treatment. Using multivariable logistic regression model with endoscope and day as random effects, the risk of an endoscope being qPCR-positive following disinfection was determined.

All samples were culture-positive and qPCR-positive after contamination. All samples were culture-negative following disinfection. Following disinfection, 22/30 OPA, 10/30 H_2O_2, and 21/30 control samples were qPCR-positive. The disinfectant used had a significant effect on the probability of being qPCR-positive. Disinfection with H_2O_2 resulted in a significantly lower probability of being PCR-positive after disinfection (32%), compared to disinfection with OPA (78%) or water (72%). The H_2O2 disinfectant is the best product to decrease the probability of a false positive qPCR result, but false positives are still common.

E38

Effect of bi-weekly administration of diclazuril on antibody kinetics to *Sarcocystis neurona* in healthy horses

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Prevention of equine protozoal myeloencephalitis (EPM) represents an important challenge and its focus relies on wildlife management, risk-factor manipulation and use of antiprotozoal medication. A recent study showed that low-dose, bi-weekly administration of diclazuril (Protazil) produced steady-state plasma drug concentrations known to inhibit *Sarcocystis neurona*. The purpose of this study was to determine if bi-weekly administration of low-dose diclazuril selen would reduce seroprevalence and magnitude of titers to *S. neurona* in healthy adult horses naturally exposed to the apicomplexan protozoal parasite.

Twenty healthy adult horses were moved from a low-risk exposure to a farm with high exposure rate to *S. neurona* in their resident horse

population. The horses were randomly assigned to either a treatment or a control group. Treatment consisted in the administration of 0.5 mg/kg body weight of diclazuril pelleted top dress (Protazil) twice weekly (Monday and Thursday of every week) for 12 months. Prior to initiation of treatment and monthly thereafter, blood was collected for the detection of antibodies to *S. neurona* using an indirect fluorescence antibody test (SarcoFluor). Further, trough plasma diclazuril levels were determined every 60 days.

All 20 horses remained healthy during the entire study period. Seroprevalence (horses with titers ≥ 40) to *S. neurona* was similar between treatment and control groups at study commencement. Seroprevalence to *S. neurona* decreased initially in the treatment group to 30% at 30 days post-treatment commencement. This was followed by a slow increase in seroprevalence in the treatment group before reaching 100% in both groups by 90 days post-treatment commencement. The seroprevalence remained 100% in both groups from 90 to 360 study days. While titer distribution between the two groups was similar at study commencement, treated horses displayed significantly lower titers throughout the 12-month treatment period ($P < 0.05$). All treated study horses had detectable plasma trough diclazuril levels at the six time points and the levels were above the concentration known to inhibit *S. neurona* in vitro (0.01 μg/mL).

In conclusion, the administration of low-dose diclazuril (Protazil) pelleted top dress twice weekly was able to maintain lower titers to *S. neurona* in healthy adult horses naturally exposed to the protozoal parasite compared to untreated controls. Further, trough diclazuril levels were in excess of the minimal concentration known to inhibit *S. neurona*.

E39

A prospective study of serum amyloid a in relation to plasma administration in neonatal foals

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Limited information is available on the change in serum amyloid A (SAA) concentrations in normal neonatal foals receiving prophylactic intravenous plasma. This study evaluated the levels of SAA prior to, and following, administration of intravenous plasma 12 hours following birth.

A prospective study was conducted with 31 foals from a veterinary hospital in Texas in a single year. Enrolled foals were part of a foaling program, where a prophylactic plasma transfusion was conducted 12 hours after birth. Blood was collected for SAA measures using the StableLab EQ-1 SAA test (Epona Biotech) at birth and at hours 12 (prior to plasma), 13 (post-plasma), 24, 48, 72, and 96.

Eight of the foals were clinically ill prior to plasma administration, and 23 foals were clinically normal. The mean SAA at birth was 1 μg/mL, rose to 11 μg/mL at hour 12, and at hour 13 (post-plasma) was 155 μg/mL. At hour 13, 15/23 (65%) of normal foals and 5/8 (63%) of sick foals had an SAA value >100 μg/mL.

Transient but substantial increases in SAA following prophylactic administration were commonly observed in this study. These substantial increases in SAA are consistent with published recommendations for identifying an infection. Evaluators of neonatal foals for clinical disease in the field should be cognizant of the timing of blood sampling in relation to plasma administration.

E40

Minimally invasive removal of obstructive ureteral stones by lithotripsy in horses: Three cases

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Reports of minimally invasive approaches for removal of obstructive ureteral stones (UO) in horses are lacking. The objective of the study was to describe the technique required to access the upper urinary tract in horses by retrograde ureteroscopy accompanied by electrohydraulic (EH) or laser (holmium:Yag) lithotripsy.

A retrospective case series documenting minimally invasive ureteral stone removal by retrograde ureteroscopy and lithotripsy in three horses presented to the equine hospital (CHUV) was performed. Signalment, physical exam findings, laboratory and diagnostic imaging results, procedures, complications, and outcomes were all recorded

Three azotemic horses were diagnosed with UO by ultrasonography. Following coccygeal epidural and sedation, a 1.1m flexible endoscope with a 2mm diameter working channel was passed into the bladder through the vulva in a mare or through a perineal urethrostomy in geldings, the ureterovesical junction was identified, a guide wire was passed through the working channel of the scope into the distal ureter. The endoscope was then slid over the wire under continuous saline flush until the ureteral stone was identified. The wire was exchanged for a lithotripsy probe. Stone fragments were flushed and larger fragments withdrawn using forceps passed through the scope. Two horses underwent EH (one procedure in total for each patient) and the last one laser lithotripsy (three successive procedures in total). Azotemia improved in 2 patients. All patients were discharged from the hospital.

UO in horses can be successfully treated in a minimally invasive manner by lithotripsy. EH lithotripsy proved superior for stone fragmentation.

E41

Comparison of cerebrospinal fluid between three collection sites in adult equids with neurologic disease

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Cerebrospinal fluid (CSF) collection is described in three sites in the horse. This retrospective study compared CSF analysis of samples

obtained from the atlanto-occipital (AO), lumbosacral (LS), and atlanto-axial (AA) sites in 115 equids aged >1 year who were presented for neurologic evaluation. Samples were analyzed objectively (WBC count, RBC count, total protein) and subjectively by a pathologist. Results were assessed between groups, then in subsets based on survival to discharge and cytopathologic categorization.

When all samples were compared, RBC contamination was increased in the LS group ($p < 0.01$, RBC/μL_{LS} =32; RBC/μL_{AA} = 10; RBC/μL_{AO} = 3). In cytopathologically normal samples, RBC contamination was increased ($p < 0.01$) in LS samples (RBC/μL_{LS} = 15, RBC/$\mu L_{AA,AO}$ = 3). Elevated WBC concentration in the non-survival group ($p < 0.01$; WBC/$\mu L_{non\text{-}survival}$ = 2.5, WBC/$\mu L_{survival}$ = 1) prompted evaluation of the receiver operating characteristic (ROC) curve for WBC count to predict non-survival. The ROC had an area under the curve of 0.77 (95% CI, 0.62-0.92) and indicated that a cutoff value of 17 cells/μL maximized specificity (80%) and sensitivity (58%). Positive predictive value was 60% and negative predictive value was 78%. All reported concentrations are median values.

While there were differences in other evaluated parameters, values fell within accepted reference intervals. This supports the application of these reference intervals to fluid from all three sites. RBC contamination in LS samples may indicate that another site should be considered if hemodilution will compromise interpretation. Furthermore, abnormal CSF cytology may have prognostic value.

E42

Magnetic resonance imaging of the normal equine pituitary gland

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The objectives of this study were to describe the magnetic resonance imaging (MRI) appearance and size of presumed normal equine pituitary glands and to correlate pituitary size with brain size and patient weight. Inclusion criteria consisted of < 16-year-old adult horses over 300 kg with no evidence of PPID and no pituitary abnormalities identified with MRI, yielding 27 horses. Histology was available on seven pituitary glands; all were considered normal by a board-certified anatomic pathologist. Imaging features of the glands were evaluated on T2-weighted and pre- and post-contrast T1-weighted images. Pituitary length, width, height and brain height, width were measured by authors EP and KH on transverse and sagittal projections in post-contrast T1-weighted images. The mean ± sd pituitary gland width was 21.1 ± 2.4 mm, height 10.4 ± 1.9 mm, and length 24.5 ± 2.7 mm. There were no significant correlations using a Pearson correlation coefficient between pituitary and brain measurements, between pituitary measurements and body weight, and brain measurements and body weight. There was no statistical difference using an unpaired t-test ($p > 0.05$) in pituitary and brain measurements between necropsied and non-necropsied horses. All pituitaries were isointense to brain white matter on T1-weighted images. A focal T1 hyperintensity was present in the caudal aspect of the pituitary on pre-contrast T1-weighted images in 88% of horses. In conclusion, pituitary dimensions did not correlate with brain size and body weight or pituitary and brain size in horses. These findings provide a reference for MRI evaluation of the equine pituitary gland.

E43

Opioid-sparing sedation for atlantoaxial cerebrospinal fluid collection in standing horses

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Cerebrospinal fluid (CSF) collection from the atlantoaxial (C1-C2) space of standing horses is an underutilized procedure in equine field practice, in part due to the widely perceived need to employ morphine-containing sedation protocols to increase sedation depth and mitigate reaction to dural puncture. The purpose of this study was to compare opioid-sparing sedation protocols to the standard detomidine + morphine combination in standing horses undergoing C1-C2 CSF collection. In this randomized crossover study, six university-owned adult horses underwent four C1-C2 CSF collections at weekly intervals. At each timepoint, horses were randomly assigned one of four sedation protocols: detomidine 5mg + xylazine 100mg (DX), detomidine 5mg + detomidine 2mg (DD), detomidine 5mg alone (DO), or detomidine 5mg + morphine 30mg (DM). Each horse received all four sedation protocols throughout the study, and personnel performing CSF collections were blinded to treatments. Procedure time (time from skin puncture to successful CSF collection) and sedations scores were compared across treatment groups using the non-parametric Friedman test. Procedural success (obtaining CSF), and horses' reactions to dural puncture were also recorded. CSF was successfully collected in all horses at all timepoints. Procedure time was significantly lower (indicating faster CSF collection) when study horses were treated with DX, compared to DO ($p < 0.05$), but not DD or DM. Similarly, sedations scores were lower (less reaction to procedure) when horses were treated with DX or DM, compared to DO ($p < 0.05$). Reactions to dural puncture were noted in three of six horses in both the DO and DD treatment groups, and none of the horses in the DX and DM treatment groups. Opioid-sparing sedation protocols result in similar procedural efficiency and success when compared to morphine-containing protocols for performing C1C2 CSF collection in standing horses.

E44

Sensorineural auditory loss associated by intravenous administration of gentamicin in healthy adult horses

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The purpose of this study was to determine if daily intravenous administration of gentamicin for 7 days would affect auditory and vestibular function in healthy adult horses. Ten horses clinically healthy based on physical and neurological examination were selected. Gentamicin sulfate (6.6 mg/kg) was administered in the jugular vein alternating sides for 7 days. Renal function was assessed, and serum creatinine concentrations remained within reference range. Brainstem auditory evoked response (BAER) testing was performed under sedation (IV detomidine hydrochloride 0.01 to 0.02 mg/kg) prior to first, after last dose, and 28 days after last dose. Peaks latencies, amplitudes, and amplitude ratios were recorded. First BAER was used as baseline and compared with results from days 7 and 28. Bone conduction was performed to rule out a conduction disorder. All horses had normal BAER prior to the first dose of gentamicin administration. Seventy percent of the horses had auditory loss: complete bilateral (N = 1), complete unilateral (N = 2), and partial unilateral (N = 4). Auditory loss was based on complete absence of identifiable peaks, increased peak latency, and decreased peak amplitude. Absent bone conduction ruled out a conduction disorder and further supported sensorineural auditory loss in horses. Dysfunction was reversible in 4 of 7 horses. Further investigations are warranted to determine if the risk of auditory loss is dependent on age, breed, sex, different dosing protocols, and states of health or disease. The clinician must be aware of this potential risk when selecting antimicrobials protocols.

E45

The comparison of two glucose monitoring systems for use in horses

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Recent advances in near-continuous interstitial glucose monitoring allow for minimally invasive sample collection, reducing stress and discomfort to patients. The objectives of this study were to: 1) to determine the accuracy of two glucose monitoring systems (GMS) for use in horses compared to a point-of-care glucometer (POC) and a standard laboratory enzymatic chemistry method (CHEM), and 2) to determine the accuracy of the devices during dextrose-induced hyperglycemia using an oral glucose absorption test (OGAT). We hypothesized that GMS would provide acceptable agreement with POC and CHEM and provide detailed OGAT glucose curves. Eight clinically healthy adult horses were enrolled in the study. One of each GMS device (Dexcom G6 and Freestyle Libre 14-day) were placed on each horse and blood glucose was measured via POC and CHEM at 33 time points and compared to simultaneous GMS readings. OGAT was performed on day 2 with glucose determination following intragastric administration of 1g/kg 20% dextrose solution. Glucose concentrations were significantly correlated with one another between all devices on days 1-5. Acceptable agreement was observed between Dexcom G6 and Freestyle Libre 14-day when compared with CHEM on days 1, 3, 4, and 5 with a combined mean bias of 10.45 mg/dL and 1.53 mg/dL, respectively. During dextrose induced hyperglycemia on day 2, mean bias values for Dexcom G6 and Freestyle Libre 14-day showed good agreement (10.49 mg/dL and 0.34mg/dL, respectively). Data supports the use of the Dexcom G6 and Freestyle Libre 14-day interstitial glucose monitoring systems to estimate blood glucose concentrations in horses.

E46

Type of metabolic acidosis and its association with survival in critically Ill horses

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In neonatal human and equine medicine, various forms of metabolic acidosis (MA) have been associated with poor outcomes. This retrospective cohort study aimed to assess different types of MA in critically ill horses and to determine the effect of different etiologies of MA in in-hospital mortality. Medical records of 314 horses admitted for acute diarrhea (n=158) or acute abdominal pain that underwent exploratory laparotomy (n= 156) were selected.

Metabolic acidosis was classified based on the predominant anion contributing to most of the acidosis: no MA (standard bases excess [SBE] ≥ 1 mEq/l); lactic acidosis (LA; L-lactate accounted for > 50% of SBE); unmeasured strong ions (USI) acidosis (USI accounted for > 50% of SBE); and hyperchloremic acidosis (SBE < -2 mEq/l not explained by L-lactate or USI). Mortality was analyzed with logistic regression models with the type of MA (independent variable), and age, disease group, sex, and breed as covariables, and their two-way interactions with MA. The prevalence of MA (pH < 7.32) was 32% (hyperchloremic acidosis: 11%; LA: 17.5%; USI 3.5%) and the overall mortality was 30%.

The odds of dying were greater in horses diagnosed with LA than in horses with hyperchloremic acidosis (OR: 3.29, 95% CI: 1.21 – 8.93, $P = 0.02$) or horses without acidosis (OR: 1.90, 95% CI: 1.02 – 3.56, $P = 0.04$). Differences between other MA categories were not statistically different.

Metabolic acidosis caused by lactic acid appears to be associated with a worse prognosis when compared with horses with hyperchloremic acidosis or horses with normal pH.

E47

Effects of vaccination with a commercially available xenogenic DNA vaccine in 15 horses with melanoma

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Equine melanomas are a common cutaneous tumor of horses. They often begin as benign lesions and, if left untreated, can lead to local invasion and widespread metastasis. Available treatment options are inconsistently effective. A xenogenic DNA vaccine encoding human tyrosinase is FDA-approved for use in dogs and has been used off-label in horses with melanoma for approximately 6 years. The objectives of this retrospective study were to evaluate 15 horses who presented to The Ohio State University between 2014 to 2020 for treatment of melanoma and report the rate of tumor regression following delivery of the manufacturer's vaccination protocol. Horses included in this study were 12 +/- 5.5 SD years old and of a variety of breeds (Arabian, Morgan, Irish Sport Horse, Thoroughbred, Andalusian, American Paint Horse, Warmblood, American Quarter Horse). Average number of doses was 5.8 +/- 4.7 SD per horse. Tumor locations included the tail base (86.6%), sheath (40%), parotid region (33.3%), and body (33.3%); other locations included ear, penis, vulva, lip commissures, jugular groove, rectum, and anus. 46.6% of horses showed no detectable response to treatment; 60% had partial or complete regression; 33.3% developed new tumors; and 6.6% displayed tumor growth. 60% of horses received adjunctive treatments, including surgical excision, cimetidine, cryotherapy, and radiation. Two transient (< 48 h) local reactions were reported. These results support further evaluation of this therapy as an adjunct for management of melanoma in grey horses.

E48

Survival proportions and risk factors for non-survival in hospitalized foals from Ontario, Canada

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A survival proportion of 50 to 65% for hospitalized foals was reported in studies conducted 30 years ago in Ontario, Canada. In those studies, alterations in the anion gap (AG) and $PaCO_2$ in those foals were associated with an increased risk of non-survival. The aim of this retrospective study was to report the current survival proportions of hospitalized neonatal foals from Ontario, Canada, and the risk factors associated with non-survival. One-hundred and two neonatal foals < 7 days of age admitted between 2016 and 2020 to a referral center in Ontario, Canada, were included. A forward stepwise logistic regression analysis was conducted to assess potential predictor variables and non-survival of the foals during hospitalization.

Overall, the survival proportion was 83% (85/102). Variables significantly associated with mortality in the univariate analysis included presence of systemic inflammatory response syndrome (SIRS), pneumonia, blood pH, base excess (BE), AG, L-lactate and total plasma protein concentrations. Multivariate model revealed that foals with pneumonia (Odds Ratio (OR): 16.0; 95% Confidence Interval (CI): 3.4 to 76.0) and high lactate concentrations (OR:1.3; 95% CI: 1.1 to 1.5) at admission were more likely to die or be euthanized.

The survival proportion of hospitalized foals in Ontario, Canada increased markedly over the last 30 years. However, acid-base imbalances, particularly metabolic acidosis, continue to be associated with a higher risk of non-survival.

E49

Pharmacokinetics and antipyretic efficacy of acetaminophen in adult horses with experimentally induced endotoxemia

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Acetaminophen (APAP, INN: paracetamol) is the most common first line antipyretic in humans and has been shown to be safe following multiple doses in healthy adult horses. This study evaluated the pharmacokinetics and antipyretic effects of acetaminophen in eight adult horses with experimentally induced endotoxemia. The study design was a randomized 3-way crossover with a 30-day washout period between trials. Each horse was administered lipopolysaccharide (LPS, *E. Coli* 055:b5) at 35 ng/kg IV and received one of the following oral treatments two hours following LPS administration: APAP (30 mg/kg), flunixin meglumine (FLU; 1.1 mg/kg), or placebo (PLAC; corn syrup). Serial physical exams were performed, and blood samples collected for determination of plasma acetaminophen concentrations using LC-MS/MS for 24 hours post-treatment. Noncompartmental analysis was used to determine APAP pharmacokinetics, and treatment effects on temperature were determined via a generalized estimating equation model, taking into account the significant effect of treatment order.

All horses obtained a minimum 2° F elevation from baseline rectal temperature by 3 hours post-LPS administration. Plasma APAP concentrations reached peak average concentrations (C_{max}) of 14.2 μg/mL within 0.72 hours of administration. The mean elimination half-life was 3.12 hr and the mean area under the curve to infinity ($AUC_{0-\infty}$) was 62.8 hr*μg/mL. Rectal temperatures decreased significantly ($p < 0.05$) with APAP and FLU compared to PLAC at 4- and 6-hours post-treatment.

The pharmacokinetics of APAP reported here demonstrate a lower C_{max} and $AUC_{0-\infty}$, when corrected for dose, compared to previously published reports of APAP in healthy adult horses. Additionally, APAP is superior to PLAC and non-inferior to FLU as an antipyretic. Acetaminophen is a suitable therapeutic option in the treatment of fever in horses.

E50

Pharmacokinetics of oral and intravenous metoprolol tartrate in clinically healthy horses

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Cardiac drugs with defined pharmacological parameters in horses are limited. The pharmacokinetics of metoprolol, a ß-1 adrenergic antagonist, have not been evaluated in the horse. The objective of this study was to characterize the pharmacokinetic properties and cardiovascular effects of intravenous and oral metoprolol tartrate in healthy adult horses. In a 2-period randomized cross-over design with 14-day washout period, metoprolol tartrate (MET) was administered IV (0.04 mg/kg) and PO (6 mg/kg) once to 6 healthy adult horses. Horses were monitored via continuous telemetry and indirect blood pressure (NIBP). Blood samples were serially collected for 72 hours post-administration and concentrations were determined by LC/MS. Pharmacokinetics were modeled using a 3-compartment model and non-linear least squares regression. The average metoprolol concentration was 112 ± 58 ng/mL in samples collected 1 minute (0.0167 hours) following a bolus IV administration. The maximum concentration (C_{max}) after PO administration was 2548 ± 1083 ng/mL at 0.5 (0.25 - 0.5) hours. The oral bioavailability was 60.2% with a volume of distribution of 0.53 L/kg, clearance of 15.24 mL/kg/min, and a mean elimination half-life of 36.9 minutes. No significant effects of IV or PO metoprolol were noted on electrocardiographic parameters, cardiac rhythm, or NIBP. Diaphoresis was the most common side effect, noted in 6/6 horses following PO and 3/6 horses following IV administration. Based on this study, intravenous and oral administration of metoprolol tartrate appear to be safe in healthy adult horses. The doses used in this study achieve plasma concentrations reported to achieve maximal ß-blockade in humans.

E51

Pharmacokinetics of thiamine hydrochloride (vitamin B1) in horses after administration of three single intravenous doses

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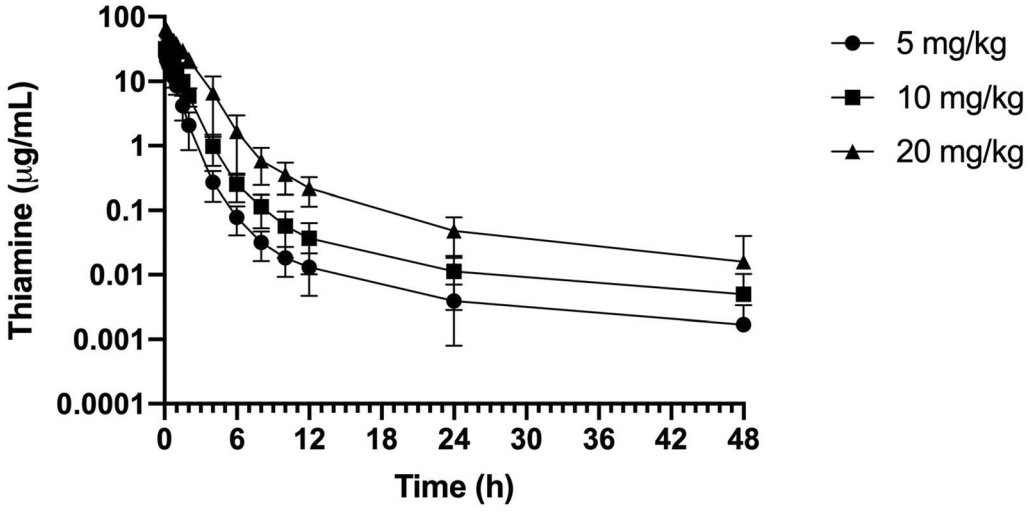
Sepsis is a common cause of morbidity and mortality in neonatal and adult horses. Thiamine deficiency is common in septic humans and is associated with refractory lactic acidosis and death. Thiamine supplementation improves neutrophil function in sheep and reduces the risk of renal damage in septic humans. Furthermore, intravenous (IV) thiamine administration in human septic patients is associated with improved lactate clearance and survival compared to controls. Thiamine hydrochloride (HCl) has been used as adjunctive treatment for several conditions in the horse, including neurological and renal



	5 mg/kg	10 mg/kg	20 mg/kg
λ_z (h^{-1})	0.905 ± 0.055	0.774 ± 0.063	0.632 ± 0.094
$t_{1/2}$ (h)	0.77 ± 0.05	0.9 ± 0.07	1.12 ± 0.18
C_0 (µg/mL)	29.81 ± 4.26	34.4 ± 3.72	69.38 ± 7.61
AUC_{obs} (h*mg/mL)	23.82 ± 4.97	42.82 ± 8.71	123.64 ± 26.85
Cl (mL/kg/h)	218.4 ± 46.4	242.3 ± 48.9	167.9 ± 32.4
V_{ss} (mL/kg)	212.7 ± 31.4	325.8 ± 50.7	317 ± 52.1

diseases, but specific therapeutic effects have not been determined. In addition, an appropriate dose of thiamine HCl for use in horses has not been established. The purpose of this study was to evaluate the single dose pharmacokinetic profile of thiamine HCl in healthy adult horses after administration at three IV doses.

A randomized cross-over study was done with a one-week washout period between each of three dosages. Nine adult horses from a university teaching herd were administered 5, 10 and 20 mg/kg thiamine HCl IV with dosages chosen based on extrapolation from large animal case reports and human sepsis studies. Heparinized blood samples were collected immediately prior to drug administration and serially over the following 48 hours. Plasma drug concentrations were quantified by high performance liquid chromatography and mass spectrometry. Non-compartmental pharmacokinetic analysis was performed for each dosage level. To determine whether thiamine is eliminated in a linear fashion in the horse or is affected by dosage, elimination rate (l_z) and clearance (Cl) were each compared between dosage levels using a repeated-measures ANOVA and Tukey's *post hoc* test for multiple comparisons.

Concentration-time curves for IV thiamine HCl administration are presented in **Figure 1** and pharmacokinetic parameters are presented in **Table 1**. In general, IV thiamine HCl administration resulted in supraphysiologic plasma concentrations with a relatively short half-life (0.77 – 1.12 h). Elimination rate significantly increased with dosage while Cl significantly decreased indicating the presence of non-linear elimination of thiamine in horses. This is further supported by non-linear increases in area under the curve with increasing dosage.

This is the first study to document the pharmacokinetics of IV thiamine HCl administration in healthy horses. Non-linear kinetics were observed suggesting saturation of elimination processes, likely renal excretion. Thus, kinetics of thiamine HCl at higher dosages (> 20 mg/kg) cannot be accurately predicted. Similarly, concentrations during chronic therapy may be difficult to predict, although thiamine's short half-life makes significant drug accumulation unlikely with intermittent dosing. Future pharmacodynamic studies are needed to identify therapeutic plasma concentrations so rational dosing regimens can be established. Subsequent studies to investigate the effects of thiamine HCl administration in septic horses can then be pursued.

E52

Potassium penicillin and gentamicin pharmacokinetics in conscious and anesthetized horses

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Potassium penicillin (PEN) and gentamicin (GENT) combinations are frequently administered to horses for the treatment of various diseases as well as perioperatively for emergency and elective orthopedic and soft tissue surgeries. The pharmacokinetics of these drugs when co-administered and when given to animals undergoing anesthesia have not been published. Therefore, the aims of this study were to determine the pharmacokinetics of PEN/GENT when co-administered to conscious and anesthetized horses, and to determine synovial fluid concentrations under each circumstance. Six healthy adult horses were administered PEN (22,000 IU/kg IV over 5 minutes) followed by 12 mL of heparinized saline, then GENT (6.6 mg/kg IV bolus) through an IV catheter on day 1. After GENT administration, plasma samples were collected over a 6-hour period and synovial fluid was collected from the left and right intercarpal joints at 30 min and 6 h respectively. After at least a 48-hour washout period, drug administration and sample collection protocols were repeated following induction of anesthesia using xylazine/ketamine and maintenance on isoflurane gas. Drug concentrations were determined using ultra-pressure liquid chromatography with mass spectrometry. A 2-compartment model was used to determine pharmacokinetics and differences were determined between conscious and anesthetized horses using paired t-tests (significance $p < 0.05$). PEN had a significantly higher minimum concentration (0.44 vs 0.11 μg/mL), longer half-life (71 vs 59 min) and slower clearance (3.41 vs 5.1 mL/kg/min) in anesthetized horses. PEN concentrations remained above the breakpoint MIC (0.5 μg/mL) for 332 min in anesthetized vs 199 min in conscious horses. GENT, had a significantly higher minimum concentration (3 vs 1.9 μg/mL), longer half-life (149 vs 109 min) and slower clearance (1.18 vs 1.48 mL/kg/min) in anesthetized horses. All horses reached GENT concentrations ten times higher than the breakpoint MIC (2 μg/mL) and maintained those concentrations for 58 vs 59 min in anesthetized and conscious states, respectively. Synovial fluid concentrations were significantly higher in conscious horses at 30 min for PEN and 30 min and 6 h for GENT. The results of this study demonstrate differences in plasma and synovial fluid concentrations of co-administered PEN/GENT between conscious and anesthetized healthy horses.

E53

Inhaled corticosteroids influence pulmonary microbiome in severe equine asthma

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Inhaled corticosteroids (ICs) affect the respiratory microbiome, but it remains difficult to separate the immunomodulatory effects of ICs from their indirect effects via improvement of lung function and ventilation. Our objective was to determine if ICs alter the lung microbiome independently from their effects on lung function. To do so, we treated horses with severe asthma with either bronchodilators (long-acting β-agonist (LABA)) alone or in combination with ICs.

Twelve horses in exacerbation were randomly assigned to receive LABA (salmeterol, 250 μg TID) or ICs/LABA (fluticasone/salmeterol, 2500/250 μg BID) by inhalation for 2 weeks. Lung function and bronchoalveolar lavages (BAL) were performed before and after

treatment. 16S rRNA gene quantification and sequencing were performed on BAL fluid, using ddPCR and the Illumina MiSeq platform. Data were processed using the software package mothur v.1.44.2.

Lung function improved with both treatments ($p < 0.05$). The relative abundance of *Bacteroidetes*, *Moraxellaceae*, and *Acinetobacter* increased with ICs/LABA, while the *Actinobacteria* phylum decreased with LABA ($p < 0.05$ for all). Beta-diversity differed from baseline after treatment only in the LABA group ($p < 0.05$, AMOVA). Alpha-diversity indices did not vary significantly with treatment but pulmonary bacterial loads decreased significantly only with LABA.

The pulmonary microbiome is influenced by treatment. The differences observe between the ICs/LABA and LABA groups suggest that the changes in bacterial communities are not only due to improved ventilation, but it is too early to determine if the effects of ICs are positive or detrimental to the lung environment.

E54

Oscillometry bronchodilator response does not differentiate horses with severe asthma in remission and healthy controls

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Oscillometry (OS) is an effortless method that allows the evaluation of lung function in unsedated and untrained horses. However, OS lacks sensitivity in horses with severe equine asthma (SEA) in a remission state. In humans, the assessment of a bronchodilation response by OS is useful to differentiate asthmatics from healthy subjects. The objective of the study was therefore to determine whether the response to an inhaled bronchodilator improves the ability of OS to detect subclinical airway obstruction in horses.

Eleven horses with SEA in remission and 10 university-owned horses without respiratory signs were studied. Oscillometry was performed at baseline, and 8 (T8) and 30 minutes (T30) after the inhalation of a β_2-adrenergic bronchodilator (salbutamol; 2 µg/kg) using the Aerohippus mask. Resistance (R) and reactance (X) from 2 to 10 Hz, resistance ratios (R2/R5, R3/R5, R3/R7 and R5/R10), and the proportional or absolute variation of these parameters after salbutamol inhalation were compared. Data were analyzed with linear mixed models and multiple comparison tests performed with Bonferroni corrections.

A significant effect of the bronchodilator was observed at T8 for R3/R7 (mean difference T0-T8 ± SD: 0.07 ± .02, $p = 0.006$), R5/R10 (0.1 ± 0.04, $p = 0.006$), X2 (-0.006 ± 0.002, $p = 0.006$), X3 (-0.007 ± 0.002, $p = 0.004$) and X5 (-0.009 ± 0.003, $p = 0.004$) and at T30 for R3/R7 (0.07 ± 0.02, $p = 0.006$) in the remission group. In the control group, an effect of salbutamol was present at T30 for R3/R5 (0.05 ± 0.01, $p = 0.002$), R3/R7 (0.1 ± 0.03, $p < 0.001$) and X5 (-0.01 ± 0.003, $p = 0.001$). OS parameters or their variation were not different between SEA horses in remission and control horses at any time points.

The results of this study indicate that horses with SEA in remission could not be distinguished from control horses by OS alone or combined with a bronchodilator response.

E55

Bronchial smooth muscle remodeling in mild and moderate equine asthma

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Airway smooth muscle (ASM) remodeling in severe equine asthma includes both hyperplasia and changes in contractility. However, ASM changes have not been studied in milder forms of the disease.

The objective of this study is to investigate bronchial smooth muscle remodeling in horses with mild and moderate asthma.

The endobronchial biopsies from 18 horses with mild and moderate asthma referred to the Equine Hospital of the University of Montreal and from 7 healthy age matched control horses were studied. The diagnosis was based on clinical signs and bronchoalveolar lavage fluid cytology. ASM cell proliferation was measured by quantifying the expression of the proliferating cell nuclear antigen (PCNA) employing immunohistochemistry and histomorphometry. The expression of the (+)insert smooth muscle myosin heavy chain (SMMHC) isoform, an hypercontractile protein, was assessed by RT-qPCR.

The (+)insert isoform in ASM was overexpressed in asthmatic horses compared to controls ($p = 0.02$). While there were no differences between groups in the proliferation of ASM cells ($p = 0.37$), the percentage of proliferating myocytes was correlated to pulmonary neutrophilic inflammation and to the expression of the (+)insert SMMHC isoform in asthmatic horses ($p = 0.01$, $r = 0.80$ and $p = 0.03$, $r = 0.66$, respectively), but not in healthy controls ($p = 0.99$, $r = -0.05$ and $p = 0.35$, $r = -0.60$).

These results confirm the presence of bronchial smooth muscle remodeling in mild forms of equine asthma and pave the way for the development of biomarkers to measure asthma progression and response to therapy.

E56

Evaluation of the safety of ciclesonide delivered via SoftMist™ inhaler in horses

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Ciclesonide is a novel glucocorticoid pro-drug with pharmacokinetic and pharmacodynamic profiles that should significantly reduce the potential for systemic effects after inhalative administration. The objective of this study was to evaluate the safety and local tolerance of ciclesonide administered via a Soft Mist™ inhaler to horses.

Thirty-two healthy horses (24 average size with maximum body weight 550 kg and 8 light weight horses with maximum body weight 300 kg) were enrolled in a placebo-controlled, randomized, blinded study with four treatment groups (0X, 1X, 2X, and 3X) using a parallel group design. Margin of safety and local tolerance was evaluated

through physical examinations, water consumption, feed consumption, clinical pathology, fecal analysis, nasal fungal culture and gross and histopathologic examination at the terminal necropsy.

All horses completed the study with minimal clinical findings. Mild nasal discharge was observed in all treatment groups including placebo and with no dose-dependent effect. No clinically significant drug-associated changes in individual animal health occurred at any dosage. No statistically significant differences were observed for clinical pathology parameters, most notably there was no clinical or statistically significant change in serum cortisol in any group.

Based on the results of this study, ciclesonide administered via Soft Mist™ inhalation up to three times the maximum recommended dose for 30 consecutive days is well tolerated and does not result in statistically significant decreases in serum cortisol. The overall lack of cortisol suppression suggests an improved safety profile over other known systemic and inhalant steroid treatments for horses.

E57

Effect of treatment with excede and azithromycin on culture of transtracheal washes from healthy foals

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Alterations of the respiratory tract microbiome have been associated with disease states in human patients and adult horses. However, the effects of antimicrobial agents on the respiratory microbiome of foals have not been described. The goal of this study was to determine the effect of treatment with two commonly used antimicrobial agents on aerobic bacteria cultured from transtracheal wash (TTW) samples. Twenty-four healthy four to six- week-old Quarter horse foals were randomly assigned to one of three treatment groups: 1) Control (no treatment), 2) Excede® (ceftiofur crystalline free acid, 6.6 mg/kg IM q96 hr for 2 doses), and 3) azithromycin (10 mg/kg PO q24 hr for 7 doses). Transtracheal wash samples were collected on days 0, 3, 7, and 35, and aerobic bacterial culture was performed using standard methods. A total of 27 bacterial genera belonging to 4 phyla were observed in the TTW samples. Independent of the treatment and collection time point, bacterial species belonged predominantly to Firmicutes phylum, followed by Proteobacteria, Actinobacteria and Bacteroidetes phyla. At the genus level, only 4 genera were present in every experimental condition; *Streptococcus* (Firmicutes), *Staphylococcus* (Firmicutes), *Rhodococcus* (Actinobacteria) and *Actinobacillus* (Proteobacteria). To determine changes in the genera over time for each treatment, a mixed-effects model with bacterial genus and time as fixed effects and foal as random effect was used. Post hoc Sidak's multiple comparisons test was then performed and significance was set at $P < 0.05$. Azithromycin decreased the number of *Actinobacillus spp.* and significantly decreased the number of *Streptococcus spp.* recovered on day 3 after treatment, while the number of *Rhodococcus spp.* found in the TTW significantly increased. This effect was also observed on day 7 after treatment but returned to baseline on day 35 (post- treatment). Interestingly, *R. equi* was the only species of *Rhodococcus* present in the sample. Excede® significantly decreased *Actinobacillus spp.* on day 7 but the genus returned to baseline by day 35. In conclusion, antimicrobial administration resulted in transient alterations in the aerobic bacteria cultured from TTW samples of healthy foal.

E58

Effect of treatment with excede and azithromycin on the respiratory microbiota of healthy foals

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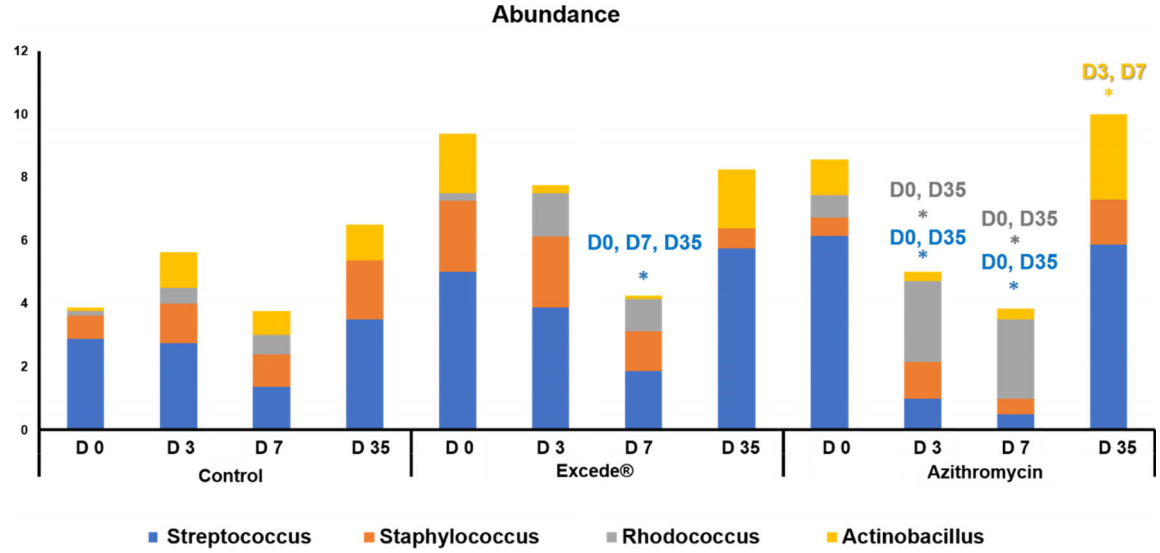

Figure 1. Abundance of Streptococcus, Staphylococcus, Rhodococcus and Actinobacillus species present in aerobic cultures of transtracheal washes from healthy foals after treatment with Excede or azithromycin. * indicated significant differences in abundances between days within the same treatment group ($p<0.05$).

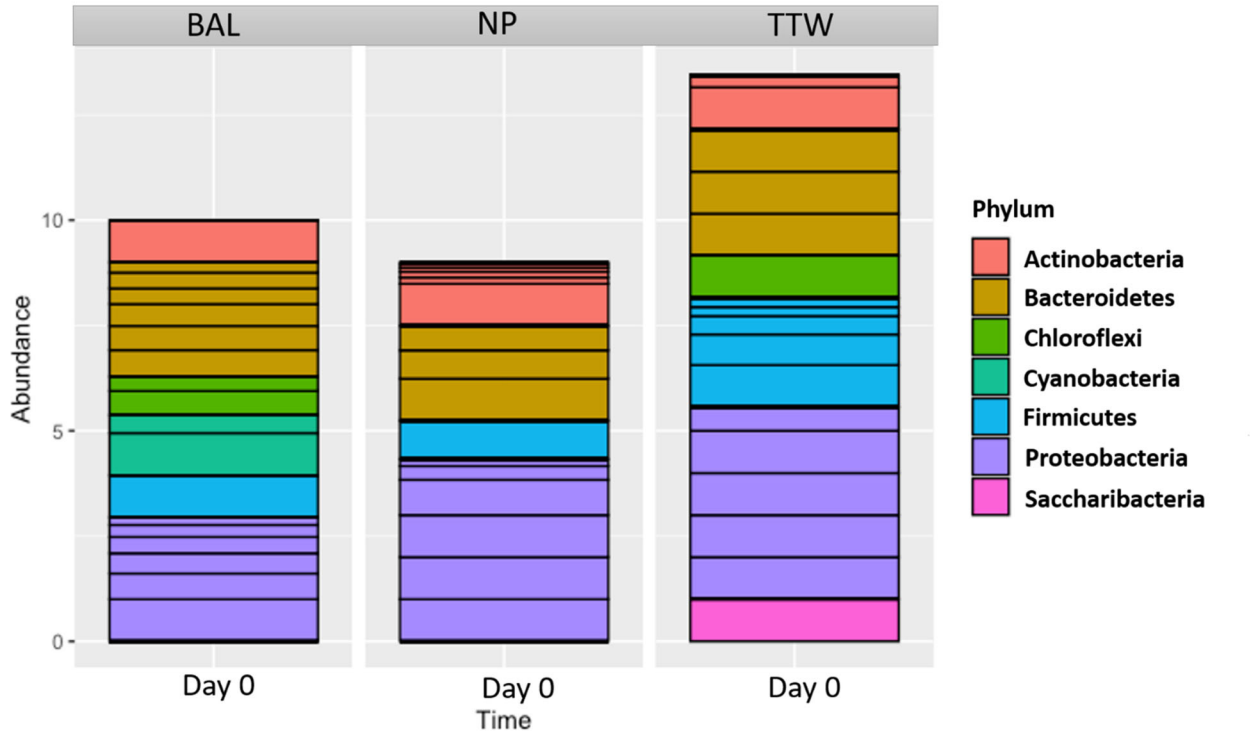

Figure 1. Relative abundance of predominant phyla in bronchoalveolar lavage (BAL), nasopharyngeal (NP), and transtracheal wash (TTW) samples from healthy foals prior to antibiotic treatment.

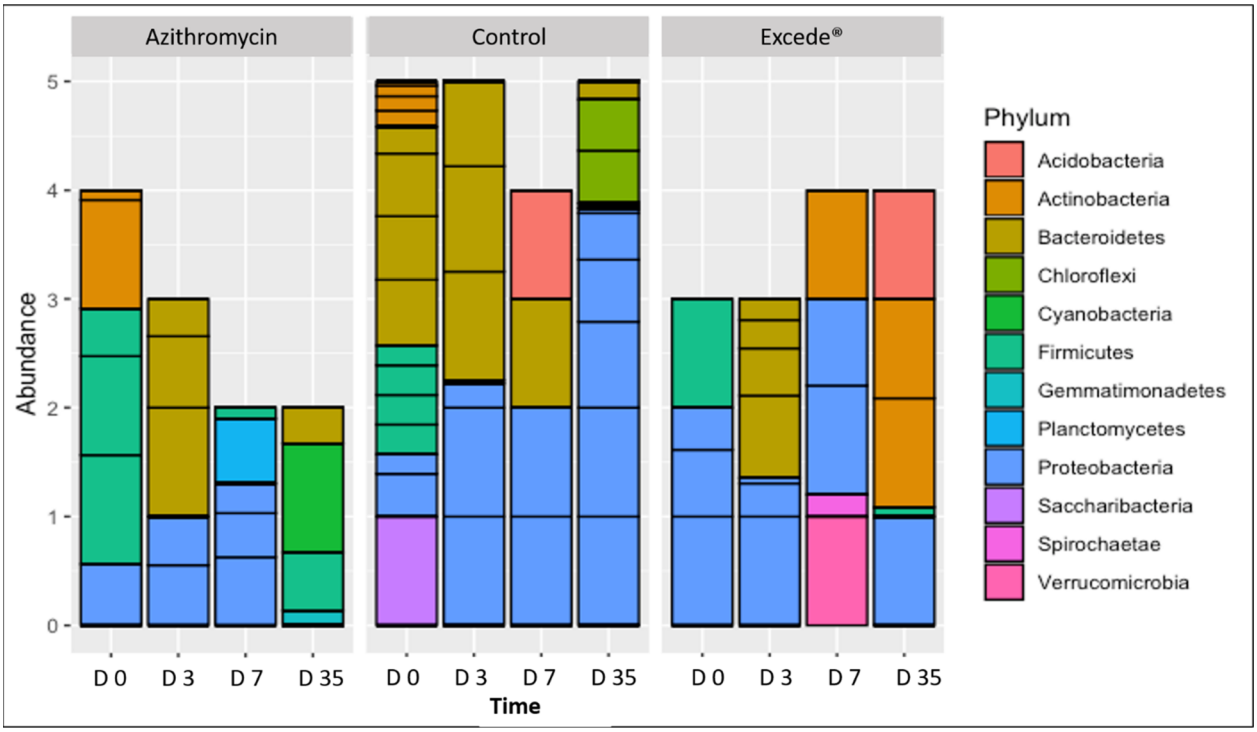

Figure 2. Relative abundance of predominant phyla in transtracheal wash samples from healthy foals treated with azithromycin and Excede, on Days 0, 3, 7, and 35.

Antimicrobial treatment affects the respiratory microbiota of children and has been associated with detrimental health effects long- term. However, the effects of antimicrobial treatment on the respiratory microbiome of foals have not been described. The goal of this study was to determine the effects of two antimicrobial agents on the respiratory microbiota of foals. Twenty-four healthy four to six- week-old

Quarter horse foals were randomly assigned to one of three treatment groups: 1) Control (no treatment), 2) Excede® (ceftiofur crystalline-free acid, 6.6 mg/kg IM q96 hr for 2 doses), and 3) azithromycin (10 mg/kg PO q24 hr for 7 doses). Nasopharyngeal swab (NP), transtracheal wash (TTW), and bronchoalveolar lavage (BAL) samples were collected on days 0, 3, 7, and 35. Bacterial microbiota were analyzed using 16S amplification (V4 region) and the Illumina MiSeq platform. Statistical analyses were performed using linear mixed-effects for α diversity metrics (Shannon diversity measure) in R package nlme (version 3.1.13770). Significance was set at $P < 0.05$. Low biomass and inefficient amplification were encountered in all samples. Consistent with reports in adult horses, bacteria from all three sites were predominantly from four common phyla; Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria at day 0 (Fig. 1), and 21 genera. Shannon indices indicated that TTW samples were significantly ($P< 0.05$) richer in bacterial communities than NP and BAL samples on day 0. However, this difference in richness could be because TTW samples generated the highest number of reads. Alterations in phylum level abundances are shown in Fig. 2. Overall, assessment of respiratory microbiota was complicated by low biomass and PCR inhibition. Phylum level differences were apparent after treatment with Excede® and azithromycin.

E59

Equine asthma management: Survey of horse owners demonstrates interest in mobile phone app

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Nonadherence to medical therapy for asthma represents a significant problem in human medicine, leading to increased asthma exacerbations and emergency room visits. Mobile phone applications have been successfully employed to increase asthma medication adherence in human patients. Our long- term goal is to design and develop a mobile phone app that will assist horse owners in adhering to Equine Asthma control measures, provide educational information to owners about Equine Asthma, and be used as a research tool. We surveyed owners of horses with respiratory disease to better understand treatments and environmental measures they use, and to gauge interest in an Equine Asthma management app. A Qualtrics survey was distributed using social media and the ACVIM listserve. A total of 195 owners from 34 US States and 12 countries in North America, Europe, and Australia completed the survey. Seventy-seven percent reported treating their horse for Equine Asthma. Horse performance was affected by the respiratory condition 86% of the time. Although 77% of owners reported abnormal breathing at rest in their horses, only 19% were soaking their horses' hay. Thirty-nine percent of owners had administered inhaled or oral steroids, 33% had administered inhaled or oral bronchodilators, and 17% had administered antibiotics. Of horses treated for asthma, 11% experienced a full recovery with no recurrence of symptoms. Seventy-three percent of respondents expressed interest in a mobile app to help manage Equine Asthma in their horses. In conclusion, survey results demonstrate interest in and need for a mobile Equine Asthma management app

E60

Effects of soaked hay on lung function and inflammation in horses with severe asthma

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Reducing inhaled dust particles improves lung function of horses with severe asthma. Soaked hay is commonly used for this purpose by owners, but its efficacy in improving respiratory function has not been documented. The objectives were to measure the effects of soaked hay in horses with severe asthma, and to compare them to those obtained with an alfalfa pellet regimen.

Ten asthmatic horses in exacerbation were housed indoors and fed soaked hay (45- min immersion, $n = 5$) or pellets ($n = 5$). Clinical scores were recorded weekly and pulmonary function measured before and after 2, 4 and 6 weeks. Tracheal mucus scores and cytologic evaluation of bronchoalveolar lavage fluid were performed before and after 6 weeks.

Clinical scores decreased in both groups over time ($p < 0.005$). Compared to exacerbation, lung resistance was significantly lower at 2, 4 and 6 weeks with soaked hay, and at 4 weeks with pellets ($p < 0.01$). Mucus scores decreased significantly with soaked hay, while pulmonary neutrophilia decreased significantly only with the pellet regimen ($p < 0.05$ for both).

Soaked hay improves clinical scores, lung function and mucus scores of horses with severe asthma. The strict protocol for soaking and discarding dried-out hay in this study could however be considered too great an inconvenience in some stables. Response to soaked hay or pellets could also differ if only a few horses in a barn are on a dust-reduction program.

F01

Gastrointestinal foreign bodies in pet pigs: 17 cases

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The aim of this retrospective study was to describe the clinicopathologic and diagnostic imaging findings, treatments, and outcome (survival) of 17 pet pigs diagnosed with gastrointestinal foreign bodies (FB). Medical records of pet pigs admitted to 4 Large Animal Hospitals between 2013 and 2019 were reviewed. Gastrointestinal FB were defined as swallowed objects that became lodged within the gastrointestinal tract distal to the cardia and were identified during exploratory laparotomy. Data was collected regarding signalment, clinical signs, hematologic/biochemical profiles, and diagnostic imaging findings from the date of admission. Information on treatments, surgical findings, duration of hospitalization, and survival was also recorded. Median and range were used to describe population characteristics. Seventeen cases met the inclusion criteria. The most frequently observed clinical signs were anorexia/hyporexia (n= 16, 94%), tachypnea (n= 15, 88%), vomiting (n= 13, 76%), dehydration (n= 11, 68%), hypomotility (n=9, 52%), tachycardia (n=8, 47%), obstipation (n= 6, 35%), and decreased demeanor (n=6, 35%). Hematologic and biochemical alteration were unspecific. Diagnostic imaging findings included moderate gastric distention (n= 11, 65%); gas-distended small intestinal loops (n= 9, 53%) and the presence of a FB in 4 cases (n= 4, 24%). Medical therapy consisted of fluid therapy via intravenous/rectal/oral administration, combination intravenous/oral or combination rectal/oral in 14 cases. Additional therapies included antimicrobial therapy (n= 16, 94%), non-steroidal anti-inflammatory drugs (n = 16, 94%), exploratory laparotomy (n= 17, 100%), and antiemetics (n= 3, 18%). The FB were located in the stomach (n=9, 53%) and small intestine (n=8, 47%). A total of 4 pigs had > 1 FB and in all cases one FB was located in the stomach and the other one in the small intestine. Common types of FB included fruit pit (n=6, 35%), diaper (n= 3, 18%) and metallic objects (n=2, 12%). Of the 17 pigs, 15 (88%) were discharged from the hospital and 2 (12 %) were euthanized during surgery because of severe peritonitis (n=1) and poor viability of the small intestine (n=1). The median time of hospitalization for surviving and non-surviving pigs was 4 days (range: 2 to 8 days) and 18 hours (range 0.5 to 1 days), respectively. Based on the observations in this study, surgical correction of FB in pigs carries a good prognosis. Diagnostic imaging findings such as moderate gastric and small intestine distention are commonly identified on radiographs of the abdomen.

F02

Comparison of transfaunate collected from two different rumen compartments in a healthy fistulated donor cow

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Rumen transfaunation is an effective treatment in ruminants diagnosed with gastrointestinal disorders. Fluid can be collected from the fiber mat (FM) and the ventral sac (VS) of a fistulated donor cow. It is unclear if collection from these different compartments has a clinical impact on the quality of fluid administered to patients. The objective of this study was to compare the quality of rumen fluid collected from the VS and the FM from a healthy fistulated donor cow. We hypothesized that the rumen fluid collected from the FM was of higher quality compared to the rumen fluid collected from the VS. This was a prospective, observational study, comparing quality of rumen fluid collected from the VS and the FM using one healthy non-lactating fistulated Holstein-Friesian cow was performed. Eighteen consecutive daily samples were collected from the VS and FM and the following variables were evaluated within 15 minutes after collection: Temperature (cowside), pH (cowside), temperature (laboratory), pH (laboratory), Methylene Blue Reduction Test (MBRT), Sedimentation Rate (SR), protozoal counts, and motility. Median pH (laboratory) was higher in the VS vs the FM (6.5 vs 6.3; $P = 0.0174$). Median MBRT was longer in the VS than the FM (441.5 vs 306.5 seconds; $P = 0.0041$). Median standard deviation of movement type was higher in fluid collected from the VS vs the FM (17.06 vs 11.39; $P = 0.0062$), indicating that the VS was less diverse. Strong correlations were present between the pH measured cow side and in the laboratory ($r = 0.98$), temperature measured cow side and protozoal counts ($r = 0.45$), total distance travelled by protozoa and speed of protozoa ($r = 0.82$), protozoal movement type and speed of protozoa ($r = 0.77$), and total distance travelled by protozoa and movement type ($r = 0.65$). Transfaunate from the fiber mat was of higher quality compared to the ventral sac. Transfaunate from the fiber mat is recommended when administration of rumen fluid is not possible within 15 minutes after collection.

F03

Agglutination and hemolytic crossmatches to determine transfusion reaction differences between large and small breed goats

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Although whole blood transfusions are frequently performed in goats, crossmatches are rarely performed due to the large variety of blood groups and associated factors in ruminants. Many universities own donor goats of both large and small breeds due to concern for increased reactions when transfusing between breeds. However, as a large breed goat can donate four times more blood than a smaller breed goat, having a majority of large breed donors may be beneficial. The objective of this study was to determine the difference in rate of agglutination and hemolytic transfusion reactions between goats of similar and varying breeds.

Ten large breed and ten small breed goats, privately and university owned, were enrolled in this study. Blood from each goat was collected via the jugular vein. Two hundred and eighty agglutination and hemolytic crossmatches were performed: 90 large breed donor to

large breed recipient (L-L), 90 small breed donor to small breed recipient (S-S), and 100 large breed donor to small breed recipient (L-S). Grading was performed using a 0 - 4 scale, with ≥ 1 considered a reaction. Differences in outcomes were evaluated using χ^2 or Fisher's exact tests and relative risk. $P < 0.05$ was considered significant.

Agglutination rates for L-L, S-S, and L-S were 6.67%, 15.6%, and 16% respectively. Differences in agglutination reactions between the three groups were insignificant ($P = 0.1$). Hemolytic reactions for L-L, S-S, and L-S were 57.8%, 26.7%, and 55% respectively. A significant increase in hemolytic reactions for L-L combinations compared to S-S combinations was seen ($P < 0.0001$) with a relative risk of 2.167. Similarly, a significant increase in hemolytic reactions for L-S combinations compared to S-S combinations was seen ($P < 0.0001$) with a relative risk of 2.1.

Clinicians should be cautious when transfusing blood from large to small breed goats due to an increased risk of hemolytic reactions and the potential for an acute hemolytic crisis. When clinically practical, blood from small breed goats should be administered.

F04

Comparison of a point-of-care hematocrit assay and an automated microcentrifuge for cattle and sheep

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The HESKA Element is a point of care (EPOC) analyzer validated for use with canine, feline, and equine whole blood samples; despite a lack of validation, these analyzers are also frequently utilized for production animal species such as cattle and sheep. However, erythrocytes of the species for which the EPOC is validated have larger volumes than those from cattle and sheep. As such, the current application of this device for calculation of hematocrit (HCT) in these species is unknown. The objective of this study was to compare HCT measured by the EPOC to spun packed cell volumes (PCV) measured using an automated microhematocrit centrifuge (HemataSTAT II).

The medical records of cattle and sheep presented to a veterinary teaching hospital between January 1, 2017 and October 1, 2020 were screened for animals that had simultaneous measurement of HCT by the EPOC and spun PCV. Results from each method were compared using Pearson's correlation, Bland-Altman analysis, and Deming regression.

90 cattle and 59 sheep samples were included. PCVs ranged 17 to 56% (HCT: 16 to 50%) for cattle and from 17 to 50% (HCT: 16 to 41%) for sheep. The EPOC vs spun PCV correlation was $r = 0.910$ for sheep and $r = 0.931$ for cattle. Average bias for cattle samples was -9.33 ± 3.98 (range: -17.13 to -1.532) and -6.915 ± 3.45 for sheep (range: -13.68 to -0.1521). Deming regression equations were $Y = 0.7147X + 1.332$ (CI: 0.6585 to 0.7709 for slope; -0.5536 to 3.217 for Y intercept) for cattle and $Y = 0.7626X + 0.9434$ (CI: 0.6641 to 0.8611 for slope; -2.345 to 4.231 for Y intercept) for sheep samples. Both constant and proportional bias was observed between EPOC and spun PCV values.

Bias between EPOC HCT and spun PCV in cattle and sheep has potential to be clinically significant, and clinicians should be aware of this bias when making medical treatment decisions based on HCT data. EPOC HCT and spun PCV data should be interpreted in light of method-specific reference values.

F05

Novel caprine coronavirus: ELISA development and serologic survey of exposed herds in Northern California

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Coronaviruses cause respiratory and gastrointestinal disease in a range of animals including humans, cattle, small ruminants, other mammals, turkey, and other avian species. A coronavirus was identified in 2017, associated with an outbreak of respiratory and gastrointestinal disease in goats in Northern California who attended two different shows. Sequencing revealed a novel coronavirus closely related to bovine coronavirus. The first objective of this study was to adapt a bovine coronavirus ELISA protocol for the novel goat coronavirus. The ELISA was used to characterize seropositivity in exposed herds and individuals over time.

A bovine coronavirus ELISA kit (Svanovir BCV-Ab, SVANOVA Biotech), was adapted using a monoclonal antigoat IgG antibody (mouse anti-goat IgG-HRP, Santa Cruz Bio tech). Due to lack of a gold standard, the mean plus three times the standard deviation of the negative control samples was used as the cut point for determining seropositivity. Seropositivity over time within three goat herds involved in the original disease outbreak was determined using the ELISA test.

The percentages of seropositive individuals within the three exposed goat herds were determined (62-100%), and there was an increase in the percent of seropositive animals within the herds over time. Serial samples from 8 individual goats existed which showed an increase in individual titer over time and persistence of positive titers from year to year. Goats born in 2020, a year when no goat shows took place, were negative for coronavirus antibodies despite contact with seropositive herd mates.

An ELISA to detect antibodies against the novel goat coronavirus was successfully adapted from a commercial bovine coronavirus assay. Herd percentages of seropositive individuals indicate that this coronavirus is a significant pathogen within the Northern California show goat population. Furthermore, tracking changes in individuals and herds over time showed that individuals maintain antibodies against this novel coronavirus for years. Further research needs to be done to determine whether this is due to the virus circulating within the herd or re-exposure to subclinical individuals during shows. Negative serology results from goats born in a year without shows indicates that intermingling animals at shows may be important in maintaining this disease in the goat population.

F06

A high plane of nutrition is a protective factor against calf diarrhea on dairy farms

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Calf mortality and morbidity is still unacceptably high worldwide. Neonatal calf diarrhea is the most important cause of calf losses in the first weeks of life. Therefore, risk factors for calf diarrhea as a herd health problem were investigated retrospectively on Bavarian dairy farms.

A total of 59 dairy farms were investigated by veterinarians of the Bavarian Animal Health Service due to problems with neonatal calf diarrhea (group P). A second group consisted of 18 farms out of the customer base of the Bavarian Animal Health Service that reported no veterinary treatments for calf diarrhea (group C) where calf management was monitored. Management factors were assessed using a questionnaire during a face-to-face interview. Serum samples were collected from up to 10 healthy calves from 2 to 10 days of age for the examination of the quality of passive transfer using total protein analysis. Up to 10 colostrum samples were assessed for immunological and hygienic quality. Data were analyzed using IBM SPSS Statistics 24.0.0.1. by means of a multivariate regression model using a stepwise backward procedure with a Wald $P < 0.05$ as selection criterion and using presence of diarrhea problems on farm as a binary outcome variable.

Of eight variables entered into the multivariate regression model the variables remaining in the final model were 3 litres or more at second feeding; ad-libitum feeding during the first week of life; and administration of an iron containing preparation after birth.

Neonatal calf diarrhea is a multifactorial disease with numerous infectious and non-infectious factors determining if calves fall sick or stay healthy. In this study, two of the three variables in the final model gave evidence for a higher plane of nutrition being a protective factor against calf diarrhea on Bavarian dairy farms. In the past decades evidence is accumulating, that early life nutrition has an important impact on calf growth, development, health and well-being. Results of this study are in line to previous findings and support the establishment of ad-libitum feeding programs in dairy calf rearing.

F07

Clinical findings of gastrointestinal parasitism in camels presenting to a veterinary teaching hospital

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While not native to North America, Old World Camelids (OWCs; *Camelus bactrianus* and *Camelus dromedarius*) routinely present to veterinary teaching hospitals for specialty care. Currently, there is a paucity of data regarding clinical findings associated with gastrointestinal parasitism of OWCs in North America, contrary to their counterparts the South American Camelids (SACs) presenting for gastrointestinal parasitism in North America.

In this retrospective study, the medical records of camels diagnosed with gastrointestinal parasitism were evaluated with respect to presentation, clinical pathology and parasite identification.

Twenty-nine camels met the inclusion criteria. Ages ranged from 3 months to 19 years (mean: 6.1 ± 4.1 years). Common presenting complaints included hyporexia/anorexia (9/29); diarrhea (8/29); and presumptive weight loss (8/29). Common clinical pathology findings included anemia (PCV < 24%; n= 8/25); Eosinophilia (> 3%; n=6/16); hypoalbuminemia (< 3.0 g/dL; n = 17/27); and hyponatremia (< 149 mEq/L; n = 10/23). Of the 40 fecal examinations performed, commonly observed parasites including: Trichostrongyle-type (34/40); *Eimeria* (24/40); *Trichuris* (19/40); and *Monezia* (3/40). Of the 40 fecal exam samples 30 included mixed infections, of which the most commonly observed mixed parasite populations were *Eimeria* and Trichostrongyle-type (7/40); *Eimeria*, Trichostrongyle-type and *Trichuris* (7/40); as well as Trichostrongyle-type and *Trichuris* (5/40).

OWCs appear to present similar to SACs with gastrointestinal parasitism and share similar clinical pathological findings. Clinicians should consider gastrointestinal parasitism as a significant differential in camels presenting with hypoproteinemia and evidence of parasites on a fecal examination. Trichostrongyle-type nematodes, *Eimeria*, and *Trichuris* appear to be the most commonly observed parasitic infections. Additional research is needed to investigate regional differences in parasite infection, as well as treatment efficacy in OWCs presented to North American veterinary hospitals for gastrointestinal parasitism.

F08

Serum haptoglobin concentrations in sick calves

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Haptoglobin (Hp) is a positive acute-phase protein and increased concentrations can be observed in cattle with infection and inflammation. Limited information is available regarding the prognostic value of Hp for survival of hospitalized sick calves. This study aimed to investigate whether admission serum concentration of Hp ([Hp]) was associated with disease severity and survival of sick calves. Medical records from 266 calves < 30 days of age presented to a teaching hospital between 2011 and 2019 were reviewed. Calves were included in the study if their history and physical exam were associated with disease and if

serum [Hp] was measured at admission. Demographic clinicopathological data, clinical diagnosis, presence of systemic inflammatory response syndrome (SIRS), and survival to discharge were recorded. Comparisons between groups were performed using a Kruskal-Wallis test. Cox proportional hazard models were constructed to assess associations between potential predictor variables and mortality during hospitalization. A total of 101 calves met the inclusion criteria, of which only 83 calves were categorized for SIRS. Serum [Hp] was similar in calves with diarrhea, pneumonia and sepsis ($P > 0.05$, for all comparisons). No difference in serum [Hp] was found between calves with (n = 48, median: 0.2, range 0 to 4.2 g/L) and without SIRS (n = 35; median: 0.2; range: 0.05 to 3.6 g/L) was observed ($P = 0.63$). Surviving calves had similar serum [Hp] (n= 79; median: 0.2, range: 0 to 4.2 g/L) when compared to non-surviving calves (n = 22; median: 0.2, range: 0.05 to 0.9 g/L) ($P = 0.49$). Calves with normal suckling reflex and normal heart rate had a higher hazard of survival, while calves with a neutropenia and increased activity of GGT had a higher hazard of non-survival. In conclusion, admission serum [Hp] was not associated with disease severity or survival of hospitalized calves.

F09

Lack of oral absorption of grapiprant in adult alpacas

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Grapiprant is a competitive antagonist of the EP4 prostanoid receptor subtype that belongs to the piprant class of drugs. It acts downstream of the arachidonic acid cascade, thereby not interfering with the constitutive function of other prostaglandins, including PGE_2. This results in a decreased risk of the adverse gastrointestinal and renal effects associated with COX-inhibiting NSAIDs. There is a need for information on the pharmacokinetics and potential use of analgesic and anti-inflammatory drugs in camelid species. The aim of this study, therefore, was to investigate the plasma concentrations and oral absorption of grapiprant in adult alpacas to determine the potential for use of this drug in this species. A single dose of 2 mg/kg of grapiprant (Galliprant®) was administered orally via syringe to four healthy adult alpacas. Plasma was collected via direct venipuncture of the jugular vein immediately prior to (time 0) and 0.5, 1, 2, 4, 8 and 12 hours after drug administration. Plasma grapiprant concentrations were determined by ultra-pressure liquid chromatography with tandem mass spectrometry (UPLC-MS/MS). Plasma concentration of grapiprant was below the lower limit of quantification (1 ng/mL) at all time points in each alpaca, and well below the therapeutic concentration in other species (160 ng/mL). Results of this study indicate the use of oral Galliprant® in alpacas, at doses recommended for dogs, should not be attempted in clinical cases. Future studies should focus on alternate routes of administration, altered formulations or higher doses.

N01

Drug-drug interaction between cannabidiol and phenobarbital in healthy dogs

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Cannabidiol (CBD) use in epilepsy is ever evolving yet drug-drug interaction studies have not been performed in veterinary medicine. The objective of this study was to assess for drug-drug interaction between phenobarbital (PB) and CBD when administered simultaneously.

Nine healthy beagle dogs were included. A three-phase prospective, randomized PK interaction study between CBD and PB was designed. Phase 1: single dose CBD tolerability and PK (5 mg/kg, 10 mg/kg, 20 mg/kg) followed by two-week chronic dosing. Phase 2: single-dose, three-way, cross-over PK of CBD (10 mg/kg), PB (4 mg/kg), or CBD + PB. Phase 3: chronic PB (4 mg/kg for 30 days) followed by single-dose CBD (10 mg/kg) and PK analysis. Adverse events and laboratory findings were recorded throughout.

No significant difference was observed in the CBD PK of dogs receiving CBD alone or in conjunction with single-dose PB or chronic PB. No significant difference was observed in the PB PK of dogs receiving PB alone or with CBD. Mild adverse events were seen in 7/9 dogs during chronic CBD administration consisting of mild gastrointestinal signs in 5 dogs and ALP elevation >2x reference range in 4 dogs. A significant increase in ALP was observed in chronic CBD of phase 1 between Day 0 and Day 14 ($P < .001$).

No significant PK interactions were found between CBD and PB. Neither dose escalation of CBD nor adjustment of PB is not recommended based on this study. Monitoring of CBD plasma levels and liver values are recommended with chronic CBD use.

N02

Handheld Raman spectroscopy for intraoperative differentiation of normal brain tissue from neoplasia in canine patients

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The aim of this study was to assess feasibility of use and accuracy of a hand-held, intraoperative Raman spectroscopy device as a neuronavigation aid to accurately detect neoplastic tissue from adjacent normal grey and white matter. Although Raman spectra are

complicated fingerprints of cell signature, the relative shift corresponding to lipid and protein content (2845 cm^{-1} and 2930 cm^{-1}, respectively) can provide a rapid assessment of whether tissue is normal white or grey matter versus neoplasia for real-time guidance of tumor resection.

Thirteen client owned dogs were initially enrolled in the study. Two were excluded from final analysis due to patient deterioration or lack of neoplastic disease. The diagnoses of the remaining 11 dogs included 6 meningiomas, 2 histiocytic sarcomas, and 3 gliomas. Intraoperatively, interrogated tissues included normal grey and/or white matter and tumor. A total of 5 Raman spectra readings were recorded from the interrogated tissues and samples were submitted for confirmation of Raman spectra by histopathology. A resultant total of 24 samples, 13 from neoplastic tissue and 11 from normal grey or white matter, were analyzed, calculating sensitivity and specificity of Raman spectra compared to histopathology.

The handheld Raman spectroscopy device had a sensitivity of 85.7% and specificity of 90% with a positive predictive value of 92.3% and negative predictive value of 81.6%. The Raman device was feasible to use intraoperatively with rapid interpretation of spectra. Applications of intraoperative Raman spectroscopy include intraoperative guidance of tumor resection, such as in situations where neuronavigation is inaccurate due to brain shift.

N03

A retrospective evaluation of the efficacy of zonisamide in controlling seizures in 59 cats

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Evidence-based recommendations for anti-epileptic drug selection in cats beyond phenobarbital is limited, and additional studies are needed for cats where seizures remain inadequately controlled on phenobarbital alone, as well as cats that cannot safely receive phenobarbital. Zonisamide is an anti-seizure medication safely tolerated in cats, but efficacy studies for controlling seizures in cats are lacking. Medical records for all cats prescribed zonisamide between 2010 and 2020 were retrospectively evaluated, and data on total number of seizures per month and number of seizure days per month were collected before and after starting zonisamide for 59 cats. The average zonisamide dose was 8 mg/kg (range 3.8 – 17.7 mg/kg). Dose frequency was SID in 23 cats and BID in 36 cats. The total number of seizures per month decreased by 38.71% ($p = 0.011$, 95% CI [47.06%, 76.32%]). A subgroup of cats (16/59) had a normal brain MRI and CSF analysis. In this subgroup diagnosed with idiopathic epilepsy, a change in the number of seizure days per month by -2.92 days on average was observed ($p = 0.004$, 95% CI [-4.85, -1.4]). Serum biochemistry panels were available before and after starting zonisamide in 27 cats, and 4/27 (15%) showed serum biochemical changes. These results suggest that zonisamide is safe and efficacious in controlling seizure activity in cats.

N05

Cervical locked facet injuries in the dog: Neurologic signs, advanced imaging findings, treatment, and outcomes

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The objective of this descriptive retrospective case series is to detail the clinical and imaging findings, treatments, and outcomes in dogs suffering from a type of rotational cervical vertebral luxation known as a locked facet.

Medical records and advanced imaging studies were reviewed for dogs diagnosed with cervical locked facets.

Twelve dogs were identified, all of which were small or toy-breed dogs with trauma-induced injury. Four dogs were tetraplegic with intact pain perception, seven were nonambulatory tetraparetic, and one was ambulatory tetraparetic. Five/8 dogs that were not tetraplegic were more severely affected in the thoracic limbs. Locked facets occurred at C5/6 (n=7) and C6/7 (n=5). Dorsal displacement of the caudal vertebra's cranial articular process was observed in all dogs. Five/12 dogs had coexistent articular process fractures. Five dogs were treated surgically, three by external splinting, three by strict restriction, and one was euthanized prior to treatment. Eight/9 (88.9%) dogs with known follow-up data had successful outcomes, as defined by independent walking without discomfort. Of these outcomes, nonambulatory dogs (n=8) returned to independent walking in a median of four weeks (IQR 1-12; range 1-28).

In conclusion, cervical locked facet injuries should be a differential for small or toy-breed dogs with a history of trauma and a subsequent cervical myelopathy. This particular luxation affects the caudal cervical spine and can be identified on imaging with the presence of dorsal displacement of a cranial articular process at the luxation site. Our small cohort had largely successful outcomes regardless of treatment choice.

N06

Using deep learning to detect spinal cord diseases on thoracolumbar magnetic resonance images of dogs

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Magnetic resonance imaging (MRI) has become readily available in veterinary medicine, but specialists who can interpret the acquired images accurately are sparse. There is a need for new technologies which can support specialists in their ever increasing daily work. The

purpose of this study was to train a convolutional neural network (CNN) with thoracolumbar magnetic resonance images of dogs in order to create an algorithm that can detect common spinal conditions in dogs.

MRI of 500 dogs with and without thoracolumbar spinal cord conditions were collected. All cases with a diagnosis of intervertebral disc extrusion or protrusion were surgically confirmed. 2693 images of 375 dogs (75%) were used for CNN training. CNN was trained with T1- and T2-weighted sagittal and transversal images. Each image was manually labelled as control for the pathology shown. To test specificity and sensitivity the images from the other 125 dogs (25%) were used.

Intervertebral disc extrusion was identified on T1 weighted images with an 80% sensitivity and 96% specificity and on T2-weighted images 93% sensitivity and 99% specificity. Intervertebral disc protrusion was diagnosed with a sensitivity of a 100% and specificity of 95.5% on T1-weighted images and sensitivity of 68% and specificity of 96% on T2 weighted images. Sensitivity for ischemic myelopathy or acute non-compressive nucleus pulposus extrusion on T2-weighted images was 62% and specificity 98%.

CNN can be trained to detect common lesions on thoracolumbar magnetic resonance images. Further studies are needed on a bigger cohort of cases to understand its full potential.

N07

Relationship between hearing and cognitive function in senior and geriatric canines

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Age-related hearing loss, or presbycusis, is a significant risk factor for dementia in humans; however, the relationship between presbycusis and cognitive function has yet to be determined in dogs. The purpose of this study was to evaluate the correlation between aging, cognitive function, and hearing in companion dogs. Senior and geriatric dogs were recruited and evaluated every 6 months. Owners completed the Canine Dementia Scale (CADES). Dogs underwent physical and neurological examinations, routine bloodwork and urinalysis. Executive control, sustained attention, and brainstem auditory evoked responses (BAER) were tested. The presence of wave V at 70 decibels (dbs) was determined. If absent in either ear, testing was repeated at 90 dbs. Dogs were grouped by hearing at 70 versus 90 dbs. Age, CADES score, sustained attention, and executive control were compared between groups using the Mann-Whitney U test. Thirty-six BAER examinations from 24 dogs (9.5 to 16 years) were analyzed. All dogs could hear. Wave V was present at 70 dbs in 30 of these evaluations. Dogs that could hear at 70 dbs were significantly younger than those that could not ($p = 0.0008$). CADES scoring identified four dogs with severe, six with moderate, five with mild cognitive dysfunction syndrome, and 21 dogs as normal. Dogs that could not hear at 70 dbs performed significantly worse on executive control tests ($p = 0.0063$), sustained attention tests ($p = 0.0009$), and CADES scoring ($p = 0.0008$). Our findings suggest hearing loss is associated with aging and may be a risk factor in cognitive dysfunction in companion dogs.

N08

Canine idiopathic vestibular syndrome: Unilateral decrease in inner ear signal in fluid-attenuated inversion recovery sequences

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The pathology of canine idiopathic vestibular syndrome (IVS) remains unclear. Characteristic magnet resonance imaging (MRI) techniques are used to reveal differences in the endolymph composition of affected and unaffected inner ears in people. The purpose of this study is to determine whether comparable procedures can help to detect changes in canine inner ears in IVS patients.

Medical records of two veterinary clinics were reviewed retrospectively. Dogs were included when they had a diagnosis of IVS, clear lateralization of clinical signs, and an MRI of the vestibular system. A region of interest (ROI) was manually outlined defining the anatomical area of the inner ear in T2-weighted and FLAIR images. In order to calculate the ratio of FLAIR suppression of each ear, the mean grey value of the ROI was determined in both sequences. If a unilateral decrease in suppression could be identified, it was evaluated in consideration of the lateralization of clinical signs.

In total, 80 dogs met the inclusion criteria. The ratio of suppression was significantly reduced on the affected side compared to the unaffected side, 0.885 versus 0.9342, respectively ($p = 0.0025$). The affected inner ear had a higher lack of suppression. In 92.5% of the cases, the affected side matched the clinical signs of the respective dogs.

The results provide preliminary evidence that the endolymph MRI appearance is altered on the affected side in the majority of the IVS cases. Further studies are needed to evaluate if the degree of changes can be used to provide prognostic information.

N09

The effect of medium-chain triglycerides on brain metabolism and neurotransmitter concentration

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Medium-chain triglycerides (MCTs) enriched diets are used in the management of cognitive dysfunction and epilepsy in dogs and humans. The anticonvulsant and cognition improving features have been described in

numerous studies. However, the mechanism of action remains not fully elucidated. The aim of the current study was to investigate the effect of MCTs on the neurotransmitter concentration in the brain.

Eight healthy Beagle dogs were included in a randomized crossover feeding trial. Dogs were fed with a commercial hypoallergenic dog diet (control), which was either supplemented with one dose of MCT (9 % of caloric requirement, 'acute', 2 h) or for two weeks (9 % of caloric requirement, 'chronic', 2 weeks) substitution with MCT-oil. 1H-MRS by single voxel Point Resolved Spectroscopy Sequence was performed with a 3.0 Tesla MR scanner in four brain regions (parietal, piriform and the occipital lobe and thalamus) and analyzed with an LCModel. The concentration of glutamate, gamma-aminobutyric acid (GABA) and glutamine was obtained relative to creatine and phosphocreatine. Cerebrospinal fluid (CSF) was analyzed by high-performance liquid chromatography for glutamate, GABA and glutamine in μg/ml.

Glutamate was significantly reduced in the piriform lobe after acute and chronic MCT consumption and glutamine after acute MCT administration only. There was no difference in the other brain regions, nor was there a difference in GABA. Furthermore, no differences of neurotransmitter concentration between feeding regimen were found in CSF. The piriform lobe plays a crucial role in epilepsy. The reduction of glutamate after prolonged MCT intake might represent one of the anticonvulsant mechanisms of MCTs.

N10

Sexual dimorphism of relative corpus callosum size in dogs as measured via magnetic resonance imaging

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Currently, assessment of corpus callosum (CC) size in dogs is limited to a subjective analysis as there is no quantitative measurement system established. The purpose of this study was to establish a repeatable quantitative methodology with which to assess CC morphology in a population of dogs undergoing brain MRI at a referral hospital. CC measurements were made on midsagittal MR images in 343 dogs using two techniques previously validated in human studies, CC Ratio (CCR) and CC Index (CCI). The intraclass correlations (ICC) for the CCI were excellent for the intraobserver and good for interobserver measurements. The ICC for the CCR were good for the intraobserver and poor for interobserver measurements. Female entire dogs had a significantly smaller CCR and CCI than all other sex and neuter statuses (all $P < 0.01$, Turkey Post Hoc Test). Using multivariable models, age was non-linearly associated with ratio. Inclusion of age did not change the direction of estimated effects of sex/neuter status. Similar sex-related differences in the size of canine CC were previously reported in a small, postmortem study. In humans, advanced imaging techniques have shown sex-specific differences in CC neural structure and function. Factors such as influence of female sex hormones on white matter development or regression are thought to be playing a role. This is the first study in dogs assessing repeatability and presence of sex differences using 2 relative CC measurements on antemortem MRI studies. Our findings are relevant for future studies concerning abnormalities in canine brain development and degeneration.

N11

Novel chemical chaperon inhibitors for aggregation of mutant canine superoxide dismutase 1 protein

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Canine degenerative myelopathy (DM) is a fatal neurodegenerative disease associated with mutant superoxide dismutase1 (E40K SOD1). Canine SOD1 protein is a stable homodimer with copper and zinc ions. Several studies have reported that E40K SOD1 forms protein aggregation in neurons and glial cells, and the de-metaled form (apo-form) might be the initial intermediate in the process of aggregation. In the present study, we screened small molecule compounds for their ability to inhibit E40K SOD1protein aggregation.

Wild type (WT) and mutant (E40K) recombinant canine SOD1 proteins were expressed in *Escherichia coli* and apo-forms of SOD1 proteins were purified. After addition of each small molecule compound No.1~16 (A patent has been applied for.), aggregate formation of SOD1 protein was measured over time using the Thioflavin-T (Th-T) fluorescent assay. Further, the denaturation midpoint (T_m) was calculated from the denaturation curve obtained by differential scanning fluorimetry (DSF) measurement under heating. The plasmids of WT-SOD1-GFP (WT-GFP) or E40K-SOD1-GFP (E40K-GFP) were transfected into mouse neuroblastoma (Neuro2a) cells. After 48 hours incubation with or without small molecule compounds, cells were observed under a confocal laser scanning microscope. Thioflavin-T fluorescence intensity was significantly increased in non-treated apo-E40K SOD1, whereas apo-WT SOD1 did not give rise to Th-T positive aggregates. Compounds No.7, 8 and 14 significantly inhibited aggregation of apo-E40K SOD1 in Th-T assay. In the DSF assay, these three compounds did not alter the T_m of apo-E40K SOD1. In Neuro2A cells, WT-GFP proteins were diffusely localized in the cytoplasm. Although E40K-GFP proteins formed aggregates in the cytoplasm of Neuro2A cells, the proportion of cells containing SOD1 aggregates was significantly lower in E40K-GFP transfected cells in the presence of compounds No.7 (mean±SD, 13.3±2.3 %), No.8 (13.3±0.6 %), or No.14 (10.0 ±1.2 %) than in non-treated E40K-GFP transfected cells (20.7±1.5 %).

Chemical chaperons are a group of small molecules, which enhance the folding or stabilization of proteins. Some chemical chaperons have been reported to be effective in inhibiting fibrillation of proteins, such as amyloid β and polyglutamine proteins. In the DSF assay, the small molecule compounds did not affect the stability of apo-E40K SOD1. Nevertheless, in the Th-T assay, three of the tested small molecular

compounds (No.7, 8 and 14) inhibited apo-E40K SOD1 aggregation. These results suggested that the compounds No.7, 8 and 14 inhibited the oligomer formation of apo-E40K SOD1. Inhibitory effects of these compounds on aggregation formation of apo-E40K SOD1 was also observed in the experiments using SOD1-transfected cell line. In conclusion, we found small molecular compounds which inhibited aggregation of E40K SOD1 proteins *in vitro* possibly by their chemical chaperon effects. Further study using high resolution techniques, such as biophysical interaction analysis, is required to elucidate the mechanism of inhibiting SOD1 aggregation.

N12

Evaluation of 18F-fluorodeoxyglucose uptake of beagle dogs in different durations of isoflurane anesthesia

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^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) is used for the evaluation of tumor. In veterinary medicine, anesthesia is essential during the scanning process of PET. However, changes in FDG uptake in dogs who undergo anesthesia for a longer duration have not been studied. This study aimed to analyze the influence of isoflurane anesthesia on FDG uptake in dogs undergoing PET.

A crossover design was implemented by exposing three groups of six dogs to different durations of anesthesia (60, 90, and 150 minutes). Inhalation anesthesia was maintained during the entire scanning process (30 minutes), and FDG was injected one hour before the initiation of PET scan. The standard uptake value (SUV) of FDG was obtained for the seven gross structures (whole brain, lung, salivary gland, liver, spleen, mediastinal blood pool, and kidney cortex) as well as for the seven intracranial structures, including the frontal, parietal, temporal, and occipital lobe, cerebellum, brain stem, and caudal colliculus.

The whole brain and the intracranial structures showed significantly lower FDG uptake in dogs with a longer duration of anesthesia, while other gross structures did not.

Our results suggest that the duration of anesthesia should be considered in the evaluation of FDG uptake by the brain.

N13

Calprotectin and canine pancreatic lipase activity in dogs treated surgically for thoracolumbar intervertebral disc herniation

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Pancreatic and gastrointestinal (GI) mucosal inflammation or injury are reportedly common in dogs with thoracolumbar intervertebral disc herniation (TL-IVDH). Data on non-invasive markers of such inflammation are minimal in this population. Our objective was to quantify fecal calprotectin (CP) and serum canine pancreatic lipase immunoreactivity (cPLI) concentration in dogs treated surgically for TL-IVDH. Additionally, we investigated the relationship between these clinicopathologic tests and clinical GI signs and the benefit of prophylactic omeprazole treatment in reducing signs of GI inflammation in this population.

A randomized double-blinded placebo-controlled clinical trial was performed in client-owned dogs undergoing hemilaminectomy for acute TL-IVDH. Dogs were orally administered 1 mg/kg omeprazole q12h or placebo during hospitalization. Fecal samples for CP were obtained daily and serum samples for cPLI were acquired twice (pre- and post-operatively) during hospitalization and at 2-4 week rechecks. GI signs were recorded during hospitalization and at rechecks. Wilcoxon rank sum tests were used to compare CP and cPLI between hospitalization and recheck samples, between dogs with and without GI signs, and between dogs treated with omeprazole or placebo during hospitalization.

Of 37 enrolled dogs, 20 received omeprazole. 17/37 developed GI signs, 10 of which were treated with omeprazole. 38% of dogs had CP > 961 ng/g and 30% had cPLI > 400 μg/L during hospitalization. Median CP during hospitalization was 594.9 ng/g (0-1522) versus 41.1 ng/g (0-1442.8) at rechecks ($p < 0.0001$). Median CP in dogs with GI signs was 751.3 ng/g (0-1470.8) versus 480.2 ng/g (0-1522.2) in dogs without GI signs ($p = 0.054$). Median CP in dogs receiving omeprazole was 696.8 ng/g (0-1516) versus 466 ng/g (0-1522.2) for placebo ($p = 0.11$). Median cPLI was 63 μg/L (30-2000) pre-surgery, 115.5 μg/L (30-1432) post-operatively, and 61.5 μg/L (30-632) at recheck evaluation ($p = 0.009$). Median cPLI was 90 μg/L (30-2000) for dogs with GI signs and 71.5 μg/L (30-231) for dogs without GI signs ($p = 0.54$). Median cPLI was 86 μg/L (30-2000) in dogs receiving omeprazole and 70 μg/L (30-1801) in dogs on placebo ($p = 0.26$).

Fecal CP concentrations during hospitalization and post-operative serum cPLI concentrations were significantly increased compared to recheck samples in dogs managed surgically for TL-IVDH, but the majority remained below the upper limit of the respective reference intervals. No significant differences were identified in CP or cPLI concentrations between dogs with or without GI clinical signs. There was no overt evidence that short-term, prophylactic omeprazole treatment reduced evidence of GI or pancreatic inflammation.

N14

Adapting laser interstitial thermal therapy for treatment of intracranial lesions in dogs

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Laser interstitial thermal therapy (LITT) is a minimally invasive surgical intervention permitting thermal ablation of brain tumors in humans. During LITT, a laser probe is stereotactically introduced through a small skull burr hole into the tumor. Data from a continuously running MRI scanner is converted to real-time temperature data, and software calculates zones of cytotoxicity as the tumor is heated.

The purpose of this study was to adapt a commercially available LITT system for use in dogs with intracranial lesions. Canine cadavers were used to optimize LITT procedures before performance in live dogs. Volumetric, T1-weighted, MRI studies were obtained, transferred to a neuronavigation system (Curve, Brainlab) and trajectories to intracranial targets were planned. Dogs were fixed to a surgical bed using a head frame (Brainsight, Rogue Research) with an attached fiducial array (Brainlab) and registered using surface matching to a volume rendered image. An integrated instrument holder (Varioguide, Brainlab) was used to create skin incisions, followed by 4.5 mm drill holes in the dogs' skulls. Self-tapping titanium "mini-bolts" (Monteris Medical) were placed into the drill holes. Dogs were transferred to the MRI suite and laser catheter probes (Monteris Medical) fed through the mini-bolts and into the lesions, followed by lesion ablation.

This method allowed rigid stereotaxy and successful neuronavigation after registration of dogs using a volume rendered surface technique. Laser ablation procedures have been performed on 3 dogs to date. All were accomplished through minimally invasive approaches (1-2 cm skin incisions), followed by successful ablation of the targeted regions.

N15

Developing a predictive model for spinal shock in dogs with acute spinal cord injury

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Acute spinal cord injury is common in dogs, where reduced pelvic limb reflexes typically suggest a lesion of the L4-S3 spinal cord segments. However, pelvic limb reflexes may also be reduced in dogs with a T3-L3 myelopathy and concurrent spinal shock, which can lead to clinical confusion. In order to help distinguish between these two localizations, we compared clinical and demographic factors in a large, prospectively identified cohort of 59 dogs with T3-L3 myelopathies and spinal shock and a group of 13 dogs with L4-S3 myelopathies. A univariable logistic regression was performed to assess influence of clinical factors on the odds of spinal shock. Independent variables were selected for a multivariable logistic regression model if they had a significant effect ($p < 0.100$) on the odds of spinal shock in univariable logistic regression. When other independent variables are held constant, the odds of spinal shock increased with decreasing weight, decreasing duration of clinical signs, presence of a cutaneous trunci cut-off, and severity of neurologic deficits (presence of paraplegia). The odds of spinal shock in paraplegic dogs is 7.87 times greater than dogs with paraparesis ($p = 0.041$). The odds of a L4-S3 myelopathy is 8.57 and 5.63 times greater in dogs with decreased pelvic limb tone and decreased patellar reflex, respectively ($p = 0.004$; 0.013). A predictive formula, as developed by the present study and using these criteria, can be useful for assisting the clinician in determining the likelihood of spinal shock in various clinical scenarios and aid clinicians in diagnostic planning.

N16

MRI visualization of the volume changes in dorsal root ganglia in dogs with degenerative myelopathy

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Canine degenerative myelopathy (DM) is a chronic, progressive and fatal neurodegenerative disease. The diagnosis is based on the observation of clinical signs, genetic testing of the SOD1 gene, and ruling out of other spinal cord diseases; however, a definitive diagnosis of DM can only be achieved by postmortem histopathological examination of the spinal cord. In a recent study, dogs with DM exhibited significant loss of sensory root axons, and sensory neurons in dorsal root ganglia (DRG) displayed evidence of degeneration. The aims of the present study were to investigate the ability of the water excitation MRI sequence to visualize DRG in dogs and whether volumetry of DRG using this technique could be of diagnostic value for DM.

In order to establish the reference value of normal DRG volume in dogs, we included 7 control dogs which had no-lesion in the spinal cord and peripheral nerve tissue. Using a 3.0-tesla MRI, water excitation images were obtained to visualize and measure the volume of DRG. In control dogs, the correlations between DRG volume and body weight, body surface area, and L2 vertebral body length were evaluated by Spearman's rank correlation coefficient. Moreover, the DRG volume between each spinal cord segment was compared by Kruskal-Wallis test. Normalized DRG volumes of each spinal cord segment between T8 and L2 were compared between dogs with DM ($n = 8$), dogs with thoracolumbar intervertebral disc herniation (IVDH) ($n = 26$), and control dogs ($n = 7$), using Kruskal-Wallis test.

The body surface area had the strongest correlation with the DRG volume in control dogs ($r = 0.622$, $p = 0.019$). In control dogs, there was no significant difference in the DRG volume between each spinal cord segment. DRG to body surface area ratios of DM dogs were significantly lower than those of control dogs at all spinal cord segment between T8 and L2 bilaterally ($p < 0.01$, respectively). DRG to body surface area ratios of DM dogs were significantly lower than those of IVDH dogs in T8, T9, T10, T11, L2 on left side and T8, T9, T10, T11, T12 on right side ($p < 0.01$, respectively).

DRG of dogs could be visualized with the water excitation MRI sequence. DRG of dogs with DM were smaller than those with IVDH and control dogs, which are consistent with the histopathological findings in previous reports. The measurement of DRG volume to body surface area ratio by this technique provides non-invasive and quantitative assessment of neurodegeneration in DRGs and may have the diagnostic potential for DM.

N17

Canine meningoencephalitis of unknown origin: The search for infectious agents via next-generation sequencing

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Canine meningoencephalitis of unknown origin (MUO) is suspected to be of multifactorial etiology, an infectious pathogen might trigger a destructive inflammatory response in susceptible individuals. Different attempts to prove the trigger failed. A relatively new metagenomic method to detect unknown infectious pathogens involves screening biological samples for viral or bacterial DNA or RNA via next-generation sequencing (NGS). NGS was recently used to screen a North American subpopulation of dogs with MUO without finding an etiologic agent. As MUO is an umbrella term for different subtypes of meningoencephalitis, it is assumed that different triggers might be responsible for different MUO subtypes. Moreover, infectious pathogens can show a specific geographic distribution and might only be detectable in acute phases of the disease. Therefore, we examined freshly frozen CSF samples of 6 dogs from Europe, suffering from MUO in the acute phase, via NGS using a modified sequence-independent, single-primer amplification protocol to detect a possible infectious trigger specific for Europe. In none of the 6 samples any infectious agents could be found.
The fact that no infectious agents could be found in the acute phase of MUO, might allow several conclusions: It is possible, that the trigger is rather toxic or environmental, or the initial trigger is already eliminated at the time of development of clinical signs, but the inflammatory process is self-maintaining. This fact might help to get understand the pathomechanism of MUO, which might be a cornerstone to develop a specific therapy or even prevent disease outbreak in the future.

N18

Evaluating the impact of canine epilepsy on their caregivers

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Growing scientific evidence suggests multiple benefits of pet ownership to human wellbeing. However, evidence is also emerging describing negative effects of dog ownership, particularly when animals are affected by chronic and/or terminal diseases. Epilepsy is the most common chronic neurological disorder in dogs, and in addition to its recognized impacts on canine quality of life (QoL), recent qualitative research from our group identified substantial, diverse negative impacts of the disease upon English caregivers of affected dogs. This study aimed to test the generalizability of these findings in a large international sample.
An online cross-sectional survey was designed, informed by our qualitative findings and caregiver/parent impact instruments from pediatric epilepsy. Caregivers of dogs diagnosed with idiopathic epilepsy were recruited via snowball sampling across social media and disease-specific forums between September-December 2020. Affected dogs' clinical variables including seizure history, epilepsy severity (e.g., history of status epilepticus/cluster seizures), and anti-seizure drug (ASD) treatment were captured. Caregivers reported whether ten domains related to their own wellbeing had improved (+1), stayed the same (0) or worsened (-1) due to their dog's epilepsy: sleep quality, tiredness levels, time to take care of their own health, anxiety/worry levels, confidence in ability to look after their dog, relationships with close family, other pet(s), and close friends, work hours and household finances. A mean score of the ten domains was calculated for each caregiver, with this variable then modelled in linear regression to identify the most influential impacts upon caregivers.
In total, 590 valid responses were received of which the majority were female (94.9%), aged 45-54 (29.7%) and from the UK (58.3%), USA (20.9%) or Germany (7.9%). The majority of dogs were ASD treated (89.3%) with 53.4% of dogs treated with 2 or more ASDs (polytherapy). On a scale of -1 to +1, their dogs' epilepsy had an overall negative effect on caregivers (mean: -0.37 ± 0.25). The greatest negative impacts were on sleep quality (worsened: 68.8% of caregivers), anxiety/worry levels (worsened: 83.7%) and household finances (worsened: 58.4%). Only caregiver confidence in their ability to look after their dog described substantial improvement (improved: 34.6%). In multivariate modelling, significant predictors of greater negative caregiver impact included a history of cluster seizures ($p = 0.035$) or status epilepticus ($p = 0.004$), shorter time since diagnosis ($p < 0.001$), ASD polytherapy ($p = 0.003$) and female caregiver gender ($p = 0.003$).
Being the caregiver of a dog with epilepsy has potentially wide-ranging negative effects on human physical health (e.g. sleep), mental health (e.g. anxiety) and overall wellbeing (e.g. social relationships, employment, finances). Greater awareness of these negative impacts may engender better support from the veterinary profession for affected caregivers; however, wider support may be required for the worse affected individuals.

N19

Prevalence of and risk factors for early postoperative seizures in dogs following intracranial surgery

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Dogs undergoing intracranial surgery for brain tumors commonly have structural epilepsy. However, seizures in the immediate post-operative period may adversely affect outcome. A retrospective case study was performed to determine the prevalence and risk factors for early postoperative seizures (EPS) in dogs undergoing intracranial surgery. Patients were included if they had rostrotentorial neoplastic masses. EPS was defined as seizures occurring within 14 days of surgical intervention. The primary hypothesis was that dogs with pre-existing structural epilepsy (SE) would be more likely to have EPS.

Of the 88 cases identified, 20 underwent stereotactic brain biopsy, 31 had a craniotomy or craniectomy, and 37 underwent both procedures. Pre-existing SE was identified in 64/88 (72.7%) of cases, while 12/64 (18.8%) of dogs with previously diagnosed SE experienced EPS. Of the 24 cases without pre-existing SE, 4/24 (16.7%) experienced EPS. The presence of previously diagnosed SE did not significantly affect the risk for EPS ($p = 1.00$).

The use of perioperative prophylactic anticonvulsants had no significant effect on EPS ($p = 0.06$). Tumor location (frontal vs temporal/piriform vs other) did not have a significant effect on EPS ($p = 0.23$). Cases with EPS were more likely to have a transient or permanent neurologic deficit than those that did not experience EPS ($p = 0.005$). The duration of hospitalization was significantly shorter in dogs that did not experience EPS ($p < 0.001$). Thus, the occurrence of EPS was not related to a previous diagnosis of epilepsy, tumor location, or prophylactic anticonvulsants, but did occur in patients with worsening neurologic signs after surgery and longer hospitalizations.

N20

Canine central nervous system metastatic melanoma: A retrospective analysis and comparative review

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Malignant melanoma (MM) makes up 7% of malignant cancers in dogs and is the most common oral malignancy. Canine oral melanoma has a high likelihood of local invasion and metastasis with up to 92% of affected dogs experiencing distant metastases. In humans, over 100,000 patients are diagnosed with MM every year, and 10-40% of those cases will develop cerebral metastases. MM usually metastasizes to the frontal lobe and causes seizures in 33% of patients – the average time to onset of neurologic signs is 3.5 years. However, in dogs, neither the incidence of CNS metastasis nor features associated with these cases, are known. Therefore, our study sought to determine the incidence and clinicopathologic features associated with canine CNS metastatic MM. We performed a retrospective review of cases evaluated at the UC Davis Veterinary Medical Teaching Hospital from 1985-2019. All cases with a diagnosis of MM and histopathological examination of the CNS were included. Cases were excluded if the dog was alive at the time of data collection or if the dog had no clinical signs related to their melanoma diagnosis prior to death. 55 cases of metastatic melanoma were identified, with 20 dogs (38%) having CNS metastasis. While most metastases affected the brain (n=18), few MM lesions were observed in the spinal column and spinal nerves (n=5), with three dogs having MM lesions in both the brain and the spinal column or nerves. Most dogs had multiple metastatic lesions (n=12), averaging at least four across the CNS. The most common site of metastasis within the brain was the frontal lobe. Of the 20 dogs with CNS MM, neurological signs were observed in 70% (n=14) of dogs. Most dogs (n=11) developed neurological signs following a primary diagnosis (median 187 days). However, three dogs developed neurological signs as the first indication of MM. Seizures were the most common sign, seen in 64% (n=9) of clinically presenting dogs. These findings suggest that canine and human MM patients share similar CNS metastasis incidence rates and clinical presentation, but may differ in time to onset of neurologic signs. Furthermore, this study reveals key features of canine CNS metastatic MM, which will guide clinical management of these patients.

N21

Region of interest compared to semi-automated diffusion tensor imaging metrics

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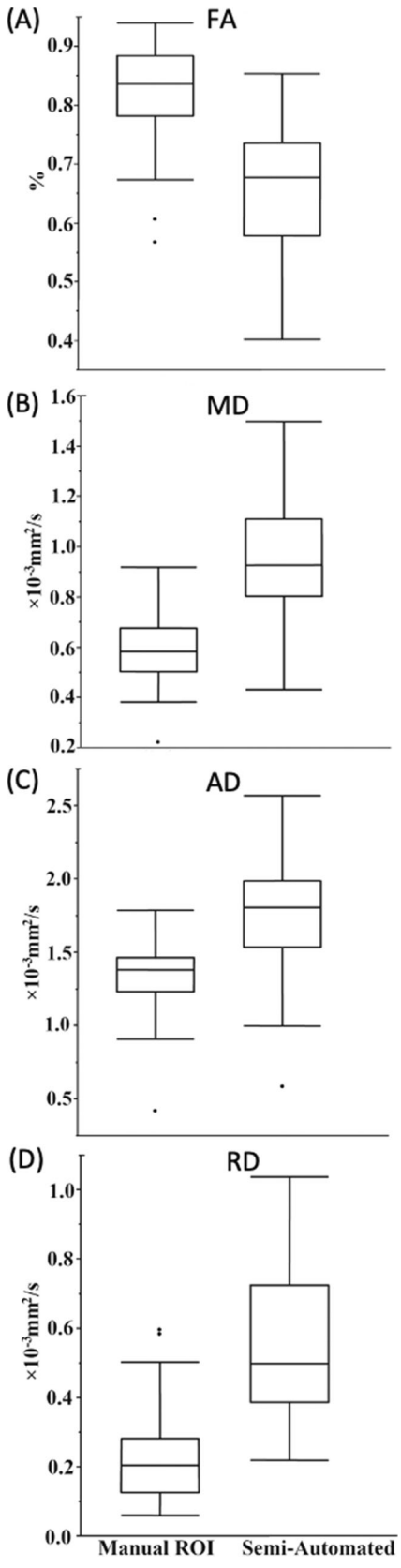
Diffusion tensor imaging (DTI) is gaining favor as a prognostic tool for dogs with spinal cord injury but there is still lack of agreement for determining which metrics are clinically relevant. We hypothesized that one reason for the discrepancies in reported DTI metrics is due to processing metric variability. To test this hypothesis, we retrospectively searched hospital medical records from 2017 to 2020 and identified 65 dogs that had DTI of the spinal cord performed. We then compared manual region of interest (ROI) and semiautomated Spinal Cord Toolbox (SCT) DTI metrics in these dogs. For ROI, the median: fractional anisotropy (FA) was 0.84 (range 0.57 - 0.94), mean diffusivity (MD) was 0.58×10^{-3} mm^2/s (range 0.22 - 0.92), axial diffusivity (AD) was 1.38×10^{-3} mm^2/s (range 0.41 - 1.79), and radial diffusivity (RD) was 0.2×10^{-3} mm^2/s (range 0.05 - 0.59). For SCT the median: FA was 0.68 (range 0.4 - 0.85), MD was 0.93×10^{-3} mm^2/s (range 0.43 - 1.5), AD was 1.81×10^{-3} mm^2/s (range 0.58 - 2.57), and RD was 0.5×10^{-3} mm^2/s (range 0.21 - 1.03). There was a significant difference between DTI metrics ($P < 0.01$) where FA was significantly higher, and MD, AD and RD were significantly lower for ROI compared to SCT. For both ROI and SCT, the DTI metrics MD, AD, and



RD were significantly lower (P < 0.01) in dogs that did not return to ambulation. In conclusion, although DTI shows promise as a prognostic tool, a standardized method for processing DTI metrics is needed.

N22

Evaluation of a novel dorsal cemented technique for atlantoaxial stabilization in 12 dogs

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Dorsal atlantoaxial stabilization (DAAS) has mostly been described using low stiffness constructs in dogs. The aim of this study was to assess the feasibility and surgical outcome of a rigid cemented DAAS. The medical records of 12 dogs treated with DAAS were retrospectively reviewed. The technique involved bi-cortical screws placed in at least 4 of 8 available bone corridors, embedded in polymethylmethacrylate. Surgical outcome was assessed via client questionnaires and review of postoperative CT images. Screw placements were graded according to their position and the amount of breach outside the intended bone corridor.

All DAAS procedures were completed successfully. Preoperative surgical planning was used in 11 cases and 3D printed guides in 2 cases. A titanium mesh affixed to the occipital bone was added to the construct in one patient suffering from a complex occipito-atlantoaxial malformation. A total of 72 atlantoaxial screws were placed with 51 (70.8%) graded as optimal and 4 (5.6%) graded as hazardous (2 minor vertebral canal breach, 1 excessively long, 1 alar foramen breach). The remainder 17 (23.6%) screws were considered safe but not perfectly placed within the intended corridors, most commonly due to monocortical position in 11 (15.3%) screws. The clinical outcome was considered good to excellent in all but one case displaying episodic discomfort despite appropriate atlantoaxial reduction. A single construct failure was identified despite a positive clinical outcome.

This study suggests the proposed DAAS is a viable alternative to ventral techniques. Prospective studies are necessary to accurately compare complication and success rates.

N23

Neurofilament light chain as a biomarker of meningoencephalitis of unknown etiology in dogs

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Neurofilament light chain (NfL) is a neuron-specific cytoskeletal protein expressed in axons. Damaged axons of the central nervous system release NfLs into cerebrospinal fluid (CSF) and blood. In humans

with neurologic diseases, NfL is used as a biomarker. To establish the utility of NfL as a biomarker for the diagnosis, prognosis, and monitoring of meningoencephalitis of unknown etiology (MUE), this case-control study was performed.

Twenty-six client-owned healthy dogs, 10 normal beagle dogs, and 38 client-owned MUE dogs were included. The levels of serum and CSF NfL were measured using single-molecule array technology.

The median NfL level was significantly higher in MUE dogs (serum, 125 pg/mL; CSF, 14,700 pg/mL) than in healthy dogs (serum, 11.8 pg/mL [$P < 0.0001$]; CSF, 1,410 pg/mL [$P = 0.0002$]). The areas under the receiver operating characteristic curves of serum and CSF NfL levels were 0.99 and 0.95, respectively. The cut-off values were 41.45 pg/mL (serum) and 4,005 pg/mL (CSF) for differentiating between healthy and MUE dogs, with sensitivities of 89.19% and 90%, respectively, and specificities of 96.97% and 100%, respectively. There was no correlation between the rate of lesion volume to the whole brain volume and NfL concentration. There were no significant differences in NfL levels between dogs with and without seizures. NfL level showed a significant decrease ($P = 0.0156$) in the good treatment-response group and a significant increase ($P = 0.0313$) in the poor treatment-response group.

Therefore, NfL could be a potential biomarker for diagnosing MUE and evaluating therapeutic effects on it.

O01

Canine cancer mutations homologous to human hotspots: Precision medicine opportunity

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The determination of the best treatment and prognosis of a cancer in companion animals is still a challenge for most tumors in veterinary medicine. In humans, the implementation of precision medicine techniques have increased the accuracy and efficacy of cancer treatment due to identification of genomic subtypes, driver mutations and the development of drugs able to block aberrant activating signals. Using the Next-Generation Sequencing (NGS) panel from FidoCure® Precision Medicine Platform, 701 client-owned tumor-bearing dogs enrolled by 200 veterinarians in clinical practice were analyzed. Mutated positions identified in canine genes were compared to the corresponding position in the human gene. We identified the human hotspot mutations based on cBioPortal on-line platform. Variants which have not been reported in dbSNP nucleotide variations database were analyzed with SIFT and PROVEAN tools to check the predicted effect of the variants on the protein function. TP53 was the most frequently mutated gene found in 30.81% of analyzed tumors, with high frequency in hemangiosarcoma and osteosarcoma, and low frequency in soft tissue sarcoma, anal sac carcinoma and urothelial carcinoma. Thirty-seven canine mutations homologous to human hotspots were identified in the following genes: PIK3CA (14), KRAS (9), NRAS (4), BRAF (3), KIT (3), ERBB2 (2), and EGFR (2). Four mutations were statistically correlated with three tumor types ($P < 0.0001$); hemangiosarcoma with N-RAS-G61R and PIK3CA-H1047R, pulmonary carcinoma with ERBB2-V659E and urothelial carcinoma with BRAF-V588E. This work corroborates several canine hotspot mutation candidates and contributes to the implementation of precision medicine tools to treat cancer in dogs.

O02

Characterization of tumor-derived genomic alterations and heterogeneity in dogs with cancer by noninvasive liquid biopsy

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This proof-of-concept study aims to demonstrate the ability of blood-based liquid biopsy testing in dogs with cancer to identify genomic alterations that originate from spatially separated tumors.

Nine dogs with a variety of confirmed cancer diagnoses, including patients with localized and disseminated disease, were recruited into the study. Informed consent was obtained from all owners. Multiple spatially separated tumor tissue samples were collected from each patient, along with a blood sample before and after the surgical resection. All samples were analyzed using a proprietary next-generation sequencing (NGS) based assay designed to interrogate multiple classes of cancer-associated genomic alterations.

Analysis of the pre-surgical blood samples revealed genomic alterations, including small nucleotide variants (SNVs) and copy number variants (CNVs), in a majority of patients that matched alterations independently detected in the patient's tumor tissue samples. Liquid biopsy collectively captured the unique alterations seen individually in multiple spatially separated tissue samples, demonstrating its ability to capture the heterogenous tumor profiles noninvasively. In blood samples collected after surgery, genomic alterations were detected in patients with incomplete tumor resection but not in those with complete resection, suggesting potential utility to noninvasively detect post-surgical residual disease.

Liquid biopsy allows noninvasive profiling of the heterogeneous genomic alterations derived from physically separated tumor lesions in dogs with cancer, overcoming the limitation of tumor-based testing posed by the physical locations of individual tumors and the complexity of the tumor genomes. It has potential utility to aid in cancer detection and cancer management in dogs.

O03

L-CHOP in combination with monoclonal antibody (AT-005, Tactress) for naïve canine intermediate/high-grade, peripheral T-cell lymphoma

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Dogs with T-cell lymphoma have a relatively poor prognosis compared to those with B-cell lymphoma; additional therapeutic options are

needed. This study evaluated if addition of AT-005, a USDA licensed caninized monoclonal antibody, to L-CHOP chemotherapy improved progression-free survival (PFS) and response rates in dogs with intermediate and high-grade peripheral T-cell lymphoma.

Randomized, placebo-controlled, investigator- and owner-masked, multicenter clinical study. Dogs were immunophenotyped via flow cytometry and randomized (1:1) into AT-005 or placebo groups. Dogs received a 19-week L-CHOP chemotherapy protocol; AT-005 or placebo was contemporaneously (beginning at Day 7) administered intravenously twice weekly for 4 weeks, then every other week for 4 treatments. Response was evaluated via RECIST criteria for canine lymphoma.

A total of 228 dogs were screened; 49 dogs with T-cell lymphoma were enrolled (24 received AT-005 and 25 received placebo). Although more dogs with stage IV and V disease were treated with AT-005 (8 in AT-005 group, 2 in placebo group), all other demographic factors were similar between groups. Median PFS was 64 days (95% CI, 36-118) in AT-005 group compared to 103 days (95% CI, 56-118) in placebo group (P = 0.8163). The objective response rate (ORR) for all dogs in the study was 98% (48/49); complete response (CR) rate in AT-005 group (67%) was not different from placebo (64%) [P = 1.0000].

Treatment with L-CHOP chemotherapy, with or without AT-005, resulted in a high ORR but relatively brief PFS in dogs with intermediate and high-grade T-cell lymphoma.

O04

Procoagulant platelet microparticles associate with regional metastatic cancer in dogs

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Platelet microparticles (PMP) are implicated in human cancer progression and metastasis. PMP concentration and procoagulant activity, assessed by phosphatidylserine (PS) expression, may differ in dogs according to cancer stage and potentially serve as a biomarker of disease severity. The purpose was to analyze PMP concentrations, including pro-coagulant PS+PMP and PS expression-level, in dogs with different stages of cancer.

Using flow cytometry, PMP and PS+PMP were analyzed in citrated whole-blood of 15 healthy dogs and 40 dogs with local (n = 19), regional metastatic (lymph node; n = 11) or distant metastatic (n = 10) cancer of various types. Influence of age, gender, weight, platelet count, white cell count, hematocrit and C-reactive protein (CRP) were investigated.

Only PS+PMP concentrations (median, range) differed among groups and was highest for regional metastasis (5,180, 1,050-28,360). This group, along with local cancer (2,790, 795-7,875), differed from controls (1,145, 250-7,525; $P = 0.0001$ and $P = 0.04$, respectively) and from distant metastasis (1,695, 250-17,845, $P = 0.01$). Platelet count did not differ among groups but positively correlated with PMP (r = 0.67, $P < 0.0001$) and PS+PMP (r = 0.38, $P < 0.006$). CRP positively influenced PS+PMP ($P < 0.0001$).

In a heterogenous group of canine cancers, increased PMP procoagulant phenotype was associated with regional metastasis and systemic inflammation, but not distant metastasis. The latter may be explained by cancer-induced systemic inflammation, ongoing platelet activation and 'platelet exhaustion'. The potential of PS+PMP as a biomarker of early canine cancer progression and prothrombotic risk warrants further investigations.

O05

Canine hepatobiliary neuroendocrine neoplasia: An immunohistochemical and proteomic study

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Hepatobiliary neuroendocrine neoplasia (NEN) occurs rarely in dogs and survival outcomes are highly variable. Improved characterization of these tumors is needed to identify prognostic biomarkers and generate informed treatment protocols. Formalin-fixed paraffin embedded tissue from two gallbladder neuroendocrine tumors and one hepatic neuroendocrine tumor were stained with immunohistochemical markers of NEN (chromogranin-A, synaptophysin, and neuron-specific enolase) and the gallbladder NENs were immunohistochemically stained to assess gastrin expression. Tumors were graded according to the WHO NEN classification system, which relies on mitotic rate and Ki-67 index. All NENs were positive for chromogranin-A, synaptophysin, and neuron-specific enolase. Unlike previously published reports, neither gallbladder NEN stained positively for gastrin. Tumor grade did not correlate with survival in the present cases. Secondarily, a proteomics strategy was applied to identify differentially expressed proteins in canine hepatobiliary NENs compared to normal canine adrenal and liver tissue. Thirty-four up-regulated, differentially expressed proteins were identified. Among these was galectin-1, a multivalent carbohydrate binding protein known to play a role in the development and progression of lung and pancreatic NEN. Drugs targeting the galectin family have shown promise as anticancer therapeutics in human cervical cancer, prostate cancer, lung and pancreatic NEN. Galectin-1 may represent a novel treatment target of hepatobiliary NEN in dogs.

O06

Cancer anorexia cachexia syndrome and associated biomarkers in dogs with lymphoma

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Cancer anorexia cachexia syndrome (CACS) in humans is characterized by weight loss, anorexia, and weakness, resulting in negative outcomes. The pathophysiology is incompletely understood but involves pro-inflammatory cytokines and hormonal dysregulation. Dogs might also develop CACS with similar negative outcomes.

We adapted human guidelines to diagnose CACS in dogs with lymphoma. We diagnosed CACS if dogs had > 5% weight loss 6 months prior to lymphoma diagnosis, with any **three** of following: appetite score < 3, muscle condition score < 3, body condition score < 4/9, CRP > 12.0 mg/l, IL-6 > 4.0 pg/ml, hemoglobin < 14 g/dl, or albumin < 3.2 g/dl. We hypothesized that inflammatory cytokines, ghrelin, leptin and survival would differ between dogs with and without CACS and healthy controls.

We included 36 dogs with lymphoma and 17 healthy controls. We measured aforementioned variables and also calculated a Glasgow Prognostic Score (GPS) for all dogs with lymphoma.

14/36 lymphoma dogs (38.9%) and 0/17 healthy controls had CACS. CACS dogs had higher serum CRP than healthy controls ($p = 0.001$) and lower albumin ($p < 0.0001$) and hemoglobin than both non-CACS or healthy controls ($p = 0.003$ and $P < 0.0001$, respectively). Ghrelin, leptin, TNF-α and γ-interferon did not differ between groups. CACS dogs had higher GPS than non-CACS dogs ($p = 0.002$) but similar survival. Dogs with CACS had similar survival to non-CACS dogs (log rank $p = 0.95$). Using only 5% weight loss and CRP > 12.0 mg/l to identify CACS had a PPV of 0.93 and a NPV of 1.0.

Dogs with lymphoma can have CACS, but this diagnosis appears to be of questionable clinical importance.

O07

Characteristic and comparison of physiologic FDG uptake in cats and dogs with positron emission tomography

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The purposes of this study were to identify the physiologic 18F-fluoro-2-deoxy-D-glucose (FDG) uptake of the cat using positron emission tomography/computed tomography (PET/CT) and to figure out its characteristics through comparing physiologic differences with the dog.

Total 7 recruited normal cats and 6 normal beagle dogs were examined with FDG-PET/CT. Regions of interests (ROIs) were manually drawn over 41 detailed structures of 5 gross structures (brain, head and neck, musculoskeleton, thorax, and abdomen), and then mean and maximum values of standard uptake value (SUVmean and SUVmax) were calculated for each ROIs. The physiologic variant was classified if increased radiopharmaceutical activity that had no evidence of clinical or radiological abnormal findings or SUVmean was greater than 2.5.

In both cats and dogs, the brain had the highest SUV compared to other gross structures. In the brain, the highest SUV was observed in the cerebellum of both cats (SUVmean: 4.90 ± 1.04, SUVmax: 6.04 ± 1.24) and dogs (SUVmean: 3.15 ± 0.57, SUVmax: 3.90 ± 0.74). Especially, cats had significantly higher intracranial uptake in comparison with dogs ($P < 0.001$). In the digestive system, the SUVs of the duodenum and the jejunum were significantly higher in dogs than those in cats ($P < 0.05$). FDG uptakes of the submandibular tip, the tonsils, the neck of the gallbladder, and the caudal colliculus were physiologically increased in cats.

This study demonstrates physiological FDG uptake in normal tissues throughout the body and its differences were interpreted between cats and dogs based on the species-specificity. This study will contribute to improve the accurate diagnosis of cancer patients and help to understand glucose metabolism in both cats and dogs.

O08

Veterinarian attitudes toward blood-based 'liquid biopsy' testing for cancer detection in dogs

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Cancer is the leading cause of canine death, affecting up to 1 in 3 dogs. Early cancer detection saves lives, but cancer in dogs is usually diagnosed when clinical signs have developed, the disease is advanced, and a cure is unlikely. Non-invasive pan-cancer blood tests are being developed for humans and may soon be available for dogs, leveraging recent advances in cancer genomics and sequencing technology. This 'liquid biopsy' testing may address multiple use cases, including cancer screening in a high-risk population; aid in diagnosis (when the clinical presentation is suspicious for cancer); and recurrence monitoring after therapy.

Nationwide quantitative (n=305) and qualitative (n=16) surveys of licensed veterinarians and veterinary oncologists were conducted by Kynetec, focusing on cancer screening, diagnosis, and management in pet dogs, and on clinician attitudes toward a blood-based liquid biopsy test across multiple use cases.

Respondents were evenly distributed nationwide, averaged 20 years in practice, were 64% female, and primarily treated dogs. One quarter of respondents defined themselves as early adopters of new tests, and 35% as proactive seekers of new tests and technology to incorporate into care. Among all respondents, there was a high perception of overall need for a liquid biopsy blood test for cancer detection, with 73% of veterinarians identifying a definite/likely need. Further, 84% of all respondents indicated they were very/extremely likely to use such a test as an aid in cancer diagnosis, and 80% were very/extremely likely to use as a cancer recurrence monitoring test. In verbatim survey comments, many general practice veterinarians reported that clients have asked for a blood test to identify cancer in dogs, and veterinary oncologists acknowledged the potential benefits of characterizing the genomic features of the cancer.

Both general practitioners and oncologists are aware of the high risk of cancer in dogs and clearly see the value of early detection for

achieving better outcomes. Novel pan-cancer liquid biopsy tests, when available, are likely to be welcomed by a considerable proportion of veterinarians and veterinary oncologists caring for pet dogs.

O09

A retrospective analysis of 12 dogs with surface osteosarcoma

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While the majority of canine osteosarcoma (OSA) arises from the medullary cavity, a rare subset arises from the surface of bone. In humans, surface OSA often has a more indolent disease course with significantly better outcomes than medullary OSA. The two most reported surface OSAs in the dog are periosteal (arising from under the periosteum) and parosteal (arising from the surface of the bone). The aim of this retrospective case series was to evaluate the clinical course and potential prognostic factors of dogs with surface OSA. Medical records from 14 dogs with surface OSA were identified and reviewed from 5 academic institutions. The histopathology of all cases was evaluated by two veterinary anatomic pathologists. The Kaplan-Meier method was utilized for the estimation of median progression free interval (PFI) and median survival time (ST). Log-rank tests were used to compare PFI and ST between groups. Six dogs were diagnosed with periosteal OSA, 5 dogs with parosteal OSA, one dog with juxtacortical OSA, and two cases were excluded after histopathologic evaluation. Three dogs were found to have metastatic disease at the time of diagnosis and four developed metastatic lesions after treatment. The median PFI and median ST for all dogs with surface OSA was 393 and 555 days, respectively. The 6 dogs diagnosed with periosteal had a median PFI of 461 days and median OST of 555 days, while the 5 dogs with parosteal OSA had a median PFI of 350 days and ST could not be calculated. Multiple prognostic factors (surgery, systemic adjunctive therapy, elevated ALP at baseline, appendicular vs axial location) were evaluated and none were found to be prognostic for PFI or OST. Dogs with surface OSA appear to be have prolonged PFI and ST, consistent with human studies of surface OSA.

O10

Investigation of the mechanism of impaired skin barrier function in dogs with malignant tumors

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Disturbances in the skin barrier function have mainly been associated with the pathogenesis of inflammatory dermatoses. In human medicine, skin barrier function has also been evaluated in patients with systemic disorders such as cancers and chronic kidney disease. However, there exist no studies on skin barrier dysfunction associated with naturally occurring systemic diseases in veterinary medicine. The study aimed to investigate the relationship between disturbed skin barrier function and increased oxidative stress and inflammatory mediators in dogs with spontaneously occurring internal diseases.

Healthy controls (n = 9, age = 4.11 ± 2.52 years, weight = 11.09 ± 8.44 kgs, 78 % male and 22 % female dogs, and 44 % sexually intact) and dogs with systemic diseases were enrolled in three different disease groups: malignant tumor (n = 10, age = 9.30 ± 3.37 years, weight = 9.52 ± 6.93 kgs, 50 % male and 50 % female dogs, and 20 % sexually intact); hyperadrenocorticism (n = 6, age = 9.00 ± 2.19 years, weight = 7.31 ± 4.57 kgs, 50 % male and 50 % female dogs, and 17 % sexually intact); and kidney disease (n = 8, age = 11.29 ± 4.50 years, weight = 4.30 ± 2.14 kgs, 50 % male and 50 % female dogs, and 25 % sexually intact). Dogs were excluded from the study if they were diagnosed with primary skin disease such as atopic dermatitis. Transepidermal water loss (TEWL), serum levels of claudin-1 and pro-inflammatory cytokines, and complete blood count (CBC) were measured to evaluate skin barrier function and the inflammatory state in each dog. Five pro-inflammatory cytokines assessed using multiplex immunoassays included granulocyte macrophage-colony stimulating factor (GM-CSF), keratinocyte chemotactic-like (KC-like), monocyte chemotactic protein-1 (MCP-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Serum levels of claudin-1, the major component of epidermal tight junction and skin barrier function were also determined by the ELISA.

It was observed that the mean TEWL value for the axilla region was significantly increased ($p < 0.01$) in the dogs with malignant tumors while serum claudin-1 concentrations were significantly lower ($p < 0.05$) compared to the healthy controls. Serum TNF-α levels were also significantly higher ($p < 0.05$) in the cancer group than in the healthy group. Also, the subgroup of cancer patients who were treated with chemotherapy showed significantly higher serum MCP-1 concentrations ($p < 0.05$), but lower IL-6 levels ($p < 0.05$) compared to the dogs who had not undergone chemotherapy. In the CBC panel, basophils were significantly increased ($p < 0.05$) in the cancer patients compared to the controls although the mean value was within the normal range (0.048 ± 0.069; reference range, 0.00 - 0.10 K/μL).

In conclusion, the skin barrier function was significantly decreased in the dogs with malignant tumors compared to the healthy dogs. The possible explanation is that the inflammatory condition and increased oxidative stress in the oncologic patients might result in a disturbed skin barrier. Indeed, canine cancer patients showed significantly higher serum concentrations of TNF-α compared to healthy controls, and the mean values of GM-CSF, KC-like, MCP-1, and IL-6 were all higher at levels that exceeded statistical difference, which might be due to the small sample size and various medical conditions in the cancer patients. Cancer patients who had received chemotherapy had significantly elevated serum MCP-1 concentrations, which suggests the increased oxidative stress in those patients. MCP-1 is released in response to oxidative

stress and inflammatory stimuli, and most of the antineoplastic agents elevate the levels of reactive oxygen species to induce the death of cancer cells. Significantly reduced circulating levels of IL-6 could be also explained based on the efficacy of chemotherapeutic drugs.

The clinical significance of this study is that the skin barrier defects in oncologic patients can be early detected with TEWL measurement. Suitable therapy might improve the skin barrier function by providing a defense ability against secondary infections and other cutaneous complications.

O11

The prognostic significance of metabolic tumor volumes F18-FDG PET/CT in dogs with appendicular osteosarcoma

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The purpose of this study was to retrospectively analyze a cohort of dogs afflicted with appendicular osteosarcoma that were staged with ^{18}F-FDG PET CT in order to determine if volumetric tumor volumes were prognostic for survival. Dogs were included if osteosarcoma was either cytologically or histologically confirmed, ^{18}F-FDG PET/CT was performed prior to initiation of treatment, and treatment consisted of a standard of care chemotherapy protocol including between 4-6 doses of carboplatin (300mg/m^2) after local treatment (SRT, surgical limb spare or amputation). Dogs were excluded if SUV_{max} of the primary tumor was less than 3.0. Metabolic tumor volumes (MTV) and total lesion glycolysis (TLG) were automatically segmented at a variety of set and fixed thresholds and calculated by 2 observers in order to be able to test for repeatability of measurements. These values, along with SUV_{max}, were evaluated for prognostic significance in this population of dogs. There were 73 dogs included in this analysis. There was excellent correlation between 2 observers for all values. Multiple volumetric parameters were significantly associated with survival. SUV_{max}, TLG 2.5, MTV (liver + 1SD), and MTV (liver + 2SD) had statistically significant hazard ratio (1.055, 1.002, 1.006, 1.007, respectively) and the p-values were 0.015, 0.006, 0.045, and 0.030. SUV_{max} had the highest sensitivity for survival (82.6%) and TLG at 2.5 SUV*cm^3 had the highest specificity for prediction of survival (74.4%) based on ROC calculations. The SUV_{max}, MTV at 2.5 SUV were significantly different between dogs that survived more than or less than 1 year (p-value of 0.004 and 0.048 respectively). This study is the first of its kind in veterinary medicine that retrospectively evaluated volumetric tumor values for prognostic significance.

O12

CCNU in combination with monoclonal antibody (AT-005, tactress) for naïve canine intermediate/high-grade, peripheral T-cell lymphoma

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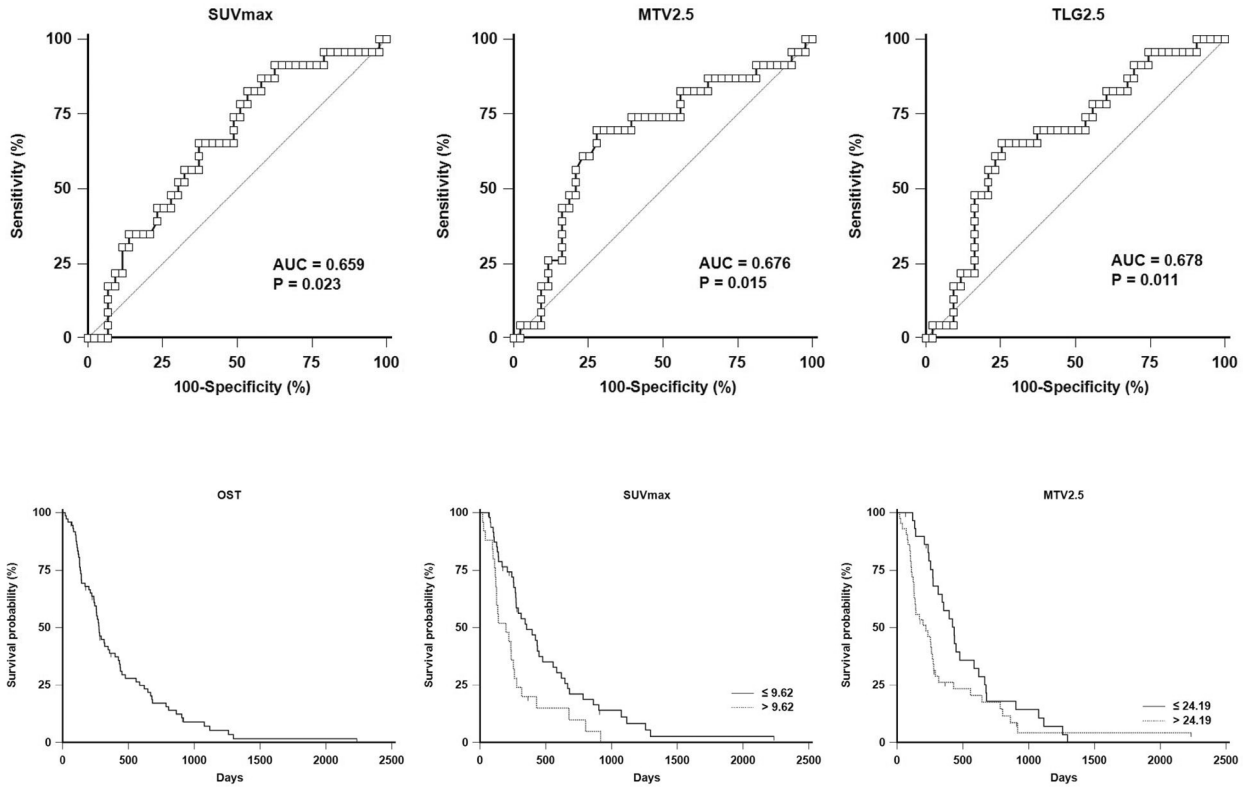

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Canine T-cell lymphoma has a relatively poor prognosis compared to B-cell lymphoma. AT-005 is a caninized monoclonal antibody licensed by the USDA. This study evaluated if addition of AT-005 to CCNU chemotherapy improved progression-free survival (PFS) and duration of response (DOR) in dogs with intermediate and high-grade peripheral T-cell lymphoma.

Randomized, placebo-controlled, investigator- and owner-masked, multicenter clinical study. Dogs were immunophenotyped by flow cytometry and received 2 treatments of CCNU (70 mg/m^2) with a 4-week tapering course of prednisone prior to randomization. Dogs achieving partial response or complete response (CR) 2 weeks following the second CCNU treatment were randomized 1:1 into AT-005 or placebo groups. AT-005 or placebo was administered intravenously twice weekly for 4 weeks, then every other week for 4 treatments. Response was evaluated via RECIST criteria for canine lymphoma. PFS was calculated from time of randomization (~Day 35) to progressive disease.

Sixty-one (61) dogs were enrolled; 46 dogs were randomized (23 to AT-005 and 23 to placebo). Demography was similar between groups. At the time of randomization, CR was observed in 19 dogs (83%) in AT-005 group and 17 (74%) in placebo group. Median PFS was 35 days (95% CI, 21-41) in AT-005 group compared to 14 days (95% CI, 12-49) in placebo group (P = 0.3714). The mean DOR was 77 days for AT-005 group and 62 days for placebo group (P = 0.6354).

Treatment with 2 cycles of CCNU and prednisone, with or without AT-005, provided limited clinical benefit to dogs with intermediate and high-grade T-cell lymphoma.

O13

Expression and prognostic value of receptor c-met in canine malignant melanoma

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Canine malignant melanoma (CMM) is an aggressive tumor with a high degree of local invasiveness and metastatic propensity. While surgery and radiation therapy can provide stage-dependent clinical benefit, CMM is resistant to chemotherapy so alternative systemic treatment is urgently needed. In many human malignancies, including cutaneous melanoma, aberrant activation of c-Met, a cell-surface tyrosine kinase receptor, has been reported and c-Met has been identified as a relevant therapeutic target. c-Met expression has not been thoroughly evaluated in canine malignant melanoma, highlighting a gap in knowledge that our study aims to address. Through Western Blots using 2 CMM cell lines, TLM-1 and CMGD-2, we confirmed expression of c-Met protein in CMM. We also validated our c-Met antibody with positive immunohistochemistry (IHC) staining of cell pellets generated from these 2 CMM cell lines. To evaluate the clinical relevance of c-Met expression in CMM, 33 cases (20 oral, 7 digits, and 6 cutaneous) are retrospectively evaluated for prognostic factors, including tumor

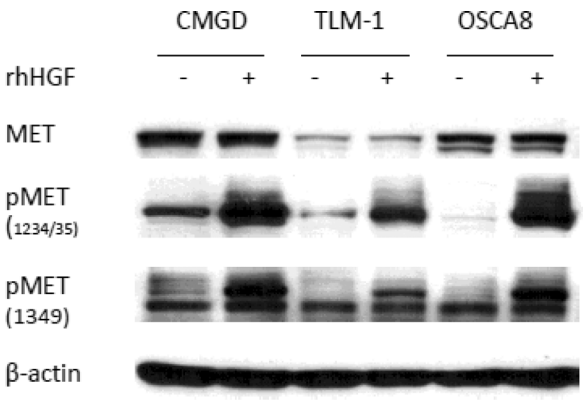

Figure 1. Recombinant human HGF (rhHGF) induces Met activation. CMGD-2 and TLM-1 are canine melanoma cell lines and OSCA-8 is a canine osteosarcoma cell line, used as positive control. All the experiments were performed after serum starvation and then stimulated with rhHGF (+) or untreated (-).

location, tumor size, stage, lymph node and distant metastasis, mitotic count, and Breslow thickness. These prognostic factors, as well as patients survival times, will be correlated with the respective score of c-Met positive cells in tissue samples. Results of this study will provide proof of concept to evaluate the potential role of c-Met as a biomarker and stratification criteria for patients diagnosed with CMM.

O14

Vaccine-induced ErB (EGFR/HER2)-specific immunity in spontaneous canine cancer

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Members of the ErbB family of proteins are overexpressed on a number of human and canine cancers, which often is indicative of a poor clinical outcome. Treatment of EGFR/HER2 overexpressing human cancers includes monoclonal antibody therapies, cetuximab (Erbitux) or trastuzumab (Herceptin), respectively, either alone or in conjunction with other chemotherapies. Herein, we describe a novel immunization approach to induce a polyclonal anti-EGFR/HER2 immune response in companion dogs with spontaneous arising tumors associated with ErbB tumor protein expression. Canine patients were immunized and boosted with a short peptide of the ErbB extracellular domain with shared sequence homology between EGFR, HER2, and HER3. Serum analyses demonstrated high titers of EGFR/HER2 binding antibodies with biological activity similar to that of cetuximab and trastuzumab. The canine serum antibodies bound both canine and human EGFR and HER2 on tumor cell lines and/or tumor tissue. CD8 T cells and IgG deposition were observed in the tumor microenvironment from EGFR peptide immunized dogs. The antibodies inhibited EGFR intracellular signaling (pEGFR), resulting in the inhibition of tumor cell growth *in vitro*. Additionally, we demonstrate preliminary objective responses in reducing tumors at pulmonary metastatic sites and 12-month survival statistics (mean of 65%; 95% CI) in osteosarcoma bearing patients. Taken together, the results of this study suggest that dogs with ErbB bearing tumors mount specific humoral and cell-mediated immune responses directed at the tumor *in vivo* and *in vitro* following ErbB peptide immunization, and responses may mediate clinically relevant outcomes in dogs with osteosarcoma. Incorporating ErbB peptide vaccination with standard-of-care osteosarcoma treatment, or in combination therapy with other immune stimulators such as checkpoint inhibitors, may expand treatment options and potentially improve outcomes of dogs with osteosarcoma.

O15

Single-agent procarbazine chemotherapy for Naïve multicentric B cell lymphoma in dogs

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Procarbazine is an alkylating chemotherapy agent commonly used in multi-agent protocols such as MOPP (mechlorethamine, vincristine, prednisone, procarbazine) for the treatment of canine lymphoma. The use of procarbazine in veterinary oncology has been directly extrapolated from human medicine, and there is inexistent data regarding safety and efficacy as a single-agent therapy for canine multicentric lymphoma. The aim of this study was to evaluate the response rate and tolerability of single-agent oral procarbazine in dogs with confirmed, substage a, treatment-naïve, multicentric B cell lymphoma. Enrolled dogs received procarbazine at a dosage of 50 mg/m^2/day orally for 14 days, and response was evaluated at day 7 and 14. All clinical and hematological toxicities were graded according to the VCOG Common Terminology Criteria for Adverse Events (VCOG-CTCAE).

Six dogs were enrolled; response evaluation at day 14 was available for 5 dogs with an overall clinical benefit of 80% (stable disease in 4/5). All six dogs experienced an adverse event (AE), however, none of the dogs had a grade III or higher AE. The most common AEs noted were weight loss (n=6) and hyporexia (n=3). The only hematologic toxicity noted was a grade 1 thrombocytopenia in one patient. One dog developed a grade 1 hypercreatinemia.

Oral procarbazine was well-tolerated and dose escalation studies should be considered to determine if additional clinical benefit can be achieved with higher dosing.

O16

An anti-canine PD-1 monoclonal antibody for immunotherapy of cancer in dogs

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Programmed cell death receptor 1 (PD-1) is a T cell co-inhibitory checkpoint molecule expressed by T cells, natural killer (NK) cells and antigen presenting cells (APCs). The interaction of PD-1 with its ligand programmed cell death ligand 1 (PD-L1) on APCs results in suppression of T cell activation and effector functions, thereby preventing autoimmunity and limiting tissue damage when T cells are activated in response to infection. Recent studies indicate that tumor cells also express PDL-1 and exploit the interaction between PD-1 and PDL-1 to turn off tumor-infiltrating lymphocyte activity and thus, evade destruction by tumor-specific T cells. This realization led to the

development of a novel class of drugs as cancer therapeutics based on monoclonal antibodies (referred to as checkpoint inhibitors) that prevent the interaction between human PD-1 and PDL-1. These antibodies have been evaluated in numerous clinical trials and produced impressive tumor responses including unprecedented levels of complete and durable responses across several tumor types. Currently, these antibodies are registered by the FDA for use in humans as first and second line therapy for multiple tumor types. To explore the potential of monoclonal antibodies (mAb) as checkpoint inhibitors for treatment of cancer in dogs, we developed mouse anti-canine PD-1 antibodies with specificity and high affinity for canine PD-1 as determined by ELISA and Biacore; respectively. Several of the mouse anti-canine PD-1 antibodies were subjected to antibody engineering to produce caninized antibodies in which two amino acid residues within the Fc domain were mutated to minimize effector functions, as demonstrated by diminished binding to CD64 and complement. The pharmacological activity of the lead antibody (gilvetmab) was confirmed in vitro by demonstrating, using a CHO cell line expressing full length canine PD-1, that gilvetmab blocks the interaction between PD-1 and its ligand PDL-1. In addition, gilvetmab stimulates IFN-gamma cytokine secretion from canine peripheral blood mononuclear cells (PBMC) after stimulation with concanavalin A (ConA) but not in the absence of stimulation. We also have shown that intravenous administration of this antibody to healthy beagle dogs leads to PD-1 target engagement on CD4 and CD8 T cell subpopulations. In conclusion, we have developed a canine PD-1 specific monoclonal antibody and demonstrated its pharmacological activity, as a first step in evaluating its potential as a novel therapeutic agent for immunotherapy of cancer in dogs.

O17

mRNA expression of the prostaglandin receptor EP4 in Canine Lymphoma

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Cyclooxygenase enzymes and prostaglandins stimulate the development of solid tumors and hematological malignancies in human and veterinary medicine. These effects are thought to be mediated through the prostaglandin receptor EP4 (EP4R). In human T-cell lymphoma (LSA), EP4R expression is increased and drives LSA cell survival. The role of EP4R in human B-cell LSA is more complicated, conferring decreased malignancy in some circumstances.

The goal of this study was to determine the EP4R mRNA expression in canine B-cell and T-cell LSA, compared to reactive lymph node tissue.

Archived B-cell LSA (n = 18), T-cell LSA (n = 18), and reactive (n = 23) lymph node biopsies were evaluated for EP4R mRNA expression via the novel *in situ* hybridization technique: RNAscope®. mRNA signal expression metrics (copy number/cell, % transcript expression, and H-index) were quantified with an advanced digital pathology image analysis system (HALO).

All B-cell LSA, T-cell LSA, and reactive lymph node samples expressed EP4R mRNA. Expression was statistically higher in B-cell LSA and T-cell LSA samples compared to reactive lymph nodes. There was no statistical difference between B-cell and T-cell LSA. Mitotic index and grade did not correlate with expression of EP4R mRNA.

These results suggest increased EP4R mRNA expression may contribute to the development of canine B-cell and T-cell LSA. This is consistent and similar to reported results in human T-cell LSA, but contrasts with the reported trends in human B-cell LSA. Additional studies are necessary to fully elucidate the potential role of EP4R in the development of canine LSA.

O18

Gold nanoparticles and photothermal ablation as a novel approach for treating canine soft tissue sarcomas

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Soft tissue sarcomas originate from a variety of mesenchymal tissues, but they are often treated as one entity. These tumors are locally invasive, have poorly defined margins and tend to follow tissue planes to dissect and invade surrounding tissue [1]. Conservative surgical removal can produce common reoccurrence and aggressive surgery with wide surgical margins is recommended. With large surgical margins, local recurrence is less than 10 % and offers years of control. The most significant factor for local recurrence were narrow or contaminated surgical margins[2]. The treatment of choice at this time is still for conventional aggressive surgical intervention and when margins are not feasible to follow with radiation therapy. But there are limited options for difficult anatomic locations or recurrent sarcomas where radiation therapy would not be desirable and new novel approaches to these tumor types should be considered [3].

Nanoparticles have been extensively researched over the years [4] and are now being used in human and veterinary clinical trials [5–7]. Current research suggests nanoparticles have a variety of diagnostic and therapeutic uses in oncology[8–10]. Nanoparticles may be made with a variety of materials. Those composed of a silica core with a gold shell and a total diameter of ~150 nm, have been previously used in animal studies[11–14], are non-toxic[15] and are designed to maximally absorb near-infrared light and convert it to heat. In this type of "nanotherapy", near-infrared absorbing nanoparticles accumulate in tumor tissue via leaky tumor vasculature (a process known as the enhanced permeability and retention effect [16]) and then are irradiated with near-infrared laser light. The tumor undergoes specific photothermal heating, resulting in selective hyperthermic cell death, while sparing adjacent healthy tissues.

Building on previous pilot studies showing efficacy in small, low-grade Mast Cell tumors in canines [17], utilizing gold nanoparticles and PTA in a spontaneous tumor model for soft tissue sarcomas was considered. If this nanotherapy is successful in treating soft tissue sarcomas, especially for tumors in locations that are not amenable for complete surgical removal, a new treatment option may be available for these patients. Our hypothesis is that photothermal ablation (PTA) using gold nanoparticles and near infrared light exposure will provide palliative and/or definitive therapy of canine soft tissue sarcomas with minimal/manageable toxicity.

Nine dogs with soft tissue sarcomas varying in size, grade, and location, were entered into a pilot study over 18 months to evaluate the safety and efficacy of utilizing gold nanoparticles and photothermal ablation with laser light to treat these tumors. Some of these tumors were advanced or recurrent after other therapies and were not excluded from participation. All dogs received a systemic intravenous infusion of gold nanoparticles followed by the application of 808 nm laser light first applied to the tumor using a diode laser with a specialized sapphire treatment probe 24 hours later. Some patients received multiple treatment applications of laser light over a period of several weeks. Patients were evaluated once a week for four weeks and then monthly. Data collected included signalment, tumor dimensions pretreatment, response to therapy, duration of response, and any toxicities.

Soft tissue sarcomas ranged in size from a measurement of 0.8 cm to 7 cm in longest dimension. At the time of this abstract, all dogs achieved either a visible partial response (33 %) or complete response (67 %) to therapy. Four (4) patients have experienced progressive disease after a period of tumor control and five (5) patients are still in remission.

Of the patients that have experienced progressive disease, the mean progression free time (PFT) was 152 days, with a median PFT of 169 days. Of the patients that are still in remission, their current mean PFT is 215 days, with a median PFT of 197 days. Patients experienced only minimal toxicities, including complete death of tumor (necrosis) or very mild self-resolving thermal burns.

Gold nanoparticle infusion with targeted photothermal ablation therapy can be applied in canine patients with soft tissue sarcomas with a low risk of toxicities while providing anticancer effects.

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O19

Molecular design of hypoxia-targeting therapy for intestinal T-cell lymphoma in dogs

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The transcription factor hypoxia-inducible factor 1α (HIF-1α) is activated in response to oxygen deficiency, and is expressed in several cancers under the intratumoral hypoxic stress that arises during pathogenic processes. We previously reported a pilot study, in which hypoxic stimulation enhanced the growth potential of canine lymphoma by activating the HIF-1α signaling pathway (Yamazaki H, et al. Plos One. 2017). The aim of the present study was to establish a molecular

Fig. 1

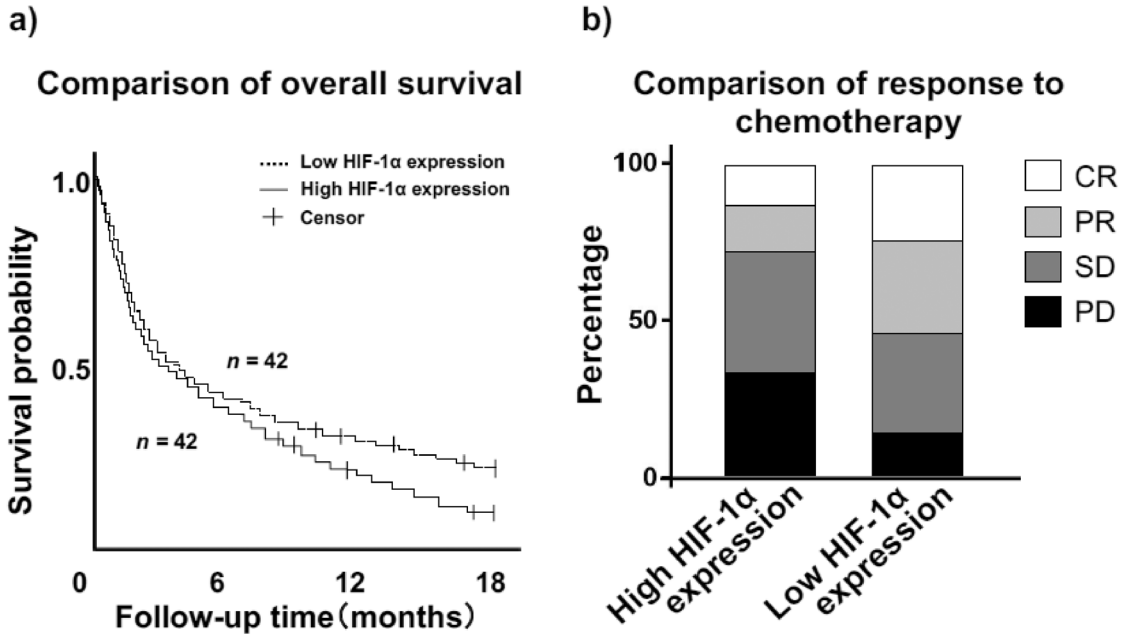

Figure 1. a) Kaplan-Meier curve of the overall survival between 42 dogs with higher HIF-1α expression and 42 those with lower HIF-1α expression in 84 dogs with intestinal lymphoma. The hazard ratio of the higher HIF-1α expression group versus the lower HIF-1α expression group was 0.72 (95% CI 0.66–0.96; $P = 0.0025$). b) Comparison of response to chemotherapy between 42 dogs with higher HIF-1α expression and 42 those with lower HIF-1α expression in 84 dogs with intestinal lymphoma.

Fig. 2

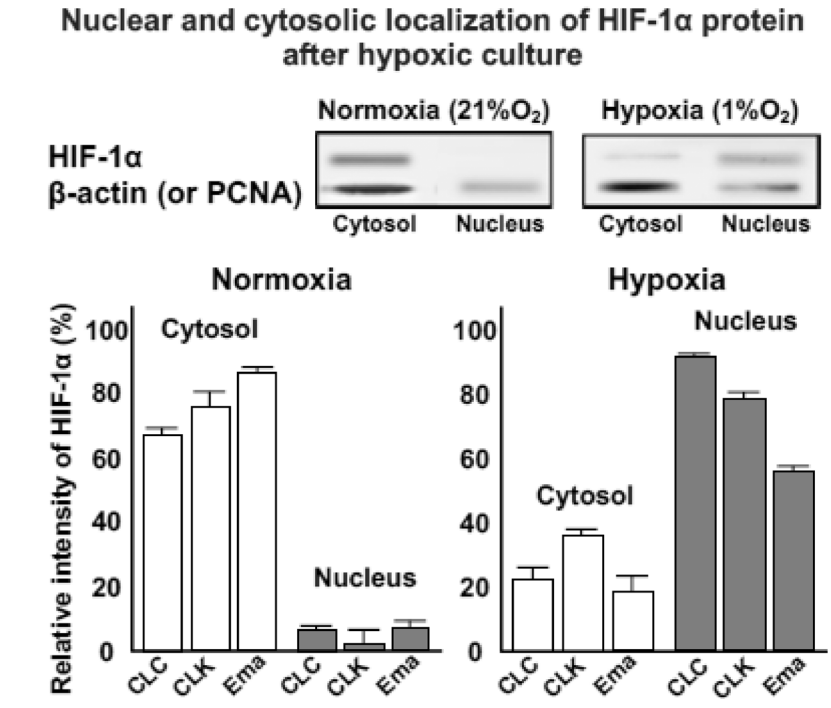

Figure 2. Nuclear and cytosolic localization of HIF-1α protein in intestinal T-cell lymphoma cell lines (CLC, CLK and Ema) after hypoxic culture. After 24 hours in culture under normoxia (21%O_2) or hypoxia (1%O_2), nuclear and cytosolic HIF-1α protein was detected with a western blotting analysis. Immunoreactive bands were quantified and are presented as relative intensities (%) normalized to those of β-actin (cytosol) or PCNA (nucleus). Each bar represents the mean ± SD of three separate experiments.

Fig. 3

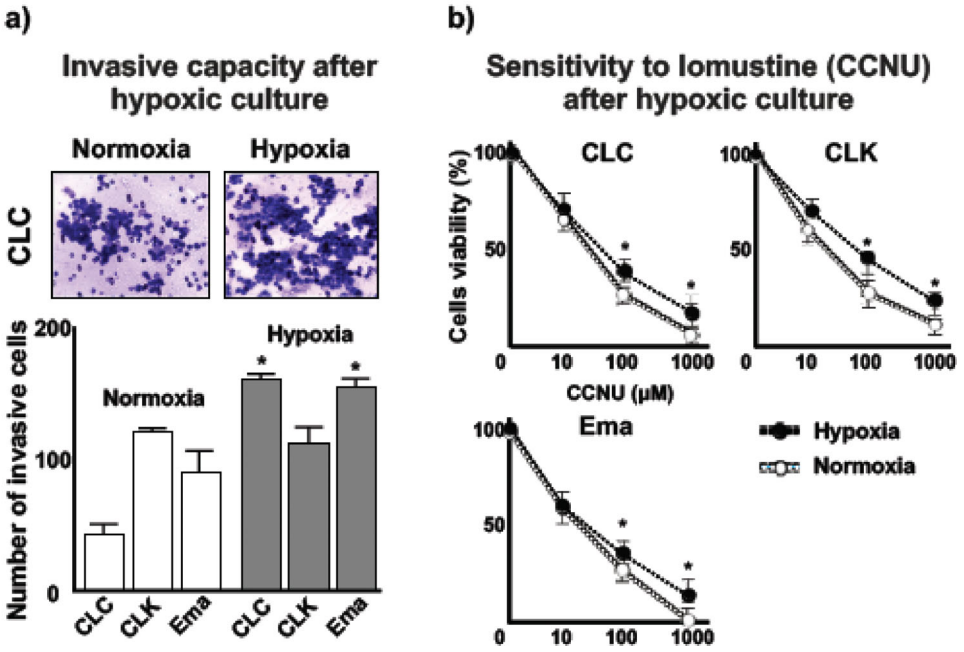

Figure 3. Invasive capacity and sensitivity to lomustine (CCNU) of intestinal T-cell lymphoma cell lines (CLC, CLK and Ema) after hypoxic culture. a) After 7 days in hypoxic culture (1% O_2), number of invasive cells were evaluated by using cell invasive assay kit. Each bar represents the mean ± SD of three separate experiments. *P < 0.05 vs normoxia (Dunnett's test). b) After 7 days in hypoxic culture (1% O_2), CCNU sensitivity was evaluated by using MTT assay kit. Relative cell viability is shown as a percentage (%) of the control (nontreatment), and each bar represents a mean ± SD. *P < 0.05 vs normoxia (Dunnett's test).

Fig. 4

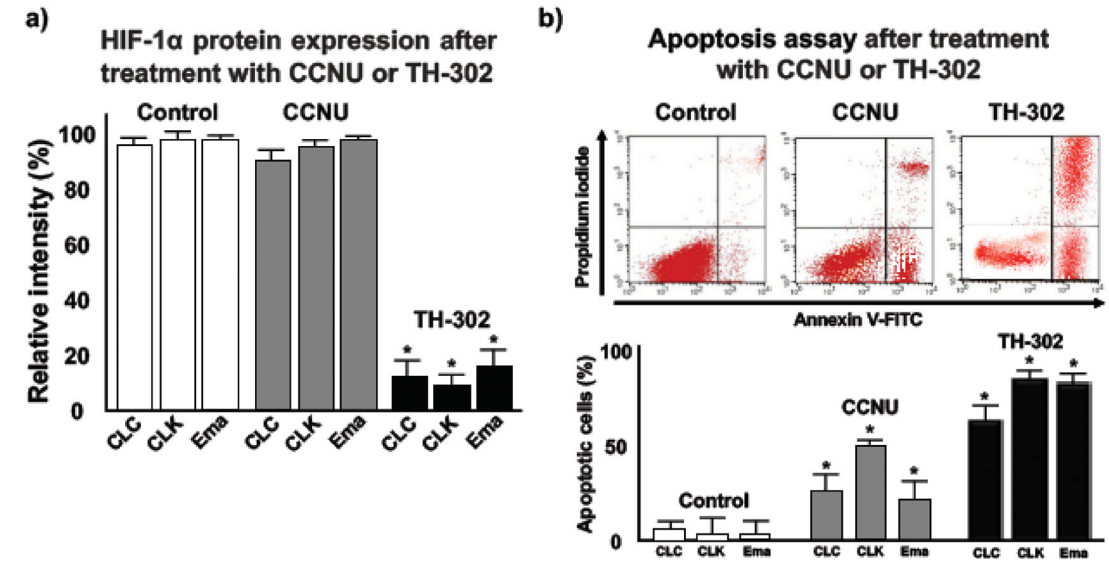

Figure 4. HIF-1α protein expression and apoptosis in intestinal T-cell lymphoma cell lines (CLC, CLK and Ema) after treatment with CCNU or TH-302. a) After treatment with CCNU (100 μM) and TH-302 (50 μM) for 24 h during hypoxic culture (1% O2), nuclear HIF-1α protein was detected with a western blotting analysis. Immunoreactive bands were quantified and are presented as relative intensities (%) normalized to those of PCNA. Each bar represents the mean ± SD of three separate experiments. *P < 0.05 vs control (nontreatment) (Dunnett's test). b) After treatment with CCNU (100 μM) and TH-302 (50 μM) for 24 h during hypoxic culture (1% O2), apoptotic cells were evaluated with flow cytometry. Each bar represents the mean ± SD of three separate experiments. *P < 0.05 vs control (Dunnett's test).

design strategy for a novel hypoxia-targeting therapy for intestinal T-cell lymphoma in dogs.

We assessed the relationship between immunohistochemistry-based HIF-1α expression and clinical information, including clinical stage, treatment response, and outcomes, using 84 tissue samples from dogs with intestinal lymphoma. We investigated the effect of hypoxic stimulation on the biological behavior of cell lines from three different types of canine intestinal T-cell lymphoma. We assessed the effects of the hypoxia-activated prodrug (TH-302) on the cell lines cultured under hypoxic conditions (exhibiting increased expression of HIF-1α).

Our data showed that treatment response and overall survival might be significantly decreased in dogs with higher HIF-1α expression than in those with lower HIF-1α expression. Hypoxic culture (1% O_2, 72 h) enhanced the growth rate and invasiveness of canine lymphoma cell lines, and decreased their sensitivity to CCNU (CeeNu, lomustine), resulting in hypoxia-dependent aggressive behavior. Sensitivity to TH-302 significantly increased in these cell lines compared to those cultured under normoxia, which exhibited hypoxia-dependent apoptosis. Additionally, TH-302 preferentially down-regulated HIF-1α expression, induced cell-cycle arrest, and enhanced double-stranded DNA breaks in the cell lines cultured under hypoxia, suggesting that TH-302 might inhibit cell growth potential by inactivating HIF-1α-dependent cell signaling.

Our results revealed the preclinical anti-tumor activity of TH-302 and provided a rationale for further treatment strategies for dogs with intestinal T-cell lymphoma.

EN01

RNA-sequencing as a novel hypothesis generating tool to unravel the pathogenesis of feline hyperthyroidism (ESVE award winner)

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Feline hyperthyroidism is highly prevalent amongst geriatric cats. Causal mechanisms in this disease are not fully understood. Identification of key driving factors in thyroid hyperplasia and autonomous production of thyroid hormone has the potential to reveal novel treatment targets.

RNA-sequencing (RNA-seq) is an unbiased technique for examining the transcriptome of a tissue, to identify genes that are differentially expressed between tissues. We hypothesised that thyroids from hyperthyroid and euthyroid cats would have a different transcriptome and that differentially expressed genes would offer novel targets for management of feline hyperthyroidism.

In this pilot study, RNA was extracted from four archived feline thyroid glands, obtained with owner consent and ethical approval, either immediately after euthanasia, or during surgical thyroidectomy and frozen at -80 degrees after fixation in RNA*later*. Two glands were from euthyroid cats, one from a medically treated hyperthyroid cat, and one from an untreated hyperthyroid cat. After a ribosomal RNA depletion step, a barcoded RNA-seq library was prepared from each sample and the pooled libraries were sequenced on a single lane of a HiSeq4000.

Sequencing reads were mapped to the feline reference genome (*FelCat9.0*) and bioinformatic differential expression analysis was undertaken using a bespoke pipeline and high-performance computing cluster. Principal component analysis (PCA) revealed that the 2 euthyroid cats clustered together, separately to the two hyperthyroid cats. The treated and untreated hyperthyroid cats were also separated by the PCA. In total, 1068 genes were significantly differently expressed between hyperthyroid and euthyroid cats. Differentially expressed genes included those related to thyroid hormone synthesis (e.g., *SLC5A5*, *PSMP8*), general cell growth (e.g., *VEGFA*, *CNTN1*), cell activity (e.g., *G6PD*, *SRXN1*) and calcium metabolism (e.g., *CYP27B1*, *CAMK1G*).

This pilot study demonstrates that RNA-seq is feasible in archived feline thyroid glands and has exciting potential to unravel the pathogenesis of feline hyperthyroidism, as well as to reveal new targets for therapy.

EN02

Blood-to-saliva glucose time lag in sedated dogs

Gal, Arnon[1], *Ioannou, Antonia*[2], *Phillips, Heidi*[2], *Keating, Stephanie*[2], *Barger, Anne*[2], *Lopez-Villalobos, Nicolas*[3], *Wilson, Macy*[4], *Dillonaire, Alyssa*[5], *Silveston-Keith, Rebecca*[6], *Ambrisko, Tamas D.*[2]

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The management of diabetes mellitus mandates sampling of blood glucose. Saliva offers an alternative to blood sampling, but the blood-to-saliva glucose time lag is uncertain. This study aimed to determine the serum-to-saliva glucose time lag in dogs' saliva.

The combined duct of the mandibular/sublingual salivary glands of 6 healthy dogs were cannulated to collect saliva and prevent glucose degradation by oral bacteria. Paired serum-saliva samples were collected at baseline and in 12, 5-minute blocks over 60 minutes following a 0.25g/kg intravenous bolus of dextrose. Serum and salivary glucose levels were analyzed with a linear mixed model for repeated measures with a compound symmetry error structure.

Mean (±SD) saliva production was 10.3±2.9μL/kg/min. The area under the curve ($AUC_{glucose}$) $_{saliva/serum}$ ratio was 0.006. Salivary glucose significantly increased by 0.0167±0.0056μmol/L (0.003±0.001μg/mL) for every 1μL increase in saliva volume. Serum glucose at 20 minutes correlated with salivary glucose at 45 ($r=0.82$, $p=0.09$), 50 ($r=0.95$, $p=0.047$), and 55 ($r=0.85$, $p=0.066$) minutes. The serum-to-saliva glucose time lag was approximately 30-40 minutes. Storage time at -80°C had a significant linear and quadratic effect on salivary glucose ($p=0.003$); this means that the rate of reduction in salivary

glucose concentration diminished over time. Glucose storage at -80°C significantly reduced serum glucose linearly (p=0.008).

Saliva is an attractive matrix for noninvasive glucose quantification; however, glucose measurements in the saliva pose challenges. Low salivary glucose levels require sensitive glucose measurement methodologies. We caution against the stability of salivary glucose secondary to rapid degradation by oral bacteria or during storage.

EN03

The use of desmopressin acetate to reduce polyuria and polydipsia associated with prednisolone administration

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Glucocorticoids are used for a variety of purposes in veterinary medicine but often come with significant adverse effects. Polyuria and polydipsia are the most frustrating adverse effects noted by owners. This aim of this study is to determine whether administration of desmopressin ameliorated the polyuria and polydipsia associated with prednisolone administration.

Seven healthy adult Walker Hounds participated in this prospective study. Daily water intake and urine specific gravity were measured in dogs under 4 separate sequential conditions: no medications (C), prednisolone only (P), prednisolone and desmopressin (PD), and prednisolone immediately after discontinuation of desmopressin (PAD).

When compared to baseline, six out of seven dogs became polydipsic after administration of prednisolone, 0.5mg/kg orally twice daily. When desmopressin, 5 mcg/dog subcutaneously twice daily was administered to dogs receiving prednisolone, there was a statistically significant decrease in water intake and sodium concentration, and a significant increase in urine specific gravity.

Administration of desmopressin to dogs receiving prednisolone significantly decreased water intake and sodium concentration, and increased urine specific gravity. This suggests that desmopressin ameliorates the most significant side effect of prednisolone noted by owners, but that hyponatremia is an important complication associated with desmopressin use.

EN04

Safety and tolerability of OKV-119: A novel exenatide long-term drug-delivery-system in cats

Gilor, Chen[1], Klotsman, Michael[2], Adin, Christopher[2], Anderson, Wayne[2]

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone that plays an important role in glucose homeostasis and food intake. In people, GLP-1 receptor agonists (GLP-1RAs) are commonly used for the treatment of obesity and type-2 diabetes; however, nonadherence to injectable medications is common. OKV-119 is an investigational drug-delivery-system intended for subcutaneous implantation and delivery of the GLP-1RA exenatide for up to 6 months.

Our objectives were to develop protocols for the subcutaneous insertion and removal of OKV-119 and evaluate its tolerability, safety of supratherapeutic doses, and weight loss effects in purpose-bred cats.

Implant insertion, removal and imaging were first studied in two cadaveric cats at three subcutaneous locations. Four purpose-bred cats were implanted with OKV-119 configured to release exenatide for a four-week duration. Body weight and plasma exenatide concentrations were measured weekly for nine and six weeks, respectively.

In anesthetized cats, implant insertion and removal were performed in under two minutes. OKV-119 was easily identified on radiographs, and well tolerated without any apparent implant site reactions. No systemic adverse effects were observed. Weekly plasma exenatide concentrations were inversely correlated to weight loss ($r = 0.8$ [95% CI = -0.9 – -0.6], $p < 0.0001$).

Our findings suggest that OKV-119 can be easily inserted and removed during a routine clinic visit. Exenatide may be an effective agent for treating feline obesity. Further study of OKV-119 for feline diabetes is also warranted to determine whether the combination of improved glycemic control, coupled with minimal impact on pet-owner's lifestyle, leads to improved patient outcomes.

EN05

Evaluation of glycemic variability in cats with diabetes mellitus and prediction of diabetic remission

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Within-day glycemic variability (GV) is a relevant parameter in monitoring humans with diabetes mellitus (DM). A recent study showed that diabetic cats achieving remission had lower GV when compared with cats not achieving remission. Therefore, it may be useful to investigate further the GV in monitoring feline DM. Our study aims at defining a threshold of within-day GV to predict diabetic remission. In addition, the relationship between GV and insulin therapy, diet, home monitoring, concurrent suspected hypersomatotropism (serum IGF-1 >1000 ng/mL), and glycemic control was evaluated. Blood glucose curves (BGCs) were retrospectively reviewed from the database of a single referral center. BGCs lasting for less than 8 hours and with less than 4 blood glucose measurements were excluded from the evaluation. Glycemic control was assessed using the serum fructosamine concentration and owner's perception of clinical signs (mental status, polyuria, polydipsia, and polyphagia). GV was determined for all BGCs using the percentage coefficient of variation [%CV=(Standard Deviation of BGC values/Mean of BGC values)*100] of the blood glucose concentrations. Data were summarized through the median (interquartile range) and analyzed using nonparametric tests. Logistic regression analysis was performed to evaluate, through a sensitivity analysis, a possible threshold for %CV. Nine

hundred twenty-seven BGCs from 98 cats were retrospectively evaluated. Median %CV was higher in cats receiving porcine lente insulin [28.6(21.9-40.4)%], in cats monitored with at-home BGCs [23.2 (18.9-31.5)%] and in cats not achieving remission [22.4(15.7-27.8)%] when compared with cats receiving other insulin products [21.3 (15.6-27)%](p < 0.01), cats monitored with in-clinic BGCs [21.3 (14.5-26.1)%](p = 0.04) and cats achieving remission [15.2(12.3-19.8)%] (p < 0.01), respectively. Median %CV was not different when comparing cats receiving a low carbohydrate diet [23.5(16.8-28.1)%] and cats with suspected hypersomatotropism [24.3(20.6-28.5)%] with cats receiving a not specific diet [21.8(20.5-23.4)%](p = 0.79) and cats without suspected hypersomatotropism [21.9(15.8-27.5)%](p = 0.43), respectively. Serum fructosamine concentration and %CV showed no correlation (r = 0.01). For all clinical signs evaluated median %CV was not different when comparing cats presenting clinical signs with cats not presenting clinical signs. A possible %CV threshold of 15% to distinguish cats likely or unlikely to achieve remission was identified (specificity 56.12% and sensitivity 76.19%). Remission probability decreased of 10% for each point over the %CV threshold [OR 0.90; CI 95% (0.84-0.97)]. Cats receiving porcine lente insulin, monitored with at-home BGCs, and not achieving remission tend to have higher glycemic excursions. Serum fructosamine concentration and clinical signs do not relate to GV. A %CV value of 15% appears to be a suitable threshold to discriminate cats likely to get into remission.

EN06

Outcome of radioiodine therapy for feline hyperthyroidism: fixed dosage versus individualized dosage

Lutz, Bérénice[1], Matos, Joana[2], Grandt, Lisa-Maria[3], Meneses, Felix[4], Francey, Thierry[3], Schweizer-Gorgas, Daniela[4], Campos, Miguel[3]

[1]VetSuisse, Bern, Bern, Switzerland, [2]Clinique Vétérinaire Animalis, Puidoux, Switzerland, [3]Small Animal Clinic, Internal Medicine, VetSuisse, Bern, Switzerland, [4]Department of Clinical Veterinary Medicine, Clinical Radiology, Vetsuisse, Bern, Switzerland

Radioiodine is considered the best curative treatment for feline hyperthyroidism. To date, there is no consensus regarding the best method to calculate the dosage of radioiodine to administer to hyperthyroid cats. The goals of this study were to compare thyroid function, renal function and survival time between hyperthyroid cats receiving a fixed dosage of radioiodine and hyperthyroid cats receiving an individualized dosage calculated using a clinical scoring system.

The medical records of 110 cats treated with radioiodine therapy between 2008 and 2020 were reviewed. The thyroid function, renal function and survival of cats treated with a fixed dosage of radioiodine (2008 - 2015; n = 50) were compared to those of cats treated with an individualized dosage (2015 - 2020; n = 60,) at different timepoints after therapy.

After treatment with a fixed dosage (mean 171 MBq; IQR 152-181), 68% of cats were euthyroid, 20% persistently hyperthyroid and 12% hypothyroid, whereas treatment with an individualized dosage (median 130 MBq; IQR: 95.3 – 140) led to 54% of euthyroid, 23% of hyperthyroid and 23% of hypothyroid cats ($P = 0.48$).

Thyroxin concentration at diagnosis was the only variable associated with euthyroidism after therapy (OR 0.99; $P = 0.025$). Indeed, for each nmol/L increase in T4 at diagnosis, the odds of treatment success (euthyroidism) decreased by 1 %.

Twelve months after treatment, the number of azotemic cats was not statistically different between those treated with a fixed dosage of radioiodine (48%) and those treated with an individualized dosage (42%; $P = 0.06$).

Median survival time after radioiodine therapy was 44 months. In a multivariate analysis, persistent hyperthyroidism was the only variable independently associated with a shorter survival time (HR= 4.04, $P = 0.03$).

In conclusion, method of calculation of radioiodine dosage (fixed vs individualized) to treat feline hyperthyroidism does not appear to be decisive for post-treatment thyroid function, renal function nor survival time.

EN07

Targeted metabolomic analysis in canine diabetes

O'Kell, Allison L.[1], Wasserfall, Clive H.[1], Guingab-Cagmat, Joy[1], Webb-Robertson, Bobbie-Jo M.[2], Atkinson, Mark A.[1], Garrett, Timothy J.[1]

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Previous studies using untargeted metabolomics identified differences in serum metabolites between diabetic and control dogs with some similarities to humans with type 1 diabetes. The purpose of this study was to validate results of previous untargeted metabolomic studies, select candidate metabolite biomarkers, and quantify these using targeted metabolomics.

Untargeted metabolomic analysis via liquid chromatography-mass spectrometry (LC-MS) was performed on serum from diabetic (n=15) and control (n=15) dogs. Results from this combined with results from our previously published studies using identical methods (12 diabetic and 12 control dogs) were used to identify metabolites consistently different between groups in all 54 dogs. Thirty-two candidate biomarkers were measured by LC-high resolution MS and compared between diabetic and control groups via multiple linear regression with age and group as co-variates. A p-value adjusted for multiple comparisons of < 0.0032 was considered significant.

Untargeted metabolomics identified multiple persistent differences in serum metabolites in diabetic dogs compared with our previous studies. Using targeted metabolomics, GABA, valine, leucine, isoleucine, and 2-hydroxyisobutyric acid were higher and indoxyl sulfate, n-acetyl-L-aspartic acid, kynurenine, and tauroursodeoxycholic acid were lower in diabetic versus control dogs (P< 0.0032).

In conclusion, targeted metabolomic analysis confirmed quantitative differences in select serum metabolites between diabetic and control dogs. Some metabolite alterations are consistent with those found in humans, including increases in GABA (type 1 diabetes) and branched chain amino acids (type 1 and type 2 diabetes). Future studies to investigate the role of these metabolites in diabetes pathogenesis or early disease markers in dogs are warranted.

EN08

Clinical and laboratory findings in dogs with low basal cortisol levels: A retrospective case-control study

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Measurement of serum basal cortisol is commonly used to rule out hypoadrenocorticism. When basal cortisol concentrations are above 2 microg/dL, hypoadrenocorticism is ruled out without the need of an ACTH stimulation test. When it is below this value, an ACTH stimulation test is essential to assess adrenal function. In clinical practice, it is not uncommon to have a basal cortisolemia below 2 microg/dL and a subsequent ACTH stimulation test showing a physiologic adrenal response. This reflects a main practical concern, slowing the medical exploration and diagnostic approach.

The aim to this study is to explore potential clinical parameters that can help the clinician to assess the likelihood of having a normal ACTH stimulation test in a dog with low basal cortisol concentration (≤ 2microg/dL).

A retrospective case-control study was conducted using the clinical data of all dogs submitted to the measurement of basal cortisol at a Veterinary Teaching Hospital from April/2018 to October/2020. Dogs were included if basal cortisol was ≤ 2 microg/dL and if, following this result, an ACTH stimulation test was available. Dogs that had received steroids for any route over the last 8 weeks were excluded. Data collected included sex, age, breed, clinical signs, hematology and biochemistry panels (including electrolytes) and functional test results. According to post-ACTH cortisol levels, dogs were divided into two groups: those diagnosed with hypoadrenocorticism (basal and post-ACTH cortisol ≤ 2 microg/dL – HAC group) and those with a basal cortisol ≤ 2 microg/dL and a physiologic post-ACTH cortisol (Non-HAC group). Shapiro-Wilk test was used to assess normality. Age was expressed using mean ± standard deviation (SD). For comparison between groups, independent Samples T-test and Fisher's exact test were used. A p-value of < 0.05 was considered significant.

Thirty-five dogs met the inclusion criteria. Among them, 26/35 (74.3%) were included in the Non-HAC group and 9/35 (25.7%) in the HAC group. Males were overrepresented (55.6% in the HAC-group and 57.7% in the Non-HAC group), mean age was similar in both groups (5.22 yo ± 3.52 in the HAC-group and 4.32 yo ± 2.77 in the Non-HAC group) and mostly were purebred dogs (55.6% in the HAC-group and 76.9% in the Non-HAC group). There were no significant differences between groups regarding sex, age, and breed (p >0.05). Chronic gastrointestinal signs were prevalent in both groups (66.7% in HAC-group and 50% in Non-HAC group). A basal cortisol < 1 mg/dL was observed in 77.8% of dogs with HAC and only in 30.8% of Non-HAC (p=0.01; odds ration=7.38). Polyuria/polydipsia was more reported by owners from HAC-dogs (55.6%) than Non-HAC dogs (7.7%) (p=0.01; odds ratio=15). Regarding hematology and biochemistry results, only BUN showed a statistically difference between groups; 55.5% of the HAC-dogs had an increased BUN (55.5%) while only 11.5% of the Non-HAC showed it (p=0.03; odds ratio=7.92). There was no significant difference concerning electrolytic parameters in both groups (p >0.05).

This study shows that dogs with low basal cortisol levels without increased BUN and polyuria/polydipsia are more likely to have a normal ACTH stimulation test. Further studies are needed to extend these conclusions to a larger number of animals and to explore the relevance of hypocortisolemia in dogs with normal ACTH stimulation test.

EN09

Impact of six minutes of physical activity on baseline cortisol concentrations in clinically healthy dogs

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Hypoadrenocorticism (HOC) is a common clinical suspicion in canine practice and baseline serum cortisol concentrations ≥ 55 nmol/L (≥ 2 μg/dL) are used to rule out the disease. Cortisol release has been associated with physical activity and/or stress. Physical activity 15-20 minutes prior to blood collection leads to increased cortisol concentrations in humans. The hypothesis of this study was that blood collection after 6 minutes of physical activity in healthy dogs would lead to cortisol concentrations ≥ 55 nmol/L, and help to rule out HOC.

Eighteen healthy medium-to-large breed dogs were enrolled into the study; Median age was 6 years (range, 2 – 12 years), and included 10 spayed females and 8 castrated males. The dogs were ≥ 1 year of age, free from any comorbidities, and were not receiving any medications that are suspected to affect cortisol production. Dogs were fitted with a smart collar (PetPace®) the night before the experiment at home, which was removed after the last blood collection was completed the next day in hospital. Dogs were randomized into three groups; A: 6 dogs had their blood collected immediately after 6 minutes of physical activity, B: 6 dogs had their blood collected 15 minutes after the physical activity, and C: 6 dogs had their blood collected at the end of the physical activity, and again at 15 minutes later. All samples were processed shortly after collection, stored at -20°C, and processed together as a batch.

Median cortisol concentrations in groups A and B were similar (p = 0.69); A: 51 nmol/L (range, 27 – 96 nmol/L); B: 54 nmol/L (range, 37 – 161 nmol/L). Only 50% of participants in group A and B had cortisol concentrations ≥ 55 nmol/L. The median cortisol concentrations in group C at T_0 and T_{15} were significantly different (p = 0.014); T_0: 54 nmol/L (range, 27 – 205 nmol/L); T_{15}: 94 nmol/L (range, 40 –

258 nmol/L). All cortisol concentrations measured at T_{15} were higher than T_0 in group C: median 40% (range, 2 – 126%). However, only 50% and 67% of the samples had cortisol concentrations ≥ 55 nmol/L at T_0 and T_{15}, respectively.

Six-minutes of physical activity did not increase baseline cortisol concentrations in healthy dogs consistently enough to improve sensitivity as a screening test for HOC. Samples collected 15 minutes after the physical activity had higher cortisol concentrations than those collected immediately after activity. This finding warrants further investigation.

EN10

The effect of capromorelin on glycemic control in healthy dogs

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Capromorelin (Entyce®) is a ghrelin-receptor agonist commonly used to stimulate appetite in dogs. This study evaluated the effect of capromorelin on blood glucose (BG) and interstitial glucose (IG) concentrations in healthy dogs.

Eight client-owned dogs were randomized to receive either capromorelin (3 mg/kg) or placebo (saline) daily for 3 days in a crossover study with one week between treatment periods. On day 1 of each period, drug was administered at time zero and a glucose-rich diet (Ensure Plus®, 21 kcal/kg) was fed 30 min later. BG was measured at -10, -1, 20, 29, 60, 90, 120 and 150 min; IG was measured on days 2-3 with a continuous glucose monitor. Mean baseline (-10 to -1 min), post-drug (20-29 min) and post-prandial (60-150 min) BG were compared between treatments over time using a two-way repeated measures ANOVA; mean glucose concentrations were compared between treatments with paired two-tailed t-tests (Bonferroni correction, $\alpha = 0.05$, $\beta = 0.2$).

There was an interaction between time and treatment ($p = 0.02$). Mean baseline and post-drug BG were unaffected by treatment. Mean post-prandial BG was higher after capromorelin than placebo (108 ± 14 vs. 95 ± 10 mg/dL, respectively; $p = 0.009$). Mean 48h IG was 98 ± 11 and 90 ± 12 mg/dL during capromorelin and placebo treatment, respectively ($p = 0.08$).

This study demonstrated that capromorelin interferes with normal glucose control in healthy dogs. Further studies are needed to determine the mechanism and whether capromorelin poses a risk in dogs predisposed to diabetes mellitus.

EN11

Determination of serum TSH using novel, feline-optimized TSH immunoassay: New diagnostic test for hyperthyroid cats?

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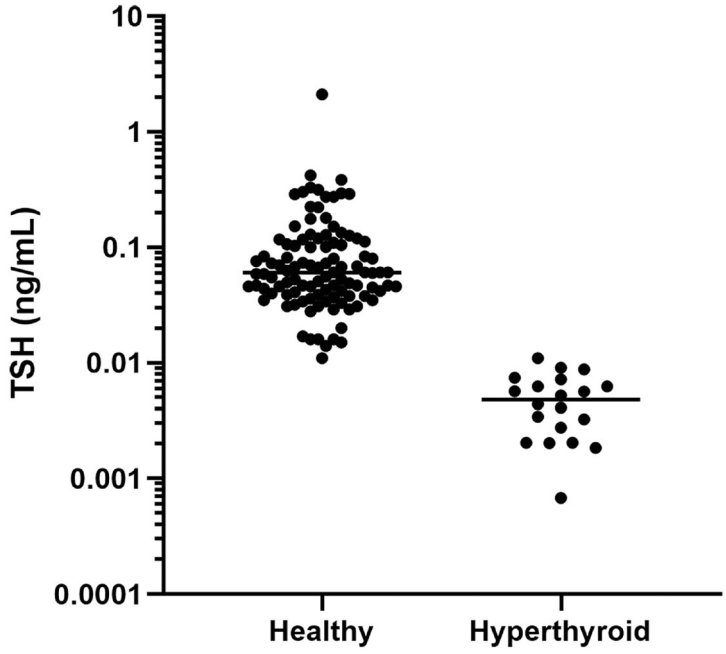
Measurement of serum thyroid-stimulating hormone (TSH) is commonly used for diagnosis of thyroid disorders in humans and dogs. In cats, we have not had a feline-optimized TSH assay, necessitating the use of canine-optimized TSH (cTSH) assays to measure serum TSH concentrations in this species. Although the cTSH assay can easily measure high TSH concentrations that develop in cats with primary



hypothyroidism (*JSAP*; 2017; 58:519), these cTSH assays cannot accurately measure low TSH concentrations (< 0.03 ng/mL) and therefore cannot distinguish between the low-normal TSH concentrations of clinically healthy cats and the suppressed TSH concentrations that develop in cats with hyperthyroidism (*J Vet Intern Med* 2015;29:1327). Therefore, in comparison to human TSH tests, which are used as the first-line diagnostic test for assessing hypo- and hyperthyroid status (*Clin Med Res* 2016;14:83), the available cTSH assays have limited clinical use as a diagnostic test for feline hyperthyroidism due the assay's poor test sensitivity.

In this study, a feline-optimized immunoassay was developed that leverages a highly sensitive bulk acoustic wave (BAW) biosensor to quantify feline TSH concentrations down to 0.008 ng/mL, significantly below canine-optimized TSH assays, which report lower limits of quantitation of 0.03 ng/mL or higher. With this assay, we measured serum TSH concentrations in 110 clinically normal cats and 21 hyperthyroid cats (disease confirmed in all cats with thyroid scintigraphy) to determine whether there was a significant separation of normal and hyperthyroid cats. We found that 95% of clinically healthy cats had a serum TSH level > 0.01 ng/mL, while 90% of hyperthyroid cats had a TSH concentration < 0.01 ng/m. Of the normal cats, 12% had a TSH level < 0.03 ng/mL but ≥ 0.01 ng/mL, which highlights the importance of utilizing a highly sensitive feline TSH assay in the differentiation of healthy and hyperthyroid cats.

These results suggest that with the enhanced sensitivity (i.e., improved detection limits) enabled by the BAW biosensor, serum fTSH concentration can be used (as in human patients using the current 3rd or 4th generation hTSH assays) to distinguish between normal and hyperthyroid cats. Therefore, measuring serum fTSH concentration using this highly sensitive feline-optimized assay may be a useful diagnostic test for mild hyperthyroidism in cats. Further studies are needed to investigate the influence of non-thyroid illness and other disease states that complicate diagnosis of thyroid disorders on serum feline-optimized TSH concentrations in cats.

EN12

Assessment of once daily dosing with ProZinc® insulin in diabetic beagle dogs

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The purpose of this study was to assess glucose control of PZI insulin (ProZinc®) given SID in dogs with laboratory-induced diabetes mellitus. The hypothesis was that ProZinc® will control hyperglycemia and clinical signs in diabetic dogs using a SID posology. Nine well-controlled diabetic dogs on twice daily lente insulin (Caninsulin®) were transitioned to ProZinc® as a single morning dose while maintaining their twice daily feeding regimen. The initial ProZinc® dose of 1U/kg was decreased to 0.4-0.5IU/kg because of asymptomatic hypoglycemia and then increased by 0.05-0.1IU/kg every 5 days as needed. Glucose control was determined by continuous interstitial glucose monitoring, glucometer, urine ketone, and fructosamine measurements. Food and water consumption, bodyweight, clinical observations, and physical exam parameters were used to assess clinical control. This was a non-randomized, non-blinded laboratory study conducted over 56 days.

ProZinc® insulin showed a clear and dose dependent biphasic glucose lowering effect over 24 hours. Onset of action was 1-2 hrs, with two peaks of activity occurring at 8-10 and 12-14 hrs. Duration of action was 20-22 hrs. At D56 average weight and water consumption was consistent with those at D0; fructosamine was slightly increased. Food consumption remained consistent. The final ProZinc® dose range was 0.45-0.8IU/kg; however, 3 dogs may have required subsequent upward adjustments. These results show a biphasic glucose lowering effect of ProZinc® over each 24-hour period, clearly demonstrating that SID dosing is appropriate when using this insulin. The starting dose should be less than 1IU/kg, but may be higher under field conditions.

GI01

Evaluation of serum zonulin in canine chronic enteropathies

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Diagnosis and treatment of canine chronic enteropathy (CE) is challenging and often limited to diet trials and endoscopic evaluation followed by immunosuppressive therapy. In people, serum zonulin has been shown to be a reliable marker of intestinal permeability correlated to underlying enteropathies, with potential application as a therapeutic target.

The objective of the study was to evaluate serum zonulin as a biomarker for canine CE using a commercially available ELISA kit ®. A power analysis was performed for sample size calculations using data from humans. Twenty-two client-owned dogs diagnosed with CE were included in the study (IACUC ASAF # 6482, 20-32, C-8-19). All CE dogs presented with at least one of the following gastrointestinal signs for greater than 3 weeks duration: anorexia, hyporexia, dysrexia, vomiting, weight loss or diarrhea. Dogs were ineligible if antibiotics or immunosuppressive medications were administered within one month of enrollment. Diets were not controlled for the dogs. All CE dogs were assigned CIBDAI and CCECAI scores and underwent a complete diagnostic evaluation including a complete blood count (CBC), serum chemistry, canine pancreas-specific lipase (sCPL), cobalamin, resting

cortisol, abdominal ultrasound and gastrointestinal endoscopy with histopathology.

Age, breed and when able, sex-matched, control dogs were enrolled for comparison. Control dogs were not on antibiotics or immunosuppressive medications, had a normal physical exam, CBC, serum chemistry and no clinical signs of GI disease. Serum samples for all study participants were collected at the time of enrollment, centrifuged, and frozen at -80° C for subsequent batch zonulin analysis using a commercial canine serum zonulin ELISA kit ®.

Serum zonulin levels of CE and control dogs were compared using a Wilcoxon rank signed test. Results showed serum zonulin levels did not significantly differ between dogs with CE and control dogs ($p = 0.97$). Although no difference in serum zonulin levels were observed in this preliminary study, further research with a larger population and more severe disease may be warranted to determine if serum zonulin has application for canine CE therapeutic monitoring or as a potential therapeutic target.

GI02

DECiSION study: Does endoscopy change clinicians' supportive interventions or not?

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Chronic enteropathy (CE), protein-losing enteropathy (PLE), and intestinal lymphoma (LSA) are common gastrointestinal conditions in dogs. The goal of this study was to determine whether gastrointestinal endoscopic evaluation or histopathologic diagnosis influenced clinician case management of these diseases. We hypothesized that interpretation of endoscopic data and histology results would affect clinician decisions regarding case management in CE, PLE, and LSA in dogs.

Ten cases of CE, 10 cases of PLE, and 9 cases of LSA were retrospectively identified by histopathology reports. Each case was curated into 3 case presentations: 1. Total case data including history, physical exam, all diagnostics including endoscopy and biopsy results, 2. Case data without biopsy results, and 3. Case data without both endoscopy and biopsy results. Four academic internists (AI), 4 private practice internists (PI), and 7 general practitioners (GP) were surveyed to evaluate the impact of these scenarios on treatment strategies.

Treatment approach in case presentation 1 and case presentation 2 were compared to case presentation 3 for consistency. Frequencies at which treatment decisions were affected or not by additional diagnostic data were compared with chi-squared between diagnoses and practice types.

The inclusion of endoscopic data altered treatment decisions in 69.6% of all case scenarios (66.6% CE cases, 68.1% PLE, 78.8% LSA). The frequency at which endoscopic data affected treatment was not significantly different between diagnoses for all practice types. The inclusion of histologic data altered treatment decisions in 79.5% of scenarios (69.3% CE cases, 80.9% PLE, 88.3% LSA). The frequency at which histologic data affected treatment was significantly different between diagnoses for AI ($p = 0.03$), PI ($p = 0.05$), and GP ($p = 0.01$). The frequency at which endoscopic or histologic data affected treatment was not significantly different between practice types for all diagnoses.

Based on these results, endoscopic and histologic data were used clinically by clinicians of various practice types and influenced the treatment strategies elected. Further evaluation is warranted to investigate the impact of these decisions on clinical outcomes.

GI03

Investigating fecal microbial transplant in dogs with inflammatory bowel disease: A pilot study

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Inflammatory bowel disease (IBD) is a frequent cause of chronic vomiting, anorexia and diarrhea in dogs. The objective of this study was to assess if the addition of fecal microbial transplant to standard therapy (corticosteroids and a hypoallergenic diet) resulted in improved outcome versus standard treatment alone.

Thirteen client-owned dogs with IBD were enrolled in this double blinded, randomized clinical trial. All patients received corticosteroid therapy and a hypoallergenic diet; patients were randomized to receive either placebo or fecal microbial transplant. Measured outcomes included the canine chronic enteropathy clinical activity index (CCECAI) along with albumin, C-reactive protein, and cobalamin levels at 1 week, 1 month, and 3 months after enrolment. Fecal microbiota was analyzed after extracting DNA from fecal samples and profiling using 16S amplicon sequencing.

The CCECAI significantly decreased over time regardless of treatment group ($p = 0.001$). There was no difference between treatment groups in the CCECAI ($p = 0.735$), albumin ($p = 0.43$), C-reactive protein ($p = 0.287$), or cobalamin ($p = 0.601$) after

90 days of treatment. No adverse effects were reported after FMT.

The alpha and beta diversity measurements (including community membership (Jaccard index), and structure (Yue and Clayton index)) were not significantly different between and within the treatment groups at all times (P > 0.05).

In conclusion, the addition of FMT did not change patient outcome as measured by the CCECAI, albumin, C-reactive protein, and cobalamin levels, as well as fecal microbiota diversity and composition, in dogs with IBD in this study.

GI04

Metoclopramide effect on capsule endoscopy evaluation of the gastrointestinal tract in 17 healthy dogs

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Capsule Endoscopy (CE) allows the evaluation of the entire gastrointestinal (GI) tract in awaken dogs. However, in about 40% of dogs the battery turns off before reaching the colon, resulting in an incomplete study.

The objective was to determine whether administration of a prokinetic drug (metoclopramide) before CE administration would affect GI transit times (TT) and completeness of a CE study.

Clinically healthy dogs with normal physical examination and no known GI signs were included in a cross-over, blinded, randomized prospective study.

ALICAM® CE was administered twice (control versus treatment group) to each dog 1-2 weeks apart, with a fasting time of 12 hours prior to and 6 hours post CE administration. Treatment group received oral metoclopramide 0.3mg/kg, 30 minutes before CE administration. Dogs' signalment, crude dietary fat percentage (fat%), esophageal, gastric, small intestinal transit times (ETT, GTT, ITT) and completeness of study were recorded. Data tested for normality using Shapiro-Wilk test; A general mixed model, logistic regression and Wilcoxon sign-rank tests were used to study the effect of treatment, age, body weight (BW), fat% on ETT, GTT, ITT, and study completeness. P < 0.05 was considered significant.

17 dogs (median age: 5.1 years [0.7 – 9.8]; median weight: 28kg [14 – 42]) were included. All studies were complete in treatment group, two studies were incomplete in control group. Treatment did not significantly increase study completion rate (p=0.22). Fat% was a predictive factor for incomplete study, for each unit increase in fat% dogs where 27% more likely to not complete the study (p=0.019). ITT significantly increased with age (p=0.012).

Metoclopramide did not significantly improve study completeness in healthy dogs, neither significantly increase GI TT. Higher fat% was a predictive factor for incomplete study.

GI05

Effect of a commercially available synbiotic on mycophenolate mofetil associated diarrhea

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Mycophenolate mofetil (MMF) is an effective immunosuppressant in dogs with immune-mediated diseases, but gastrointestinal (GI) toxicity is common. Based on a retrospective study in our laboratory, 24.4% of 135 client owned dogs experienced GI toxicity (median dose 17.5 mg/kg/day). One of the possible mechanisms for MMF induced GI toxicity is GI dysbiosis, but our previous experiment was unable to document that syndrome in healthy beagles. However, in that experiment, all dogs developed a fecal score of ≥ 4 over the course of the study.

The purpose of this study was to supplement 9 additional beagles that were on the same facility diet as the dogs in the previous experiment with the synbiotic (FortiFlora® SA, Nestlé Purina) while being administered MMF at 10 mg/kg, PO, twice daily. Individuals masked to the objectives of the study applied a score to each dog's feces daily, using a standardized fecal scoring system. In this system, fecal scores of ≥ 4 are considered diarrhea.

In the 28-day study, the dogs administered MMF alone frequently had a fecal score ≥ 4 (68/252). In contrast, the dogs administered MMF with the synbiotic only had a fecal score of 4 detected twice (2/252). The frequency of fecal scores ≥ 4 (P < 0.0001) was statistically different between groups.

These results suggest that the mechanism of diarrhea associated with MMF may involve effects on the gastrointestinal microbiota and support the use of this synbiotic when MMF is used clinically.

	Treatment n=17 Median (range)	Control n=17 Median (range)	p value
ETT (seconds)	8 (2.00 - 348.00)	7 (4.00 - 80.00)	0.21
GGT (minutes)	84.5 (0.90 - 345.93)	71.9 (3.55 - 940.50)	0.7
ITT (minutes)	110.8 (15.40 - 581.00)	154.7 (79.00 - 393.55)	0.25
Complete study (total)	17	15	0.22

GI06

Incidence of relapse of inflammatory protein-losing enteropathy in dogs and associated risk factors

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Inflammatory protein-losing enteropathy (PLE) in dogs is associated with a guarded prognosis, as disease-associated death occurs in approximately 50% of cases. Currently, it is unknown what percentage of dogs that attain remission subsequently relapse. Therefore, the aims of our study were to determine the incidence of relapse of inflammatory PLE in dogs that have previously attained remission and identify associated risk factors.

Medical records of dogs diagnosed with PLE between August 2009 and August 2019 were retrospectively searched. Dogs were included if hypoalbuminemia was documented at presentation and an inflammatory enteropathy was diagnosed on histopathology of intestinal biopsies. Dogs were excluded if a neoplastic cause was identified on histopathology or if initial serum biochemistry, abdominal imaging and urinalysis results failed to exclude a hepatic or renal cause of hypoalbuminemia. Follow-up information for eligible cases was obtained from the records of referring veterinarians. Remission was defined as a resolution of clinical signs and normalisation of serum protein and albumin concentrations. Relapse was defined as a recurrence of clinical signs and hypoalbuminemia.

In total, 66 dogs met the inclusion criteria; 20 dogs (30%) achieved sustained remission without documentation of relapse (SR), 13 dogs (20%) achieved remission but then subsequently relapsed (RR) and 33 dogs (50%) never achieved remission (NR). A Chi-squared test was used to compare categorical data between groups. For quantitative data, Shapiro-Wilk test was used to determine normality with normally distributed data compared using 1-way ANOVA or independent *t* test and non-normally distributed data compared using Wilcoxon signed-rank or Mann-Whitney U test.

There was no significant difference between the 3 groups (SR, RR, NR) when comparing age at diagnosis, breed, sex and neuter status, body weight, presenting signs, treatment prior to presentation, serum total protein, albumin, cholesterol and cobalamin concentrations at diagnosis, histopathologic diagnosis including WSAVA histopathology scores, type of dietary treatment, treatment with prednisolone, time from diagnosis to commencement of prednisolone treatment, starting prednisolone dose or commencement and number of days from diagnosis to starting a second immunosuppressive agent (P >0.125). However, serum globulin concentration at diagnosis was significantly higher in the RR group compared to the NR group (P =0.046, mean +/- SD: RR 19.4g/L+/- 5.4, NR 16g/L+/- 4.1).

Of the 50% (n =33) of dogs that initially achieved remission, 39% (n =13) subsequently relapsed. For these dogs, the median (range) number of days from diagnosis to relapse was 374 (70 to 1,498) and from initial remission to subsequent relapse was 180 (21-1,470). The mean +/- SD for serum albumin concentration at subsequent relapse was 19.6g/L +/- 4.3. Forty-six percent (n =6) of dogs that subsequently relapsed achieved a second remission within a median of 64 days (range 21-94). For the 20 dogs with sustained remission, the median (range) number of days from diagnosis to last follow-up was 2,068 (388-4,063). Dogs that subsequently relapsed had significantly higher poor dietary compliance, as defined by frequent scavenging or changing from the recommended diet compared to dogs with sustained remission (P =0.025). The number of days from diagnosis to initial remission was significantly longer in dogs with remission-relapse compared to sustained remission (P =0.044, mean +/- SD: RR 236 days +/- 304, SR 85 days +/- 86).

Our study suggests that ensuring owners adhere to dietary recommendations and inducing remission within a shorter period of time might help to prevent subsequent relapse of dogs with inflammatory PLE that attain initial remission. However, prospective studies are needed to confirm the role of dietary compliance and time to initial remission on subsequent relapse rates in canine PLE.

GI07

Detection of entero-invasive *Escherichia coli* in dogs with granulomatous colitis using immunohistochemistry

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Histiocytic granulomatous colitis in dogs is often associated with the presence of entero-invasive strains of *Escherichia coli (E. coli)* in the colonic mucosa. Fluorescent *in situ* hybridization (FISH) is the current gold-standard method to assess intramucosal and intracellular bacterial invasion. FISH requires expensive fluorescence microscopy equipment and is therefore not widely available. This study aimed to assess immunohistochemistry (IHC) as an alternative method to detect entero-invasive *E. coli* in dogs with granulomatous colitis.

Archived paraffin-embedded blocks were selected from dogs with colitis, in which FISH had been previously performed at an outside laboratory. A total of ten cases were selected; five of them positive for entero-invasive *E. coli* and five of them negative. IHC was performed for *E. coli* using a commercial polyclonal antibody on sections cut from the same blocks, and the presence of entero-invasive *E. coli* was recorded.

All five specimens in which *FISH had detected E. coli* were positive on IHC, with strong immunolabeling in the cytoplasm of macrophages and extracellularly in the lamina propria. All five specimens which were negative for entero-invasive bacteria on FISH were negative on IHC, with bacteria immunolabeled as *E. coli* seen on the mucosal surface with the intestinal contents and in the lumen of the crypts, but not in the lamina propria. The Cohen's Kappa (K) statistic indicated agreement between both methods (K=1.0; standard error=0.0).

This pilot study shows that entero-invasive *E. coli* can be successfully detected with IHC in dogs with granulomatous colitis. IHC may be a viable alternative to FISH to detect entero-invasive *E. coli*.

GI08

The effect of a hydrolyzed diet on the fecal microbiota of cats with chronic enteropathy

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The effect of a hydrolyzed diet on the fecal microbiota has not been extensively studied in cats with chronic enteropathy (CE). Thus, the aims of our study were to 1) compare the fecal microbiota of cats with CE to control cats with no gastrointestinal (GI) signs and 2) to determine the effect of a hydrolyzed diet on the fecal microbiota of cats with CE, and to compare this between cats improving clinically (responders) and non-responders.

Cats with confirmed or suspected CE (n = 39) and non-GI signs (n = 14) were prospectively enrolled from 2 referral hospitals in the U.K. Their fecal microbiota were characterized using 16S RNA sequencing on samples collected from the time of diagnostic investigations (all cats) and from cats with CE after receiving a minimum of 6 weeks of the same commercial hydrolyzed diet (n = 25).

Three cats were excluded due to subsequent diagnosis of small cell lymphoma (n = 2) or feline infectious peritonitis (n = 1). Three cats did not eat the diet and were excluded from responder/non-responder analysis. Fifteen cats had full remission of signs (responders) and 18 cats had partial/no response after 6 weeks of exclusive feeding (non-responders).

The fecal microbiome of cats with CE showed decreased a-diversity in terms of genus richness (P = 0.04) and increased b-diversity in terms of Bray-Curtis Dissimilarity (P < 0.001) compared to control cats. The relative abundance of 15 bacterial genera were significantly different been cats with CE and control at the time of diagnosis (P < 0.05). In particular, cats with CE had more fecal *Enterococcus* and *Clostridium* compared to control cats at the time of diagnosis. The distribution of several differentially abundant taxa was bimodal in cats with CE, which was explained by the presence or absence of these taxa. For example, the genus *Enterococcus* was observed only in a subset of cats with CE and none of the control cats.

Shannon's Diversity Index for a-diversity decreased over time in both responders and non-responders (P < 0.05). At the genus level, b-diversity was different between the responders and non-responders at diagnosis (P = 0.004), but not between the 2 groups after dietary intervention (P = 0.35). However, at the family level, non-responders became increasingly dissimilar after dietary intervention (b-diversity, P = 0.012). Thirty-eight genera were associated with dietary response and included 12 of the 15 genera that were significantly different in cats with CE at diagnosis compared to controls.

Univariable logistic regression models showed no significant association between the presence of fecal *Clostridia* or *Enterococcus* in cats with CE and referral center, signalment, duration and nature of signs, body condition score, laboratory parameters (albumin, cobalamin, folate, alanine aminotransferase and alkaline phosphatase), results from imaging and intestinal histopathology, and feline chronic enteropathy activity index (FCEAI) (P > 0.081). Although, cats with a FCEAI of 6 or above were more likely to have fecal *Enterococcus*, this did not reach significance (P = 0.081). No clinicopathologic variables were significantly associated with response to a hydrolyzed diet (P > 0.117).

As with human inflammatory bowel disease studies, our study suggests *Enterococcus* is associated with the pathogenesis of feline CE. However, further studies are needed to determine the functional significance of the taxa identified in our study, to explain the bimodal microbiome in feline CE, and to determine the underlying cause for the increasing dissimilarity of the fecal microbiota family in non-responders following intervention with a hydrolyzed diet.

GI09

Effect of different storage conditions of fecal samples on some diagnostic markers of canine dysbiosis

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Gut microbiota alteration (dysbiosis) is one of the chief factors which involved in the pathogenesis of canine inflammatory bowel disease (IBD), and associated with change in the abundance of immunoglobulin A-coated microbiota (IgA). Fecal samples are commonly used to identify patients with intestinal dysbiosis; therefore, it is important to consider delays in transit times or incorrect storage conditions during domestic or international shipment to the diagnostic laboratory, which may negatively impact the results. The canine fecal dysbiosis Index (DI) is a diagnostic marker that quantifies the abundances of major bacterial taxa and summarizes them in a single number based on a mathematical algorithm. Fecal IgA-coated microbiota can be quantified to evaluate the intestinal immune response induced by intestinal dysbiosis with a recently developed flow cytometry research assay.

The aim of this study was to evaluate the effect of different storage conditions of fecal samples on the dysbiosis index and the IgA-coated/non-IgA-coated microbiota ratio.

For evaluation of the effect of storage conditions on the dysbiosis index, fecal samples from 9 dogs were used to prepare 9 aliquots from each. DNA was extracted immediately from 1 aliquot. Two aliquots were stored for 1 week at each of the following 4 storage conditions: room temperature, 4°C, -20°C, and -80°C; one aliquot with and one without the addition of 95% ethanol. Quantitative PCR assays were performed for total bacteria, Faecalibacterium, Turicibacter, Escherichia coli, Streptococcus, Blautia, Fusobacterium, and Clostridium hiranonis, and the DI was calculated. For evaluation of the effect of storage conditions on the abundance of IgA coated microbiota, fecal samples from another 7 dogs were used to prepare 3 aliquots from each sample. A single cell suspension was prepared from one fresh fecal aliquot for immediate analysis by flow cytometry, a second aliquot was stored at 4°C for one week, and the third was stored at -80°C for one week prior to analysis by flow cytometry. Statistical analysis was performed for the

DI and the IgA-coated/non-IgA-coated microbiota ratio for all aliquots with Friedman tests. Significance was set at P < 0.05.

The DI for all aliquots stored at different conditions with and without 95% ethanol for one week revealed no statistically significant differences (P = 0.656) compared to fresh fecal aliquots. The percentage of fecal IgA coated microbiota, the percentage of non-IgA coated microbiota, and the IgA-coated/non-IgA-coated microbiota ratio showed no significant difference (P = 0.486, 0.237, and 0.185, respectively) in aliquots stored at either 4°C or -80°C for 1 week compared to fresh fecal aliquots.

In conclusion, storage of fecal samples at room temperature, 4°C, -20°C, or -80°C, with or without addition of 95% ethanol for one week prior to DNA extraction had no effect on the dysbiosis index. Also, storage of feces at 4°C or -80°C for one week prior to analysis by flow cytometry had no effect on the IgA-coated/non-IgA-coated microbiota ratio. These findings increase the confidence to clinicians in collecting samples and then shipping it to the diagnostic laboratory, both domestic and international.

GI10

Clinical signs, histopathology and serum high-mobility group box-1 concentrations in dogs with inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a common intestinal disease in dogs. In humans, clinical improvement is not necessarily accompanied by histopathological improvement. High-mobility group box 1 (HMGB1) is a nuclear non-histone DNA-binding protein that mediates the inflammatory response contributing to the pathogenesis of IBD in humans. Thus, the objectives of the present study were to compare the severity of clinical signs and histopathological grade and to evaluate serum HMGB1 and C-reactive protein (CRP) concentrations in dogs with IBD. Group 1: Thirty IBD dogs. Group 2: Seventeen IBD dogs and 25 healthy dogs.

Group 1: Clinical signs of IBD were assessed using the canine IBD assessment index (CIBDAI), and histopathological samples obtained by endoscopic biopsy were evaluated before and after treatment. The CIBDAI score was compared along with the histopathological grades. Group 2: Serum CRP and HMGB1 concentrations were analyzed by enzyme-linked immunosorbent assays. Clinical signs of IBD were assessed using the CIBDAI. In addition, the IBD dogs were divided into 2 groups by histopathological grade (mild and moderate to severe) and further analyses were performed between the subgroups.

Total CIBDAI scores decreased significantly after a 70-day treatment in all IBD dogs compared to those of dogs at baseline, but IBD histopathological grade did not improve. No difference in the CIBDAI score was observed with histopathological grade. Serum CRP and HMGB1 concentrations were significantly higher in dogs with IBD than those in healthy dogs. Serum CRP and HMGB1 concentrations and the CIBDAI score decreased significantly after the 30-day treatment in all dogs with IBD. Serum HMGB1 concentration was significantly higher in IBD dogs and a moderate to severe histopathological grade than those in IBD dogs and a mild histopathological grade. A positive correlation was observed between serum CRP concentration and CIBDAI score in IBD dogs.

This study showed no improvement in the histopathology of IBD dogs, although clinical signs were ameliorated after the treatment, indicating that clinical improvement is not accompanied by histopathological improvement in IBD dogs. In addition, serum CRP might reveal the severity of clinical signs and HMGB1 might be related to histopathological severity in IBD dogs.

GI11

The effects of amoxicillin/clavulanic acid or doxycycline on the fecal microbiota in young cats

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Antibiotic treatment (AT) affects the composition of the gastrointestinal (GI) microbiota. Microbial shifts due to antibiotic therapy early in life are considered important because they have been associated with an increased risk for disease development later in life. No studies have investigated the effects of antibiotic treatments on the fecal microbiota in young cats. The aim of this study was the investigation of the effects of antibiotics on the fecal microbiota of young cats.

Naturally passed feces were collected from 15 kittens that received amoxicillin/clavulanic acid (20 mg/kg q12h) for 20 days (AMC), and 15 kittens that received doxycycline (10 mg/kg q24h) for 28 days (DOX) as part of the standard treatment of upper respiratory tract infection. In addition, fecal samples were collected from 15 healthy control kittens that did not receive antibiotics (CON). All kittens were approximately 2 months of age at enrolment and were randomly allocated to receive one of the two antibiotics. In addition, all kittens were on the same diet and antiparasitic treatment protocol for the duration of the study period. Fecal samples were collected on days 0 (baseline), 20 or 28 (AMC and DOX, respectively; last day of treatment), 60, 120, and 300. DNA was extracted and analyzed using sequencing of the 16S rRNA gene. Data were analyzed using Quantitative Insights Into Microbial Ecology v.2 (QIIME2). Beta diversity was evaluated with the phylogeny based unweighted UniFrac distance metric and statistics for beta diversity were performed on ANOSIM. Alpha diversity and bacterial abundancies were assessed for normality and those not normally distributed were transformed into ranks. Statistical analysis was performed using a linear mixed model after adjustment for the False Discovery Rate (FDR). Statistical significance was set at p < 0.05 and correction for multiple post hoc comparisons was used where appropriate.

In cats of the AMC group, the fecal microbial composition was significantly different from DOX (p = 0.011, R = 0.109) and CON (p = 0.001, R = 0.188) on the last day of treatment. The number of observed

species (p = 0.025) and Chao1 (p = 0.029) were significantly increased in the DOX group compared to the CON group on the last day of treatment. Cats in the AMC group had a significantly higher abundance of Enterobacteriales (p = 0.010) compared to CON on the last day of treatment. On days 120 and 300, cats in the AMC group had a higher abundance of unclassified *Collinsella* spp (day 120; p = 0.047, day 300; p = 0.022) compared to CON. Cats in the DOX group had a higher abundance of Proteobacteria on days 60 (p = 0.001) and 120 (p = 0.026) and a lower abundance of unclassified *Bulleidia* spp. (p = 0.023) on day 300 compared to cats in the CON group.

In conclusion, amoxicillin/clavulanic acid caused microbial shifts with most of them having subsided by 1 month after discontinuation of treatment. Doxycycline did not cause microbial disturbances during its administration, yet significant changes appeared 1 month after withdrawal and persisted for at least 2 months. Only few changes were identified 9 months after antibiotics withdrawal. Further research is required to determine the clinical significance of these antibiotic-associated alterations of the fecal microbiota in cats.

GI12

Profiling of the fecal microbiome in cats with chronic enteropathies using quantitative PCR

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Intestinal dysbiosis has been documented in cats with acute diarrhea, chronic diarrhea, chronic inflammatory enteropathies, small cell lymphoma, obesity, or diabetes mellitus using next generation sequencing (NGS). Although NGS can provide comprehensive microbiome data, the high cost, long turnaround time, and lack of reference intervals limit its clinical application. Therefore, a cost-effective, standardized, and targeted quantitative method to evaluate the gastrointestinal microbiome of cats is needed. The aims of this study were to establish reference intervals for selected quantitative PCR (qPCR) assays for analysis of the fecal microbiome in cats and compare the fecal microbiome in cats with chronic enteropathies (CE) and healthy control cats.

A panel of qPCR assays was established to measure the abundance of total bacteria and nine bacterial taxa (i.e., *Faecalibacterium*, *Turicibacter*, *Escherichia coli*, *Streptococcus*, *Blautia*, *Fusobacterium*, *Clostridium hiranonis*, *Bifidobacterium*, and *Bacteroides*). These bacterial targets were identified from a literature review as frequently altered in cats with CE. Fecal DNA samples from 46 healthy control cats and 33 cats with CE were analyzed. Results from the healthy cats were used to establish reference intervals of absolute abundances using the freeware (i.e., Excel add-on Reference Value Advisor v2.1). Bacterial abundances were compared between cats with CE and healthy cats using Mann-Whitney tests. Statistical significance was set at $p < .05$.

The abundances of short chain fatty acid (SCFA)-producing bacteria, including *Faecalibacterium* ($p < .0001$), *Turicibacter* ($p = .0326$), and *Bacteroides* ($p = .0012$) were significantly lower in cats with CE, while those of *Streptococcus* ($p = .0069$) and *E. coli* ($p = .0001$) were significantly higher in cats with CE than in healthy cats. Although the abundance of *C. hiranonis* was not statistically different between groups, 9/33 (27%) of cats with CE had an abundance of *C. hiranonis* below the lower limit of the reference interval, suggesting abnormal intestinal bile acid metabolism.

Differences in the abundances of bacterial groups between cats with CE and healthy controls were documented using qPCR assays. These changes resembled those previously reported in studies of cats with CE using NGS and the pattern associated with dysbiosis in dogs with CE.

GI13

Diabetic dogs had higher gall bladder volume than healthy dogs

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Endocrinopathies and hyperlipidemia are risk factors to gall bladder (GB) diseases. Biliary motility and fluidity modifications are associated to biliary sludge, GB mucocele and cholelithiasis. In this study we compared GB volume (V) and GB emptying in diabetic and healthy dogs. GB volume (V) was obtained according to the ellipsoid method by ultrasonography using formula: V = (π/6) x length (cm) x width (cm) x height (cm). Measurement of length and height were

Table 1 Mean (standard deviation) of gall bladder volume and ejection fraction of healthy (n=22) and diabetic dogs (n=17).

Time after meal (min)	Gallbladder volume (ml/kg)			Ejection fraction (%)		
	Diabetic	Healthy	p-value	Diabetic	Healthy	p-value
0	1.34 (1.00)	0.70 (0.30)	0.02*	-	-	-
30	1.25 (0.98)	0.57 (0.26)	<0.01*	3.54 (27.62)	17.12 (26.76)	0.13¥
60	1.13 (0.91)	0.55 (0.26)	<0.01*	15.16 (25.55)	19.15 (22.43)	0.61¥
120	1.11 (0.96)	0.50 (0.27)	<0.01*	19.21 (21.97)	30.71 (19.33)	0.09¥

* p-value obtained by Wilcoxon test or ¥Welch Two Sample t-test.

determined from long axis images, and width was determined from transverse images. Seventeen diabetic dogs, without comorbidities, with body condition score (BCS) 4 to 7 (nine-point scale) were selected from referral veterinary hospital. Twenty-two healthy dogs were selected after announcement at the university. All dogs were fed the same high-fiber, high-protein, moderate-starch and moderate-fat diet indicated to diabetic dogs for 3 months. Dogs were fasted for 12h before examination. Once the fasting (baseline) gallbladder volume (V_0) was measured sonographically, they were fed half daily ration (diabetic dogs received habitual insulin dosage) and postprandial gallbladder volume was recorded at 30, 60 and 120 min after the meal. GB volumes were then indexed for body weight in kilograms. Gallbladder ejection fraction (EF) of each postprandial time (y) was calculated: $EF = (V_0 - V_y) \times 100/V_0$ (%). All measurement was realized in triplicate. Statistical tests were performed and $p < 0.05$ was considered significant. There was no difference ($p < 0.05$) in age, body weight and BCS between groups. Healthy dogs had smaller GB volume in all measurement times than diabetic dogs, without difference in gallbladder emptying (table 1). This was the first comparation of GB volume and emptying between healthy and dogs with endocrinopathies. Further research is needed to explain association of endocrinopathies and gall bladder diseases.

GI14

Comparison of gastrointestinal pH and transit times in the fasted state between cats and dogs

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Understanding feline gastrointestinal (GI) pH and transit times is necessary for developing and selecting appropriate drug formulations for cats, particularly those administered orally. Dogs are commonly used for preclinical studies for humans, resulting in an abundance of pharmacologic data for the dog, but there is a paucity of data for the cat. Comparative studies using the same methodology in cats and dogs can help to identify interspecies differences and advance our ability to extrapolate drug data between species. Our main study objective was to measure GI pH and transit times in the fasted and fed state in dogs and compare this to data recently obtained in cats using an identical methodology.

Seven healthy, colony-housed dogs were transitioned to the same diet, used in a previous study in cats, for 2.5 weeks prior to study onset. Food was withheld from the dogs for 20 hours prior to oral administration of a continuous pH monitoring capsule and 15 mL of water. Five hours after capsule administration, dogs were meal fed for a period of 1 hour by offering them their daily allowance of food. Fasted GI pH and transit times were recorded and compared to previously collected feline data using nested t tests.

In this study, dogs had faster intestinal ($P = 0.0024$) and total transit times ($P = 0.0032$) and lower small ($P = 0.0281$) and large intestinal pH ($P = 0.0318$), compared to cats. Study dogs showed similar intra- and inter-individual variability in transit times and pH compared to cats, except for esophageal pH, for which cats showed a wider intra-individual variability. Based on our results, we believe that cats and dogs have dramatically different GI transit times and intestinal pH. These differences could have profound effects on dissolution and permeability of oral drugs, which are the most important determinants of their bioavailability.

GI15

Clinical utility of fecal scoring and evaluation of daily fecal scoring fluctuations in healthy dogs

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Various fecal scoring systems exist to characterize canine bowel movements (BMs) in clinical settings. The most commonly utilized scoring systems have been developed by Nestle Purina PetCare Company and The Waltham Petcare Science Institute. These scoring systems categorize feces based on a number of characteristics, including shape, color, consistency, and visual features. The data from these systems can assist in monitoring gastrointestinal disease and therapeutic responses. Currently, the use of these scoring systems requires accurate in-person scoring, which can limit the potential for use in clinical trials. Moreover, little is known about the daily variation in fecal scores amongst healthy dogs. Therefore, this study aims to:

	Transit time (mins) median (range)		pH median (range)	
	Dog	Cat	Dog	Cat
Esophageal	1 (1 – 24)	11 (1 – 317)	6.6 (5.8 – 7.2)	7.0 (3.5 – 7.8)
Gastric	1 (1 – 197)	94 (1 – 4,101)	2.5 (1.4 – 5.7)	2.7 (1.7 – 6.1)
Intestinal	1,039 (188 – 1,428)	1,350* (929 – 2,961)	7.9 (7.2 – 8.4)	8.2 (7.6 – 8.7)
Total	1,070 (244 – 1,430)	1,733* (1,115 –5,741)	N/A	N/A
1hr Small intestinal	N/A	N/A	7.6 (6.1 – 8.3)	8.2* (7.4 – 8.7)
1hr Large intestinal	N/A	N/A	7.9 (6.8 – 8.9)	8.5* (7.0 – 8.9)

* P-value ≤ 0.05 compared to the dogs.

1. Determine if a digital photo can be scored in place of an in-person fecal score; 2. Evaluate daily variability of fecal scores in healthy dogs. For Aim 1, a collection of 113 BMs were scored in person by two veterinarians using both the Purina and Waltham scoring systems. Digital images were obtained during the in-person scoring. These images were randomized and scored by three veterinarians and scores were analyzed using Cohen's and Fleiss' kappa statistics and Bland-Altman agreement plots. Kappa scores can be interpreted as: < 0 indicates poor agreement, 0.0 to 0.2 indicates slight agreement, 0.21 to 0.41 indicates fair agreement, 0.41 to 0.60 indicates moderate agreement, 0.61 to 0.80 indicates substantial agreement and 0.81 to 1.0 indicates almost perfect agreement. For Aim 2, 21 client-owned, healthy dogs were enrolled in a 4-week study where clients were trained on utilizing the Purina and Waltham scoring systems to score daily defecations and obtain digital images of each BM.

The results for Aim 1 demonstrated agreement between in-person scoring and image scoring, using Bland-Altman plots, with more than 50% of the data falling within the interquartile ranges along the mean. Moderate to substantial agreement between veterinarian raters and the in-person scores among both scoring systems based on kappa statistics was observed. The Purina scoring system showed higher agreement when compared to the Waltham scoring system (Cohen's kappa 0.54-0.73 and 0.40-0.43 respectively). For Aim 2, interim analysis of 10 dogs, reveal that over the 28-day course of the study most dogs (n=7) had minimal variability in their daily fecal scoring. In these dogs, 83% or more of the first BMs of the day were assigned the same fecal score with the exception of a few BMs. Some healthy dogs (n=3) showed considerable daily variation in fecal scores.

Overall, these findings support the scoring of digital fecal images in lieu of in-person fecal scoring in dogs. This provides proof of concept that artificial intelligence could be used to automatically perform fecal scoring using a digital fecal image, which is directly applicable to clinical and research settings. Most dogs have consistent fecal scores over a 28-day period with little variation, while some dogs have considerable daily variation in their fecal scores. The clinical significance of fecal score variation in healthy dogs is unknown at this time and further studies assessing how fluctuations in the fecal score and microbiome impact health and wellness monitoring are currently underway.

HM01

Novel cold storage method using platelet additive solution to prolong storage of canine platelet concentrates

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Stored platelet concentrates are not readily available for transfusion in critically thrombocytopenic dogs. The long-term storage of platelets will increase the availability of platelet concentrates to canine patients that are at risk for catastrophic hemorrhage due to thrombocytopenia. The objective of the study was to assess the viability of a novel cold storage method to minimize platelet storage lesion development and to maintain function of canine platelet concentrates (PC) in a platelet additive solution (PAS) compared to a plasma control at 4°C for 21 days.

In this prospective, *in vitro* controlled study, 10 units of PC were obtained from blood donors and separated in two bags, one containing 100% plasma and another containing 35% plasma and 65% of a PAS (Plasma-Lyte A). Both bags were stored at 4°C without agitation for 21 days. At days 0, 7, 14 and 21, the samples were analyzed for the presence of swirling, aggregate formation, platelet counts, platelet indices, glucose, lactate, lactate dehydrogenase, PvCO2, PvO2, maximum percent aggregation via light aggregometry, activation percentages using flow cytometry for the measurement of surface P selectin, and for bacterial growth via culture.

Cold stored PC in both PAS and plasma maintained a mean pH > 6.8 ($p < 0.05$) and mean lactate values < 9.0 mmol/L over 21 days. Cold storage was able to maintain average platelet counts above 250 x 103/mcL except for day 21 in the PAS ($p < 0.05$). Glucose utilization rate was not different between plasma and the PAS. Mean platelet volume was lower in the PAS than plasma on day 0 but higher in PAS on days 7 and 14 ($p < 0.05$), however it remained within reference range. Mean platelet component was significantly lower in the PAS on days 0 and 7 ($p < 0.05$). Platelet component distribution width was lower in the PAS on day 0 ($p < 0.05$) but was not different on other days. The PvCO2 was higher in plasma compared to PAS on all days ($p < 0.001$) and there was no difference for PvO2. Platelet distribution width was different on day 7 ($p < 0.05$), but the same on all other days. The platelet indices did not reflect significant platelet storage lesion development in cold stored PC in either plasma or PAS. There was no difference for lactate dehydrogenase between both solutions, but it increased overall from days 7 to 21. Platelet function as measured by mean maximal aggregation percentage was reduced but maintained in both PAS and plasma at day 21 at 14.9% and 13.7% respectively, with no significant difference found between the solutions. No bacterial growth developed during the 21 days of cold storage in either solution. The highest mean activation percentage was seen in PAS on day 7 (4.5%) which was significantly higher than plasma ($p < 0.05$), but there was no difference between solutions on any other day.

Cold storage allowed PC to be stored for up to 21 days with minimal storage lesion development, maintenance of platelet function, limited platelet activation, and no bacterial growth within stored bags. Overall, PAS and plasma were comparable for long term cold storage of PC. Additional studies are needed to determine utility of cold stored platelets *in vivo*.

HM02

Characterization of post-transfusional anti-FEA 1 alloantibodies in transfusion-naive FEA 1 negative cats

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Discovery of the *Mik* antigen in 2007 led to further research on non-AB group-related feline erythrocyte antigens (FEA) and their potential

role in transfusion reactions. Recently, five new FEA have been identified (FEA 1, 2, 3, 4, 5). This study's objective was to characterize anti-FEA 1 alloantibodies following sensitization of FEA 1 negative cats, including their appearance rate, agglutination titer over time, and immunoglobulin class. A secondary objective was to obtain polyclonal anti-FEA 1 alloantibodies for future blood typing.

Thirty-five type A cats from the hospital blood donor and teaching colonies underwent extensive blood typing for FEA 1-5. Two FEA 1– cats without natural anti-FEA 1 alloantibodies were identified and transfused with 25 ml of FEA 1+, but otherwise compatible, packed red blood cells. Using FEA 1 positive and negative controls, the production of anti-FEA 1 alloantibodies was monitored daily for the first week, weekly for the first month and monthly thereafter. If alloantibodies were not produced or fell below detection level, subsequent 5 ml transfusions were administered. Anti-FEA 1 alloantibodies were detected as early as 5 days post-transfusion. Recipient I required three additional transfusions (maximal titer 1:8), while recipient II maintained alloantibody levels for over 200 days after a single transfusion (maximal titer 1:32). Based on mercaptoethanol and dithiothreitol treatments, alloantibodies were predominantly IgM.

FEA 1's immunogenicity was documented, but with significant inter-individual variability. Further studies are needed to better characterize its role in feline blood transfusion medicine, which will be facilitated by storage of polyclonal anti-FEA 1 antibodies.

HM03

Anticoagulant effects of rivaroxaban, prednisone, alone and in combination, in healthy dogs

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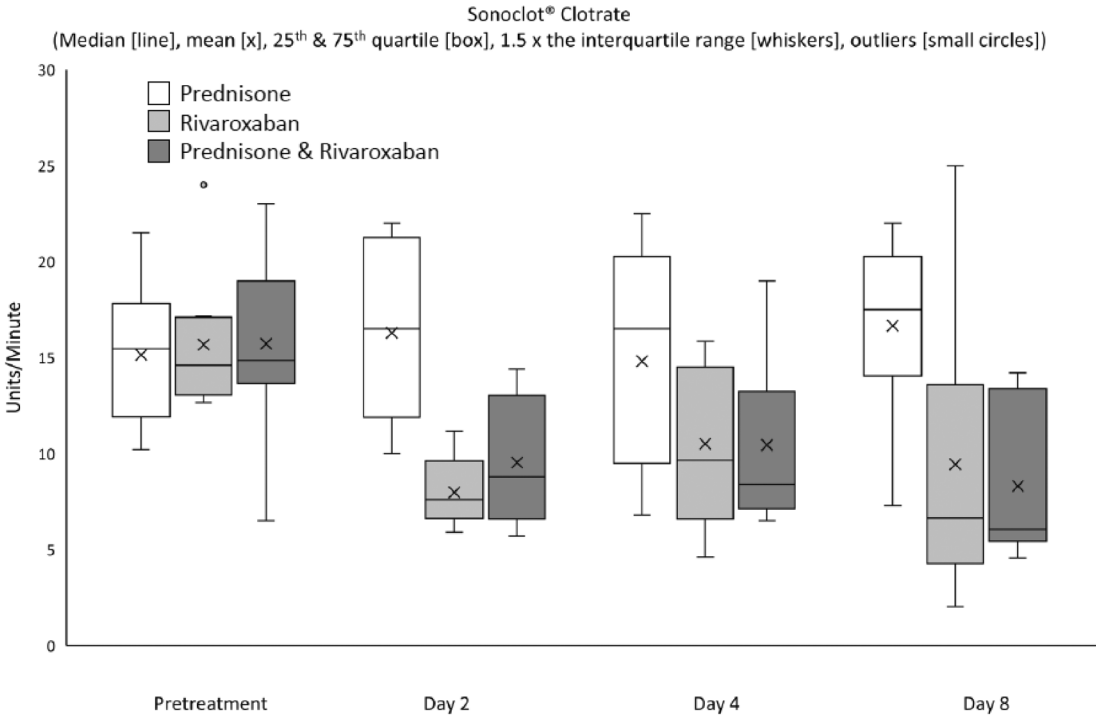
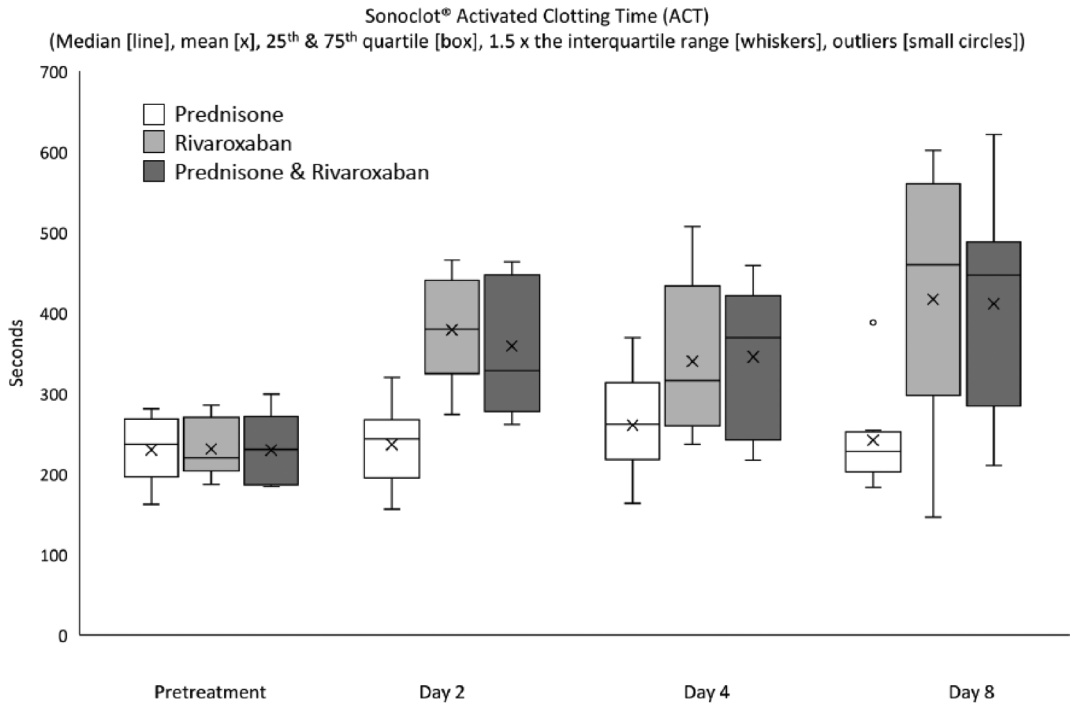
Management of canine immune-mediated hemolytic anemia includes immunosuppression with glucocorticoids and thromboprophylaxis. Glucocorticoids can, however, cause hypercoagulability in dogs. Rivaroxaban is a promising oral anticoagulant, but it is unknown if rivaroxaban ameliorates glucocorticoid-induced hypercoagulability or if glucocorticoids limit the anticoagulant effects of rivaroxaban. Our objective was to determine if glucocorticoids influence rivaroxaban anticoagulant effects. Our hypotheses were that rivaroxaban would provide appropriate anticoagulation, and the concurrent administration of glucocorticoids would have minimal effects on coagulation parameters.

In a randomized, crossover study, 9 healthy dogs were treated with rivaroxaban (1.5 mg/kg, PO, q12hrs), prednisone (2 mg/kg, PO, q12hrs), or prednisone *and* rivaroxaban, for 8 days. Blood samples were collected pre-treatment, and on Days 2, 4, and 8 of drug administration and analyzed using viscoelastometry (Sonoclot®). Following a 2-week recovery period, dogs switched groups and the study was repeated until all dogs received each treatment.

Compared to pre-treatment, when administered rivaroxaban, the mean Sonoclot® activated clotting time (ACT) increased by 64%, 47.1%, and 80.4% on Days 2, 4, and 8. In the rivaroxaban/prednisone group, the mean ACT increased by 56.2%, 50.4%, and 79.2% on the same days. Compared to pre-treatment, when administered rivaroxaban, the ClotRate decreased by 49%, 33.1%, and 40.1% on Days 2, 4, and 8. In the rivaroxaban/prednisone group, the mean ClotRate decreased by 39.4%, 33.8%, and 47.1% on the same days. Differences were not statistically significant.





Prednisone does not alter the anticoagulant effects of rivaroxaban in healthy dogs, but additional studies are needed to determine effects in hypercoagulable dogs.

HM05

Validation of the use of bedside agglutination card for dal blood typing in dogs

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While 98% of the canine population is *Dal*-positive, *Dal*-negative dogs are more common in some breeds such as Dalmatians (11.7%) and Dobermans (42.4%), for whom finding compatible blood may be challenging notably given the limited access to *Dal* blood typing. This study's objectives were: (a) to validate a bedside agglutination card for *Dal* blood typing and (b) to determine the lowest packed cell volume (PCV-threshold) at which interpretation remains accurate.

A total of 128 dogs were recruited: 38 blood donors, 18 Dalmatians, 51 Dobermans and 21 anemic dogs (PCV < 25%, negative saline test). Dal blood typing was performed on EDTA samples (< 48 hours) using the agglutination card according to the manufacturer's instructions (DMS Laboratories), as well as a gel column technique as previously described (gold standard). PCV-threshold was determined using plasma-diluted blood samples (n=3; undiluted sample and PCV of 25, 20, 15 and 10%). All results were read by two observers, blinded to each other's interpretation, and to the sample's origin.

The interobserver agreement was 97.7% and 100% using the card and gel column assay, respectively. Overall, 11/117 samples were mistyped using the agglutination cards (8/11 by both observers): one false-positive (Doberman), and 10 false-negative samples including 6 anemic dogs (range: 5-24%; mean PCV: 11%). Similarly, PCV-threshold allowing a reliable interpretation was determined at ≤15%. Sensitivity and specificity of the cards were respectively 89.9-92% and 96.6-100%, depending on the observer.

Dal agglutination cards are a reliable bedside test, although results should be interpreted cautiously in severely anemic patients.

HM06

A pilot study of sample tube temperature changes in three simulated 24-hour shipping conditions

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In multi-center studies involving biological sample analysis at centralized laboratories, ensuring that the shipping process does not spuriously alter the results is important. This study's major objective was to observe the temperature change of six different blood collection tubes packaged in a shipping container in three different temperature conditions over 24 hours. Additionally, suitable non-biological sample fluids and thermometers were assessed.

Temperature measurements of sucrose solutions (0% to 40%) were compared with plasma and whole blood samples in three temperature conditions across six timepoints. Differing tube types were assessed using both immersion and infrared thermometers in two conditions at two timepoints. Absolute temperature differences were averaged in each condition.

Various sucrose solutions and distilled water changed temperatures similar to blood and plasma in conditions tested ranging from best (0.69 ± 0.70°C, 30% sucrose vs. plasma) to worst approximation (1.99 ± 1.36°C, distilled water vs plasma). The average of all absolute temperature differences between immersion and infrared thermometers was 1.50°C. For shipping container assessment, the average temperatures of all blood tube types at the 24-hour timepoint were -7.30 ± 2.84°C, 19.03 ± 0.56°C, and 35.20 ± 2.29°C for freezer (cold), room temperature (ambient), and outdoor vehicle (hot) incubation conditions, respectively.

Diverse sucrose solutions and water performed similarly to blood and plasma, and thermometer types performed similarly. Major differences in the 24-hour sample temperatures between different simulated shipping conditions indicate the shipping container did not maintain consistent sample temperature. Investigating the effects of these temperature differences on sample analysis results is warranted.

HM07

Production and characterization of an anti-DAL murine monoclonal antibody for blood typing in dogs

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Considering the strong immunogenicity of the Dal antigen, and that >98% of dogs, including blood donors, are Dal-positive, finding compatible blood for a previously transfused Dal-negative patient may be challenging. This is exacerbated by limited access to typing reagents, which currently rely on polyclonal antibodies (PAb) produced following sensitization of dogs. Therefore, the objective of this study was to produce and characterize an anti-Dal murine monoclonal antibody (MAb).

Conventional hybridoma technology was used to produce MAb directed against canine red blood cells (cRBC). Briefly, female BALB/c mice were immunized via repeated intraperitoneal injections of 0.5mL of washed Dal-positive cRBC (10^9cells/mL; DEA 1,3,7 negative; DEA 4,5 positive) until serologic titers were sufficient (>1:1000). Following fusion with myeloma cells, 573 hybridoma cell culture supernatants were obtained and screened for MAb of interest using a gel column agglutination technique, and known Dal-negative and Dal-positive cRBC. Fifteen supernatants led to cRBC agglutination, but only one had the desired pattern (ie.anti-Dal). To assess its specificity and sensitivity, Dal blood typing of 62 canine EDTA-samples was performed using the anti-Dal MAb and two canine PAb:45 Dal-positive and 17 Dal-negative were identified with 100% agreement between reagents. Based on mercaptoethanol and dithiothreitol treatments, the anti-Dal MAb produced is an IgM.

Conventional hybridoma technology, aided by a gel column technique, has enabled the production of a murine MAb specific against the canine Dal antigen. This will ensure long-term perennity of Dal blood typing, facilitate clinical management and research, as well as avoid resorting to repeat dog sensitization.

HM08

Pharmacodynamic monitoring of clopidogrel therapy in dogs with thrombosis or risk for thrombosis

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This study investigated high-on-treatment platelet reactivity (HOTPR, drug resistance) in clopidogrel-treated dogs with thrombosis (n = 117), or at risk for thrombosis (n = 119), using Plateletworks® (PW, ADP agonist), and Platelet Function Analyzer – 200® (PFA, P2Y and Collagen/ADP cartridges).

Results are reported as median (range) in Table 1. Pre-treatment results were significantly different ($P \leq 0.05$) from results during treatment (Post) by Mann Whitney U and Chi-Squared tests.

Clopidogrel dose in dogs with HOTPR was 1 – 5 mg/kg. In 7 of 8 dogs, dose increase partially or completely overcame HOTPR. In summary, the rate of HOTPR was 12%, 22%, and 41% based on PW, PFA P2Y, and PFA CADP, respectively. Some HOTPR was overcome by dose escalation.

Table 1

Plateletworks	***Aggregation (%)***
Target response	≤ 20
Pre, normal response (n = 50)	84 (21-98)
Pre, abnormal response (n = 2)	4 (3-4)
Post, good drug effect (n = 66)	0 (0-20)
Post, HOTPR (n = 9)	57 (21 – 89)
PFA – 200 P2Y	***Closure Time (sec)***
Target response	> 300
Pre, normal response (n = 68)	68 (36 – 226)
Pre, abnormal response (n = 32)	> 300
Post, good drug effect (n = 69)	> 300
Post, HOTPR (n = 20)	78 (52 – 279)
PFA – 200 Collagen/ADP	***Closure Time (sec)***
Target response	> Reference Interval (45 – 109)
Pre, normal response (n = 82)	69 (51 – 109)
Pre, abnormal response (n = 4)	262 (194 – > 300)
Post, good drug effect (n = 52)	262 (112 – > 300)
Post, HOTPR (n = 36)	78 (52 – 279)

HM09

Characterization of a flow cytometric assay for anti-canine erythrocyte IgG utilizing novel standardized assay controls

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Immune-mediated hemolytic anemia (IMHA) is a common cause of severe anemia in dogs that is associated with high morbidity and mortality. Rapid diagnosis of IMHA is crucial for patient management and ultimate recovery, but this can be challenging as no "gold-standard" test currently exists for IMHA in veterinary patients. Ideally, IMHA should be diagnosed by detection of surface-bound immunoglobulin or complement on erythrocytes. However, the traditional direct antiglobulin test (DAT) for anti-erythrocyte antibodies/complement lacks sensitivity. Flow cytometric (FC) detection of surface-bound immunoglobulin not only confirms the presence of anti-erythrocyte antibodies but also provides quantitative information on the proportion of

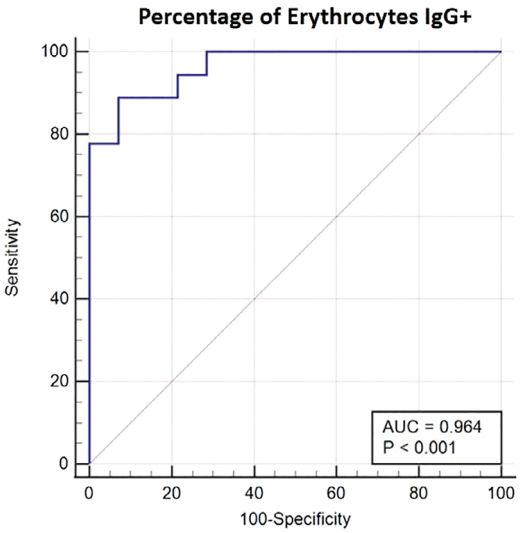

Figure 2. Receiver-operator curve for % IgG+ erythrocytes for diagnosing immune-mediated hemolytic anemia.

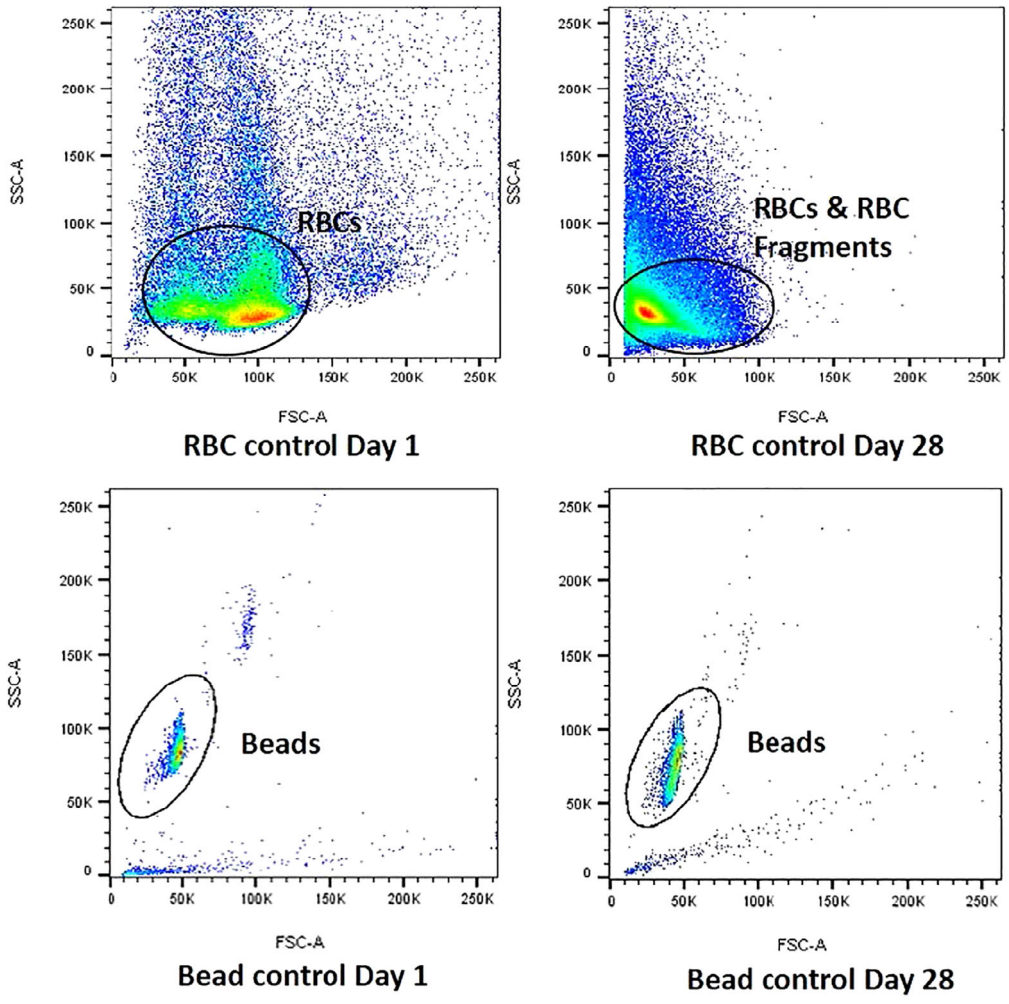

Figure 1. Stability of IgG-RBC and IgG-bead controls over 1 month.

erythrocytes affected and density of bound immunoglobulin. However, previously used FC assays do not have controls or utilize complex biologic controls requiring production of IgG-coated erythrocytes. Silica microspheres coated with canine immunoglobulin could be an alternative positive control that are easy to produce with a long shelf-life. As such, we aimed to a) develop and evaluate a FC assay for anti-erythrocyte IgG using canine IgG-coated silica bead controls and b) compare the performance of this FC assay to an immunochromatographic DAT for diagnosing canine IMHA.

Using DEA 1+ erythrocytes from donor dogs incubated with anti-DEA 1 antisera (RBC positive control), we first optimized an FC protocol to detect surface-bound IgG using an anti-dog IgG fluorochrome-conjugated antibody. We then coated microspheres with canine IgG (bead positive control) and analyzed RBC and bead positive control stability weekly for 4 weeks. We also performed spiking experiments with positive and negative RBC controls and positive and negative bead controls to determine the accuracy, precision, and linearity of the assay. While RBC controls remained IgG positive for 4 weeks, decreasing RBC size and increasing in RBC fragmentation was evident at 2 weeks. Bead controls retained integrity and IgG signal intensity for the 4-week period and remained analytically useful for at least 18 months (Figure 1). The assay using the RBC controls had a mean accuracy of 93% and within run and between run precisions of 2.01% and 5.49%, respectively. The assay using the IgG-conjugated control beads had a mean accuracy of 100% and within run and between run precisions of 1.57% and 2.61%, respectively. The time to perform the assay was on average 2 hours shorter for the beads than the red cell controls.

For method comparison, FC assay and DAT were performed on 18 dogs with IMHA (diagnosed following ACVIM Consensus Statement guidelines) and 14 non-IMHA anemic dogs. The FC assay had an optimal diagnostic threshold of >1.87% IgG+ erythrocytes; FC assay receiver-operator curve AUC was 0.964 ($p < 0.001$) for IMHA diagnosis and the assay was 89% sensitive and 93% specific for IMHA (Figure 2). Using this threshold, the FC assay properly classified 16/18 IMHA and 13/14 non-IMHA dogs while the DAT classified 13/18 IMHA and 14/14 non-IMHA dogs correctly. Agreement between assays was good ($K = 0.63$; 95% CI 0.37–0.89).

Immunoglobulin-coated microspheres appear to be acceptable and stable positive controls for anti-erythrocyte immunoglobulin FC assays. The FC assay may be more accurate for diagnosing IMHA than the immunochromatographic DAT. Further studies are warranted to determine if the quantitative metrics of this FC assay, such as % IgG+ erythrocytes or IgG signal intensity, may be useful for evaluating response to therapy or predicting survival in dogs with IMHA.

HM10

Prevalence, severity, and concurrent clinical characteristics of microcytosis in cats

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Decreased red blood cell (RBC) size, or microcytosis, is associated with conditions leading to iron deficiency or altered iron metabolism, bone marrow disorders, or altered RBC structure. The prevalence of microcytosis and associated medical conditions in client owned cats have not been described.

Retrospective study over a 20-year period. In the study period, 15,704 individual cats had at least one complete blood count (CBC) performed. Of these cats, 3,502 (22%) had at least one CBC with documented microcytosis (MCV < 42fL). Microcytosis was categorized as mild (40-41.9fL), moderate (38-40fL), or severe (< 38fL) in 2,112 (60%), 845 (24%), and 545 (16%) cats respectively with a mean MCV of 39.8fL. Anemia was documented in 1,351 of the 3,502 (39%) cats, with a mean hematocrit of 31%. A reticulocytosis (>60,000/ul) occurred in 209 (6%) cats, 111 of which were also anemic.

Medical records of 1,266 cats with microcytosis were evaluated and primary disease processes were recorded for each cat. The most commonly observed disease processes in cats with microcytosis include gastrointestinal (26%), neoplasia (22%), and renal disease (20%), which was consistent across all microcytosis severity categories. Based on patient hematological data and concurrent primary diseases, the physiologic cause of microcytosis was classified as anemia of chronic disease, portosystemic shunt, iron deficiency, or unknown etiology in 244 (19%), 7 (0.8%), 5 (0.4%), and 1010 (80%) respectively.

In conclusion, microcytosis was a common finding in this population and was frequently observed in the absence of anemia. The physiological cause underlying this abnormality was often not identified.

HM11

Comparison of i-STAT® point-of-care blood gas parameters between non-anticoagulated and heparinized whole blood in cats

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Measurement of ionized calcium (iCa) may be affected by the presence of heparin anticoagulant in the specimen, a phenomenon that may be of clinical importance for evaluation of hypercalcemia. This study evaluates agreement in blood parameters, including iCa, between non-anticoagulated whole blood (NAB) and heparinized whole blood samples (HEP) in cats.

Blood samples were obtained by jugular venepuncture from 22 senior (≥ 9 years) client-owned cats. Whole blood (125 μL) was transferred into a capillary tube containing 70 IU/mL electrolyte balanced heparin. NAB samples were immediately analyzed on an *i-STAT® 1* Analyzer followed by the HEP samples (≤ 5 minutes) quantifying iCa, sodium, potassium, glucose, venous pH, bicarbonate and base excess. Agreement was evaluated using intra-class correlation coefficient and Bland-Altman plot; NAB and HEP measurements were compared using paired *t*-test and percentage bias was evaluated against their respective allowable total error (TEa).

Glucose and iCa concentrations from HEP were significantly lower compared to NAB (percentage bias -0.84% (-0.06 ± 0.09 mmol/L; $P = 0.006$) and -0.49% (-0.007 ± 0.013 mmol/L; $P = 0.022$)

respectively). No significant difference was identified for other parameters and the intra-class correlation for all parameters was excellent (r = 0.94–0.99; $P < 0.001$). One sample exceeded human TEa for iCa (defined as 2%).

Despite significant underestimation of [glucose] and [iCa] obtained from HEP samples, the low magnitude of the negative bias is not deemed clinically important. In practice, NAB and HEP samples may be used interchangeably for blood gas parameters, including iCa, on *i-STAT® 1* Analyzer.

HP01

Ultrasonographic characteristics of the portal venous system of 37 healthy unsedated student-owned cats: Prospective study

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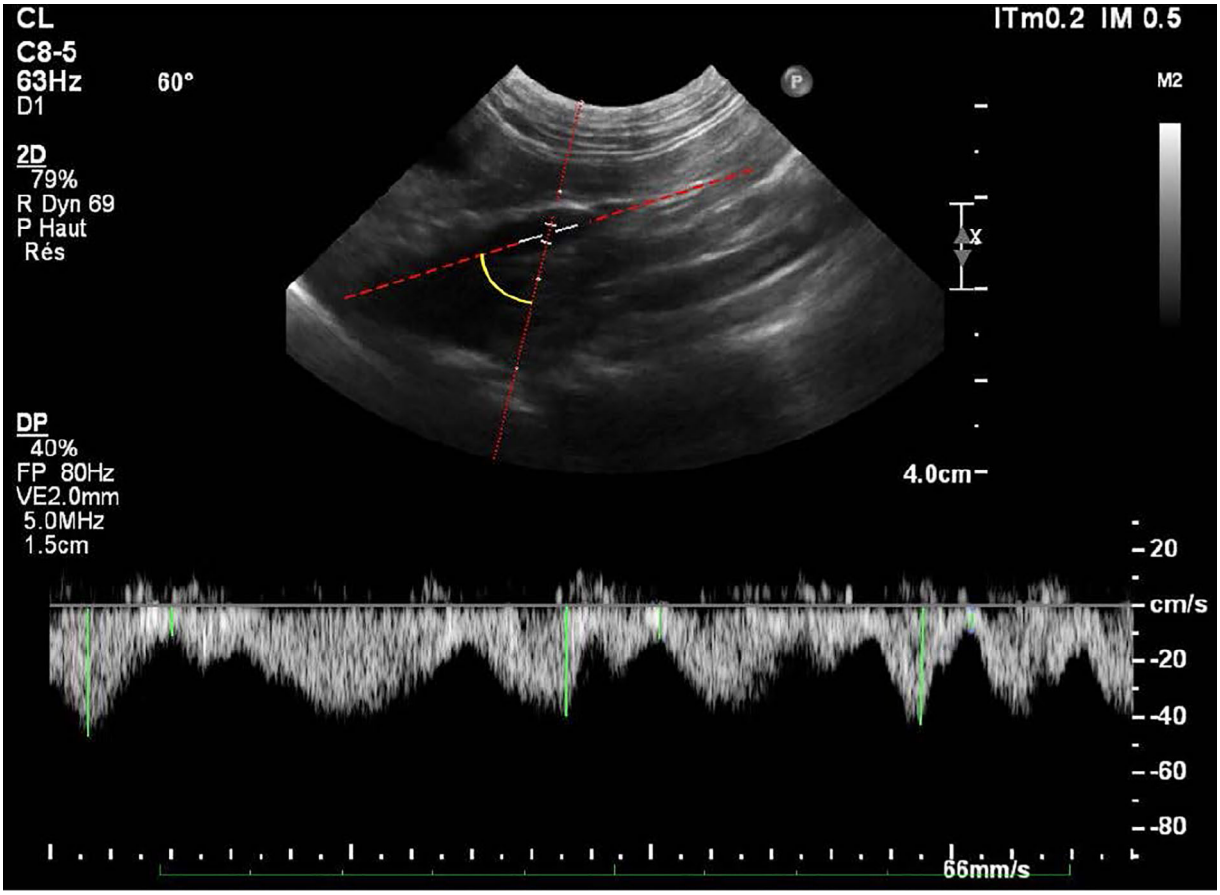
The aim of this study was to obtain normal values of B-mode and Doppler ultrasound parameters of the portal vein in healthy, unsedated, client-owned cats.

A clinical protocol was designed and 37 student-owned, healthy, adult cats were prospectively enrolled in the study. Longitudinal and transverse sonographic planes were evaluated and measurements were performed by a single investigator. Three anatomic sites were assessed, including the extra-hepatic portion of the portal vein, the intra-hepatic branches of the portal vein and the aorta at the level of the porta hepatis. R was used for statistical analysis.

The porta hepatis and the extra-hepatic portion of the portal vein were identified in all cats (37/37). The left and right intra-hepatic branches of the portal vein were identified in 29 cats (29/37) and the aorta was identified in 31 cats (31/37). Pulsed Doppler ultrasound of the portal vein was obtained in 32 cats (32/37). On the longitudinal view, the mean maximal diameter of the extra-hepatic portal vein was 3.6 mm (SD +/- 0.7) and the mean portal flow velocity was 14.6 cm/s (SD +/- 4.3). On the transverse view, the mean maximal diameter of the extra-hepatic portal vein was 4.8 mm (SD +/- 0.8). On the transverse view, the mean maximal and minimal diameters of the intra-hepatic divisions of the portal vein were respectively 2.6 mm (SD +/- 0.7) and 1.8 mm (SD +/- 0.6) for the right branch, and 3.1 mm (SD +/- 0.8) and 2.1 mm (SD +/- 0.7) for the left branch. The mean portal-vein-to-aorta ratio was 1.2 (SD +/- 0.2).

Our study provides sonographic data of the portal vein in healthy, unsedated cats. Values outside these established intervals should prompt the clinician to further investigate the liver status of the patient.



Vascular segment	Variable	Effective	Normal distribution	Mean [standard deviation] *or* *median (interquartile range)*
Extra-hepatic portal vein	Maximal diameter in transverse view (mm)	27	Y	4.8 [4.0-5.5]
	Minimal diameter in transverse view (mm)	27	N	*3.5 (3.3-4.2)*
	Maximal diameter in longitudinal view (mm)	37	Y	3.6 [2.9-4.3]
	Maximal velocity (cm/s)	32	Y	25.7 [18.1-33.3]
	Minimal velocity (cm/s)	32	Y	9.2 [5.7-12.8]
	Portal flow (ml/kg/min)	23	Y	29.9 [18.4-41.4]
	PV/Ao ratio	24	Y	1.2 [1.0-1.4]
	Pulsatility index	32	N	*0.65 (0.57-0.7)*
	Congestive index (cm.s)	25	Y	0.012 [0.006-0.017]
Right intra-hepatic portal branch	Maximal diameter in transverse view (mm)	28	Y	2.6 [1.9-3.2]
	Minimal diameter in transverse view (mm)	28	Y	1.8 [1.3-2.4]
Left intra-hepatic portal branch	Maximal diameter in transverse view (mm)	29	Y	3.1 [2.3-4.0]
	Minimal diameter in transverse view (mm)	29	Y	2.1 [1.4-2.8]
Aorta	Maximal diameter in transverse view (mm)	11	Y	4.1 [3.1-5.0]
	Minimal diameter in transverse view (mm)	11	Y	3.6 [2.7-4.5]
	Maximal diameter in longitudinal view (mm)	31	Y	3.9 [3.2-4.7]

HP02

Morphometric characteristics of liver masses in dogs: A Promising tool for predicting malignancy

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The size of liver masses is one of the predicting criteria for malignancy. However, there are discrepancies for the measurement of the maximum size of the lesion, resulting in contradicting results among studies and incidences of false positive outcomes. To our knowledge, the morphometric changes of liver masses for distinguishing malignancy from benignancy remains undocumented. Therefore, this study aimed to investigate morphometric characteristics of liver masses using computed tomography (CT).

CT images of 43 dogs with histopathological confirmation of 53 liver masses, including 39 hepatocellular carcinomas (HCCs) and 14 benign liver diseases between December 2016 and December 2018 were retrospectively reviewed. The morphometric parameters including size (long and short axis diameters), shape (measured by long to short axis (L/S) ratio), volume, and margin of a liver mass were evaluated and analyzed using univariate and stepwise multivariate analyses.

From univariate analysis, long and short axis diameters, L/S ratio, volume and margin of a mass were significantly different between HCCs and benign liver diseases. The multivariate analysis showed that short axis diameter (> 3.34 cm; OR: 24.5, 95% CI: 3.62 – 166.37, $P = 0.0001$) and L/S ratio (> 1.23; OR: 26.2, 95% CI: 2.44 – 281.94, $P = 0.0006$) were independent predictors of malignancy with the area under the curve of 0.904.

These findings reveal that short axis diameter and L/S ratio on CT are promising tools for predicting liver malignancy and that a liver mass with a L/S ratio > 1.23 might be at an increased risk for malignancy.

HP03

Using big data to compare single versus paired bile acid testing in dogs

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A dynamic bile acids stimulation test is used diagnostically to evaluate liver function by estimating the efficiency of enterohepatic circulation. Submission of a single bile acids test is a common practice likely due to the practical difficulties associated with collecting both fasted and 2-hour postprandial blood samples. While an increased result on a single test is suggestive of liver dysfunction, there is concern that omitting the second test decreases the sensitivity of the testing. Past evidence for this is based on studies evaluating a limited number of patients. This study used clinical results of bile acids testing in a cross-sectional design intended to identify the proportion of dogs with discordant pre- and postprandial bile acid test results where liver dysfunction would have been unrecognized if only one of the bile acids tests was performed. All paired bile acids stimulation test results for dogs submitted to IDEXX Reference Laboratories from veterinary practices located in the United States, Canada, and Europe from 2001-2019 were included, resulting in 301,766 canine bile acids stimulation test results for analysis. Test results were classified into buckets with

cutoffs based on the concentration of the pre- and postprandial result. For the purposes of this study, liver dysfunction was defined as having either the preprandial result or postprandial result or both above the cut-off. The reference interval cutoff was 15 μmol/L for preprandial results and 30 μmol/L for postprandial results.

In the study population, 43.6% of dogs met the definition of liver dysfunction when using the reference interval as a cutoff. Approximately half of those had discordant pre- and postprandial results which could have led to under identification of liver dysfunction had a single bile acids test been performed. The potential for unrecognized liver dysfunction with a single bile acids test continued at higher cutoffs. For example, at a cutoff of 40 μmol/L, 29.4% of dogs had results consistent with liver dysfunction but approximately 3 out of 5 of these results were discordant. Another key finding is that the postprandial result was increased more often than the preprandial result across all thresholds. Sixty-nine percent of dogs in this population had a preprandial bile acids result within the reference interval. Of those approximately 1 in 5 had elevated postprandial results and would have been misclassified had the postprandial test been excluded. When using the cutoff of 40 μmol/L to define liver dysfunction, 15.5% of dogs were identified as consistent with liver dysfunction if evaluating the preprandial bile acids test alone, while 25.8% were identified as consistent with liver dysfunction when evaluating the postprandial result alone. One limitation of a big data approach is that information about the individual patient, including reason for testing and definitive diagnosis, is unknown. Therefore, these data should be used with caution to determine the utility of a single bile acids test when evaluating for a specific disease process. The results of this study support the findings of previous studies suggesting that paired bile acids tests identify more dogs with potential liver dysfunction than a single bile acids test and that if a single bile acids test is performed, a postprandial result will be above the diagnostic threshold more often than a fasting bile acids result.

HP04

Evaluation of outcomes using various treatments for extrahepatic biliary obstructions in dogs and cats: 2012-2019

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To compare clinical outcomes for extrahepatic biliary obstruction (EHBO) using medical, endoscopic retrograde cholangiopancreatography (ERCP), or surgery. The hypothesis is that medical management and ERCP are associated with lower intra- and peri-operative morbidity and mortality rates than surgery.

Fifty-three animals (29 dogs and 24 cats) with EHBO.

Retrospective review of medical records of patients with EHBO. Data, including clinical signs, biochemical abnormalities, imaging, procedure, complications (short- and long-term), and survival times were collected.

Median total bilirubin at presentation was 8.5mg/dL(range,0.1-29.3), ALP(1190U/L, 9–10691), ALT(824U/L,38–4400), GGT(33U/L,1–540), cholesterol(1190U/L,123–1743). Common bile duct median diameter was 8mm(range,4–30). Intrahepatic duct dilation was noted in 17/51 animals. EHBO resulted from stricture(13/53), cholelithiasis(10/53), MDP mass(10/53), or pancreatitis(8/53). Medical management, ERCP, or surgery were pursued in 20(37.7%;10 dogs,10 cats), 13(24.5%;12 dogs,1 cat), and 20(37.7%;7 dogs, 13 cats) animals, respectively. Intra-operative complications, including hypotension, hemorrhage, or biliary leakage, occurred more frequently in the surgical group versus the ERCP group (100% and 38%, respectively). Forty-six of 53(86.8%) animals survived to discharge (medical–100%, ERCP–92.3%, surgical–70%). Median survival time (MST) for all animals was 366 days (range,5–2005) and 769 and 505, 343 and 202, and 21 and 402 days in each group (medical, ERCP, surgery) for dogs and cats, respectively. Animals with cholelithiasis that were successfully treated with medical management alone had the longest MST (1258 days).

Results suggest that medical management alone is often highly successful for various EHBO etiologies. Results support that traditional surgical decompression is associated with a higher mortality rate than medical management or ERCP.

HP05

Evaluation of serum MicroRNAs 15b, 181a and 150 as biomarkers for chronic hepatitis in dogs

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The diagnosis of canine chronic hepatitis is a challenge due to the cost and invasiveness of a liver biopsy. MicroRNAs (MiRs) are noncoding

Table 1: Mann-Whitney U tests to compare differences in copy numbers between groups and Spearman's Rank Correlations between serum MicroRNAs copy numbers and serum ALT activity, hepatic copper concentration, inflammatory activity, as well as fibrosis stage.

Comparison with healthy controls		*Correlation with other findings (ρ / P-value)*			
MiRNA	*P-value*	***ALT activity***	***Copper concentration***	***Inflammatory activity***	***Fibrosis stage***
15b	0.23	-0.09 / 0.82	0.20 / 0.63	-0.04 / 0.91	0.14 / 0.75
181a	0.79	-0.28 / 0.50	0.02 / 0.97	0.01 / 0.98	0.51 / 0.20
150	0.47	0.16 / 0.70	0.64 / 0.09	0.40 / 0.32	-0.25 / 0.54

RNA sequences that regulate gene expression, and have been shown to be promising non-invasive markers for hepatocellular injury in dogs. Our previous untargeted study described the increased expression of MiRs 15b, 181a, and 150 in hepatic tissue from dogs with chronic hepatitis compared to healthy control dogs. The objective of this study was to determine if these MiRs have potential as serum markers in dogs with chronic hepatitis.

Stored serum samples from 8 dogs with histologically confirmed chronic hepatitis and 12 healthy control dogs enrolled in our previous study were used. MiRs 15b, 181a, and 150 were quantified by RT-qPCR and absolute quantification was calculated using standard curves. Mann-Whitney U tests were used to compare differences in copy numbers between groups. Correlations between serum MiR copy numbers and serum ALT activity, hepatic copper concentration, inflammatory activity, as well as fibrosis stage were assessed using Spearman's rank correlation. Statistical significance was set at $P < 0.05$.

No significant differences in serum copy numbers were observed between groups for the 3 MiRs evaluated. No significant correlations were found between copy numbers of any of the 3 MiRs and ALT activity, hepatic copper concentration, inflammatory activity, or fibrosis stage (Table 1).

Circulating serum MiRs 15b,181a, and 150 copy numbers did not differ in this population of dogs with chronic hepatitis compared to healthy controls. Despite promising results from our previous untargeted study of hepatic expression, these subsequent results did not support the utility of these MiRs as serum markers for chronic hepatitis in dogs.

Table 1: Mann-Whitney U tests to compare differences in copy numbers between groups and Spearman's Rank Correlations between serum MicroRNAs copy numbers and serum ALT activity, hepatic copper concentration, inflammatory activity, as well as fibrosis stage.

HP06

Gallbladder motility in dogs with hyperlipidemia

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The pathogenesis of gallbladder mucoceles is unknown in the dog. It has been proposed that hyperlipidemia could impair gallbladder motility and contribute to gallbladder mucocele formation.

The objective of this study was to compare gallbladder motility in dogs with hyperlipidemia to healthy, control dogs using ultrasonography. We hypothesized that hyperlipidemic dogs will have decreased gallbladder motility, specifically increased fasting gallbladder volume (GBV) and decreased gallbladder ejection fractions at 60 (EF_{60}) and 120 (EF_{120}) minutes compared to controls.

Twenty-six hyperlipidemic and 28 healthy, age-matched control dogs were prospectively enrolled. Hyperlipidemia was defined as hypercholesterolemia (>332 mg/dL) and/or hypertriglyceridemia (>143 mg/dL). Primary and secondary causes of hyperlipidemia were included. All dogs were fasted for at least 12 hours prior to collection of plasma biochemistry and pre-prandial ultrasound. Ultrasound was performed on dogs in the fasted state as well as 60 and 120 minutes after being fed 10g/kg of Hill's a/d. GBVs and EFs were calculated using the following formulas: $GBV = (0.52 \times L \times W \times H)/kg$ and $EF = ((GBV_0 - GBV_{60,120})/GBV_0) \times 100$, respectively. Normal gallbladder motility was defined as EF > 25%. GBV_0, GBV_{60}, GBV_{120}, EF_{60} and EF_{120} were compared between dogs with hyperlipidemia and controls using the Wilcoxon rank sum test. Statistical significance was set to $p < 0.05$.

Hypercholesterolemia and hypertriglyceridemia were present in 15/26 and 21/26 hyperlipidemic dogs respectively, and 10/26 had elevations in both values. The median age in both groups was 10 years. Median cholesterol concentrations were 346 mg/dL (181-1372) and 238 mg/dL (153-324) in hyperlipidemic and control dogs, respectively. Median triglyceride concentrations were 330 mg/dL (52.0-2213) and 65.5 mg/dL (32-142) in hyperlipidemic and control dogs, respectively. Eleven (42%) hyperlipidemic dogs were deemed severely hyperlipidemic if either triglyceride, cholesterol, or both concentrations were >500 mg/dL.

There were significant differences in GBV_0 and GBV_{60} between hyperlipidemia and control dogs. Dogs with severe hyperlipidemia had significantly larger GBVs (0,60,120). Dogs with hypercholesterolemia also had significantly greater GBVs at all times compared to dogs without hypercholesterolemia. Median EF_{60} and EF_{120} were not significantly different between hyperlipidemic dogs and control dogs nor severely hyperlipidemic dogs and non-severely hyperlipidemic dogs. EF_{120} was < 25% in 9 (35%) and 8 (29%) of hyperlipidemic and control dogs, respectively.

Hyperlipidemic dogs have significantly greater fasting and postprandial GBVs than control dogs but similar ejection fractions. Gallbladder contractility is unaltered in hyperlipidemic dogs, but gallbladder volume remains higher in hyperlipidemic dogs even after feeding. This distention could contribute to retention of bile and gallbladder disease.

ID01

Anti-erythrocyte and anti-platelet antibodies in dogs co-infected with *Babesia gibsoni* and Candidatus *Mycoplasma haematoparvum*

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Canine babesiosis is an erythrocytic protozoal disease in domestic dogs. *Babesia gibsoni* (Bg) is considered a small form of *Babesia* spp. and is believed to be less pathogenic than *B. canis*. However, Bg can trigger immune-mediated hemolytic anemia (IMHA) which can be a fatal condition in dogs and also has been associated with thrombocytopenia. Canine hemoplasmosis is also a blood-borne erythrocytic bacterial disease. Both *Mycoplasma haemocanis* and *Candidatus* M. haematoparvum (CMhp) are only mildly pathogenic in dogs unless immune suppression is present. This experiment was part of ongoing

work attempting to determine the value of erythrocyte and platelet antibody assays in differentiation of infectious and primary immune mediated cytopenias. The primary aim of this study was to determine if experimental Bg infection induces anti-erythrocyte and anti-platelet antibodies as detected by flow cytometry assays. The secondary aim was to compare the results of the anti-erythrocyte flow cytometry with those of the direct Coombs test.

Blood from a client-owned Pitbull terrier in North Carolina was currently positive for DNA of Bg and historically, positive for DNA of CMhp. Additional samples were collected into EDTA and shipped overnight to Colorado State University on cold packs. The infected blood was inoculated into 5 purpose bred research beagles with IACUC approval on Day 0. Prior to inoculation and on various times over the 8-week study, blood samples were collected for performance of complete blood counts, indirect immunofluorescent assay (IFA) for Bg antibodies, flow cytometry for anti-platelet and anti-erythrocyte antibodies, and PCR assays for vector borne disease agents including Bg and CMhp. If clinical illness or cytopenias developed, approved treatment plans were to be initiated.

All 5 dogs became PCR positive for Bg and CMhp and developed anemia, thrombocytopenia, anti-erythrocyte antibodies (both by flow cytometry and Coombs test), and anti-platelet antibodies. Positive Coombs test results were detected sooner than the detection of anti-erythrocyte antibodies by flow cytometry assay in two of five dogs. The antimicrobial rescue protocol (doxycycline, azithromycin, and atovaquone) was applied to all dogs between Day 21 to Day 28 post-infection based on clinical signs and worsening anemia. Presence of anti-erythrocyte and anti-platelet antibodies rapidly resolved after initiation of treatment. One dog unexpectedly acutely died on Day 31 from either vasculitis or a thromboembolic complication. Bg antibodies were first positive for 4 dogs on Day 14 and Day 21 for 1 dog. After treatment, peak Bg titer magnitudes dropped 2-fold (1 dog), 4-fold (1 dog) or 8-fold (2 dogs) but the 4 dogs available for testing were still positive at Day 56.

The results support that Bg and CMhp co-infections induce transient anti-erythrocyte and anti-platelet antibodies that may play a role in the development of hemolytic anemia and thrombocytopenia in infected dogs. The results provide further documentation that antibodies directed against platelets or erythrocytes do not prove primary immune mediated disease. Further experiments are planned to assess the differences in results between the anti-erythrocyte antibody assays.

ID02

Disseminated I complex infections in 8 dogs

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Clinical features, treatment and outcome of opportunistic infections with *Rasamsonia* spp. are not well documented in dogs.

To describe the clinical, radiographic, pathologic features and outcome of dogs with disseminated *Rasamsonia* sp. infections.

8 client-owned dogs.

Case series. Medical records were reviewed to describe signalment, history, clinicopathologic and imaging findings, microbiologic and immunologic results, cyto- and histopathologic diagnoses, treatment and outcome.

Presenting complaints were vague with anorexia (n=5) and back pain (n=4) most common. Five dogs were German shepherd dogs or mixes. Six dogs had multifocal discospondylitis and 2 had pleural effusion. Two dogs had *Rasamsonia argillacea* and 6 had *Rasamsonia piperina* infections with isolates identified using DNA sequencing. *Rasamsonia* spp. were isolated following urine culture in 5 of 7 dogs (n=4 fungal and n=1 aerobic bacterial). Five of 6 dogs had positive serum *Aspergillus* galactomannan antigen EIA results. Median survival time was 35 days, and 161 days for dogs who survived to discharge. Four died during initial hospitalization (median survival 6 days). All isolates had low MECs to echinocandins with variable MICs to amphotericin B and azole antifungals.

Rasamsonia infection in dogs results in multisystemic disease involving the vertebral column, central nervous system, kidneys, spleen, lymph nodes, lungs and heart. The infection shares clinical features with other systemic mold infections and can be misidentified when using phenotypical microbiologic methods. Molecular techniques are required to identify the organism and guide appropriate antifungal therapy.

ID03

Computed tomographic findings of dogs and cats with cryptococcal mycotic rhinitis

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Cryptococcosis is the most common cause of fungal rhinitis in cats and occurs in dogs. Limited information is available regarding the computed tomographic (CT) appearance of nasal cryptococcosis in dogs and cats. The objective of this retrospective study was to describe the CT features of nasal cryptococcosis in cats and dogs.

Medical records and CT images of 10 cats and 12 dogs diagnosed with cryptococcal mycotic rhinitis between 2010 and 2020 were evaluated retrospectively. Cases were diagnosed based on identification of *Cryptococcus* on cytology or histopathology; in most cases, a *Cryptococcus* serum antigen test was performed and was positive (dogs, n=8; cats, n=9). Computed tomographic images were obtained using a helical CT scanner with contrast and evaluated by a board-certified radiologist (SC).

In this subset of cats, the most common features included the presence of a discrete nasopharyngeal (NP) mass (10/10), middle ear soft tissue attenuation (6/10), and frontal sinus soft tissue attenuation (6/10). The NP masses were located caudal near the tympanic bullae

in 8 cats (in 2 cats the mass extended cranially in the NP) and cranial near the choanae in 2 cats. In addition to a NP mass, 2 cats had a mass in the caudal nasal cavity. No cats had lysis of the cribriform plate, maxillary bone, or hard palate; 1 cat had severe lysis of the nasal turbinates and orbit. In this subset of dogs, the most common features were frontal sinus soft tissue attenuation (9/12) and a nasal mass (8/12). The most common mass location was in the caudal nasal passage with extension into the NP (6/8). Lysis of the nasal turbinates (11/12); lysis of the orbit, maxilla, or hard palate (10/12); and lysis of the cribriform plate with meningeal enhancement (9/12) was common in dogs. Mass extension into the intracranial cavity was found in 4 dogs.

In conclusion, a nasal mass is a common feature in dogs and cats with nasal cryptococcosis, especially a discrete mass in the NP of cats. Nasal cryptococcosis in dogs is more destructive in comparison to cats and lysis of the bones of the nasal cavity with intracranial extension is common in dogs. Biopsy of nasal masses in dogs and cats is encouraged to exclude cryptococcosis.

ID04

Susceptibility of imipenem-resistant Pseudomonas aeruginosa isolated from canine otitis

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Multidrug resistant *Pseudomonas* can cause otitis in dogs that is non-responsive to empirical therapy. This study determined susceptibility profiles of canine otitis *Pseudomonas aeruginosa* isolates known to be imipenem-resistant, to enhance clinical success and antimicrobial stewardship.

Imipenem-resistant *Pseudomonas aeruginosa* isolates (N=16) originating from canine ear swabs submitted to the Kansas State Veterinary Diagnostic Laboratory from 2018-2020 were reviewed. Imipenem resistance was defined as minimum inhibitory concentration (MIC) ≥ 8μg/mL in accordance with Clinical and Laboratory Standards Institute (CLSI) standards. Percent of isolates susceptible to systemic antimicrobial administration was determined. Canine *Pseudomonas* CLSI breakpoints were used when available (ceftazidime, gentamicin, piperacillin-tazobactam, and amikacin). Feline *Pseudomonas* breakpoints (enrofloxacin), human *Pseudomonas* breakpoints (imipenem), and canine non-*Pseudomonas* breakpoints (marbofloxacin, orbifloxacin, and pradofloxacin) were also used.

Susceptibility was highest for ceftazidime (94%, Susceptibility ≤ 8μg/mL), gentamicin (88%, S ≤ 2μg/mL), piperacillin-tazobactam (88%, S ≤ 8/4μg/mL) and amikacin (81%, S ≤ 4μg/mL). Isolates were less susceptible to fluoroquinolones: marbofloxacin (31%, S ≤ 1μg/mL), enrofloxacin (19%, S ≤ 0.5μg/mL), pradofloxacin (19%, S ≤ 0.25μg/mL), and orbifloxacin (13%, S ≤ 1μg/mL).

Aminoglycosides retained high *in vitro* susceptibility in this sample of imipenem-resistant *Pseudomonas* isolates despite being present in many topical otic therapies. It is important to recognize *in vitro* data might not correlate well with clinical outcomes. As exudate interferes with aminoglycoside efficacy, proper cleaning of ears prior to topical treatments should continue to be emphasized. When empirical therapy fails, susceptibility testing and thorough otic cleaning are essential to optimize treatment success.

ID05

Clinical accuracy of cryptococcal antigen lateral flow assay for diagnosis of canine and feline cryptococcosis

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Fungal infections with *Cryptococcus* spp. are commonly diagnosed by serological detection of circulating antigen. Available assays are the latex cryptococcal antigen agglutination test (LCAT) offered by commercial laboratories and the cryptococcal antigen lateral flow assay (CLFA) as a point of care test. Two recent veterinary studies revealed an excellent sensitivity but variable specificity of the CLFA when compared to LCAT. The accuracy of the CLFA for diagnosing canine and feline cryptococcosis in a clinical setting is unknown.

The aim of the study was to investigate clinical performance of a CLFA for dogs and cats presented to a referral hospital. It was hypothesized that clinical data demonstrate suitability of the CLFA as a screening test to exclude cryptococcosis but would not allow reliable confirmation of the disease.

A hospital patient database was searched for dogs and cats that had a CLFA (IMMY, Immuno-Mycologics Inc, Oklahoma) performed between August 2012 and December 2020. Eligible animals were divided into cryptococcosis positive (Cr+) and negative (Cr-) based on their clinical records. All Cr+ patients had confirmation of diagnosis by cytology, histopathology and/or culture. The Cr- patients had a follow-up of at least 3 months with stable, improved or resolved clinical signs and no treatment with antifungal medication.

A total of 170 patients were included. Nine patients (4 dogs, 5 cats) were assigned to the Cr+ group and all had a positive CLFA result. The remaining 161 patients (120 dogs, 41 cats) were classified as Cr- with a median follow-up time of 15 months (range 3-101) for dogs and 14 months (range 3-55) for cats. Of 120 Cr- dogs, 110 had a negative CLFA result and 10 a positive CLFA result (median follow-up 15 months, range 3-31). Two positive CLFA results were recorded for the cats in the Cr- group (follow-up 28 months each). Disease prevalence in this study was 3.3% and 11.1% for dogs and cats respectively. Sensitivity and negative predictive value of the CLFA were 100% for both dogs and cats. Specificities were 91.7% (CI 85.2-95.3%) for dogs and 95.1% (CI 83.5-99.4%) for cats with corresponding positive predictive values of 29% (CI 18.5-42.6%) and 71.9% (CI 39.9-90.8%).

The clinical performance of the CLFA reflects the previously established laboratory data and enables the clinician to reliably exclude cryptococcal infections in dogs and cats. However, a positive result requires further confirmation of the presence of the disease in both dogs and cats.

ID06

Effect of a novel liposome-TLR immune therapeutic on ocular FHV-1 infections in experimentally inoculated cats

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Feline herpesvirus 1 (FHV-1) is the most common cause of feline conjunctivitis in most studies. Clinical signs can be recurrent in FHV-1 infected cats and neither vaccination nor prior infection confers complete immunity. A new immunotherapeutic (liposome-TLR agonist complexes; LTC) was shown to lessen clinical signs of FHV-1 infection when administered by the intranasal and oropharyngeal routes prior to FHV-1 challenge. The LTC was shown to be a potent stimulant of toll like receptor 3 (TLR3) and TLR9, inducing production of type I (IFN-α, IFN-β) and type II (IFN-γ) interferons by feline peripheral blood mononuclear cells in vitro. In unpublished data, our group evaluated an ophthalmic gel preparation containing this LTC (LTC-O) and showed it to be safe for use as an eye drop in cats and dogs. The objectives of this study were to determine the effect of the LTC-O on clinical signs and FHV-1 shedding in a FHV-1 experimental model.

Young adult purpose bred research cats (n = 18) were evaluated in this IACUC approved protocol. All cats were clinically normal, had been exposed to the same strain of FHV-1 in a previous study, and were shown to have FHV-1 serum neutralization titers that varied from 1:16 to 1:64 at the start of the current study. The cats were randomized into 2 groups of 9 cats; one group was to be administered 1 drop of the LTC-O twice daily and the alternate group was to be administered 1 drop twice daily of a liquid tear replacement (GenTeal Tears; Alcon). All cats were administered the same dose of the same strain of FHV-1 into the conjunctival fornix of both eyes to attempt to induce conjunctivitis. A standardized scoring system was applied to each cat once daily in the morning by 2 trained individuals masked to the treatment groups. Treatment was initiated on the day one or both eyes had a combined ocular score of > 2. Cats that had not achieved a score of > 2 by Day 5 were treated with their randomized product. All cats were treated for 10 days. Samples were collected from the conjunctival fornix of both eyes for quantitative FHV-1 PCR assay twice weekly for 21 days after FHV-1 infection.

One cat was intractable and was removed on Day 5 of the study. The proportion of days that each cat had FHV-1 associated illness in the 10 days following initiation of treatment was shown to be significantly (P = 0.017) lower for the LTC-O group (16.3%) than the control group (33.8%). None of the cats were positive for FHV-1 DNA by PCR on samples collected prior to challenge. After challenge, both eyes of all cats were positive for FHV-1 DNA at least once. The proportion of FHV-1 positive ocular samples from the 6 PCR sample dates following initiation of treatment was shown to be significantly (P = 0.016) lower for the LTC-O group (32%) than the control group (51.2%). FHV-1 shedding was not detected in any of the ocular samples on the last sample collection day. The cats were adopted or returned to the colony at the end of the study.

The results suggest that the LTC-O has clinically relevant activity against FHV-1 when applied using the protocol described. Further studies in cats with naturally occurring conjunctivitis are indicated.

ID07

Current trends on antileishmanial treatment: A questionnaire-based survey among general practitioners from portugal

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Canine leishmaniosis (CanL) is an endemic disease in several countries, being emergent in North America. Its zoonotic potential highlights the need to control the disease in dogs, which are important reservoir hosts. CanL can be appropriately staged following current international guidelines such as those from the LeishVet group (available on http://www.leishvet.org). According to these guidelines, infected dogs can be classified into four stages – from I (mild disease) to IV (very severe disease) – bearing in mind the clinical presentation, laboratory findings and serologic titers. On stage II, substages A and B are based on the absence or presence of proteinuria, respectively. This clinical staging affects the medical approach and concurrent prognosis. In subclinical infection and mild disease (stage I), clinical approach does not necessarily rely on medical treatment and is only based on close monitoring and preventive measures, while in stages II to IV, antileishmanial treatment is essential for the therapeutic success. In these later cases, the association of allopurinol + meglumine antimoniate (MA) or allopurinol + miltefosine are the first-choice protocols. Despite these guidelines, only a few studies have assessed which are the most common antileishmanial treatment protocols in endemic regions. This survey aimed to assess the preferred antileishmanial treatment trends among general practitioners working in an endemic country (Portugal), in face of different stages of infection and disease.

A questionnaire-based cross-sectional study was conducted online, using an electronic platform, including 24 to 64 questions, depending on the answering pathway of each respondent. Part of the items surveyed the preferred antileishmanial protocols, considering different clinical scenarios such as subclinical infection and clinical leishmaniosis in LeishVet stages II, III and IV. After internal validation, it was uploaded and diffused online, for 2 months, via Portuguese social network veterinary groups.

Eighty-six answers were obtained from 15 Portuguese geographic regions. Concerning subclinical infection/stage I, 51.2% (44/86) of the respondents do not prescribe medical treatment, but 98.8% (85/86) would not only monitor these patients (including repeating serology within 3-6 months) but also apply appropriate preventive measures. Among those who treat it, domperidone is the preferred choice in

47.6% (20/42), followed by single therapy with allopurinol (23.8%; 10/42), allopurinol + domperidone (19.0%; 8/42), allopurinol + miltefosine (7.1%; 3/42) and allopurinol + MA (2.4%; 1/42). Stage II (including substages A and B) was mostly treated with the association of allopurinol + MA (69.8% [60/86] and 73.3% [63/86], respectively), followed by allopurinol + miltefosine (20.9% [18/86] and 19.8% [17/86]). For stage III, the preferred choice was also the association of allopurinol + MA (51.2%; 44/86), followed by allopurinol + miltefosine (38.4%; 33/86). In contrast with stages II and III, the respondents prefer treating stage IV with allopurinol + miltefosine (48.8%; 42/86) rather than allopurinol + MA (23.3%; 20/86). Non-evidence-based protocols were evoked by a minor percentage of respondents in stages II to IV (stage IIA: 9.3%[8/86]; IIB: 7.0% [6/86]; III: 10.5%; [9/86]; and IV: 22.1% [19/86]).

These results emphasise that most respondents follow the international guidelines for antileishmanial treatment, electing allopurinol + MA or allopurinol + miltefosine as first-choice protocols. While allopurinol + MA is the preferred antileishmanial treatment for stage II and III, the use of allopurinol + miltefosine is the first choice for stage IV. This latter association increases in line with disease severity, possibly due to the unpredictable effect of MA on renal function. This study contributes to a better understanding of the current trends and practical approach of CanL cases among general practitioners from Portugal.

ID08

New information on skin fold dermatitis in dogs under primary veterinary care the UK

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Skin fold dermatitis (SFD) is a surface dermatitis that can cause severe welfare impacts from pruritus and pain. Brachycephalic breeds are commonly considered as predisposed but there is little published information on veterinary clinical care on the wider dog population. This presentation will describe the prevalence, risk factors and clinical management of SFD in dogs under primary veterinary care in the UK.

The study used a cohort design for anonymised clinical records from dogs within the VetCompass Programme in the UK. Candidate SFD cases were identified by searching the clinical free-text records using search terms. Risk factor analysis used random effects multivariable binary logistic regression modelling.

From 905,553 dogs under veterinary care in 2016 at 887 veterinary clinics, the one-year period prevalence for SFD was 0.37% (95% CI: 0.36-0.38). The most frequently recorded locations for SFD were lips (38.58%), facial (23.06%), vulva (14.87%), nasal (9.59%) and tail (6.03%). The most commonly recorded clinical signs were erythema (34.36%), inflammation (24.18%), moistness (20.55%), malodour (18.55%) and pain (18.00%).

Laboratory tests were not used to support SFD diagnosis in 95.79% cases. The most common medical treatments used to treat SFD were systemic antibiosis (42.30%), antibacterial +/- antifungal shampoo/cleanser (39.22%), antibacterial +/- antifungal +/- glucocorticoid topical creams/gels (30.39%), antibacterial +/- antifungal wipes (15.50%) and systemic glucocorticoids (13.55%). A surgical approach was undertaken 1.54% of cases. Only 0.21% of SFD cases were referred for advanced clinical management.

Compared with crossbreds, breeds with highest odds included British Bulldog (odds ratio [OR] 49.07, 95% CI 37.79-63.7), French Bulldog (OR 25.92, 95% CI 19.62-34.26) and Pug (OR 16.27, 95% CI 12.20-21.69). Compared with crossbred dogs, purebred dogs had 2.54 times increased odds (95% CI 2.06-3.13). Breeds with brachycephalic skull conformation had 4.51 times the odds (95% CI 3.90-5.22) compared with breeds with mesocephalic conformation. Spaniel types had 2.11 times the odds (95% CI 1.79-2.48) compared with non-spaniel types. Older animals had increased odds of SFD.

These results confirm high breed predisposition for brachycephalic types in general and for some individual brachycephalic breeds in particular. These suggest that conformational reforms may need to be considered for some current breeds to reduce negative welfare impacts. The results also provide an evidence base for current clinical management in first opinion practice that can support measures to improve future management protocols.

ID09

Outcome and prognostic factors in canine infective endocarditis: 113 cases (2005-2020)

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Factors associated with outcome in dogs diagnosed with infective endocarditis (IE) are not well characterized. The objective of this study was to evaluate outcome and prognostic factors in dogs with IE.

Medical records were reviewed retrospectively to identify dogs that fulfilled modified Duke's criteria for a diagnosis of IE. A total of 113 dogs were discovered and categorized as survivors (n=47), non-survivors (n=57), or lost to follow up (n=9). Signalment, pre-existing conditions, clinicopathologic findings, treatment regimens, and outcomes were recorded. Univariate logistic regression was performed to identify categorical factors associated with mortality.

Survival to discharge and at 1 month was documented in 79/113 (70%) and 56/104 (54%) dogs, respectively, with median survival time (MST) of 72 days. Risk factors associated with mortality included development of congestive heart failure (OR 13.9; 95% CI 2.6 – 257.9), thromboembolic events (OR 5.9; 95% CI 2.5 – 15.1), proteinuria (OR 2.9; 95% CI 1.1 -8.3), and acute kidney injury (OR 6.4; 95% CI 2.3 – 20.3). Administration of antithrombotic medications was associated with survival (OR 0.25; 95% CI 0.087 – 0.64). Dogs that were not administered antithrombotics had a MST of 92 days, while dogs administered antithrombotics did not reach a MST during the

study period. The heart valves involved and etiologic agent identified did not correlate with outcome.

In this study, dogs that had thromboembolic events, acute kidney injury, or congestive heart failure had a higher risk of mortality. Administration of antithrombotics was associated with prolonged survival time.

ID10

Prevalence of dirofilaria immitis and ehrlichia canis infection in dogs of San Andres Island, Colombia

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Canine vector-borne diseases (CVBD) are a threat to dog populations worldwide, some CVBD pathogens have zoonotic potential, including pathogens like *Dirofilaria immitis and Ehrlichia spp*. *D. immitis* is a nematode that affects domestic and wild canines and felines around the world. These nematodes frequently infect canines and represent a zoonotic risk, since they can infect man, producing pulmonary and subcutaneous dirofilariasis. *Ehrlichia canis* infects many dogs in Colombia, South America and is the most common CVBD pathogen diagnosed in practice with reported prevalence between 30% to 80%. The Island of San Andrés in Colombia, is considered a biosphere reserve according to UNESCO, a unique ecosystem where human and animal communities interact in a 26 km^2 island. The Isla de San Andrés is an important destination for international tourists in which local vulnerable communities and free roaming dogs interact under conditions that can favor some CVBD infection. There is no prevalence information of *Ehrlichia canis* and *Dirofilaria immitis* in dogs of insular regions of Colombia like Isla de San Andres. The objective of the presented study was to estimate the seroprevalence of *Ehrlichia canis* and *Dirofilaria immitis of* infection in dogs from the island of San Andrés, Colombia. Serum samples were collected during vaccination campaigns of Secretaria de Salud Departamental of San Andrés, and were obtained by routine venipuncture and data such as seropositivity, age, sex, breed, and physical examination findings characteristic of CVBD infection were collected. The samples were centrifuged to obtain serum in the laboratory of Secretaria de Salud Departamental of San Andrés and immediately processed for commercial immunochromatography (Uranotest Dirofilaria ®, Uranovet) to detect *Dirofilaria immitis* antigens according to manufacturer instructions. In addition, larvae of mosquitoes associated with transmission of *D. immitis* were collected accordingly to the entomology program of the Government of the Department of San Andrés, Providencia and Santa Catalina. The sites with stagnant waters of the proximities of dogs included were evaluated, and larvae were collected using meshes and submerged in alcohol. Later, the larvae were sent to laboratories of secretaria de salud de Barranquilla in mainland Colombia for taxonomic evaluation. Remaining serum samples were then sent on dry ice to the molecular biology laboratory of the University Corporation of Santa Rosa de Cabal UNISARC for further analysis. 6 samples were discarded and only 44 were for commercial chromatographic immunoassay kit (Anigen E. canis, Bionote) for *Ehrlichia canis* antibody detection following the manufacturer's instructions. Proportons of seropositivity were tabulated and the association of the binomial proportions of seropositivity with age, sex and D. immitis vector proximity (positive larva colection or negative) were analyzed using the chi-square test with the JASP Team software (2019). JASP (Version 0.11.0). The variables were taken as significant when the value of $p = < 0.05$. A total of 25 males and 25 females were sampled, 22 dogs were considered free roaming dogs. 35 (70%) samples were positive for *Dirofilaria immitis*, the positive male canines were 17 (34%) and 18 (36%) the positive females. The estimated prevalence of *D. Immitis* in canines on San Andres isla was 70% and a statistically significant association was found between the presence of larvae and the seroprevalence of *D. immitis* (X^2 12.963 $p = < .001$). The most abundant *D. immitis* vector was *Aedes aegypti in* 95% of samples, others included *Culex spp* and *Anopheles spp*. Of the 44 remaining samples analyzed, 30 (68,2%) were positive to *Ehrlichia canis* antibodies and 31.8% (14/44) were negative for *Ehrlichia canis*. This study is the first report of insular prevalence of the *Dirofilaria immitis* and *Ehrlichia canis* infection of Colombia wich was very high compared with mainland regions of Colombia. *D. immitis* antigen positivity is associated with *Aedes aegypti* occurrence in the island, which was very common in urban areas. This study is the first prevalence report of *Dirofilaria immitis*, a zoonotic parasite of importance for public health in the Colombian Caribbean. San Andres Archipelago is an ecoregion with presence of CVBD pathogens with zoonotic potential.

ID11

Urinary tract infections in dogs and cats: How can we optimize empirical antibiotic therapy?

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Urinary tract infections (UTIs) in cats and dogs are a common reason for empirical use of antibiotics in veterinary practice. Both international and local Australian guidelines recommend the use of amoxicillin or trimethoprim-sulfonamide (TMS) for 3 to 5 days for sporadic UTI, however the utility of these antibiotics in urinary pathogens in Australian cats and dogs has not previously been established.

We analysed the bacterial genera and antimicrobial susceptibility results from 5614 culture-positive cat and dog urine samples submitted to a single veterinary laboratory between 2015 and 2019, mostly from first opinion veterinary clinics (78% of samples). *Escherichia coli* was by far the most prevalent genus, found in 67% of dog and 55% of cat samples. *Enterococcus faecalis*, *Staphylococcus pseudintermedius*, *Proteus* spp, *Enterobacter* spp., coagulase-negative staphylococci, and *Streptococcus canis* were also found with significant frequency. Overall, there was relatively little acquired antimicrobial resistance in the urinary isolates in this large sample, with the notable exception of high fluoroquinolone resistance in *Enterococcus faecalis* and *Streptococcus*

Table 1: Antimicrobial susceptibility and impact factors for the most prevalent urinary isolates from dogs

			Low importance antimicrobials					Medium importance antimicrobials			High importance antimicrobials	
Organism	n	% of dog isolates	Amox (AMX)	TMS (SXT)	Tetra (TET)	Doxy (DOX)	Erythro (ERY)	Gent (GEN)	Amox-clav (AMC)	Ceph (LEX)	Cefo (CVN)	Enro (ENR)
E. coli	2058	55%	75%	93%	82%	98%	*IR*	93%	97%	90%	90%	95%
Proteus mirabilis	401	11%	91%	94%	IR	IR	*IR*	*100%*	99%	95%	97%	99%
Staphylococcus pseudintermedius	380	10 %	91%	93%	76 %	85%	75%	89%	98%	95%	95%	98%
Enterococcus faecalis	351	9.3%	96%	IR	54%	91%	76%	95%*	95%	IR	IR	0.9%
Proteus spp.	288	7.7%	87%	92%	*1.6%***	*0%***	*IR*	*100%*	98%	96%	94%	98%
Enterobacter spp.	157	4.2%	IR	86%	*86%*	*100%*	*IR*	*100%*	IR	IR	IR	96%
Streptococcus canis	111	2.9%	96%	96%	84%	97%	89%	IR	97%	90%	89%	4.8%
Coagulase-neg. staphylococci	89	2.4%	87%	93%	86%	*86%*	*93%*	*95%*	97%	94%	92%	98%

Amox = Amoxicillin; TMS =Trimethoprim-sulfonamide; Tetra = Tetracycline; Doxy = Doxycycline; Erythro = Erythromycin; Gent = Gentamicin; Amox-Clav = Amoxicillin-clavulanate; Ceph = cephalexin, Cefo = cefovecin, Enro = Enrofloxacin. Note: Not all isolates tested against all antimicrobials

*Enterococci tested against higher potency gentamicin disc of 120µg to detect high-level resistance

**some Proteus spp are intrinsically resistant and others are not, unable to separate species with available data

IR - = intrinsic resistance

Grey = fewer than 30 isolates of this species/group were tested against this antibiotic and/or intrinsically resistant

Green = more than 80% susceptible

Yellow = 60-80% susceptible

Pink = less than 60% susceptible

Table 2: Antimicrobial susceptibility and impact factors for the most prevalent urinary isolates from cats

			Low importance antimicrobials					Medium importance antimicrobials			High importance antimicrobials	
Organism	n	% of cat isolates	Amox (AMX)	TMS (SXT)	Tetra (TET)	Doxy (DOX)	Erythro (ERY)	Gent (GEN)	Amox-clav (AMC)	Ceph (LEX)	Cefo (CVN)	Enro (ENR)
E. coli	1236	67%	80%	94%	82%	*82%*	*IR*	100%	99%	92%	93%	97%
Enterococcus faecalis	285	15%	98%	IR	53%	87%	77%	95%*	99%	IR	IR	1.4%
Staphylococcus pseudintermedius	133	7.2%	91%	97%	*89%*	*100%*	*100%*	*100%*	100%	93%	94%	100%
Coagulase-neg. staphylococci	88	4.8%	98%	100%	100%	*100%*	*100%*	*100%*	99%	98%	98%	100%
Proteus mirabilis	39	2.1%	87%	92%	IR	IR	*IR*	*0.0%*	100%	95%	95%	97%
Enterobacter spp.	33	1.8%	IR	76%	*100%*	*100%*	*IR*	*100%*	IR	IR	IR	97%
Proteus spp.	22	1.2%	*82%*	*73%*	*0%***	*0%***	*IR*	*100%*	*96%*	*87%*	*82%*	*100%*
Streptococcus canis	14	0.8%	*100%*	*86%*	*86%*	*100%*	*100%*	IR	*100%*	*100%*	*100%*	*0%*

Amox = Amoxicillin; TMS =Trimethoprim-sulfonamide; Tetra = Tetracycline; Doxy = Doxycycline; Erythro = Erythromycin; Gent = Gentamicin; Amox-clav = Amoxic llin-clavulanate; Ceph = cephalexin, Cefo = cefovecin, Enro = Enrofloxacin. *Note: Not all isolates were tested against all antimicrobials*

*Enterococci tested against higher potency gentamicin disc of 120µg to detect high-level resistance

**some Proteus spp are intrinsically resistant and others are not, unable to separate species with available data

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canis. The prevalence of multi-drug resistance was highest in *Escherichia coli* (9.7%) due primarily to resistance to multiple (non-potentiated) beta-lactam antibiotics, however 84% of these MDR isolates remained susceptible to amoxicillin-clavulanate. Using the formula for rational antimicrobial therapy, the most effective empirical antibiotic choice is the medium-importance antibiotic amoxicillin-clavulanate. However, when isolates were divided into two groups by bacterial morphology, low-importance amoxicillin was almost as effective as amoxicillin-clavulanate against cocci, and low-importance TMS was almost as effective against bacilli, highlighting the usefulness of in-clinic urinary microscopy in selecting empirical therapy. We also performed a novel population analysis to incorporate antimicrobial importance ratings with susceptibility results, which suggest that where morphology is unknown, TMS is the best first-line empirical choice and amoxicillin the best second-line choice.

Our results support current international and Australian guideline recommendations of TMS or amoxicillin for empirical treatment of sporadic UTIs in dogs and cats. We also show that in-clinic urinary microscopy can greatly improve antibiotic selection, with amoxicillin a superior choice for UTIs caused by cocci and TMS a superior choice for bacilli.

ID12

Hand hygiene compliance and antimicrobial-resistant microorganisms on the hands of veterinary healthcare worker

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Hand hygiene (HH) is one of the most important measures to prevent nosocomial infection. Data on HH compliance in companion animal clinics in Europe is scarce and no studies have assessed hand contamination of companion animal healthcare workers with antimicrobial-resistant microorganisms (ARM) in relation to HH procedures.

The study assessed HH compliance according to the WHO five moments of HH in five different clinical areas in a Swiss companion animal clinic, compared the use of an online application and the WHO evaluation form, and evaluated the use of gloves. Hand contamination with selected ARM (extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae, vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* (MRSA) and *pseudintermedius*) and total viable count (TVC) was investigated before and after patient contact.

A total of 202 hand swabs from 87 staff members and 1165 HH observations were analyzed. HH compliance was low (36.6%, 95% CI 33.8–39.5) and was similar when assessed with the two observation methods. HH compliance differed significantly between hospital areas ($p = 0.0035$) and indications ($p < 0.0001$) but not between professional groups. Gloves were worn in 22.0% (95% CI 18.0–26.6) of HH observations and of those 37.2% (95% CI 27.3–48.3) were indicated according to WHO recommendations. Three hand swabs (1.5%, 95% CI 0.4–4.3) were contaminated with MRSA. Mean TVC before patient contact was lower (0.52 log CFU/cm^2) than after patient contact (1.02 log CFU/cm^2), but there was no difference in TVC regarding the use of gloves before patient contact.

The study highlights the need to foster hand hygiene compliance in companion animal medicine to combat the spread of ARM in veterinary clinics.

ID13

Effect of discussion of antimicrobial guidelines on prescribing habits in a small animal first-opinion practice

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Antimicrobial stewardship is a cornerstone of efforts to combat antimicrobial resistance. This study aimed, for the first time, to evaluate the impact of a formal discussion of antimicrobial stewardship for dogs and cats ('the intervention') on systemic antimicrobial prescribing behaviors among small animal veterinary surgeons.

Electronic health records including information about the prescription of systemically administered antimicrobials were collected from a multi-site, UK veterinary practice before and after the intervention between April 2017 and March 2020. We undertook interrupted time series analysis using a quasi-Poisson model to compare the pre-intervention and post-intervention change in level and slope for each discrete-valued outcome.

After the intervention, there was an immediate marked reduction in the antimicrobial prescription rate of cefovecin to cats (-22.3%; 95% CI: -36.0%, -5.6%) and of metronidazole to dogs (-41.6%; 95% CI: -53.6%, -26.4%); however, compensatory increases in the prescribing of co-amoxiclav were noted. In cats, the prescribing rate increased by 18.7% (95% CI: 0.9%, 39.8%) immediately after the intervention, with a transient reduction in the likelihood of underdosing (P=0.03). For dogs, there was an immediate reduction in the prescribing rate of off-license antimicrobials (P=0.002) and in underdosing (P=0.049). There was a transient increase in the likelihood a dog would have their weight recorded at the time of prescription (P=0.01).

This study suggests that discussion of antimicrobial stewardship is more likely to influence the antimicrobial choice than whether to prescribe or not. Interventions may benefit by focusing on appropriate antimicrobial selection rather than overall prescription frequency.

ID14

Prevalence of SARS-Cov-2 in domestic cats presenting to a teaching hospital during the COVID-19 pandemic

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As the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic ensues, public health concerns regarding

transmission modes are rising. Insight into potential zoonotic transmission of SARS-CoV-2 from companion animals to humans is essential for establishing public health recommendations during the current pandemic. In order to expedite our knowledge, it is critical to characterize the prevalence of SARS-CoV-2 (representing zooanthroponosis or reverse zoonosis) in companion animals. Similar to humans, domestic cats have the ACE2 receptor utilized by SARS-CoV-2 to enter host cells; thus, providing evidence that infection with SARS-CoV-2 is possible in domestic cats. We aimed to characterize the prevalence of SARS-CoV-2 in medically compromised, sick domestic cats presenting to a teaching hospital during the COVID-19 pandemic. Our *working hypothesis* was the prevalence of SARS-CoV-2 in medically compromised, sick domestic cats was low.

Client-owned sick cats presenting to the Ohio State University Veterinary Medical Center Emergency Department (6/1/20-9/30/20) were recruited for this study. Enrolled cats underwent a conjunctival, deep oropharyngeal, and nasal swabs during hospitalization. Viral RNA was extracted and PCR performed to determine if SARS-CoV-2 was present in each sample. The LIPS assay was performed in a subset of serum samples for detection of antibodies against SARS-CoV-2. Metadata including complete history (household COVID-19 status), chief complaint, clinical signs, diagnostics performed, final diagnosis and ultimate outcome from each enrolled cat was obtained.

Of the cats sampled (n = 83), two cats were from COVID positive households and one cat was from a suspected COVID positive household. Cats were sampled during a peak in SARS-CoV-2 within the state of Ohio, with cumulative human SARS-CoV-2 cases totaling 122,147 over the study period. Despite high human COVID cases, SARS-CoV-2 was not detected in any of the cats. Additionally, no feline serum was positive for antibodies against SARS-CoV-2.

In conclusion, SARS-CoV-2 was not detected in medically compromised, sick domestic cats presenting to a teaching hospital in Ohio during the COVID-19 pandemic. This project is part of an Ohio State University initiative known as eSCOUT (Environmental Surveillance for COVID19 in Ohio: Understanding Transmission). eSCOUT aims to conduct a large-scale surveillance of SARS-CoV-2 in the "wild" to *discover* the environmental and animal reservoirs that harbor the virus long-term, *assess* the hazard of virus entry to the human population from the environment (spillover), and *study* the virus adaptions/mutations in real-time to inform public health responses and preparedness. Through eSCOUT, active surveillance of animals, including domestic cats, and the environment is ongoing.

IM01

Antiviral immune responses and in vitro suppression of FIPV Replication by novel liposome-TLR immune therapeutic

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Effective immunotherapeutics for cats are greatly needed to aid in the management of intractable viral diseases, including infections with the feline coronavirus that causes feline infectious peritonitis (FIP). A new immunotherapeutic (liposome-toll-like-receptor (TLR) agonist complexes; LTC) which has been shown previously to elicit potent antiviral and antibacterial activity was evaluated for its ability to activate antiviral activity in feline leukocytes and suppress FIPV replication. The specific objectives of this study were to determine the ability of LTC to stimulate induction of type I (IFN - α, IFN - β) and type II (IFN - γ) interferon immune responses, as these responses are key to controlling viral infections, including coronaviruses. A second objective was to assess the ability of LTC to activate feline leukocytes to secrete factors capable of suppressing feline infectious peritonitis virus (FIPV) replication in vitro.

In vitro assays were conducted using peripheral blood mononuclear cells (PBMC) prepared from blood of healthy, young adult research cats. Cells were incubated with several dilutions of LTC. Production of IFN - α and IFN - β was assessed by RT-PCR and production of IFN - γ was assessed by both RT-PCR and ELISA. Lipopolysaccharide (LPS) was included as a positive assay control. To assess the ability of LTC to activate release of FIPV suppressive factors, an FIPV permissive feline macrophage cell line (fcwf-4) was infected with FIPV and treated with conditioned medium from activated or control feline leukocytes. The impact of treatment on FIPV replication and cytopathicity was assessed 72h after infection.

The LTC treatment induced significant expression of IFN - α ($p = 0.0441$), IFN - β ($p = 0.0060$), and IFN - γ ($p = 0.0152$) by feline leukocytes, at all 3 concentrations evaluated, compared to untreated cells (Figure 1). In addition, we observed that there was significant protection from FIPV-induced cytopathic effects when fcwf-4 cells were treated with conditioned medium from LTC-activated leukocytes (Figure 2).

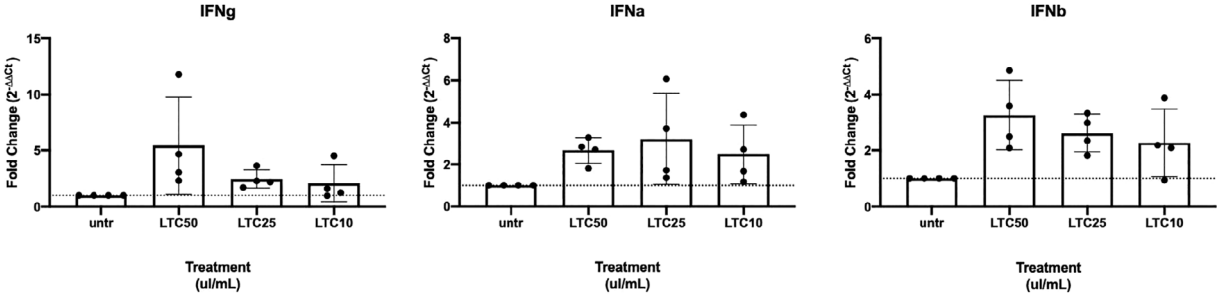

Figure 1: In vitro activation of expression of IFN - α, IFN - β, and IFN-γ measured by RT-PCR of specific mRNA after an 8-hour incubation of feline leukocytes with 3 different concentrations of LTC. LTC induced significant expression of IFN - α ($p = 0.0441$), IFN - β ($p = 0.006$), and IFN - γ ($p = 0.0152$).

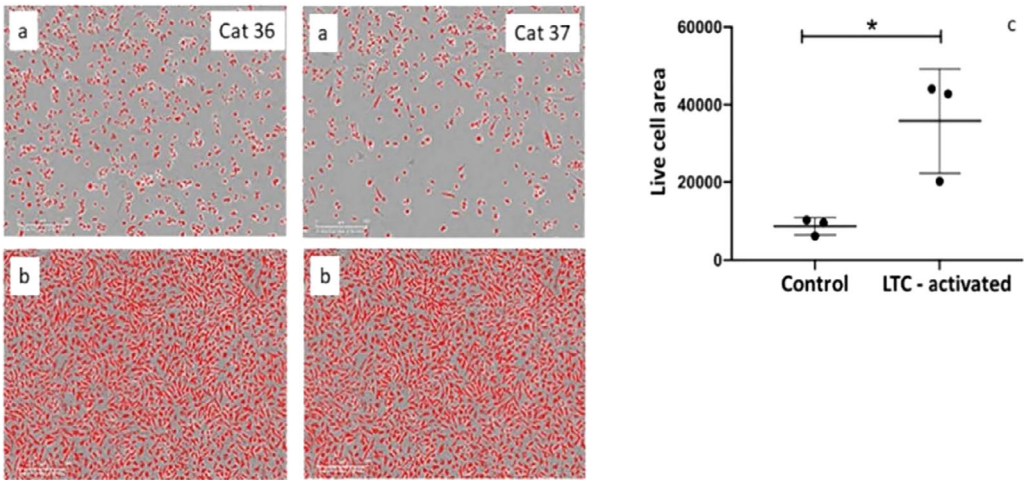

Figure 2: LTC activation of feline leukocytes induces secretion of antiviral cytokines. Untreated cells (A), or cells treated with LTC overnight (B), were inoculated with virulent FIPV to assess cytopathic effects and cell survival. (Live cells appear as red dots in these images). Cell survival data are summarized in C, illustrating significant protection from FIPV induced killing by factors from LTC activated leukocytes.

These findings are important because they indicated that the novel LTC immunotherapeutic is uniquely able to stimulate broad production of 3 key antiviral cytokines (IFN - α, IFN - β, IFN - γ). This effect was not observed with TLR2, 4 or 6 agonists (not shown), indicating that the TLR pathways activated by LTC (TLR3 and TLR9) are critical for inducing effective antiviral immunity. Importantly, these studies also demonstrate that the cytokines triggered by LTC activation are able to potently suppress FIPV replication in permissive macrophages, a key target cell for FIP pathogenesis. These studies provide the rationale for additional studies to more broadly assess the immune stimulatory properties of LTC, and to rigorously evaluate its potential effectiveness as a new treatment for FIP.

IM02

Feline leukocyte antigen class I global survey via SMRT sequencing reveals prevalent alleles and supergroups

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The domestic cat MHC contains 12 full-length Feline Leukocyte Antigen class I (FLAI) loci. Based on polymorphisms, *FLAI-E*, *-H* and *–K* are designated as classical (class Ia) and thought to restrict conventional CD8+ T cells. Four loci with limited variation, *FLAI-A*, *-J*, *-L* and *–O*, are considered non-classical (Ib), likely regulating effectors with invariant receptors. For both classes, identifying prevalent alleles can illuminate specific genotypes to be prioritized for immune studies, as shared allomorphs direct shared responses. We've developed a targeted approach to FLAI genotyping, using the Single Molecule Real-Time (SMRT) platform, which allows full-length (3.5kb) reads without assembly. In a proof-of-principle study (17 cats), 49 new FLAI sequences were found, with unambiguous locus assignment, validating this high-throughput method for large-scale genotyping. This technology was used to test our hypothesis that, while outbred, cats carry highly-shared alleles at ≥1 class Ia and Ib loci. FLAI amplicons from peripheral blood gDNA from 184 additional cats from diverse geographical regions were sequenced in 4 cohorts. Long amplicon analysis of contigs yielded many new protein-coding alleles at *FLAI-E* (n=45), *-H* (n=18), *-K* (n=29), *-J* (n=21), and *–L* (n=8). Increasing allele redundancy in later cohorts implied adequate recovery of all major variants. Prevalent alleles (19-31% frequency) were found at all loci, as well as class Ia supergroups and globally-dispersed haplotypes. These discoveries should facilitate high-resolution studies of feline T-cell responses to viral infections and cancer. The large allele library will assist allotransplantation, MHC-disease association studies, and furnish insights into origins of the domestic cat.

IM03

Cytokine dysregulation and effect of corticosteroids during treatment in cats with acute cytauxzoonosis

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Cytauxzoonosis is a tick-borne infectious disease of domestic cats with high mortality and narrow therapeutic window. Recent studies have indicated that increased mortality and severity of clinical signs are correlated with overt immune activation during acute infection, yet immunopathologic features have not been fully evaluated. Thus, the objective of this study was to characterize immunologic parameters during acute cytauxzoonosis and evaluate the effect of short-term corticosteroids in treating cats with *Cytauxzoon felis* infection. Domestic cats (n=28) from 9 veterinary clinics in 3 states (Arkansas, Oklahoma, and Missouri) were included. Acute cytauxzoonosis was

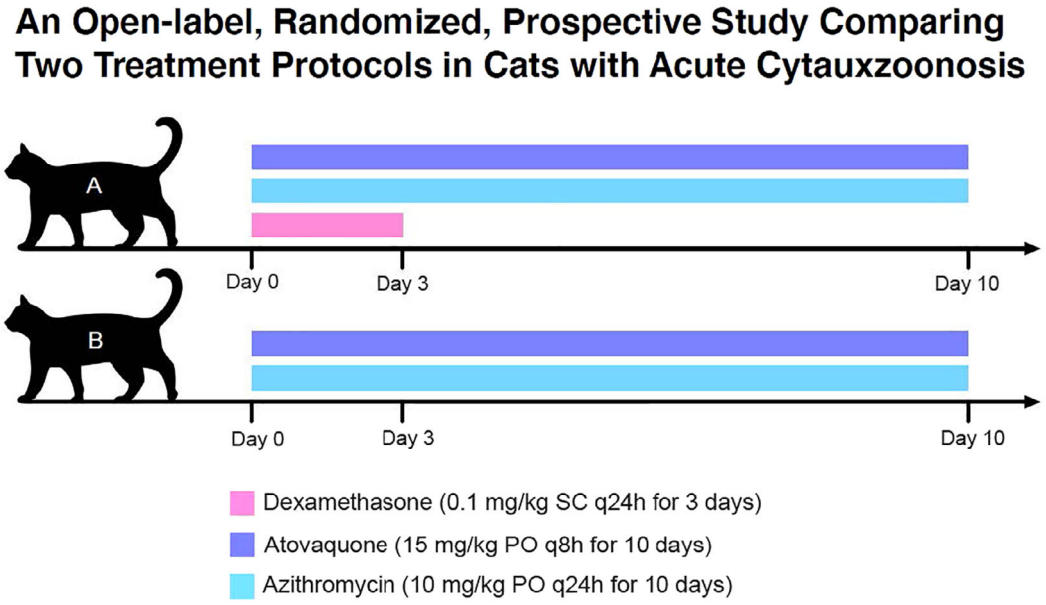

Figure. 1 Cats diagnosed with acute cytauxzoonosis were randomly assigned to one of the following treatment groups. Group A received atovaquone, azithromycin and dexamethasone and group B received atovaquone and azithromycin. Blood samples were collected at day 0, day 3 and day 10 after initiation of treatment. All cats received fluid therapy and supportive care.

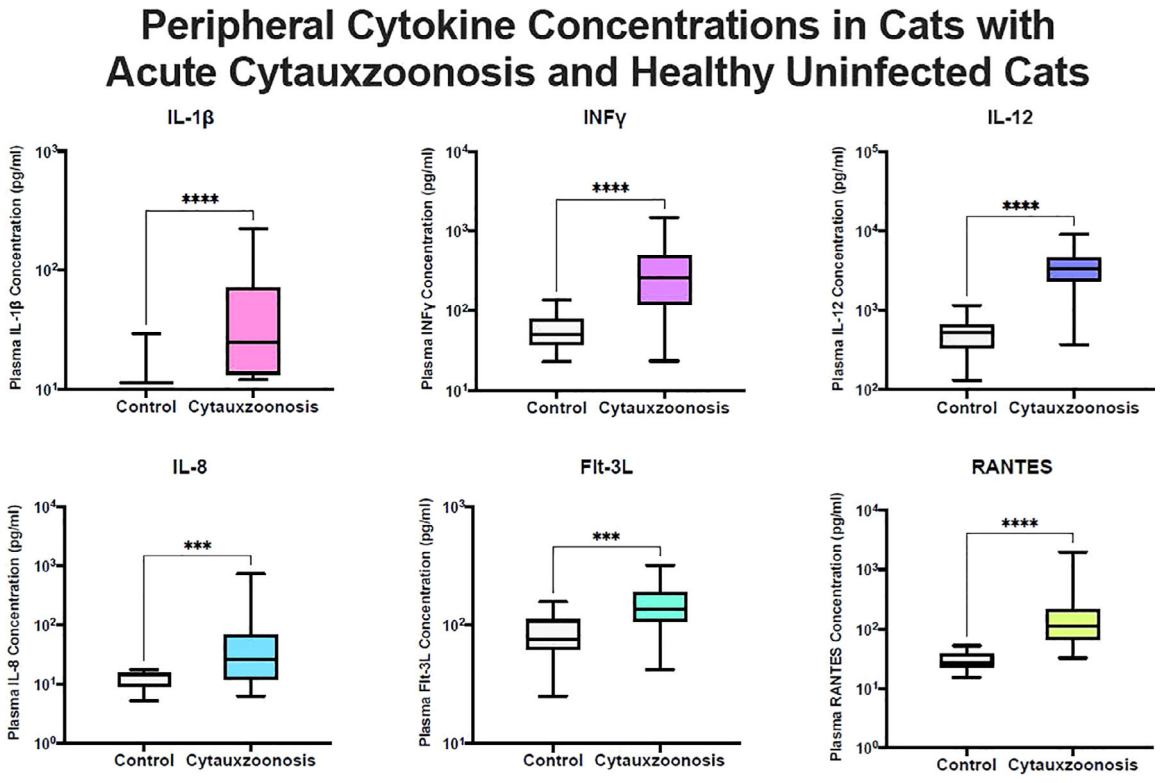

Figure. 2 Elevation of pro-inflammatory cytokines including IL-1β, INFγ, IL-12, IL-8, Flt-3L and RANTES were noted in plasma samples from cats with acute cytauxzoonosis as compared to 18 healthy uninfected cats.

confirmed by PCR and an open-label, randomized, prospective clinical trial was conducted comparing immunologic parameters and clinical outcome in cats treated with standard anti-protozoal therapy for 10 days ± dexamethasone (0.1 mg/kg SC q24h for 3 days). Blood samples were collected at day 0, and at days 3 and 10 after treatment. Peripheral concentrations were quantified by multiplex immunoassay and compared to healthy uninfected cats (Figure. 1). Increased concentrations of pro-inflammatory cytokines (IFNγ, IL-1β, IL-8, IL-12, RANTES and Flt-3L) (Figure. 2) and decreased concentrations of PDGF-BB, SDF-1, MCP-1, and IL-18 were detected in cats with acute cytauxzoonosis compared to healthy cats ($p < 0.05$). In cats that received antiprotozoal therapy, IL-12 and Flt-3L concentrations decreased and PDGF-BB increased over time ($p < 0.05$), while addition of dexamethasone showed significant effect in suppressing PDGF-BB elevation over time ($p=0.0031$). Although the addition of dexamethasone to anti-protozoal therapy did not alter survival ($p=0.7589$) in this cohort, studies are ongoing to determine the efficacy of immunomodulatory therapy in a larger study set.

Collectively, these results provide new insights into pathogenesis and reveal potential immunotherapeutic targets to treat feline cytauxzoonosis.

IM04

Differential modulation of innate immune response by primary and secondary bile acids in dogs

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Canine chronic inflammatory enteropathy is associated with disruption of the microbiome and the metabolome, but how these changes may influence local innate immune responses has not be well described. Our aim was to investigate and compare the immune modulatory properties of primary and secondary bile acids on macrophage cytokine response.

A canine macrophage cell line (MH588) was used to model innate immune responses and their modulation by bile acids. Cells were incubated with primary (cholic acid [CA], chenodeoxycholic acid [CDCA]) or secondary (lithocholic acid [LCA]) bile acids for 2 hours prior to activation with lipopolysaccharide (LPS). After 48 hours of bile acid and LPS co-culture, supernatants were harvested and cytokine concentrations were measured via canine-specific ELISA. Differences between treatments were assessed with one-way ANOVA with *P* values adjusted for multiple comparisons. An asterisk (*) indicates $P < .05$. Expression of TGR5 (bile acid receptor) was assessed by immunocytochemistry (ICC) and flow cytometry.

Incubation with bile acids alone did not induce measurable tumor necrosis factor-a (TNF-a) or interleukin-10 (IL-10) production by MH588 cells. Compared to LPS alone (mean pg/mL±SD: 720.0± 20.1), incubation with LCA at 5 (342.0±59.4*) and 50 μM (261.9±29.3*), and CDCA at 5 (645.9±16.7*) and 50 μM (427.4±33.2*) was associated with lower TNF-a concentrations. Conversely, IL-10 concentrations were higher when cells were preincubated with LCA at 5 (176.6 ±50.7*) and 50 μM (175.8±63.9*) versus LPS alone (62.8±30.3*). Neither CA nor CDCA exposure influenced IL-10 concentrations. ICC confirmed TGR5 expression in MH588 cells.

These results indicate that primary and secondary bile acids interact differently with canine innate immune cells, modulating immune responses in divergent directions. This information is important because it provides a mechanism linking the microbiome, bile acid metabolism, and regulation of inflammatory responses within the gut.

NM01

Effect of *Bifidobacterium longum* 999 supplementation on stress associated findings in cats with FHV-1 infection

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Bifidobacterium longum strain 999 (BL999) is a probiotic (Purina® Pro Plan® Veterinary Supplements; Calming Care) that has been shown to lessen anxiety in dogs and is known to be safe in cats. Feline herpesvirus 1 (FHV-1) is the most common infection of cats and clinical disease can be exacerbated by stress. The primary hypothesis was that cats supplemented with the BL999 containing product would have higher relaxation scores, lower stress markers, and lower FHV-1 clinical scores than cats supplemented with the same product, but without BL999 as a placebo when mild stress was induced by changing the type of housing. This 12-week study enrolled 24 cats with chronic subclinical FHV-1 infection that were randomly divided into two groups. The cats were supplemented with BL999 (group 1) or placebo (group 2) daily. After BL999 was supplemented for 42 days to achieve probable maximal effects, the cats were moved from the individual gang rooms into cages, back into gang rooms, and then back into cages to induce stress over the next 42 days while behavioral, clinical, and biochemical markers were measured. All cats ate a minimum of 75% of both supplements and there was no obvious vomiting or diarrhea. During the stress periods, the cats supplemented with BL999 were significantly less likely to have abnormal serum cortisol concentrations ($P = 0.0059$) or sneezing ($P < 0.00001$). During the times cats were housed in cages, those supplemented with BL999 were significantly more likely ($P < 0.0001$) to reach out to the scorers through the cage bars and were significantly less likely ($P < 0.0003$) to pace in the cages. The results of the study suggest that BL999 is well tolerated by cats, reduces stress, reduces stress associated problems like activated FHV-1, and increases social interactions between cats and people.

NM02

Effects of macronutrient composition on adipose deposition and body weight in healthy dogs fed westernized-diet

Iennarella-Servantez, Chelsea A.[1], Kathrani, Aarti[2], Bedos, Leila[1], Sahoo, Dipak K.[1], Long, Elizabeth K.[1], Petrovsky, Brian[1], Stokes, Rebecca[1], Zdyrski, Christopher[1], Gabriel, V[1], Resop, Mara J.[1], Rund, Lili R.[1], Mao, Sichao[1], Bourgois-Mochel, Agnes[1], Jergens, Albert E.[1], Allenspach, Karin[1]

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High-fat, high-energy Western diets (WD) in humans have been associated with a multitude of metabolic diseases including obesity, diabetes mellitus, colorectal cancer, cardiovascular disease. Similarly, energy and nutrient dense commercial diets have increased in popularity in the petfood industry. Dogs have been utilized as a physiologically relevant model for human nutrition due to similarities in gastrointestinal anatomy/physiology, relative prevalence of common metabolic diseases, and substantial overlap in intestinal microbial populations. Due to the high rates of obesity and metabolic dysfunction in both canine veterinary patients and humans alike, dogs may play a pivotal role in evaluation of diet-related effects on individual health. Further, both canine and human nutrition rely on the use Atwater factors for calculation of dietary energy but there is a paucity of data on the effects of macronutrient composition (independent of energy) on body composition and the associated implications for metabolic health. As a result, this study aims to evaluate the effects of isocaloric diets differing in macronutrient composition (predominately fiber and fat content) on adipose tissue deposition and body weight in the healthy dog model.

Healthy beagle dogs (n=10; 5/sex) were fed a (1) control diet (CON) followed by (2) a high-fat, high-energy, low-fiber Western diet (WD) for approximately 2 months each in a crossover design. Macronutrient inclusions levels were based on (A) National Academies of Science recommendations for adult humans or (B) National Health and Nutrition Examination Survey dataset parameters for CON and WD, respectively, and confirmed to be nutritionally balanced for maintenance of adult dogs according to National Research Council profiles. Diets were prepared in a research prep kitchen with identical ingredients (beef, egg white, and rice base) but differed in ingredient inclusion rates in order to meet target nutrient/energy profiles. Diets were fed isocalorically based on calculated metabolizable energy (ME) values using Atwater factors (9, 4, and 4kcal/g for fat, protein, and carbohydrate, respectively). Body weights were recorded on weekly basis throughout feeding periods and laparoscopic adipose tissue biopsies were collected under anesthesia at the conclusion of each feeding period.

In spite of isocaloric feeding based on calculated ME values, mean body weights at the conclusion of each feeding period were 8.8 kg and 9.3 kg for CON and WD diets, respectively ($P = 0.0018$) indicating a likely difference in diet digestibility. H&E stained slides of FFPE adipose tissue biopsies revealed differential deposition of fat in response to each diet. Mean adipocyte diameter of omental fat biopsies was 58.6 and 62.5μM for CON and WD, respectively ($P < 0.0001$). Conversely, this increase in adipocyte diameter was not seen in subcutaneous fat biopsies. Independent of diet, mean adipocyte diameters (60.7 and 65.65 μM) and adipocyte heterogeneity (variance: 177.0 and 302.45 μM^2); differed between omental and subcutaneous fat biopsies, respectively ($P < 0.0001$).

Collectively, these data indicate that differences in macronutrient composition (especially fat and fiber content) may result in differential effects on adipose tissue deposition and maintenance of body weight even if diets are fed isocalorically based on calculated ME. As Atwater factors are used to calculate ME content of diets in both human and canine nutrition, these results indicate that estimating intake requirements based on energy alone may not be adequate in minimizing metabolic risk to an individual. Future studies should aim to characterize these effects further in both the healthy dog model and obese canine veterinary patients in order to maximize progress in attenuating the effects of diet-related disturbances on both human and animal health.

NM03

Fortetropin safety and tolerability in cats

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Sarcopenia and cachexia are major problems for aging cats and in cats with chronic kidney disease, diabetes and obesity. The hormone myostatin plays a major role in inhibiting muscle growth. Fortetropin® (MYOSCORP, Cedar Knolls, NJ) is a proteo-lipid complex made from fertilized, chicken egg yolk. Fortetropin® (~0.3 g/kg) lowers myostatin concentrations, reduces muscle atrophy in dogs and increases muscle mass in people. Some rare episodes of diarrhea have been reported in dogs particularly those that were given Fortetropin® at doses > 3x the therapeutic dose.

The dose range of Fortetropin® in cats is currently unknown. This study investigated the safety and tolerability of Fortetropin® in cats.

Twelve purpose-bred cats were single-housed and randomly divided into 3 treatment groups (n = 4 per group), and were treated for 2 weeks with either a placebo (cheese powder, 1 g/day) or Fortetropin® at 1 or 2 g/day. The placebo group was treated for an additional 2 weeks with Fortetropin® at a 4 g/day dose.

No adverse events were observed in cats treated with 1 g/day and 2 g/day of Fortetropin®. A single episode of diarrhea was reported in one placebo treated cat at day 6, and a single episode of vomiting was reported in one Fortetropin® (4 g/day) treated cat at day 8.

Our data suggest that cats tolerate Fortetropin® at doses that are three times the therapeutic dose in other species. Further studies are needed on the efficacy of Fortetropin® in terms of the treatment of muscle atrophy in cats.

NM04

Evaluation of blood thiamine concentration in hospitalized dogs with and without critical illness

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Thiamine deficiency is associated with increased morbidity and mortality in human ICU populations. Thiamine deficiency has only been described in severe, late stages of illness in veterinary patients; however, neither baseline thiamine levels nor presence of thiamine deficiency have been established in hospitalized dogs.

The primary objective of this study was to prospectively investigate blood thiamine levels in systemically healthy and critically ill

hospitalized dogs. Additional aims were to measure change in thiamine over time of hospitalization and to identify any associations with patient morbidity and mortality.

Whole blood EDTA samples were collected from 31 hospitalized healthy dogs and 37 hospitalized dogs with non-septic (n = 24) and septic (n = 13) critical illness, with samples collected at time of admission and again at 72 hours. Blood samples were frozen and batch analyzed for thiamine concentration. Total thiamine (tB1) and thiamine diphosphate (TDP) were measured by high performance liquid chromatography (HPLC) and free thiamine (fB1) was determined using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Patient data, including CBC, biochemistry profile, lactate, shock index, $APPLE_{fast}$ score, comorbid conditions, and patient outcome were recorded.

Approximately 92% of total B1 present in whole blood is measured as TDP. TDP concentration was strongly correlated with tB1 ($R = 0.97$, $p < 0.0001$). Thiamine concentration, as represented by TDP, was not significantly different at admission or over time in healthy, non-septic, or septic dogs. Blood thiamine concentration was significantly lower in septic dogs requiring surgery both at admission ($p = 0.044$) and 72 hours later ($p = 0.008$), compared to dogs who did not have surgery. Thiamine concentration was not significantly different between dogs with and without stable underlying chronic diseases at any time point. Older dogs had lower blood thiamine concentration. Positive correlations were seen between thiamine concentration and body weight, $APPLE_{fast}$ score, and WBC count. Critically ill dogs with lower admission blood lactate concentration were more likely to have an increase in thiamine over time ($p = 0.0142$), when compared to healthy dogs.

Thiamine deficiency was not identified in hospitalized healthy or non-septic critically ill dogs. Further investigation into the clinical relevance of thiamine deficiency is indicated in septic dogs undergoing surgery.

NM05

Gut dysbiosis and its association with microbiota-derived metabolites in myxomatous mitral valve disease in dogs

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Gut microbiota-derived metabolites, including bile acids (BAs), short-chain fatty acids (SCFAs), and trimethylamine N-oxide (TMAO), are implicated in the development of cardiovascular disease. Our aim is to evaluate gut microbial dysbiosis and its relationship with these metabolites in myxomatous mitral valve disease (MMVD) in dogs. Fecal samples from 92 client-owned healthy, stages B1, B2, C or D MMVD dogs, were analyzed by 16S rRNA gene sequencing. Alpha and beta diversities were different between healthy and MMVD dogs (adjusted $p < 0.05$). The means of PCR-based dysbiosis index (DI) were -1.48, -0.6, 0.01, and 1.47 for healthy, B1, B2, and C/D dogs, respectively ($p = 0.07$), and DI was negatively correlated with *C. hiranonis* (-0.79). *E. coli*, capable of trimethylamine production in the gut, had an increased abundance (adjusted $p < 0.05$) and may be responsible for the increased circulating TMAO levels in B2 and C/D dogs. *C. hiranonis* was the key BA converter, with strong but opposite associations with primary and secondary BAs (-0.94, 0.95, respectively). Secondary BAs appeared to promote the growth of *Fusobacterium* and *Faecalibacterium*, but inhibited that of *E. coli*. Beta diversity analysis showed significant clustering based on the levels of DI and *C. hiranonis*, both of which were associated with alpha diversity. Multivariate analysis revealed significant but weak associations between gut microbiota and several circulating metabolites, including short-chain acylcarnitines and TMAO. In conclusion, our data suggest that gut microbial dysbiosis takes place at the preclinical stage before Congestive HF, and support the hypothesis of an interplay between gut microbiota, gut microbiota-derived metabolites, and MMVD pathophysiological progression.

NM06

Ghrelin sample storage and stability in healthy cats

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Ghrelin, a key hormone involved in the regulation of appetite, circulates in acylated (active; AG) and desacyl (inactive; DG) forms. AG initiates and stimulates appetite whereas DG is anorexigenic. DG is renally eliminated, accumulates in renal failure, and positively correlates with serum creatinine in humans. AG has a volatile acyl group that can be disrupted during sample collection, processing, or storage, increasing degradation to DG and potentially confounding interpretation. The purpose of this study was to compare the effect of sample processing and storage on the preservation of AG in plasma of healthy cats.

Plasma was collected from 5 healthy cats fasted overnight and processed three ways: 1) ethylenediaminetetraacetic acid (EDTA), 2) EDTA with aprotinin (EA) and 3) EDTA with aprotinin acidified with hydrochloric acid to a pH of 4.0 (EAH). AG concentrations were measured for each processing method at 1 month and 3 months using radioimmunoassay for AG. AG concentrations in EDTA and EA were proportionally normalized to that of EAH. A Friedman's test was used to compare the 3 sample storage methods at each time point with Dunn's post-hoc comparison. Results are presented as medians and ranges.

Four cats were included in data analyzed at 1 month. After 1 month AG concentrations in EDTA samples were 76.2% (range 70.4-78.9 %) of EAH sample concentrations ($p = 0.04$), and AG concentrations in EA samples were 86.4% (range 68.0-87.5 %) of EAH sample concentrations (not significantly different). At 3 months AG concentrations in EDTA samples were 66.3 % (range 62.7-80.3 %) of EAH sample concentrations ($p = 0.005$) and AG concentrations in EA samples were 75.4 % (range 63.0-82.3 %) of EAH sample concentrations (not significantly different). AG concentrations after 3 months for EDTA, EA, and EAH were 72.3% (range 62.5-81.2 %), 68.6% (range 63.1- 84.7 %), and 75.5% (range 71.2-88.2 %) less than the concentrations at 1 month, respectively.

In conclusion, storage of plasma in EDTA alone results in a significant loss of AG as compared to plasma stored with EAH demonstrating the importance of acidification combined with a protease inhibitor in stabilization of the acyl group and maintenance of AG within the sample. Subjectively, some sample loss occurred between 1 and 3 months regardless of sample storage method. Future studies of appetite regulation involving AG will benefit from effective storage methods.

NU01

Diagnostic yield of cystoscopy compared to ultrasonography for evaluating lower urinary tract disorders in dogs

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Cystoscopy is an adjunct tool for diagnosing lower urinary tract disorders (LUTD), but its clinical utility has not been compared to ultrasonography (US). The purpose of this retrospective study was to evaluate agreement between cystoscopy and US in dogs with LUTD.

Records of dogs presenting between 2014–2019 for LUTD were considered. Dogs that had cystoscopy and US, which was performed within the preceding 60 days, were enrolled. Dogs were categorized based on their primary presenting complaint: 1) urinary incontinence (UI), 2) stranguria, and 3) recurrent urinary tract infection (rUTI). Pertinent cystoscopic findings included any information besides those suggestive of inflammation.

Two-hundred and forty dogs were included. Cystoscopy provided adjunct information in 91/240 (37.9%) of cases. Agreement within LUTD categories was UI: 63/147 (42.8%; $\kappa = 0.35$); stranguria: 53/147 (36.0%; $\kappa = 0.27$); rUTI: 31/147 (21.0%; $\kappa = 0.38$). Cystoscopy identified pertinent clinical findings in 7/31 (22.6%) rUTI, 25/63 (39.7%) UI, and 29/53 (54.7%) stranguria cases. The proportion of pertinent cystoscopic findings was lower in rUTI cases compared with UI and stranguric cases combined ($P = .013$). Pertinent information identified by cystoscopy in rUTI cases included urethral masses, ectopic ureter, absence of ectopic ureter, fistula, and abnormal ureterovesicular junctions.

Agreement between these two diagnostics was moderate in all LUTD groups. Cystoscopy provided additional information as well as concurrent therapeutic interventions for dogs with UI; the absence of abnormalities in dogs with stranguria should be considered clinically relevant. The utility of cystoscopy for dogs with rUTI should be further investigated.

NU02

Prophylactic use of tetrasodium EDTA in 95 subcutaneous ureteral bypass devices in 66 cats

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The objective of this study was to compare rates of mineralization and positive urine culture results in cats following placement of a subcutaneous ureteral bypass (SUB) device for benign ureteral obstruction(s) (UO) with different flushing solutions (tetrasodium ethylenediaminetetraacetic acid [tEDTA] and sterile saline).

Medical records of 264 cats were reviewed. Cases were excluded if: (1) the SUB was not placed and managed by the authors; (2) there were fewer than 180 days of follow-up available unless mineralization or a positive culture was documented prior to 180 days; or (3) there were greater than 180 days between SUB flushes. Cats were placed in groups: (1) saline-only, (2) mixed-saline/tEDTA, and (3) tEDTA-only. Ninety-five SUB devices in 66 cats were included (44% bilateral). The median follow-up time was 772, 942, and 289.5 days in groups 1-3, respectively. Documentation of at least one positive urine culture occurred in 33.3% (7/21), 58.3% (7/12), and 18.2% (6/33) of cats at a median of 131, 251, and 222 days post-SUB placement, and chronic infections (>1/year) in 19.0% (4/21), 25% (3/12), and 6.1% (2/33) cats in groups 1-3, respectively. Cats with chronic infections were statistically more likely to be positive pre-operatively (p=0.0002). Mineralization was documented in 32.1% (9/28), 43.8% (7/16), and 17.6% (9/51) of devices in groups 1-3 at a median of 481.5, 301, 201 days post-SUB placement, respectively. Device exchange was needed in 14.3% (4/28), 31.3% (5/16), and 3.9% (2/51) in groups 1-3, respectively.

The prophylactic use of tEDTA may be an effective method to reduce the rate of symptomatic infection and mineralization post-operatively in cats with a SUB device.

NU03

Urine bacterial culture growth and association with clinical findings in cats with acute kidney injury

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Quantitative urine cultures (QUC) are often recommended in the diagnostic evaluation of cats with acute kidney injury (AKI) to rule out infection, even in the absence of an active urinary sediment or signs of lower urinary tract disease. The prevalence of bacterial growth in urine of cats with AKI and its association with findings on urine sediment examination and reported lower urinary tract signs (LUTS) is unclear. The objective was to determine the association between bacterial growth in urine and the presence of LUTS and an active urinary sediment in cats with AKI.

Records of cats presented between 2008 and 2018 were retrospectively reviewed. Cats with a diagnosis of AKI that had a urinalysis and urine culture prior to antibiotic therapy were included. Cats were excluded if they had malignant neoplasia, were receiving an immunosuppressant, had urine collected not by cystocentesis or had received antibiotics within 2 weeks of presentation. An active urinary sediment was defined as either pyuria (>5 WBC/high-power field [HPF]) or hematuria (>5 RBC/HPF). Lower urinary tract signs were defined as the presence of pollakiuria, stranguria, periuria, or dysuria. Significant bacterial growth on QUC was defined as > 1,000 colony forming

unit/ml of bacteria. A Fisher's exact test was performed to examine the relationship between urine culture positivity and the presence of LUTS or an active urine sediment.

A total of 83 cats met the inclusion criteria, of which 24 cats (29%) had significant bacterial growth on QUC. *Escherichia coli* (21/24; 88%) was the most common bacteria isolated on QUC. Twenty-nine cats had bacteriuria, 49 cats had an active urinary sediment, and 34 cats had an inactive urinary sediment on microscopic examination, of which 79% (23/29 cats), 37% (18/49), and 18% (6/34 cats), respectively, had significant bacterial growth on QUC. Cats with an active urinary sediment were more likely to have significant bacterial growth on QUC than cats with an inactive urinary sediment (P=.049). Thirteen of the 83 cats (16%) cats had LUTS reported at presentation, of which a majority had an active urine sediment (10/13; 77%) and significant bacterial growth in urine (8/13; 62%). Cats with LUTS were significantly more likely to have a positive urine culture than cats without LUTS (P=.008).

In conclusion, cats diagnosed with AKI with either lower urinary tract signs or an active urinary sediment are more likely to have significant bacterial growth in urine. A urine culture is recommended in all cats with AKI, even those cats with an inactive urinary sediment, to assess the need for antibiotic therapy.

NU04

Reliability of Crit-Line® monitors in predicting hematocrit and change in blood volume during canine hemodialysis

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Ultrafiltration is an extra-corporeal therapy utilized for treatment of fluid overload and can be monitored with non-invasive blood volume monitors.

The primary study objective was to determine the reliability of Crit-Line® III and IV non-invasive blood volume monitors to correctly estimate canine PCV and therefore their reliability in reflecting changes in relative blood volume during ultrafiltration. A secondary aim was to determine reliability of targeted ultrafiltration rates through the hemodialysis system by comparing them with delivered ultrafiltration rates.

An ex vivo study using the Gambro Phoenix® X36 platform with expired canine packed red blood cells was performed. Through dilution and ultrafiltration, clinically applicable PCV values (n=140, median 26%, range 8%-50%) were obtained. PCV values were compared to their respective Crit-Line® hematocrit and showed good agreement based on Bland Altman analysis (Crit-Line® III bias 1.8%, 95%CI: -2.5 to +6.2%; Crit-Line® IV bias 1.0%, 95%CI: -3.3 to +5.4%).

Targeted and delivered ultrafiltration rates (UFR, measured in ml/h) were compared. At UFR0, a median of +40ml/h (range +20 to +120ml/h) was added to the simulated patient. Less than targeted volume was removed (median; range) at UFR100 (-60ml/h; +10 to -90ml/h), UFR200 (-150ml/h; -120 to -210ml/h), UFR300 (-250ml/h; -220 to -280ml/h), and UFR400 (-340ml/h; -320 to -360ml/h), respectively.

Crit-Line® monitors provide reliable estimates of canine PCV and thus reliable means of estimating change in blood volume during ultrafiltration. Lower ultrafiltration rates may be less accurate, with minimum ultrafiltration potentially adding volume to a patient, an important consideration in ultrafiltration prescriptions for small veterinary patients.

NU05

Characterization of the urinary microbiome in miniature schnauzers with calcium oxalate urolithiasis

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Emerging evidence suggests that urine is not sterile as previously thought, but instead is comprised of diverse microbial communities. Alterations in the urinary microbiome are associated with several disease states in humans, including calcium oxalate (CaOx) urolithiasis. The purpose of this study was to characterize and compare the urinary microbiome of dogs with and without CaOx urolithiasis.

Stored (-80° C) urine samples from Miniature Schnauzers with (n = 10) or without (n = 10) a history of CaOx urolithiasis were included. Sample volumes ranged from 1 to 17 mL. Microbial DNA extraction and 16S rRNA sequencing of the V3-V4 regions were performed (Norgen Laboratory) using a no template control of nuclease free water. Diversity indices and taxa abundance were compared between cases and controls, and cluster analyses were performed.

No significant differences existed in alpha or beta diversity between cases and controls. *Lachnospiraceae* and *Clostridiaceae* were significantly overrepresented in case samples. Two distinct beta diversity clusters were identified in this population, which were distinguished by the relative abundance of *Proteobacteria*. No differences in sex, age, stone status, diet, medications, urinalysis parameters, genetic ancestry, or technical processing were identified to explain this division.

Miniature Schnauzer dogs with CaOx urolithiasis did not have significant alterations in the overall diversity of the urinary microbiome. However, certain bacterial taxa were more abundant in cases than controls. This study population contained two distinct urinary microbiome subtypes that were not explained by known patient or sample variables; the origin of these discrete subtypes remains undetermined.

NU06

Effect of prazosin on rate of recurrent urethral obstruction in cats: Preliminary results

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Urethral obstruction is a common condition in cats. In addition to relieving the mechanical obstruction, a variety of medical therapies have been employed to attempt to prevent re-obstruction, which contributes significantly to morbidity and mortality. Prazosin is an alpha-1 adrenergic

antagonist that selectively competitively inhibits post-synaptic alpha-1 adrenergic receptors, directly producing vascular smooth muscle relaxation. Prazosin has been proposed to act as a urethral relaxant via its smooth muscle activity, therein potentially reducing urethral spasm that may be associated with increased risk of re-obstruction. Potential drawbacks of the use of Prazosin in cats include the need to orally medicate a cat, the potential for side effects (notably, hypotension), and added costs. The primary objective of this study was to compare the rate of recurrent urethral obstruction (rUO) in cats treated with Prazosin compared to cats treated without Prazosin.

Clinicians who either always or never gave prazosin for UO were recruited via the ACVIM and ACVECC list serve. Data from cats were enrolled via an electronic survey (Qualtrics) which included demographic data, treatment data, and if the cat developed a rUO either before discharge or within 14 days. A Fisher's exact was used to compare groups, with a p value of < 0.05 considered significant.

Three hundred and nine cats have been enrolled. Sixty-one (19.7%) cats did not receive prazosin, while Two hundred and forty-eight (80.3%) did receive prazosin. Overall, thirty-three cats (10.7%) developed rUO before hospital discharge and 36 cats (11.7%) developed rUO before 14 days. There was no significant different between the groups in the occurrence of rUO before discharge (11.3% with prazosin, 8.2% without Prazosin, $p = 0.64$) or within 14 days after discharge (14.6% with Prazosin, 17.6% without Prazosin, $p = 0.61$).

While further enrollment is on-going, there is no apparent benefit to prazosin administration for reduction of incidence of rUO in the immediate hospitalization period or in the 14 days following discharge from the hospital.

NU07

Is glucosuria in dogs fed jerky treats associated with excessive intake of soluble phosphorus?

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Acquired proximal renal tubulopathy characterized by renal glucosuria and increased urinary fractional excretion of phosphorus occurs intermittently in dogs. Affected dogs have typically consumed commercial dried meat treats, and signs resolve with treat withdrawal. Glucosuria has been reported in cats fed high phosphorus diets (1). We hypothesized that consumption of commercial dried meat treats might result in excessive intake of highly available phosphorus that increases risk of proximal renal tubular injury. Our aim was to measure total and soluble phosphorus content of treats from the same packets as treats recently consumed by dogs with acquired proximal renal tubulopathy.

Total phosphorus (P_{total}) content of 7 different commercial dried meat treats was determined photometrically after microwave digestion. The amount of highly soluble (after 1 minute in water) phosphorus (P_{sol1}) was measured applying the previously published method (2). The results were related to metabolizable energy (ME) using the method described by the National Research Council (3).

Table 1: Concentration of total (P_{total}) and soluble (P_{sol1}) phosphorus in jerky treats for dogs [mg/1000 kcal ME].

Product	P_{total}	P_{sol1}
Kramar Supanaturals	2141	1647
Natural Pet Company duck jerky	2464	1236
Nature's Gift mini treats -chicken reward meat balls	5165	1200
Yours Droolly duck tenders	2371	959
Jerhigh chicken sticks	1079	697
Pet's Own chicken meat balls	837	348
Coles pig ear strips	487	149

In 6/7 of the tested treats the amount of P_{total} was above the recommended allowance of 750 mg/1000 kcal (3; Table 1), in one case 7-fold. In 5/7 samples the amount of highly available P_{sol1} exceeded the recommended daily intake of phosphorus, in one case 2.5-fold.

Table 1: Concentration of total (P_{total}) and soluble (P_{sol1}) phosphorus in jerky treats for dogs [mg/1000 kcal ME].

Consumption of commercial dried meat treats potentially results in excessive intake of highly available phosphorus increasing risk of proximal renal tubular injury. In dogs, high intake of P_{sol1} can cause disruption of calcium and phosphate homeostasis (4) and adverse effects on the kidneys (5). It is recommended to limit both the overall daily intake and the intake per meal of P_{sol1}, and so additional intake via treats with high P_{sol1} content must be taken into account when assessing risk. It is recommended to check both P_{total} and P_{sol1} intake in dogs with acquired proximal renal tubulopathy.

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NU08

Centrifugal mononuclear cell collection in dogs: Safety and efficacy of treatment

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Centrifugal apheresis platforms allow for the collection of specific cell types based upon cell density. This provides opportunity for removal of specific cells to either deplete a patient or obtain a viable cell product. Centrifugal mononuclear cell collection (MNC) has previously been reported in a small number of dogs as part of a therapeutic approach to canine osteosarcoma involving autologous cancer cell vaccination and adoptive T-cell transfer. The methodology and efficacy of MNC in canines has not been fully evaluated at this time.

Five study sites prospectively performed MNC as part of ongoing clinical trials for three canine cancer indications (26 osteosarcoma, 13 B cell lymphoma, 1 hemangiosarcoma) using centrifugal apheresis machines. Apheresis data from these clinical trials were pooled for purposes of this study. Regional citrate anticoagulation was performed as previously described. Operating parameters and patient biochemical as well as physical examination findings were recorded. Adverse effects of treatment were noted when they occurred. Descriptive statistics were used to define collected data. Data were tested for normality through Shapiro-Wilk testing. Normally distributed data were reported as mean and standard deviation while non-normally distributed data were reported as median and range.

A total of 40 dogs were evaluated for this study. The median weight was 32.1 kg (range 17.1-70 kg). Dogs were sedated and temporary vascular access was obtained via a dual lumen jugular catheter; 33/40 (82.5%) were placed in the right jugular vein. A median of 2.9 total blood volumes (range 0.9-3.5) were processed during treatment. An average of 175.7mL (± 47.0mL) of T-cell product was collected during the treatment. The T-cell product contained a median of $4.1x10^9$ cells (range $5.6x10^8$ – $1.0x10^{13}$) with a median cell viability of 95.35% (range 80.0-100%). Throughout treatment a median of 653mL (range 310-1665mL) of ACD-A citrate solution was administered to the patient for anticoagulation. Patients were monitored for hypocalcemia during treatment and managed with calcium supplementation. None developed hemodynamic instability or severe consequences of their hypocalcemia.

Centrifugal MNC is a safe procedure in dogs and provides a concentrated T-cell product with excellent viability. This procedure may be effectively utilized for collection of T-cell product to be used in other immunomodulatory procedures.

NU09

Complications and outcome in female dogs with ectopic ureter treated by laser ablation or surgery

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Cystoscopic-guided laser ablation (CLA) or surgical techniques are treatment options available for dogs diagnosed with intramural ectopic ureter. The purpose of this study was to compare complication rates and post-procedural owner-reported continence between female dogs receiving either surgical repair or CLA for treatment of intramural ectopic ureter.

Medical records of female dogs that underwent either surgical or transurethral CLA (Holmium:YAG laser) correction of an intramural ectopic ureter at Oregon State University (OSU) and Colorado State University (CSU) between 2008 and 2019 were retrospectively reviewed. Last follow-up information (in some cases, the appointment prior to artificial urethral sphincter placement) was obtained either during an appointment or from a documented client communication in the medical record. Patients were excluded if they were diagnosed with an extramural ectopic ureter or if they had previous surgery involving the urinary tract. Mann-Whitney test was used to compare continuous data between surgery and CLA dogs. Descriptive data is provided as median and range.

Fifty-three dogs met the inclusion criteria, of which, 33/53 (62%) underwent surgery (CSU, 29 cases; OSU, 4 cases) and 20/53 (38%) underwent CLA (all cases from OSU). Of the 33 dogs undergoing surgery, 21 dogs had neoureterostomy, 9 dogs had ureteroneocystostomy, and 3 dogs with bilateral ectopic ureters had both surgical procedures performed (1 on each ectopic ureter). No intraoperative complications directly related to the procedure were noted for dogs that underwent surgery. In 1 dog with bilateral ectopic ureters that underwent CLA, only 1 ectopic ureter was successfully ablated. Post-surgical complications were noted in 17/33 dogs (52%) and included pollakiuria (10/33), stranguria (8/33), hematuria (6/33) and incisional infection and dehiscence or uroabdomen (1 dog each). Post-CLA complications were noted in 4/20 (20%) dogs and included pollakiuria (2/20), stranguria (3/20), hematuria (1/20), and self-resolving post-procedural abdominal fluid (1/20). Follow-up information regarding continence status of the dog was available for 27/33 surgery dogs (19; 2-1767 days). At last follow-up, owners reported improvement in urinary incontinence in 13 dogs (48%) or resolution in 14 dogs (52%). Follow-up information was available for 17/20 CLA dogs (71; 2-796 days). At last follow-up, owners reported improvement in urinary incontinence in 7 dogs (41%), resolution in 5 dogs (29%), no change in 4 dogs (24%), and worsened in 1 dog (6%). The number of nights in hospital after the procedure was significantly higher in surgery dogs (1; 1-7 days) compared to CLA dogs (1; 0-2 days; $P = .0005$).

In conclusion, dogs that underwent surgery had a higher risk of post-operative complications, spent more time in hospital after the procedure, and had higher rates of owner-reported improvement or resolution of urinary continence compared to dogs that underwent CLA for treatment of intramural ectopic ureter.

NU10

A retrospective evaluation of neutrophil and platelet ratios in cats with obstructive uropathy

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Neutrophil to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are markers that have been studied in many cancers and infections, with higher ratios indicating poorer overall disease and prognosis. Recently, the veterinary literature has reflected growing interest in these ratios as diagnostic and prognostic markers for both neoplastic and inflammatory conditions. However, scattered information exists in feline medicine. This study aimed to evaluate and compare NLR and PLR with clinical, clinico-pathological features and length of hospitalization (LH) in a population of cats with obstructive uropathy. The following information at hospital admission was recorded and studied: age, weight, body temperature, NLR, PLR, serum concentrations of creatinine, blood urea nitrogen and potassium, presence of lower urinary tract infection (LUTI), urethroliths, and hemorrhagic cystitis (HC). In addition, need for surgery and LH were recorded and studied. NLR and PLR were calculated as the ratio between absolute neutrophils and platelets and lymphocytes. One hundred twenty-four cats were retrospectively enrolled. Thirty-two cats presented abnormal temperature. Creatinine, potassium and blood urea nitrogen were increased to a variable extent. Concurrent HC, urethroliths and LUTI were observed in 47, 11 and 2 cats, respectively. Eight cats required surgery. The median LH was 4.5 days. Median NLR and median PLR were 10.28 and 158.11, respectively. Although increased ratios were observed, supporting the inflammatory nature of obstructive uropathy, no significant relationships emerged between NLR or PLR and any of the considered variables. Further research is needed to confirm these preliminary results.

NU11

Evaluation of the automated FIRSTract rapid urine culture to detect canine and feline bacteriuria

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Automated urine culture for the rapid diagnosis of urinary tract infections (UTIs) is common in human medicine throughout Europe and Asia. Given the frequency of UTIs in small animal medicine and the increasing threat of antibiotic resistance, ANTECH has modified and evaluated the Alfred 60/AST and HB&L® analyzer (Alifax, Italy) for use in the detection of bacterial growth in canine and feline urine samples. This method (FIRSTract) uses 250 µL of urine, inoculated into a proprietary culture broth optimized for aerobic bacterial pathogen growth. Samples are maintained in aseptic vials with pierceable hermetic seals, incubated at a controlled temperature and continuously mixed for up to five hours. The amount of light scattered through the inoculum is measured every five minutes via spectrophotometry, after obtaining a baseline reading of turbidity. An increase in turbidity is displayed in a real time growth curve with quantitative bacterial counts in CFU/mL. An exponential increase consistent with the presence of viable bacteria in patient urine samples is reported as positive growth. The purpose of this study was to compare the results of automated FIRSTract to traditional culture of canine and feline urine.

565 urine samples (423 canine, 142 feline) with volumes greater than 500 µL were selected from samples submitted for urine culture to Antech Laboratories (Lake Success, NY) over four weeks. FIRSTract and traditional culture (TSA II Sheep Blood/MAC bi-plate incubated for 48 hours for canine samples and 72 hours for feline samples) were completed for each urine sample. A cut off of 50,000 CFU/mL was used to define positive bacterial growth.

The FIRSTract results were compared to those from traditional urine culture. 200 samples were positive for growth by FIRSTract compared to 199 by traditional culture. Sensitivity was 97.0% and specificity was 97.9%.

The FIRSTract test yields accurate results for the detection of bacteria in canine and feline urine samples. When combined with traditional plating for identification and antimicrobial sensitivity, this test will provide rapid and reliable results to ensure veterinary patients with UTIs are treated in an expedited and appropriate manner.

NU12

Canine urine ammonia-to-creatinine ratio reference interval

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Renal ammonia metabolism is critical to maintaining acid-base homeostasis. Inadequate ammonia excretion appears to drive development of metabolic acidosis in people with chronic kidney disease (CKD) and has been correlated with worse clinical outcomes. Metabolic acidosis is also recognized in dogs with CKD. However, urinary ammonia excretion has not been evaluated in healthy dogs or those with disease. The purpose of this study was to generate a reference interval (RI) for canine urine ammonia-to-creatinine ratio (UAC) in a population of healthy adult dogs from a veterinary teaching hospital. Sixty adult, client-owned dogs were assessed via history, physical examination, serum chemistry, and urinalysis and 12 were excluded. Urine samples (n=48) were processed and stored at -80°C within four hours of collection. Ammonia and creatinine concentrations were measured using commercially available enzymatic assays. To establish RI for UAC, 2.5% and 97.5% limits were determined using nonparametric methods. The Dixon method was used to detect and exclude outliers. The RI for UAC was 0.16 - 23.69 with 90% confidence intervals for lower and upper limits of (0.13 - 1.17) and (20.50 - 23.75), respectively. General linear models did not detect significant relationships between UAC and serum bicarbonate, sex, weight, or age. Statistical significance was set at $P < 0.05$ for all analyses. Having an RI for UAC in a healthy adult dog population will provide a foundation for future research to determine whether alterations in ammonia excretion are associated with specific disease states which may provide prognostic information or guide clinical management strategies.

NU13

Nephroureterolithiasis in dog breeds predisposed to calcium oxalate urolithiasis

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The objective of this study was to determine the prevalence of and risk factors for nephroureterolithiasis in breeds predisposed to calcium oxalate (CaOx) urolithiasis.

Medical records were searched for dogs with abdominal radiographs performed from 2016-2020. Dogs were selected of 5 small breeds (Bichon Frise, Lhasa Apso, Miniature Schnauzer, Pomeranian, and Shih Tzu) and 3 medium-to-large breeds (Doberman Pinscher, Standard Poodle, and Standard Schnauzer) reportedly at-risk for CaOx uroliths. Mixed breed dogs were included for comparison. Nephroureterolithiasis prevalence was compared between CaOx risk and mixed breed dogs, dogs with and without lower urinary tract (LUT) urolithiasis, and males versus females. Sex, age, breed, LUT urolithiasis and weight were included in a regression analysis for nephroureterolithiasis.

The population comprised 251 CaOx risk and 68 mixed breed dogs. Nephroureterolithiasis was more common in CaOx risk breed (23%, 58/251; OR = 4.8, 95% CI 1.7 - 18.9, $P < .001$) than mixed breed (6%, 4/68) dogs. Nephroureterolithiasis was also more common in dogs with LUT urolithiasis (41%, 53/130; OR = 13.6, 95% CI 6.3 - 33.1, $P < .001$) than those without (5%, 9/189). In the regression, LUT urolithiasis was the strongest predictor of nephroureterolithiasis ($P < .001$), followed by increasing age ($P < .001$) and lower body weight ($P = .0071$). Differences in nephrolithiasis prevalence between sexes were not significant ($P = .77$).

In conclusion, nephroureterolithiasis is common in dogs with LUT uroliths, supporting a shared etiology. Unlike with LUT urolithiasis, nephroureterolithiasis has a similar prevalence in male and female dogs.

NU14

Cystatin C and neutrophil gelatinase-associated lipocalin as early biomarkers for chronic kidney disease in dogs

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Chronic kidney disease (CKD) is the most common kidney disease in dogs and early diagnosis of CKD would facilitate timely and appropriate monitoring and therapy. Traditional biomarkers of CKD for diagnosis and monitoring are divided into markers of glomerular filtration rate (GFR) or markers of glomerular or tubular damage. Recently, the most commonly used markers of GFR are creatinine (CREA) and symmetric dimethylarginine (SDMA). However, CREA demonstrates insensitivity in the detection of early CKD, and more research is needed regarding its nonrenal influences on SDMA. Meanwhile, a few studies have demonstrated better sensitivity and specificity with cystatin C (CysC) compared to other biomarkers in the detection of kidney damage in humans and dogs with early CKD. A recent study revealed that neutrophil gelatinase-associated lipocalin (NGAL) can probably predict the risk of progression of CKD in dogs. The present study aimed to identify the biomarkers that could accurately detect early kidney damage in dogs with CKD.

A total of 41 dogs that were presented to the Veterinary medical teaching hospital of Chonnam National University between 2019 and 2020 were included in the study. Of these dogs, 9 were identified as healthy. Plasma CREA, SDMA, and urine creatinine concentrations were measured with the Catalyst Dx Chemistry Analyzer (IDEXX Laboratories, Inc., Westbrook, ME, USA). Serum CysC was measured using the Roche enzymatic method (Roche-Hitachi Modular P analyzer; Hoffmann-La Roche, Ltd., Basel, Switzerland). Urine NGAL concentration was determined using a dog-specific sandwich enzyme-linked immunosorbent assay (ELISA) (Bioporto, Gentofte, Denmark). The urinary NGAL: creatinine ratio (UNCR) was calculated as urine NGAL/urine creatinine. Data analysis was performed employing the Mann-Whitney U test, Pearson's correlation, and receiver operating characteristic (ROC) curve. Statistical significance was set at $P < 0.05$.

SDMA ($P = 0.003$), CysC ($P = 0.0002$), and UNCR ($P = 0.0003$) were identified to be significantly elevated in patients with CKD IRIS stage I compared to healthy controls except for CREA ($P = 0.07$). CysC demonstrated strong correlation with CREA ($r = 0.8097$, $P < 0.0001$). CysC (sensitivity 93.6% and specificity 100%) and UNCR (sensitivity 88.9% and specificity 100%) exhibited higher sensitivity and specificity compared to CREA (sensitivity 11.1% and specificity 100%) and SDMA (sensitivity 55.6% and specificity 100%) in dogs with CKD IRIS stage I.

These findings suggest that the use of CysC and UNCR as biomarkers for CKD leads to early detection of kidney dysfunction and damage in dogs compared to CREA and SDMA. Earlier detection might be desirable for initiation reno-protective interventions that slow the progression of kidney disease.

NU15

Isolation of urinary extracellular vesicles from healthy cats and cats with chronic kidney disease

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Extracellular vesicles are a heterogenous group of membranous structures, including exosomes, microvesicles and apoptotic bodies, that are actively released from cells. Urinary extracellular vesicles (uEVs), originating primarily from the kidney and urinary tract epithelium, contain proteins, mRNAs and microRNAs, and thus they are a potential source of biomarkers. Our aim was to optimise a methodology for isolating feline uEVs and to characterise the uEV population in cats with normal renal function in comparison to cats with normotensive or hypertensive chronic kidney disease (CKD).

In an initial experiment, feline uEVs were isolated from pooled aliquots of frozen urine (4mL) via three different methods: precipitation, precipitation followed by size exclusion chromatography (precipitation + SEC), and ultrafiltration followed by SEC (UF + SEC). EV preparations were characterised using transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). Protein concentrations of EV preparations were measured by bicinchoninic acid assay. Particle: protein ratio was calculated as a measure of purity and compared between methods using one-way ANOVA (results reported as mean ± standard deviation). Records from 2 first opinion practices were then searched for cats >9 years old with and without renal azotaemia. Cats with normal renal function (n = 9), cats with normotensive CKD (NT-CKD, n = 10) and cats with hypertensive CKD (HT-CKD, n = 10) were identified. uEVs were isolated from frozen patient urine samples (4mL) via the UF+SEC method. EV concentration (per mL and normalised to urine creatinine concentration) and mean EV diameter were determined using NTA, and groups were compared using the Kruskal-Wallis test (results reported as median [25th, 75th percentiles]).

uEV preparations obtained via all three methods contained particles of the expected size and with expected (cup shaped) morphology on TEM. uEV preparations obtained via UF+SEC had a significantly higher mean particle to protein ratio ($5.6 \times 10^8 \pm 2.7 \times 10^8$) than preparations obtained via precipitation alone ($9.0 \times 10^6 \pm 1.9 \times 10^6$) or precipitation + SEC ($4.1 \times 10^7 \pm 4.8 \times 10^6$; P = 0.009). There was no significant difference in uEV concentration per mL between cats with normal renal function (8.1×10^9 [2.8×10^9, 1.9×10^{10}] particles/mL), NT-CKD (2.1×10^9 [8.3×10^8, 9.8×10^{10}] particles/mL) and HT-CKD (1.8×10^9 [9.2×10^8, 5.6×10^9] particles/mL; P = 0.26). There remained no significant difference between groups when EV concentration per mL was normalised to urine creatinine (P = 0.68). Mean particle size did not differ between groups (normal renal function: 115.9 [106.2, 116.8] nm, NT-CKD: 112.2 [107.2, 122.8] nm, HT-CKD 118.7 [110.1, 126.9] nm, P = 0.42).

In conclusion, feline uEVs can be successfully isolated from frozen urine of cats with and without CKD, with ultrafiltration followed by size exclusion chromatography resulting in the purest uEV preparations. Further work to catalogue the EV proteome may result in the identification of novel therapeutic targets and biomarkers of disease.

NU16

Use of telmisartan to address proteinuria in dogs in various clinical contexts: A retrospective study

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Use of telmisartan for the treatment of proteinuria in dogs has not been largely investigated. Telmisartan can be effective for the treatment of proteinuria in dogs in various clinical contexts.

Forty-four client-owned dogs with proteinuria. Retrospective study; dogs diagnosed with significant proteinuria (non-azotemic dogs with a urine protein creatinine ratio (UPC) value ≥2 and azotemic dogs with a UPC value ≥ 0.5) were separated into 3 groups: telmisartan alone, with benazepril or combined with mycophenolate. UPC values were recorded prior to treatment and at subsequent follow-ups (1, 3, 6 and 12 months when available). Response to treatment was categorized as complete (UPC <0.5), partial (UPC value decreased by ≥ 50% but ≥ 0.5) or no response (UPC value decreased by < 50%). Serum creatinine, potassium and arterial pressure were also recorded.

In the telmisartan group, therapeutic response (UPC <0.5 or value decreased by ≥ 50%) was observed in 70%, 68%, 80% and 60% of individuals at 1, 3-, 6- and 12-months follow-up respectively. A similar trend was observed in the 2 other groups. No significant changes were noted in serum creatinine, potassium, or arterial blood pressure at all follow-up times. Side effects observed were mild self-limiting gastro-intestinal signs for 5 dogs. Two dogs developed significant azotemia that required discontinuation of the treatment prior to the first follow-up.

Telmisartan could be considered for management of proteinuria in dogs in various clinical contexts, alone or in combination with other common treatments for proteinuria.

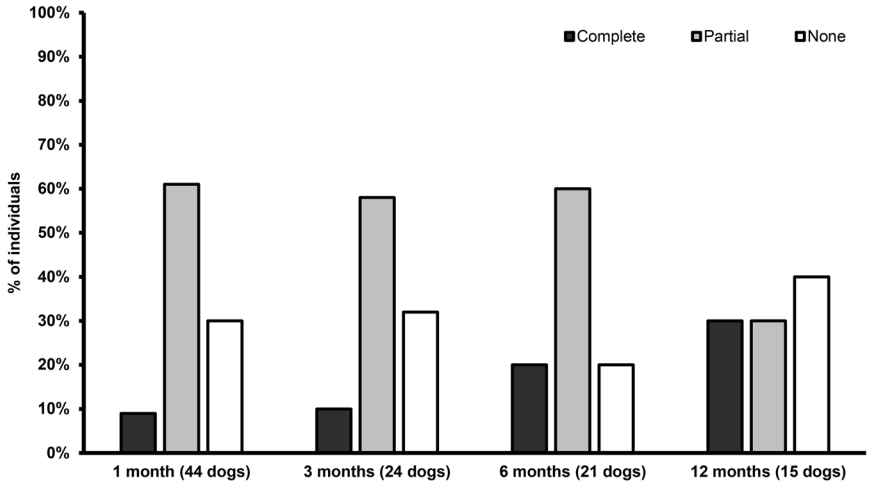

Figure 1- Percentage (%) of individuals with complete (UPC <0.5) (dark grey bars), partial (UPC ≥50% decrease from baseline but ≥0.5) (light grey bars) and no response (UPC decrease >50% from baseline) (white bars) after receiving telmisartan at 1, 3, 6- and 12-month follow-ups.

Figure 1- Percentage (%) of individuals with complete (UPC <0.5) (dark grey bars), partial (UPC ≥50% decrease from baseline but ≥0.5) (light grey bars) and no response (UPC decrease >50% from baseline) (white bars) after receiving telmisartan at 1, 3, 6- and 12-month follow-ups.

NU17

An updated relative supersaturation program, EQUIL-HL21, compared to EQUIL93 applied to healthy dogs and cats

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Urolithiasis occurs commonly in both dogs and cats and measurement of urinary relative supersaturation (RSS) can help predict risk. Nevertheless, an up-to-date program for measuring RSS is not available because older freeware programs are not compatible with newer hardware and software, or programs are protected by intellectual property and remain unshared. An early freeware program to measure relative supersaturation was developed in BASIC computer language and was published by Werness et al in 1985 and was called EQUIL2. This program was distributed free of charge by the Mayo Clinic and could be altered by the end user resulting in variations between laboratories. A compiled version running on PC's, wherein the formulas could not be read or altered, was published and distributed by Brown et al in 1994 and was called EQUIL93. Comparisons between the original EQUIL2 and EQUIL93 programs have never been reported in the veterinary literature.

For this study, the original EQUIL2 BASIC program was embedded into a spreadsheet program so it could be used on modern computers with up-to-date software and will henceforth be referred to as EQUIL-HL21. A comparison between EQUIL-HL21 and EQUIL93 calculated RSS using dog and cat urine was then performed. Urine was obtained from 10 dogs and 10 cats fed the same food for 14 days. On the last day of feeding all urine produced in a 24-hour period was collected by free catch into a closed system and was analyzed for NH_4^+, Ca^{+2}, Cl^-, citrate, creatinine, Mg^{+2}, oxalate, PO_4^{-3}, K^+, Na^+, $SO4^{-2}$ and urine pH. RSS calculation for calcium oxalate (CaOx) and struvite (MAP) was performed using both programs and an analysis of variance was used to compare the results. Results are shown in the table below and demonstrate that the RSS for MAP was significantly higher using the EQUIL93 program for both dog and cat; p=0.02 and p=0.0005 respectively, whereas they were similar for CaOx for dogs and cats; p=0.3 and p=0.9 respectively.

Results of this work support that the two most commonly used RSS programs may not produce identical results depending upon the thermodynamic stability constants used in the calculations. The original EQUIL2 was open source and this work creates a foundation wherein the modernized EQUIL-HL21 program could be used by multiple users using cat and dog specific data on a platform which can be shared with the veterinary community.

NU18

Urine and renal cultures from geriatric cats with and without chronic kidney disease

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Chronic kidney disease (CKD) is common in geriatric cats and bacterial infection is thought to be a contributing cause in some cats. However, in one recent study, progression of CKD was not linked to positive urine cultures, suggesting that not infections ascend to cause pyelonephritis. There are few studies of cats with CKD from which culture results from samples collected directly from the kidneys have been reported. In addition, it is also possible that unculturable bacteria in renal tissues could be associated with CKD in cats. While 16S rRNA PCR and next generation sequencing have been performed on renal tissues or renal swabs from people and urine from cats, use of these molecular techniques have not been performed on swabs collected from the renal pelvis of cats with and without CKD. The primary objective of the study was to aseptically collect urine samples from the bladder and both renal pelves of cats > 10 years of age with and without CKD. The secondary objective was to collect materials for molecular evaluation to determine whether cats > 10 years of age have a renal microbiome and to determine if patterns of positive results correlate to CKD.

With owner consent and humane euthanasia, cadavers were transported on ice for sample collection using aseptic technique. Urine was collected from the bladder via cystocentesis and new sterile scalpel blades were used to penetrate the pelvis of both kidneys to aid placing a sterile culture swab from a commercially available transport system to collect samples. A second swab was passed into the renal

Table 1: Relative Supersaturation (RSS) as calculated by EQUIL93 and EQUIL-HL21

		Calcium Oxalate			Magnesium Ammonium Phosphate (MAP-Struvite)		
		EQUIL93	EQUIL-HL21	Ratio EQUIL93/ EQUIL-HL21	EQUIL93	EQUIL-HL21	Ratio EQUIL93/ EQUIL-HL21
Dog n= 10	mean ± SD, CV%	25.2 ± 11.9[a] 47.4%	20.6 ± 49.5[a] 46.1%	1.2 ± 0.07 5.8%	18.7 ± 14.5[c] 77.5%	6.5 ± 4.9[c] 76.0%	2.9 ± 0.1 3.5%
Cat n= 10		2.4 ± 0.9[b] 36.8%	2.4 ± 0.8[b] 35.5%	1.0 ± 0.1 5.8%	20.7 ± 10.3[d] 49.9%	6.2 ± 3.3[d] 53.6%	3.4 ± 0.3 7.3%

[a]p=0.3, [b]p=0.9, [c]p=0.02, [d]p=0.0005

pelvis of each kidney of each cat to collect urine and cells which were stored at -80C for the bacterial nucleic acid study. All samples were collected within 2 hours of euthanasia. Aerobic, anaerobic, and *Mycoplasma* spp. cultures were performed by the Bacteriology Section of the CSU Veterinary Diagnostic Laboratory. Blood was collected and was used to evaluate BUN and creatinine concentrations by the CSU Clinical Pathology Department. Serum creatinine concentration, urine specific gravity, and urine protein/creatinine ratio were used to classify cats as normal or different International Renal Interest Society (IRIS) stages of CKD (http://www.iris-kidney.com/guidelines/staging.html). Results from cats that were classified as IRIS Stage 1 were grouped with the normal cats for analyses, and cats that were classified as IRIS stages 2, 3, or 4 were grouped together for analyses.

Among the 10 cats classified as normal or IRIS Stage 1, one was positive for *Mycoplasma* growth in urine but neither kidney. Among the 12 cats classified as IRIS stages 2, 3 and 4, bacteria were grown by aerobic culture (4 cats; *Escherichia coli* or *Enterococcus* spp.) or anaerobic culture (1 cat; [> 4 isolates] were isolated from the urine of 5 cats (41.7%). The cat with anaerobic bacteria in urine also yielded *E. coli* from the left kidney. The same bacterium in urine was cultured from one or both kidneys in 3 other cats. One additional cat had a heavy growth of *E. coli* from the left kidney but not urine.

These results suggest that bacterial culture of urine should be considered in the diagnostic workup of cats classified as having IRIS stages 2-4 CKD to evaluate the presence of pyelonephritis, and that a negative urine culture may not rule out infection. Since most of the cats did not have sample collection until up to 2 hours after death, there could be concern for bacterial translocation to renal tissues. However, since the cadavers were kept on ice and very few cats were positive for bacterial growth, we believe translocation is an unlikely explanation for the positive samples. The DNA extraction methods to be used in the second objective are being optimized. After optimization, it will be determined by 16S rRNA PCR and next generation sequencing whether there is a renal microbiome in cats > 10 years of age and if there are associations among the results of different bacteria and CKD.

NU19

Assessment of capillary rarefaction in cats with and without CKD

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Feline chronic kidney disease (CKD) is histologically characterized by tubulointerstitial inflammation, tubular atrophy and fibrosis. Hypoxia is a key driver of fibrosis in CKD and is associated with capillary rarefaction (reduction in vascular density) in humans. The purpose of this study was to determine if capillary rarefaction occurs in cats with CKD.

Seventy-eight archived formalin-fixed paraffin-embedded kidney tissues collected at autopsy from cats with CKD (16 IRIS Stage 2, 18 IRIS Stage 3, and 24 IRIS Stage 4) and without CKD (10 young cats (≤9 years old) and 10 geriatric cats (≥10 years old)) were assessed. Percentage glomerulosclerosis, inflammation and fibrosis were scored as absent, mild, moderate or severe. Vascular structures were identified with a combination of CD31 immunohistochemistry and visual assessment. Consecutive high-power fields (40x), ten from the cortex and five from the corticomedullary junction (CMJ) were examined and an observer (masked to clinical data) identified, counted and colored the capillary area. Image analysis of the colored capillaries was used to determine average capillary size, and average % capillary area (capillary area/total area). Differences between cats with and without CKD were assessed with Kruskal-Wallis test, Mann-Whitney U test, and Ordinary one-way ANOVA where appropriate. The correlation between capillary size or % capillary area and serum creatinine, % glomerulosclerosis, fibrosis, and inflammation was assessed with Spearman rank.

There was no significant difference in capillary count between cats with and without CKD in the cortex or CMJ. Capillary size was significantly smaller in CKD cats than cats without CKD in both the cortex and CMJ ($P = 0.0001$ and $P = 0.0013$ respectively). Percent capillary area was significantly smaller in CKD cats than cats without CKD in both the cortex and CMJ ($P = 0.0005$ and $P = 0.008$ respectively). Capillary size in the cortex and CMJ and % capillary area in the cortex were negatively correlated with creatinine levels ($P = 0.0003$, $r = -0.4$; $P = 0.0067$, $r = -0.3$; $P = 0.0013$, $r = -0.36$, respectively). Capillary size in the cortex was negatively correlated with % global glomerulosclerosis, fibrosis, and inflammation within the cortex ($P < 0.0001$, $r = -0.44$; $P = 0.0007$, $r = -0.38$; $P = 0.0001$, $r = -0.42$ respectively). The capillary size in the CMJ was negatively correlated with fibrosis and inflammation within the CMJ ($P = 0.0036$, $r = -0.33$; $P = 0.023$ $r = -0.26$ respectively). The % capillary area in the cortex was negatively correlated with % global glomerulosclerosis, fibrosis, and inflammation within the cortex ($P = 0.0004$, $r = -0.39$; $P = 0.0074$, $r = -0.3$; $P = 0.0089$, $r = -0.3$). There was no correlation between % capillary area in the CMJ with fibrosis and inflammation within the CMJ.

Capillary rarefaction was observed in cats with CKD and was associated with severity of disease. Further exploration of novel treatments directed towards ameliorating capillary rarefaction is warranted.

NU20

Effect of dietary protein concentration on serum concentrations of gut-derived uremic toxins in healthy cats

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The major gut-derived uremic toxins indoxyl sulfate (IS), p-cresol sulfate (pCS), and trimethylamine-n-oxide (TMAO) are metabolites of bacterial protein fermentation in the colon of cats. In people and dogs, a low protein diet reduced serum concentrations of one or more of these uremic toxins. The objective of the study was to determine the

effect of a low protein diet in comparison to a high protein diet on the serum IS, pCS, and TMAO concentrations in healthy cats.

Twenty healthy 3-year-old mixed sex research-bred cats were enrolled in a prospective randomized controlled diet trial. Cats were fed a commercial cat food for at least 3 months prior to enrollment (protein 34% dry matter [DM]). For study purposes, low protein (LPD) and high protein (HPD) dry diets were formulated to be as similar to one another as possible except for protein concentration (LPD, 31% DM; HPD, 45% DM). Cats were randomized into 2 groups and fed ad libitum either the LPD or HPD for 12 weeks. A fasted serum sample was collected at the same time of day at baseline and 4, 8, and 12 weeks after feeding the study diets. Serum was analyzed for concentrations of IS, pCS, and TMAO by liquid chromatography tandem mass spectrometry. A mixed model, repeated measures analysis for each response variable (TMAO, IS and PCS) was performed followed by Bonferroni multiple comparisons test to compare serum concentrations between the LPD and HPD groups at each time point (Week 0 [baseline], 4, 8, 12) and to compare serum concentrations at baseline compared to subsequent weeks within each group.

At Week 8, cats had higher serum IS concentrations after consumption of a HPD (1537 [1201-2982 ng/mL) when compared to a LPD (1167 [688-1710 ng/mL). No significant difference in serum IS concentrations was found between groups at Week 0, 4, and 12. No significant difference was found in serum pCS and TMAO concentrations between groups at any time point. Serum pCS concentrations increased the first 8 weeks of feeding a HPD and were significantly higher at Week 4 (3264 [1651-5734 ng/mL]; P=0.028) and 8 (3295 [1473-5234 ng/mL]; P=0.007) when compared to baseline (2629 [7390-5122 ng/mL). In contrast, serum pCS concentrations at Weeks 4, 8, and 12 did not differ from baseline in the LPD. Serum IS and TMAO concentrations were not statistically different at Weeks 4, 8, and 12 when compared to baseline values in either groups.

In conclusion, consumption of a HPD can increase serum concentrations of pCS, however the effect may be temporary. A HPD may contribute to the accumulation of IS in the serum of healthy cats. Serum TMAO concentrations were unaffected by variations in dietary protein concentrations. The effect of dietary protein on gut-derived uremic toxin burden may differ in cats with renal compromise.

NU21

Evaluation of a rapid immunoassay for detection of UTI in dogs

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Point of care UTI tests are marketed to help guide empirical antimicrobial therapy. The objective of this study was to evaluate a veterinary rapid immunoassay (RIA) for canine urinary tract infection (UTI) and to correlate these results with urine sediment and clinical signs.

Twenty-two dogs with clinical indication for a urinalysis and culture were enrolled.

Urine was assayed using the RIA test per manufacturer's instructions. Signalment, urine sediment, and urine culture results, including bacterial species and colony forming units [(CFU)/mL], were recorded alongside a survey regarding the dog's clinical signs. RIA results were compared to results reported from the microbiological laboratory and were correlated to urine sediment findings and clinical sign scores.

12 of 22 sample (55%) were positive for growth from the microbiology laboratory. Overall sensitivity and specificity was 73% and 91% respectively (95% CI: 46-99; 73-100). The sensitivity and specificity was 0% and 91% (95% CI=0=0; 73-100) for < 1,000CFU/mL and 80% and 91% when ≥1,000CFU/mL (95% CI=55-100;74-100). For gram negative positive RIA tests, gram negative bacteria were correctly identified in 5/5 of samples. No association was seen between RIA status and level of pyuria (P = 0.09) or hematuria (P = 0.61).

While our preliminary data had a higher prevalence of positive cultures compared to previous larger studies,the RIA had lower sensitivity but similar specificity. Further samples are needed to determine the accuracy of RIA for < 1,000 CFU positive urine cultures and to assess the accuracy of the RIA for correctly identifying gram negative bacteria.

NU22

Progression of chronic kidney disease in dogs with concurrent myxomatous mitral valve disease

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The aim of this study was to evaluate whether the presence of myxomatous mitral valve disease (MMVD) is associated with the rate of progression of chronic kidney disease (CKD) in dogs.

We classified 115 dogs with CKD into two groups: dogs with MMVD (n = 63, concurrent group) and dogs without MMVD (n = 52, CKD group). The concurrent group was further divided into two subgroups based on the American College of Veterinary Internal Medicine guidelines (B1 group, n = 24; B2 group, n = 39). We evaluated the time from initial diagnosis to International Renal Interest Society (IRIS) stage worsening and the time to the occurrence of hyperphosphatemia and isosthenuria.

The time to CKD progression was shorter in the concurrent group than in the CKD group (worsening from IRIS stage 1 to 2 [$P = 0.002$], and from IRIS stage 2 to 3 [$P = 0.046$]; occurrence of hyperphosphatemia [$P = 0.003$]; occurrence of isosthenuria [$P = 0.004$]). Similarly, CKD progression was faster in the B2 subgroup than in the CKD group (worsening from IRIS stage 1 to 2 [$P = 0.002$]; occurrence of isosthenuria [$P = 0.003$]); however, there was no significant difference between the B1 subgroup and the CKD group.

The results of this study suggest that stage B2 MMVD could be a risk factor for the progression of CKD. Furthermore, our findings may help to predict the prognosis of dogs with both CKD and MMVD and provide insight into the proposed mechanism of cardiovascular-renal disease.

OT01

Comparison between non-contact handheld cutaneous infrared thermometer and standard rectal thermometer in dogs and cats

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Rectal thermometers (RT) are routinely used to assess body temperature (BT) in dogs and cats, but this method is often a source of significant stress for the animal. Non-contact infra-red thermometers (NCIT) have gained popularity during the SARS-COV2 pandemic as a rapid non-invasive method for assessing BT. The aim of this prospective, multi-centre observational study was to compare the agreement of standard rectal temperature with temperature measured with NCIT in a population of dogs and cats.

NCIT readings were taken in triplicate, using a PC868 Infrared Thermometer *(Shenzhen Pacom Medical Instruments Co. Ltd.)*. from the hairless skin on the medial aspect of the pinna. RT was then taken using a standard digital thermometer. Ambient room temperature, signalment, presence of icterus, skin and coat colour, reason for presentation and final diagnosis (if known) were recorded. Patients were classified as normothermic if their BT (determined by RT) was within reference interval (38 - 39.2°C), hyperthermic if BT was > 39.2°C and hypothermic if BT was < 38°C. Performance of the NCIT was determined by assessing both correlation (using Kendall'tau) and agreement (using Kappa analysis, and Bland-Altman plots).

One hundred and fifty-nine dogs and sixty-three cats were recruited. In dogs, median (range) BT measured by RT and NCIT was 38.4°C (33.4 - 40.3°C) and 36.3°C (30.8 - 40.0°C), respectively whilst, in cats, median (range) BT measured by RT and NCIT was 38.3°C (36.2 - 40.0°C) and 35.7°C (31.8 - 38.0°C) respectively. Temperature measured using an NCIT was weakly positively correlated with rectal temperature in dogs (Kendall's tau 0.154, $P = 0.004$), but there was no correlation in cats (Kendall's tau -0.01, $P = 0.91$). On Kappa analysis, a significant, albeit weak, agreement was seen between temperature measured by NCIT and RT in dogs (Kappa 0.05, $P < 0.001$), but not cats (Kappa -0.08, $P = 0.704$). Further, in both species, Bland-Altman plots revealed a tendency for the NCIT to under-read BT compared with RT in both dogs (mean bias -2.2°C [SD 1.51], $P < 0.001$) and cats (mean bias -2.7°C [SD 1.44], $P < 0.001$), with the degree of underread lessening as BT increased.

Given both poor correlation and agreement in BT measured by NCIT and RT (especially in cats), the NCIT cannot be recommended at the current time.

OT02

Short-term effects of early-age ovariohysterectomy in cats: A preliminary report

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The purpose of this study is to identify the effects of early-age ovariohysterectomy performed at 6 weeks and 8 weeks on body weight, some blood and physiological parameters and behavior in female kittens.

20 clinically healthy kittens were randomly allocated into two groups as Group I (neutered at 6 weeks, n=10) and Group II (neutered at 8 weeks, n=10). All kittens in groups were neutered by midline ovariohysterectomy under general anesthesia. Incision was made in the middle third and then both ovaries and cornu uteri were removed. Blood analyses (complete blood count, glucose, BUN, creatinin, calcium, phosphorus), physiological parameters (heart rate, respiratory rate, body temperature) and behavior (general activity, aggression, interest in humans and other kittens) were evaluated starting from one week before surgery until two weeks following the surgery.

During the study period, no significant differences were observed on body weight and, physiological and behavioral parameters in both groups. Although group I kittens were higher body weight compared to group II, body weight increase in both groups were similar during the study. The serum concentrations of glucose, BUN, creatinine, calcium, phosphorus and complete blood counts in each group were within reference ranges and no significant changes were observed throughout the study.

In conclusion, data obtained from this preliminary study revealed that body weight, blood and physiological parameters as well as behavior were similarly affected in both groups of kittens performed early-age ovariohysterectomy. Early-age ovariohysterectomy does not affect body weight, some blood and physiological profiles as well as behavior negatively.

OT03

Evaluation of the diagnostic utility of serum amyloid A in 206 diseased cats

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Various studies have previously reported the elevation of feline Serum amyloid A (SAA) in acute inflammatory diseases. However, there are limited studies that compared SAA with other markers to evaluate the diagnostic utility of feline SAA. In this study, SSA concentration was measured in cats with various diseases and compared them with the values of other markers for each disease to evaluate the diagnostic utility of feline SAA.

This study was a prospective study. A total of 206 cats diagnosed using various tests were included; 128 cases with acute inflammation and 78 cases without acute inflammation.

In the comparison of each disease group to the group without any disease, a significant increase of the SAA concentration was found in the infectious, respiratory, gastrointestinal, and urogenital disease groups ($P < 0.05$). No significant differences were observed among the disease groups.

A significant correlation was found between SAA concentration and white blood cell and neutrophil counts ($P < 0.0001$). In the comparison of acute inflammation cases with non-acute inflammation cases for each marker, a significantly strong correlation was found for SAA ($P < 0.0001$, sensitivity, 66%; specificity, 78%), and a significant correlation was found for white blood cell count ($P = 0.026$, sensitivity, 43%; specificity, 75%) and neutrophil count ($P = 0.036$, sensitivity, 44%; specificity, 72%).

As a marker of acute inflammation, SAA has a higher sensitivity and specificity than white blood cell and neutrophil counts. Therefore, SAA can be a useful inflammatory marker for the diagnosis of acute inflammation.

OT04

Developing an end-of-life survey to capture cause of death and reasons for euthanasia in dogs

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No published validated survey exists to capture cause of death and reasons for euthanasia from dog owners. Thus, the Dog Aging Project (DAP) created a novel survey titled End of Life Survey (EOLS). Here we describe EOLS development, qualitative assessment, and preliminary results. EOLS was created by a veterinary internist, veterinary resident, veterinary epidemiologist, and human epidemiologist. Item domains include cause of death, observed changes associated with aging or illness, perimortem veterinary care, quality of life assessment, and reasons for euthanasia. Items were optimized for clarity and comprehensiveness through several pilots with 42 total bereaved dog owners. The final survey was evaluated for accuracy and consistency by conducting verbal participant interviews with 28 bereaved dog owners before survey completion and comparing to subsequent survey responses to confirm reliability. Among interviewees, the most common causes of death were cancer (n=9; 33%), weakness secondary to old age (n=4; 15%), and kidney disease (n=3; 11%). Euthanasia was performed in most dogs (n=23; 85%), and the most common reason for euthanasia was poor quality of life (n=12; 52%). However, average quality of life in the two weeks prior to death was more commonly reported as good than bad (n=16; 59%). We have demonstrated EOLS is an objective instrument capable of accurately capturing detailed information from owners about companion dog death. This instrument will be deployed in the DAP longitudinal study of aging to elucidate cause of death as well as other important factors such as reasons for euthanasia and quality of life assessment.

OT05

Development and reliability of a survey instrument used to create a canine multimorbidity index

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A well-recognized prognostic indicator in human medicine is multimorbidity, which is defined as the co-occurrence of two or more chronic conditions. As such, physicians and researchers have developed numerous multimorbidity indices to improve their prediction of patient prognosis which enables them to identify interventional targets, prioritize treatment options, determine treatment efficacy, anticipate patient needs, and develop comprehensive health policies. No such index exists for veterinary patients. Here we report the development and reliability of a novel Canine Multimorbidity Index (CMI), which was modeled after current human multimorbidity indices and adapted for use in canine patients. The index was created by a veterinary internist, veterinary epidemiologist, and human health epidemiologist. Pilot studies to assess reliability by comparing inter- and intra-rater agreement were conducted with primary care veterinarians who utilized the instrument to score multimorbidities in selected canine medical records. The initial pilot revealed substantial intra-rater agreement (kappa = 0.69) but poor inter-rater agreement (kappa = 0.34). Verbal feedback from pilot testers indicated lack of clarity surrounding disease definitions (e.g., what duration constitutes "chronic" gastrointestinal disease?) and uncertainty of diagnosis requirements (e.g., is thoracic imaging required to diagnose chronic bronchitis?). The instrument was modified by adding specific definitions for indexed diseases and offering response variables that encompassed presumptive and definitive diagnoses. A pilot of the updated instrument is ongoing, and following pilot testing, multimorbidity data from a long-term longitudinal cohort, the Dog Aging Project, will be collected in order to assess CMI's ability to predict canine prognosis, namely mortality and clinical outcomes.

OT06

Prevalence of burnout, depression and excessive daytime sleepiness in academic veterinary intern and resident trainees

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Burnout, depression and sleep disturbances in the medical field are widespread and well-recognized, particularly in medical residency trainees. The objective of this study was to investigate the prevalence of burnout, depression and excessive daytime sleepiness in veterinary intern and resident trainees at a single academic institution.

In the fall of 2020, all small animal rotating interns, specialty interns, fellows and residents were invited to participate in a voluntary,

anonymized survey in association with a new institution-led house officer wellness program. Self-reported data on variables including average clinical duty hours, frequency of overnight phone calls and hours of sleep/night were collected, among others. Validated survey assessment tools administered included the Maslach Burnout Inventory, PHQ-2 depression screening question set, and Epworth Sleepiness Scale.

Response rate varied by question between 36/55 (65.4%) to 37/55 (67.3%). Eighteen of 36 (50%) respondents reported averaging >60 hours of clinical duty/week while only 6/36 (16.7%) reported sleeping 7-8 hours or more per night on average. Overnight work-related phone calls woke 19/36 (52.8%) respondents 1-2 times/night on average, with 4/36 (11.1%) and 3/36 (8.3%) respondents woken 3-4 or 4-5 times/night on average, respectively. Nineteen of 37 respondents (51.4%) met criteria for burnout. Fifteen of 36 (41.7%) had a positive PHQ-2 depression screen, while 17/37 (45.9%) had excessive daytime sleepiness based on an Epworth Sleepiness Scale score >10.

Veterinary intern and resident trainees have a high prevalence of burnout, depression and excessive daytime sleepiness, with poor sleep and high workload commonly reported. Targeted, proactive wellness initiatives are needed.

P01

Influence of Feeding on IL-2 expression and peak blood concentration in dogs administered oral cyclosporine

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Cyclosporine A (CsA), a calcineurin inhibitor used to treat numerous diseases in dogs, blunts interleukin-2 (IL-2) expression in activated T lymphocytes. A previous study has shown a decrease in oral bioavailability when CsA is administered with food in dogs, leading to the recommendation to give CsA when fasted. However, in practice, CsA is often administered with food to reduce gastrointestinal side effects. The objectives of this study were to compare the pharmacodynamic and pharmacokinetic effects of oral CsA when administered with and without food, and to determine whether its administration with food decreases gastrointestinal side effects in healthy dogs.

In a randomized, open-label, crossover design, 5 healthy dogs received oral modified cyclosporine (Atopica; median dose rate 3.5 mg/kg; range 3.4 - 3.9 mg/kg) with food for 7 days or one hour before food for 7 days (or vice versa), separated by a 21-day washout period. Peak CsA blood concentration and IL-2 mRNA expression by activated T-cells as assessed by quantitative reverse transcription PCR were measured at baseline, on the seventh day of each treatment phase, and after the washout period. Dog owners recorded daily appetite (scored from 0 - 4; from no food consumed to all food consumed) and fecal consistency scores (1 - 7; Purina fecal score), and any adverse events throughout the study.

The effectiveness of the washout period was assessed by comparing baseline IL-2 expression before each treatment phase using a Wilcoxon signed-rank test. The effect of treatment and time on IL-2

FIGURE 1: PERCENT SUPPRESSION OF IL-2 EXPRESSION IN DOGS FED VERSUS DOGS FASTED AT TIME OF CYCLOSPORINE ADMINISTRATION

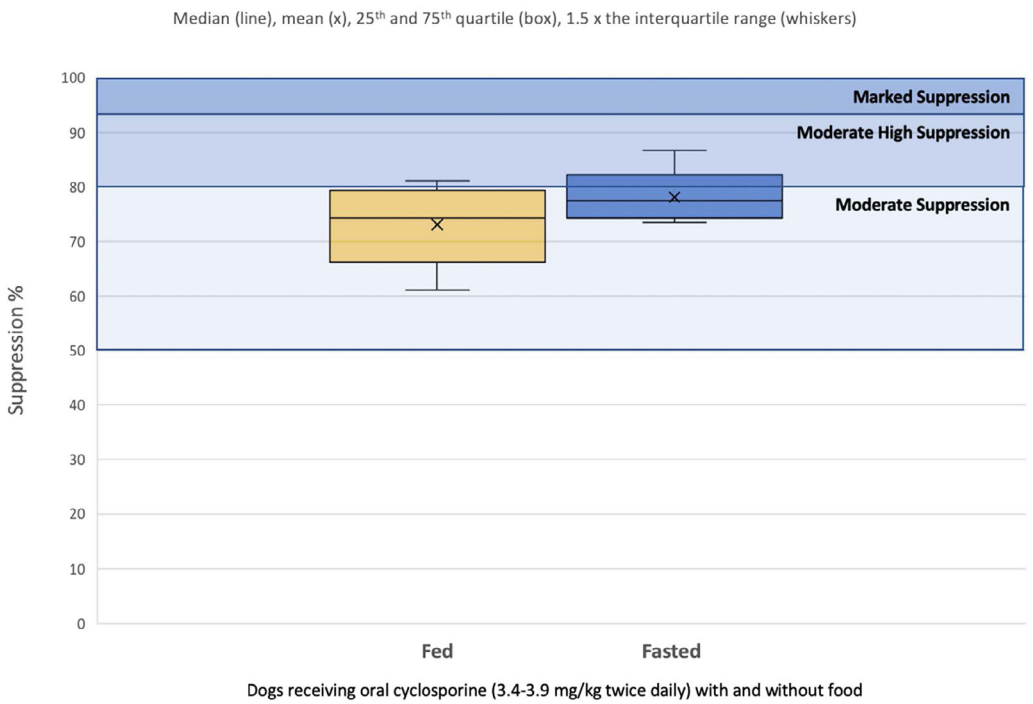
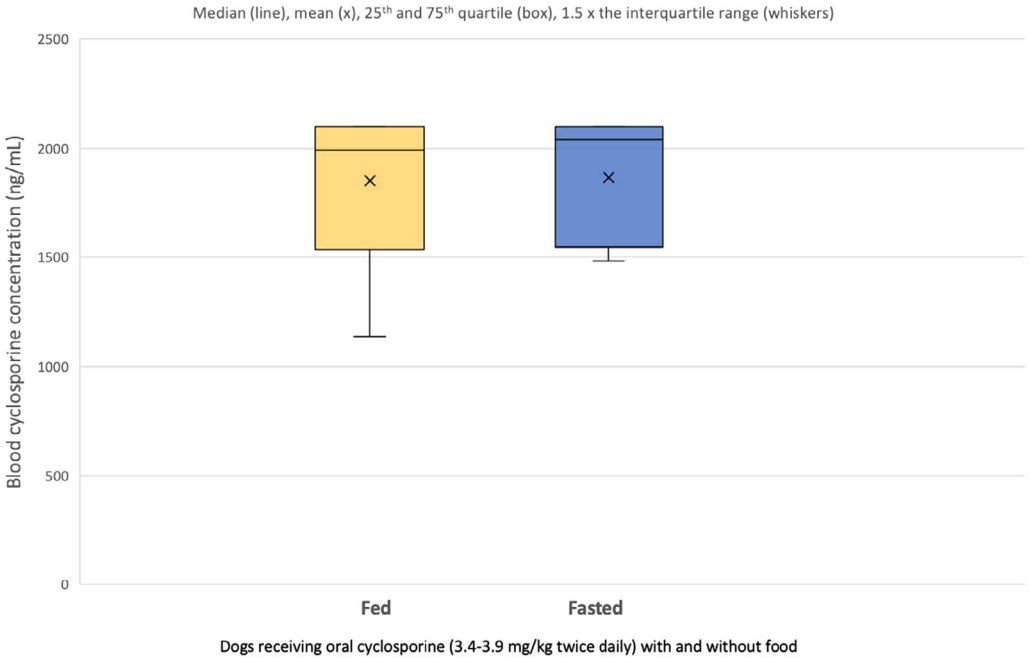


FIGURE 2: PEAK BLOOD CYCLOSPORINE CONCENTRATION IN DOGS FED VERSUS DOGS FASTED AT THE TIME OF CYCLOSPORINE ADMINISTRATION



expression was assessed by a linear mixed model. Daily scores for appetite and fecal consistency were averaged for each phase. The average of the daily scores and the blood CsA concentrations were analyzed using a method similar to the non-parametric Friedman's test using ranked data.

Lack of difference in baseline IL-2 expression between treatment phases confirmed the washout was adequate ($P = 0.3125$). CsA had an effect on all dogs ($P = 0.0001$), and all dogs achieved comparable post-treatment IL-2 expression corresponding with moderate to moderate-high levels of immunosuppression in both treatment phases {Figure 1}. There was no significant difference in IL-2 expression ($P = .9130$) or peak CsA blood concentration ($P = 0.7387$) {Figure 2} between dogs that received food at the time of CsA administration and dogs that were fasted.

Median (Interquartile Range) daily appetite and fecal consistency scores in dogs that were fed concurrently with cyclosporine were 4.0 (2.5) and 4.0 (5.0), and in fasted dogs were 4.0 (0) and 2.5 (2.0), respectively. An additional adverse gastrointestinal side effect (vomiting) was seen once in each treatment group. There was no significant difference in appetite ($P = 0.338$) and fecal consistency ($P = -0.8052$) scores between dogs that were fed or fasted at the time of drug administration.

In conclusion, in healthy dogs, modified cyclosporine can be administered with food without affecting its pharmacodynamic or pharmacokinetic profile, and can achieve levels of IL-2 expression that correspond to adequate immunosuppression. However, the administration of cyclosporine with food may not reduce gastrointestinal side effects.

P02

Genetic analysis of the cannabinoid receptor: 1 gene in three beagle dogs

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Cannabidiol (CBD) oil and associated products are rapidly growing in popularity not only in human medicine, but also in veterinary medicine. Cannabidiol's described therapeutic benefits are largely anecdotal, and efficacy in animals has yet to be confirmed. The goal of this research project is to identify genetic variation within the cannabinoid receptor 1 (*CBR-1*) gene in three healthy beagle dogs and to understand the role of this genetic variation in various disease processes and in variation of clinical response to CBD use.

Genomic DNA from whole blood samples was isolated, and the full length of the *CBR-1* gene was analyzed using the primer walking method. A reference of canine *CBR-1* genetic data was obtained from the NCBI database (accession no. NC_006594.3, accessed June 1, 2020) and was utilized to design primers with the NCBI Primer program. The *CBR-1* gene is 23,484 bp in length, comprising 1 intron and 2 exons. More than 200 primer pairs were designed, and from those, 94 succeeded in PCR amplification of the *CBR-1* gene fragments. The specificity of the PCR amplicons was confirmed with gel electrophoresis, and the amplicons were sent to the commercial Sanger sequencing service (Psomagen, Rockville, MD). Data generated from Sanger sequencing was in FASTA format and was transferred to Molecular

Evolutionary Genetics Analysis across computing platforms (Mega X) version 10.2.2 for mutation analysis.

More than 90% of the *CBR-1* gene sequences have been analyzed. We found two sets of mutations in the canine model. First, a silent mutation C.20543C >T was identified in 2 dogs located within the region of the X2 exon. Second, a novel 128 bp insertion was found in intron 1 at C.7623.

This is the first report of an intronic *CBR-1* mutation due to a short interspersed element insertion, and it is necessary to validate this insertion mutation at the RNA level. Characterization of this mutation's impact on transcription and protein translation is necessary to fully understand its influence on receptor function. Further studies are necessary to determine whether the variation in clinical outcomes associated with CBD treatment can be attributed to this genetic variation. In the future, we hope to see that genetic variation within the endocannabinoid system is utilized when forming a treatment plan for a patient.

P03

Comparison of pharmacodynamic effects of different modified cyclosporine formulations in dogs on comparable oral doses

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Cyclosporine is an immunosuppressive agent used to treat conditions such as atopy, inflammatory bowel disease, and immune-mediated hemolytic anemia in dogs. Modified cyclosporine is available in a variety of proprietary and generic formulations, with widely varying costs. The Mississippi State University Pharmacodynamic Laboratory has been evaluating an IL-2 based assay as a pharmacodynamic (PD) marker of drug effect over many years, and has tested more than 1,000 samples in dogs on a variety of cyclosporine formulations. This retrospective study was designed to utilize the available data to determine if there was any significant difference in PD effects between different modified cyclosporine formulations.

Samples from dogs on oral cyclosporine were submitted by veterinarians for measurement of IL-2 RNA expression in activated whole blood by reverse transcription quantitative PCR. The PD results from all dogs on modified cyclosporine within a dosage range of 4-6 mg/kg, PO, q12hr were extracted and compared to the PD results for different formulations using a linear mixed model with an alpha level of 0.05.

A total of 317 results were analyzed, with 269 dogs on proprietary (Novartis, Elanco, Atopica, Neoral) modified cyclosporine and 48 dogs on generic modified cyclosporine. Our analysis revealed no significant difference (p-value of 0.67) between PD assay results in dogs on proprietary or generic drug at the same oral dose rate.

Our results suggest that generic modified cyclosporine may be an acceptable substitute for the proprietary product in dogs. Use of generic modified cyclosporine can represent a significant cost saving for owners.

P04

Effect of inhaled albuterol on whole blood potassium concentrations in dogs

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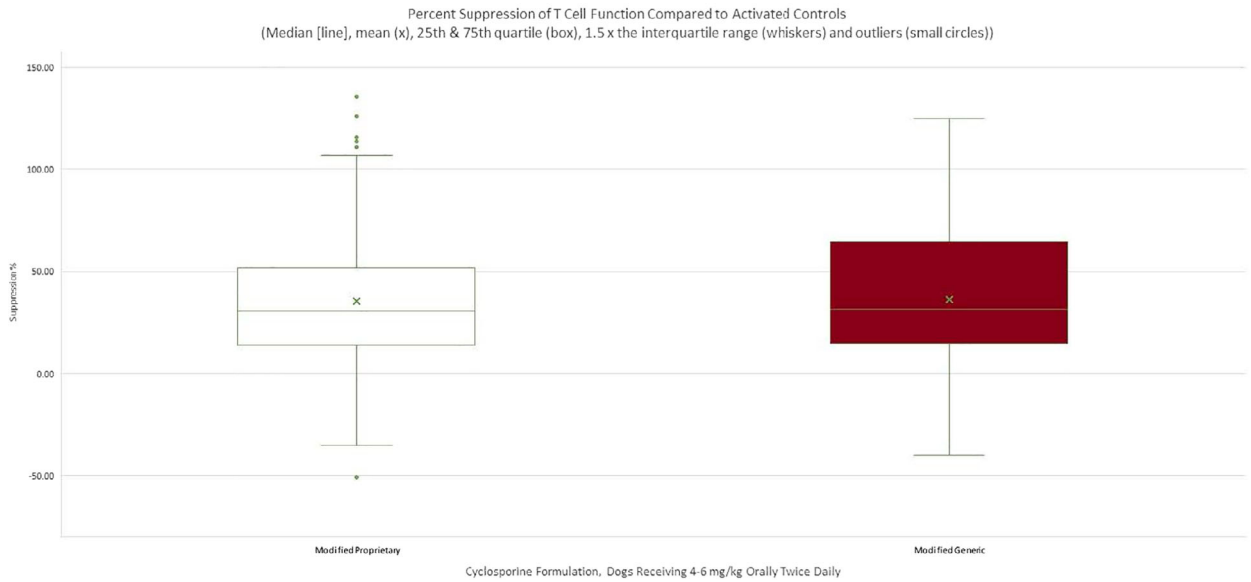
Inhaled albuterol is a common intervention used to treat moderate-to-severe hyperkalemia in humans; however, its effect on blood potassium concentrations in dogs has not been investigated.



This study had two objectives: (1) to determine if inhaled albuterol decreases blood potassium concentrations in dogs and if a difference in effect exists dependent upon albuterol dose, and (2) to assess if inhaled albuterol effects heart rate or blood glucose concentrations because tachycardia and hyperglycemia can occur in humans. Pharmaceutical grade albuterol sulfate (90 μg/actuation) was administered to normokalemic dogs via "AeroDawg" chamber device at a low-dose (90 μg) in Phase I and high-dose (450 μg) in Phase II executed seven days later. Whole blood potassium and glucose concentrations as well as heart rates were obtained at baseline and at 3, 5, 10, 15, 30, 60, 90, 120, 180, and 360 minutes (min) after the tenth breath following inhaler actuation. Whole blood potassium and glucose concentrations were measured on a blood gas analyzer. The nadir of the potassium concentrations for each dose was compared to its baseline using a two-tailed paired t-test, while sign rank tests were used to compare the peak heart rate and glucose concentrations to their respective baselines. Linear regression clustered by dog was used to determine the response based on dose. $P \leq 0.05$ was significant. Ten dogs were enrolled in this crossover clinical trial. The median low-dose delivered was 7.3 μg/kg (interquartile range [IQR], 4.9-8.7) and high-dose was 36.5 μg/kg (IQR, 24.6-43.7). The nadir of the low-dose occurred at 60 min with a mean potassium concentration of 4.07 mmol/L (standard deviation [SD], 0.40) that was significantly decreased from baseline (mean, SD; 4.30 mmol/L, 0.30; $t(9) = 2.40$, $P = 0.04$). The nadir of the high-dose occurred at 30 min with a mean potassium concentration of 3.96 mmol/L (SD, 0.39) that was decreased from baseline (4.34 mmol/L, 0.40; $t(9) = 2.22$, $P = 0.05$). The nadir potassium concentration decreased by 0.01 mmol/L for each one-unit μg/kg increase in dose ($P = 0.01$), controlling for baseline potassium concentration. There was not a significant difference in the peak heart rate or glucose concentration compared to baseline for either albuterol dose. Inhaled albuterol decreases blood potassium concentrations without significant changes in heart rate or glucose concentrations in dogs. Nadir potassium concentrations decreased with increasing dose of albuterol.

P05

Cytochrome p450 reaction phenotyping of itraconazole hydroxylation in dogs

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Itraconazole (ITZ) is a commonly used azole antifungal in dogs. The major plasma metabolite of ITZ, hydroxy-ITZ, is generated by the hepatic cytochrome P450 (CYP) enzyme system, but the specific isoform(s) responsible is unknown. Understanding species-specific metabolism of this drug is important in predicting toxicity as well as potential drug-drug interactions. Therefore, the purpose of this study was to identify the CYP enzyme(s) that hydroxylates ITZ in canine liver. Reaction conditions for hydroxy-ITZ generation were first optimized by incubating ITZ with dog liver microsomes. Then, ITZ was incubated with recombinant canine CYP enzymes (CYP1A1, CYP1A2, CYP1B1, CYP2B11, CYP2C21, CYP2C41, CYP2D15, CYP3A12, and CYP3A26) and Michaelis-Menten kinetics for ITZ hydroxylation were established for each. After incubation with ITZ, the following recombinant enzymes produced hydroxy-ITZ: CYP1A1, CYP1B1, CYP2B11, CYP2C41, CYP2D15, and CYP3A12. However, after correction for relative expression within canine liver microsomes, CYP2D15 and CYP3A12 appear to be the isoforms quantitatively important for ITZ hydroxylation. These isoforms also had k_m values (5.95 mM and 4.36 mM, respectively) most similar to the k_m in dog liver microsomes (5.43 mM). Therefore, CYP2D15 and CYP3A12 should be considered the top candidate enzymes for conversion of ITZ to hydroxy-ITZ in dog liver. Confirmatory studies using CYP isoform-specific inhibitors are currently underway.

P06

Preoperative administration of cannabidiol (CBD) in healthy dogs undergoing elective surgery in Colombia: 16 Cases

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Phytocannabinoids like Cannabidiol (CBD) is the main non-psychotropic phytocannabinoid studied for medicinal purposes and currently considered legal for medicinal use for both humans and animals in many countries in South America. Several studies in dogs about CBD considered its administration safe in a wide range of low or high doses in healthy animals, and is currently studied for its analgesic and antiepileptic effects in some canine chronic conditions. The delta-9-tetrahydrocannabinol (THC) is the main psychotropic phytocannabinoid, and is commonly found in a limited range of concentrations (< 1%) in several pharmaceutical preparations in conjunction with CBD. Although accidental exposure to high doses of THC is known to cause neurologic signs and depression in dogs, safety studies of dogs exposed to preparations with a THC:CBD ratio 1:20 reported few adverse effects in low doses (0.1 mg/kg). Veterinary prescription of CBD with low concentrations of THC (< 0,2%) is currently legal in Colombia. Although many dogs are increasingly being prescribed with phytocannabinoid compounds, no information exists about anesthetic considerations in elective surgical and anesthetic procedures. The objective of this study is retrospectively review the effect of a single dose administration of two pharmaceutical grade CBD oils on basic cardiorespiratory parameters and anesthetic recovery of dogs undergoing elective surgery. Complete medical and anesthesia records of healthy dogs of the spay/neuter program of Corporaciòn Universitaria Santa Rosa de Cabal UNISARC whose owners approved a preoperative single dose of CBD were included for analysis. Dogs were considered clinically healthy based on physical examination, complete hemogram and serum creatinine and ALT concentration. Two types of CBD oils were used, a full spectrum cannabis

oil with 200 mg/ml and THC up to 1 mg/ml (THC:CBD 1:20 ratio from Clever Leaves, Colombia) and a THC Free CBD oil with only 100 mg/ml (CBD only formula of Neviot ®, Procaps Colombia). Cannabidiol dose used was 2 mg/kg, the amount of product was calculated based on CBD concentration and randomly applied to dogs orally, 1 hour before any anesthetic premedication and closely monitored. Dogs then were routinely pre-medicated with standard care analgesia with meloxicam, tramadol and sedation with xylazine, after 20 minutes were induced and maintained with ether ketamine and/or propofol according to individual needs. Basic monitoring of heart rate, respiratory rate, pulse, rectal temperature was used during surgery and in recovery period. Once awake post-surgical pain was graded using the Glasgow Composite Pain Scale (GCPS) and any adverse effects or any other reaction was documented. Average heart rate, respiratory rate and temperature during surgery was calculated and compared between CBD and CBD:THC 20:1 doses using independent samples T test analysis for normal distributed variables and Mann-Whitney U test for nonparametric variables. Any adverse effect, anesthetic complication was documented. After retrospective analysis, 20 owners of dogs approved the CBD administration, 4 medical records were incomplete and excluded from the analysis and 16 dogs were included. There were 11 females and only 3 male dogs with a median age of 4,4 years (range 0,5-9 years). None of the dogs experience adverse effects during the first hour of observation. According to the type of CBD used, 7/16 animals received the THC:CBD ratio 1:20, no anesthesia complication was observed during surgery. In 9/16 dogs a CBD without THC was administered, only one dog experienced epileptiform activity during anesthetic induction. Average heart rate of dogs during surgery was not significantly different between CBD preparations ($p = 0,39$), also average temperature ($p = 0,52$) and respiratory rate ($p = 0,056$) during surgery was not statistically different between groups. During recovery 2 dogs in CBD: THC group show mild ataxia and only 1 dog in the CBD group. average pain GCPS scores were not statistically different between groups ($p = 0,32$). All dogs recovered well and discharged the same day and no one experienced moderate or severe adverse events. In this retrospective study a preoperative single oral administration of 2 mg/kg of Cannabidiol (CBD) oil in dogs was not associated with severe adverse effects. Some dogs experienced mild ataxia during recovery and no differences were observed between heart rate, respiratory rate and temperature between groups of CBD oils. The oil of THC:CBD with ratio of 1:20 was well tolerated in dogs receiving < 0,1 mg/Kg of THC. There was no difference between pain post-surgical pain scores between CBD oils.

R01

Comparing nasal sampling techniques for culture and mycoplasma polymerase chain reaction in canine nasal disease

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The most appropriate method to optimize aerobic culture and *Mycoplasma spp* PCR yield from dogs with nasal disease is unknown. The purpose of this study was to compare results of canine aerobic culture and *Mycoplasma spp* PCR by nasal swab, nasal flush, and nasal biopsy, submitted in Liquid Stuart-W (LSW), liquid Ames ESwab (ES), and red top tube with no additive (RT).

Twenty-nine client-owned dogs with naturally-occurring nasal disease were enrolled and underwent general anesthesia for sampling. Nasal swabs were performed using LSW and ES culturettes. A nasal flush was performed with recovered fluid submitted in a RT. Nasal biopsies were collected and submitted in an ES tube and RT. Each sample underwent aerobic culture and *Mycoplasma spp* PCR. Three blinded investigators interpreted culture results to determine clinical significance. Sampling techniques were compared using McNemar's and Cochrane Q analyses.

Primary bacterial rhinitis was rare (n=1). Nasal swabs resulted in more bacterial isolates interpreted to be clinically relevant and led to recommending antimicrobial treatment more often than nasal flushes or biopsies ($p < 0.001$). Nasal ES swab was most likely to identify clinically relevant bacteria and yielded more positive *Mycoplasma spp* PCR results than LSW swab ($p < 0.001$).

A full diagnostic workup should be considered rather than culture alone or empirical antimicrobial treatment for dogs with nasal disease. A nasal ES swab collected under anesthesia at the site of most severe disease should be sufficient to identify clinically relevant bacteria and *Mycoplasma* and to determine if antimicrobial therapy is warranted.

R02

Identification and prevalence of aerodigestive disease in dogs with hiatal hernia

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Aerodigestive disorders, hybrid disorders between the respiratory and gastrointestinal tracts, may present in the absence of GI signs. Hiatal hernia (HH) is an important aerodigestive disorder in brachycephalic dogs linked to respiratory pathology. The spectrum of other aerodigestive disorders and respiratory clinical signs in brachycephalic and non-brachycephalic dogs with HH is unknown.

Characterize clinical signs of aerodigestive disease in dogs with HH, compare clinical features between brachycephalic and non-brachycephalic dogs, and compare thoracic radiographs and videofluoroscopic swallow study (VFSS) for diagnosing HH.

Sixty-seven client-owned dogs with HH.

Medical records of dogs with HH presenting to the veterinary teaching hospitals at Auburn University and University of Missouri between 1/1/2009-12/31/2020 were retrospectively reviewed. Between

group comparisons were made by Mann-Whitney Rank Sum test ($P < 0.05$).

Dogs with HH presented with exclusively respiratory (17/67), mixed respiratory and GI (22/67), or GI signs alone (28/67). While brachycephalic dogs were not significantly more likely to present with respiratory clinical signs ($P=0.145$), they were statistically younger ($P< 0.001$), more likely to present in respiratory distress ($P=0.023$), and with radiographic evidence of aspiration pneumonia ($P< 0.001$) compared to non-brachycephalic dogs. Normal thoracic radiographs were noted in 5/12 dogs with clinical signs of respiratory disease. HH was detected by thoracic radiography in 54% of cases versus 95% by VFSS.

Dogs with HH may present with exclusively with respiratory signs. Respiratory signs may be more severe in brachycephalic compared to non-brachycephalic dogs. VFSS is superior to thoracic radiographs for the detection of HH in dogs.

R03

Distribution of allergens in cats with feline asthma: Clinical Experience in Ankara, Turkey

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Feline asthma (FA), a common serious lower respiratory system disease, is triggered by multiple aeroallergens and is driven by the stimulation of T helper 2 (TH2) response 1,2. TH2 mediated immunity results in increased production of cytokines that regulate the allergic inflammatory response and immunoglobulin E (IgE) production, leading to airway remodeling and obstruction1,2.

The aim of present study was to identify retrospectively the most prevalent allergens in cats with FA presented from 2017 to 2020. Study materials were consisted of 14 indoor cats diagnosed with asthma. Of 14 cats; 7 were female (1 intact and 6 spayed) and 7 were male (5 intact and 2 neutered). The mean age of cats at the time of diagnosis was 3.5 years old ranged from 6 months to 8 years. In vitro veterinary Polycheck allergy test was used to detect allergen-specific IgE concentrations (kU/l) against 20 different allergens in the serum samples. According the IgE concentrations (kU/l), it was classified in four levels; < 0.5 Level zero (negative); 0.5-2.0 Level 1 (Weak), 2.0-20 Level 2 (moderate), and >20 Level 3,4 (strong).

Study results indicated that allergen specific IgE levels revealed as moderate or strong positive reaction against to allergens of house dust mite (HDMAs) [Dermatophagoides farinae (n=7) and Dermatophagoides pteronyssinus (n=6)], fungi [Alternaria/Cladosporium (n=4) and Aspergillus/Pennicilium (n=3)], and mites [Acarus siro (n=4)]. Of 14, 11 cats developed a reaction against multiple allergens.

In summary, HDMAs seem to be the significant potential sources of sensitization that are underpinning the asthmatic response in cats. Therefore, dust free home environment can be the most important contributing factor in prevention and treatment of asthma disease in cats.

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R04

Development and evaluation of a novel respiratory airway model of a cat

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Feline asthma is treated with aerosolized drugs delivered through a chamber and mask. However, limited testing accounting for cats' unique anatomical features often leads to repurposed human devices being used without evidence. We describe the development of a novel Cat Anatomical Respiratory Inhalation Needs (CARIN) model that facilitates real-world characterization of airway deposition for optimization of the chamber/mask therapeutic approach in felines.

The CARIN model simulates the facial features, fur, and naso-oropharynx of a cat. The model was developed by Trudell Animal Health, and the Ontario Veterinary College and Department of Engineering at the University of Guelph from MRI and CT scans of an adult male domestic shorthair cat. 3D stereolithographic techniques were used to reconstruct the bony-structure and airways down to the carina. To mimic a mucosal lining a gel coating was used inside the airways. Physical models included replaceable fur to evaluate different fur types.

An initial validation experiment measured delivery of fluticasone proprionate (Flovent-HFA 110mcg) after a 2-second delay using a chamber (AeroKat*, Trudell Animal Health) with the CARIN model connected to a breathing simulator (ASL 5000: tidal volume of 50ml, 20-cycles/min, I:E 1:2). Drug was collected from all components and assayed by HPLC UV-Vis spectrophotometry.

Drug collected at the exit of the CARIN model is representative of deposition in the lower airways. Results showed 17.5 ± 2.1% of the label claim was delivered with the balance deposited in the device, interface, and model.

While parallels exist in human and feline respiratory systems, drug delivery mechanisms should be species specific. The CARIN model enables investigation into feline aerosol drug delivery (ex. chamber/mask design, fur type, and facial and airway features) that will facilitate optimization of therapeutic treatment. These preliminary results establish a baseline for further model refinement and evaluation of aerosol delivery improvements to cats. Future work is needed to corelate with in-vivo deposition data.

R05

Patient-specific three dimensional-printed nasopharyngeal stents in dogs

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The objective of this study was to develop and assess patient-specific 3D-printed nasopharyngeal stents in canine cadavers of varying skull types.

Prototype stents were developed by generating a digital 3D model of the nasopharynx for each dog based on computed tomography (CT) images. Three-piece molds were printed from these models and silicone was injected into the molds to produce the stents. Stents were placed in three cadavers using endoscopic- and fluoroscopic-guidance. Repeat CT scans were performed post-placement to analyze fit by use of the open polygon function in Osirix.

Mechanical testing was performed on cylindrical stents produced in the same manner and with identical wall thicknesses to the patient-specific stents in compression platens in accordance with standards defined by the American Society for Testing and Materials. These results were compared to commercially available stents currently used in the treatment of nasopharyngeal stenosis.

Stent placement was successful in all dogs and aided by modifications to the stent design during the development process. Goodness of stent fit varied among dogs. The stent in the smallest dog (7kg) was oversized causing in-folding of the stent.

The 3D-printed stents exhibited comparable stiffness and force required for compression compared to commercial stents, and also showed the least post-load deformation.

Production and placement of 3D-printed patient-specific nasopharyngeal stents is feasible and these stents offer comparable mechanical properties to commercial stents. Future studies in live animals are indicated.