### ORAL PRESENTATIONS – Thursday, June 5

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<td><strong>SMALL ANIMAL - CARDIOLOGY</strong></td>
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<tr>
<td>9:00 AM</td>
<td>C-1</td>
<td>Lance Visser</td>
<td>ECHOCARDIOGRAPHIC ASSESSMENT OF RIGHT VENTRICULAR SYSTOLIC FUNCTION IN CONSCIOUS HEALTHY DOGS: REPEATABILITY AND REFERENCE INTERVALS</td>
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<tr>
<td>9:15 AM</td>
<td>C-2</td>
<td>Lance Visser</td>
<td>ECHOCARDIOGRAPHIC ASSESSMENT OF RIGHT VENTRICULAR SYSTOLIC FUNCTION FOLLOWING A SINGLE DOSE OF PIMOBENDAN VERSUS ATENOLOL IN CONSCIOUS HEALTHY DOGS: A PROSPECTIVE, BLINDED, RANDOMIZED, CROSSOVER STUDY</td>
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<td>9:30 AM</td>
<td>C-3</td>
<td>Nicole LeBlanc</td>
<td>M-MODE, 2-DIMENSIONAL, AND 3-DIMENSIONAL VOLUMETRIC ANALYSIS OF LEFT ATRIAL SIZE AND FUNCTION IN A POPULATION OF NORMAL DOGS</td>
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<td>9:45 AM</td>
<td>C-4</td>
<td>Randolph Winter</td>
<td>BIOLOGIC VARIABILITY OF N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE AND CARDIAC TROPONIN I IN HEALTHY DOGS AND DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE</td>
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<tr>
<td>10:30 AM</td>
<td>C-5</td>
<td>Romain Javard</td>
<td>COMPARISON OF PEAK LEFT VENTRICULAR OUTFLOW TRACT VELOCITY AND INDEXED EFFECTIVE ORIFICE AREA IN GOLDEN RETRIEVER PUPPIES TO PREDICT DEVELOPMENT OF SUBAORTIC STENOSIS IN ADULTS</td>
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<tr>
<td>10:45 AM</td>
<td>C-6</td>
<td>Rebecca Cervenec</td>
<td>INOTROPIC EFFECT OF L-LYSINE IN HEALTHY CATS</td>
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<tr>
<td>11:00 AM</td>
<td>C-7</td>
<td>Sara Johns</td>
<td>HIGH-LEVEL SEROTONIN-BINDING IN SUBPOPULATION OF HIGHLY-ACTIVATED PLATELETS IN CAVALIER KING CHARLES SPANIELS WITH MYXOMATOUS MITRAL VALVE DISEASE</td>
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<tr>
<td>11:15 AM</td>
<td>C-8</td>
<td>Signe Cremer</td>
<td>HIGH-LEVEL SEROTONIN-BINDING IN SUBPOPULATION OF HIGHLY-ACTIVATED PLATELETS IN CAVALIER KING CHARLES SPANIELS WITH MYXOMATOUS MITRAL VALVE DISEASE</td>
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<tr>
<td>11:30 AM</td>
<td>C-9</td>
<td>Marlos Goncalves Sousa</td>
<td>BLOOD LACTATE IN DOGS WITH CONGESTIVE HEART FAILURE OWING TO MITRAL VALVE DISEASE</td>
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Bolded type indicates ACVIM Resident Research Award eligibility.
11:45 AM  C-10  Amanda Coleman  ATTENUATION OF THE PRESSOR RESPONSE TO EXOGENOUS ANGIOTENSIN BY ANGIOTENSIN RECEPTOR BLOCKERS IN NORMAL DOGS

12:00 PM  C-11  Arine Pellegrino  SENSITIVITY AND SPECIFICITY OF ELECTROCARDIOGRAPHIC EXAMINATION IN DETECTING VENTRICULAR OR ATRIAL OVERLOADS IN PERSIAN CATS WITH HYPERTROPHIC CARDIOMYOPATHY

12:15 PM  C-12  Johnny Li  METABOLOMIC AND TRANSCRIPTOMIC PROFILING OF DEGENERATIVE MITRAL VALVE DISEASE IN DOGS

BREAK

4:30 PM  C-13  Elizabeth Rozanski  PROSPECTIVE EVALUATION OF THE ENDOTHELIN-1 RECEPTOR ANTAGONIST BOSENTAN IN FELINE ARTERIAL THROMBOEMBOLISM

4:45 PM  C-14  Rachel James  ALDOSTERONE SERUM CONCENTRATION AND SPIRONOLACTONE (SP) PHARMACOKINETICS FOLLOWING ORAL ADMINISTRATION OF SP IN CATS WITH HEART FAILURE: INTERIM RESULTS OF THE SEISICAT STUDY

5:00 PM  C-15  Kota Kizaki  ECHOCARDIOGRAPHIC PARAMETERS INDICATED DISPLACEMENT OF PAPILLARY MUSCLES AS A CAUSE OF SYSTOLIC ANTERIOR MOTION OF THE MITRAL VALVE IN CATS

5:15 PM  C-16  Tatsuyuki Osuga  VITAMIN D STATUS AND DISEASE PROGRESSION IN CANINE CHRONIC MITRAL VALVULAR HEART DISEASE

5:45 PM  C-18  Chih Chien Lu  ENDOTHELIAL TO MESENCHYMAL TRANS-DIFFERENTIATION IN CANINE MYXOMATOUS MITRAL VALVE DISEASE

6:00 PM  C-19  Jennifer Myers  PHARMACOKINETICS AND PHARMACODYNAMICS OF THE FACTOR XA INHIBITOR APIXABAN IN CATS: A PILOT STUDY

** Also See Small Animal Cardiology abstracts C-20 - C-27, Friday, June 6, 8:00 AM - 10:00 AM.

SMALL ANIMAL – NEUROLOGY

9:00 AM  N-1  Amanda Taylor  INFLAMMATORY CHEMOKINES AND CYTOKINES IN CEREBROSPINAL FLUID OF DOGS WITH ACUTE SPINAL CORD INJURY


9:30 AM  N-3  Renee Barber  IDENTIFICATION OF GENETIC RISK LOCI IN MALTESE DOGS WITH NECROTIZING MENINGOENCEPHALITIS

9:45 AM  N-4  Daniel Krull  EVALUATION OF TRANSDERMAL ADMINISTRATION OF PHENOBARBITAL IN HEALTHY CATS

BREAK

10:30 AM  N-5  Dani Powers  DYNAMIC LUMBOSACRAL INSTABILITY; CLINICAL EVALUATION AND OWNER PERCEIVED OUTCOME OF A NOVEL APPROACH FOR SURGICAL DISTRACTION-STABILIZATION OF L7-S1: 51 CASES (2008-2013)

10:45 AM  N-6  Rob Daniel  FELINE MYOTONIA CONGENITA: CLINICAL, ELECTROPHYSIOLOGIC AND HISTOPATHOLOGIC CHARACTERISTICS WITH A NOVEL MUTATION IN CLCN-1

11:00 AM  N-7  Mario Dolera  NECK PAIN IN DOGS AND CATS
11:15 AM N-8 Mario Dolera
ATLANTO-OCCIPITAL DISLOCATION IN 4 DOGS: MRI FINDINGS AND SURGICAL TREATMENT

11:30 AM N-9 Daegi An
DYNAMIC SUSCEPTIBILITY CONTRAST IMAGING OF NORMAL CANINE BRAIN

11:45 AM N-10 Betty Chow
COMPARATIVE GENOMIC AND HISTOLOGIC ANALYSIS OF CANINE AND HUMAN MENINGIOMAS

12:00 PM N-11 Maria Perez
DESIGN, DEVELOPMENT, AND TESTING OF AN INTRACRANIAL PRESSURE BOLT FOR USE IN VETERINARY PATIENTS

12:15 PM N-12 Amanda Jurkoshek
THE EFFECT OF ANGLE SLICE ACQUISITION ON COMPUTED TOMOGRAPHIC CERVICAL VERTEBRAL COLUMN MORPHOMETRY IN GREAT DANES

BREAK

2:15 PM N-13 Brian Zanghi
COGNITIVE DOMAINS IN THE DOG: INDEPENDENCE OF MEMORY, SELECTIVE ATTENTION AND MOTOR LEARNING

2:30 PM N-14 Juliet Armstrong
CERVICAL VERTEBRAL TRABECULAR BONE MINERAL DENSITY IN GREAT DANES WITH AND WITHOUT CERVICAL SPONDYLOMYELOPATHY

2:45 PM N-15 Paula Martin-Vaquero
BODY CONFORMATION IN GREAT DANES WITH AND WITHOUT CLINICAL SIGNS OF CERVICAL SPONDYLOMYELOPATHY

3:00 PM N-16 Naris Thengchaisri
ENTROPY INDICES ARE PREDICTIVE OF AN AWAKE RESPONSE ELICITED DURING SEVOFLURANE ANESTHESIA IN DOGS

3:15 PM N-17 Devon Hague
CONGENITAL SPONGIFORM LEUKODYSTROPHY IN TWO FEMALE LITTERMATE GERMAN SHEPHERD PUPPIES

SMALL ANIMAL - ONCOLOGY

9:00 AM O-1 Lindsay Donnelly
PRECLINICAL EVALUATION OF COMBINATION LUTETIUM 177 BB2R ANTAGONIST TARGETED RADIOTHERAPY AND CHEMOTHERAPY FOR THE TREATMENT OF CASTRATION RESISTANT PROSTATE CANCER (VCS Award Winner)

9:15 AM O-2 Chris Fulkerson
PHARMACOKINETICS AND TOXICITY OF THE NOVEL ORAL DEMETHYLATING AGENT ZEBULARINE IN LABORATORY DOGS AND DOGS WITH TRANSITIONAL CELL CARCINOMA (VCS Award Winner)

9:30 AM O-3 Vanessa Rizzo
THE EFFECT OF PALLADIA ON THYROID FUNCTION

9:45 AM O-4 Melissa Tollett
A RETROSPECTIVE ANALYSIS OF HYPOFRACTIONATED RADIOTHERAPY FOR TREATMENT OF SOLID TUMORS IN DOGS

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10:30 AM O-5 Susie Kang
DNA DAMAGE IS A FEATURE OF FELINE INJECTION SITE SARCOMA

10:45 AM O-6 Roberta Portela
INVESTIGATING THE PRO-TUMORIGENIC EFFECTS OF TRANSFORMING GROWTH FACTOR BETA 1 (TGFβ1) IN CANINE OSTEOSARCOMA

11:00 AM O-7 Aubrey Bishop
COMPARISON OF HEMOSTATIC AND CLINICAL FINDINGS IN DOGS WITH SPLENIC HEMATOMA VERSUS HEMANGIOSARCOMA: 71 CASES (2006-2013)

11:15 AM O-8 Ingrid Goodman
TREATMENT OF CANINE MULTICENTRIC NON-INDOLENT T-CELL LYMPHOMA USING VELCAP-TSC PROTOCOL: 75 CASES (2003-2013)

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<td>2:15 PM</td>
<td>O-9</td>
<td>IGHV USAGE AND SOMATIC HYPERMUTATION ANALYSIS IN CANINE B CELL CHRONIC LYMPHOCYTIC LEUKEMIA</td>
<td>Stacey George</td>
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<td>2:30 PM</td>
<td>O-10</td>
<td>BONE TUMORS IN DOGS AND CATS: A PERSPECTIVE FROM ANKARA UNIVERSITY (2001-2010)</td>
<td>Irem Gul Sancak</td>
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<td>2:45 PM</td>
<td>O-11</td>
<td>FLOW CYTOMETRIC ANALYSIS OF CD4+CD25+FOXP3+ REGULATORY T CELLS IN 8 DOGS WITH CANINE LYMPHOMA</td>
<td>Dae-seung Baek</td>
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<td>O-12</td>
<td>CLINICAL CHARACTERISTICS OF THE RETINOID DERIVED NOVEL EXCIPIENT XR-17</td>
<td>Henrik von Euler</td>
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<td><strong>SMALL ANIMAL - ENDOCRINOLOGY</strong></td>
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<td>9:00 AM</td>
<td>EN-1</td>
<td>COMPARISON OF TWO DOSES FOR ACTH STIMULATION TESTING IN DOGS SUSPECTED OF OR TREATED FOR HYPERADRENOCORTICISM</td>
<td>Charles Aldridge</td>
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<td>EN-2</td>
<td>HYPOCOBALAMINEMIA AND COBALAMIN DEFICIENCY IN CATS WITH HYPERTHYROIDISM</td>
<td>Brian Geesaman</td>
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<td>9:30 AM</td>
<td>EN-3</td>
<td>PHARMACOLOGY OF THE GLP-1 ANALOG LIRAGLUTIDE IN HEALTHY CATS</td>
<td>Melanie Hall</td>
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<td>9:45 AM</td>
<td>EN-4</td>
<td>THE PHARMACOLOGY OF EXENATIDE EXTENDED-RELEASE IN HEALTHY CATS</td>
<td>Adam Rudinsky</td>
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<td>10:30 AM</td>
<td>EN-5</td>
<td>SERUM AND PLASMA GLUCOSE MEASUREMENTS WITH A POINT-OF-CARE GLUCOMETER IN DOGS AND CATS</td>
<td>Barbara Tauk</td>
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<td>10:45 AM</td>
<td>EN-6</td>
<td>NPH AND LISPRO INSULIN FOR TREATMENT OF DOGS WITH DIABETES MELLITUS</td>
<td>Abigail Bertalan</td>
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<td>11:00 AM</td>
<td>EN-7</td>
<td>MICRNORNA BIOMARKERS OF CANINE DIABETES MELLITUS</td>
<td>Caroline Mansfield</td>
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<td>EN-8</td>
<td>ANALYSIS OF P450 SIDE-CHAIN CLEAVAGE ENZYME AUTOANTIBODIES IN DOGS AFFECTED WITH HYPOADRENOCORTICISM</td>
<td>Alisdair Boag</td>
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<td>EN-9</td>
<td>HYPOADRENOCORTICISM IN COCKER SPANIELS IS ASSOCIATED WITH POLYMORPHISMS IN THE CYTOTOXIC T-LYMPHOCYTE-ANTIGEN 4 PROMOTER</td>
<td>Alisdair Boag</td>
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<td>EN-10</td>
<td>EXOGENOUS THYROTOXICOSIS SECONDARY TO THE CONSUMPTION OF COMMERCIALY AVAILABLE ALL-MEAT DOG FOOD OR TREATS</td>
<td>Michael Broome</td>
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<td>12:00 PM</td>
<td>EN-11</td>
<td>CONCENTRATIONS OF POLYBROMINATED DIPHENYL Ethers (PBDES) IN MATCHED SAMPLES OF SERUM AND HOUSE-DUST OF HYPERTHYROID CATS</td>
<td>Keshuan Chow</td>
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<td>EN-12</td>
<td>USE OF VETERINARIAN-DIRECTED VARIABLE INSULIN DOSAGE CHART RESULTS IN IMPROVED GLYCEMIC CONTROL WHEN COMPARED WITH FIXED INSULIN DOSE</td>
<td>Sara Ford</td>
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<td><strong>SMALL ANIMAL – GASTROENTEROLOGY</strong></td>
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<td>2:15 PM</td>
<td>GI-1</td>
<td>DETERMINATION OF THE SENSITIVITY AND SPECIFICITY OF A RAPID UREASE TEST FOR THE DETECTION OF GASTRIC HELICOBACTER SPECIES IN DOGS WITH CLINICAL SIGNS OF UPPER GASTROINTESTINAL DISEASE</td>
<td>Bryn Hoffman</td>
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<td>SAFETY OF ADMINISTRATION OF 25% HUMAN ALBUMIN TO DOGS DIAGNOSED WITH A PROTEIN-LOSING ENTEROPATHY</td>
<td>Kimberly Loyd</td>
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<td>GI-3</td>
<td>ROLE OF OROPHARYNGEAL BACTERIA IN ESOPHAGEAL FEEDING TUBE PERISTOMAL INFECTIONS IN CATS</td>
<td>Rebekah Mack</td>
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3:00 PM GI-4 Karin Allenspach  NO CORRELATION BETWEEN MUCOSAL IMMUNOGLOBULIN A POSITIVE PLASMA CELL NUMBERS AND TLR5 GENOTYPES IN GERMAN SHEPHERD DOGS


3:30 PM GI-6 Rachel Lavoue  LIPASE, cPLI, AND cTLI IN THE DOGUE DE BORDEAUX. IS THERE A NEED FOR BREED-SPECIFIC REFERENCE INTERVALS?

3:45 PM GI-7 Blake Guard  SERUM AND URINE METABOLITES IN DOGS WITH ACUTE DIARRHEA

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4:30 PM GI-8 Blake Guard  THE FECAL MICROBIOME IN DOGS WITH ACUTE DIARRHEA

4:45 PM GI-9 Nicole Luckschander-Zeller  FLOW CYTOMETRY AS A DIAGNOSTIC TOOL IN GASTROINTESTINAL ENDOSCOPIC BIOPSIES IN DOGS WITH CHRONIC ENTEROPATHY

5:00 PM GI-10 Liza Koster  PREVALENCE OF ACUTE PANCREATITIS IN CANINE BABESIOSIS CAUSED BY BABESIA ROSSI - A MODEL FOR THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

5:15 PM GI-11 Sarah Caddy  IDENTIFICATION OF ASTROVIRUSES ASSOCIATED WITH CANINE GASTROENTERITIS IN THE UK

5:30 PM GI-12 SueYee Lim  QUANTITATIVE CONTRAST-ENHANCED ULTRASONOGRAPHIC ASSESSMENT OF NATURALLY OCCURRING PANCREATITIS IN DOGS

5:45 PM GI-13 Rosana Lopes  ANALYTICAL VALIDATION OF TARGETED AMINO ACID ANALYSIS IN DOG SERUM BY GAS-CHROMATOGRAPHY/ MASS SPECTROMETRY

6:00 PM GI-14 Rosana Lopes  EVALUATION OF SIX DIFFERENT AMINO ACID CONCENTRATIONS IN SERUM OF HEALTHY DOGS AND DOGS WITH HYPOCOBALAMINEMIA

SMALL ANIMAL – HEMATOLOGY

9:00 AM HM-1 Jillian Haines  IN VITRO AND IN VIVO ASSESSMENT OF PLATELET FUNCTION IN HEALTHY DOGS DURING EXPOSURE TO LOW-DOSE ASPIRIN

9:15 AM HM-2 Kimberly Ho  REFERENCE INTERVAL GENERATION OF THREE PLATELET FUNCTION TESTS IN HEALTHY CATS

9:30 AM HM-3 Anna Threlfall  INVOLVEMENT OF CYTOTOXIC T-LYMPHOCYTE ANTIGEN 4 PROMOTOR POLYMORPHISMS IN GENETIC SUSCEPTIBILITY TO PRIMARY IMMUNE MEDIATED HAEMOLYTIC ANAEMIA IN COCKER SPANIELS

9:45 AM HM-4 Kimberly Ho  ASSESSMENT OF PLATELET FUNCTION IN HEALTHY CATS IN RESPONSE TO COMMONLY PRESCRIBED ANTI-PLATELET DRUGS USING THREE POINT-OF-CARE PLATELET FUNCTION TESTS

BREAK

10:30 AM HM-5 Margaret Scuderi  OUTCOME BASED ON TREATMENT PROTOCOL IN PATIENTS WITH PRIMARY CANINE IMMUNE-MEDIATED THROMBOCYTOPENIA: 58 CASES (2006-2013)

10:45 AM HM-6 Rachel Blake  EICOSANOIDS LEVELS IN STORED UNITS OF CANINE PACKED RED BLOOD CELLS

11:00 AM HM-7 Alice Defarges  STERNAL BONE MARROW ASPIRATION IN 69 DOGS WITH CLINICAL DISEASE (2008-2013)
11:15 AM HM-8 Stephanie Goulet  
PREVALENCE OF THE DAL BLOOD TYPE IN DOBERMAN PINCHERS AND IN CANINE BLOOD DONORS

11:30 AM HM-9 John Thomason  
EFFECTS OF IN VITRO EXPOSURE OF CANINE PLATELETS TO PENTOXIFYLLINE ON PLATELET AGGREGOMETRY

11:45 AM HM-10 Haruhiko Maruyama  
IDENTIFICATION OF A MISSENSE MUTATION IN THE FACTOR XII GENE IN SIBLING CATS WITH FACTOR XII DEFICIENCY

12:00 PM HM-11 Eun-Pil Son  
HEMATOPOIETIC STEM AND PROGENITOR CELLS MOBILIZATION AFTER ADMINISTRATION OF RECOMBINANT HUMAN GRANULOCYTE-COLON STIMULATING FACTOR IN DOGS

12:15 PM HM-12 Elizabeth Spangler  
INVESTIGATION OF THE EFFECTS OF ANEMIA ON THROMBOELASTOGRAPHY

SMALL ANIMAL - INFECTIOUS DISEASE

9:00 AM ID-1 Beth Licitra  
CHARACTERIZATION OF CANINE CORONAVIRUS ASSOCIATED WITH CANINE NEONATAL ENTERITIS AND MORTALITY IN THE UNITED STATES

9:15 AM ID-2 Mayur Patel  
COMPARATIVE EFFICACY OF FELINE LEUKEMIA VIRUS INACTIVATED WHOLE VIRUS VACCINE AND CANARYPOX VIRUS-VECTORED VACCINE BY MODERN MOLECULAR ASSAYS AND CONVENTIONAL PARAMETERS

9:30 AM ID-3 Sarah Kirk  
EFFICACY OF AZITHROMYCIN AND COMPOUNDED ATOVACUONE FOR TREATMENT OF BABESIA GIBSONI IN A LARGE-SCALE DOGFIGHTING CASE

9:45 AM ID-4 J Scott Weese  
EVALUATION OF THE ORAL AND CONJUNCTIVAL MICROBIOTA IN CATS WITH FELINE IMMUNODEFICIENCY VIRUS INFECTION AND UNINFECTED CONTROLS

BREAK

10:30 AM ID-5 Staci Cannon  
INFECTIOUS DISEASES IN DOGS RESCUED DURING DOG FIGHTING INVESTIGATIONS

10:45 AM ID-6 Anne M Gaynor  
IS CANINE CIRCOVIRUS ASSOCIATED WITH DISEASE?

11:00 AM ID-7 Leah Cohn  
LONGITUDINAL STUDY OF VECTOR-BORNE PATHOGEN SEROPREVALENCE IN MID-MISSOURI DOGS

11:15 AM ID-8 Marit Gaastra Maaland  
MINOCYCLINE PHARMACOKINETICS AND PHARMACODYNAMICS FOR TREATMENT OF METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS INFECTIONS IN DOGS

11:30 AM ID-9 Silvia Corrêa  
MOLECULAR DETECTIONS OF A HIGHLY PATHOGENIC PIROPLASMA IN DOGS: RANGELIA VITALI

11:45 AM ID-10 Richard Goldstein  
PERFORMANCE COMPARISON OF SNAP® 4Dx® PLUS AND ACCUPLEX®4 FOR THE DETECTION OF ANTIBODIES TO B. BURGDORFERI AND A. PHAGOCYTOPHILUM

12:00 PM ID-11 Lauren Demos  
PRELIMINARY DATA ARISING FROM A NOVEL FLORESCENCE IN SITU HYBIDIZATION ASSAY TO DETECT FELINE PIROPLASMATAVIRUSES

12:15 PM ID-12 A. Rick Alleman  
PREVALENCE OF BORRELIA SPP. IN HOST-SEEKING TICK POPULATIONS AND WHITE-TAILED DEER IN NORTH CENTRAL FLORIDA

SMALL ANIMAL - NEPHROLOGY/UROLOGY

2:15 PM NU-1 Jenny Cho  
ASSESSMENT OF IN VITRO OXALATE DEGRADATION BY LACTOBACILLUS SPECIES
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<td>2:30 PM</td>
<td>NU-2</td>
<td>Maura Duffy</td>
<td>COMPARISON BETWEEN URINE PROTEIN:CREATININE RATIOS OF SAMPLES OBTAINED AT HOME AND IN A HOSPITAL SETTING: A PILOT STUDY</td>
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<td>2:45 PM</td>
<td>NU-3</td>
<td>Jacqueline Gest</td>
<td>IRON PARAMETERS OF CATS WITH CHRONIC KIDNEY DISEASE</td>
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<td>3:00 PM</td>
<td>NU-4</td>
<td>Sean Hulsebosch</td>
<td>EVALUATION OF CANINE PANCREATIC LIPASE (SPEC CPL) AND LIPASE ACTIVITY IN AN EXPERIMENTAL CANINE MODEL OF ACUTE KIDNEY INJURY</td>
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<td>3:15 PM</td>
<td>NU-5</td>
<td>Romain Javard</td>
<td>ACUTE PHASE PROTEINS AND IRON METABOLISM IN FELINE CHRONIC KIDNEY DISEASE</td>
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<td>3:30 PM</td>
<td>NU-6</td>
<td>Catherine Vachon</td>
<td>PASSIVE URETERAL DILATION AND URETEROSCOPY FOLLOWING URETERAL STENT PLACEMENT IN NORMAL DOGS</td>
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<td>3:45 PM</td>
<td>NU-7</td>
<td>William Whitehouse</td>
<td>RELATIONSHIP BETWEEN URINARY F2-ISOPROSTANES AND IRIS STAGE OF CATS WITH CHRONIC KIDNEY DISEASE</td>
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<td>4:30 PM</td>
<td>NU-8</td>
<td>Esther Bijsmans</td>
<td>PSYCHOMETRIC VALIDATION OF A GENERAL HEALTH QUALITY OF LIFE TOOL FOR CATS (CatQoL) AND ITS USE TO COMPARE HEALTHY AND CKD CATS</td>
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<td>4:45 PM</td>
<td>NU-9</td>
<td>Andreanne Cleroux-Gaboury</td>
<td>ASSOCIATION BETWEEN UROLITHS AND RENAL DISEASE IN CATS</td>
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<td>5:00 PM</td>
<td>NU-10</td>
<td>Michelle Domingues</td>
<td>COMPARING DIRECTLY MEASURED BLOOD PRESSURE FROM ARTERIES IN DIFFERENT ANATOMICAL LOCATIONS IN ANESTHETIZED DOGS</td>
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<td>5:15 PM</td>
<td>NU-11</td>
<td>Yasuhito Kuwahara</td>
<td>ENDOSCOPE-ASSISTED SURGICAL NEPHROLITHOTOMY FOR NEPHROLITHIASIS IN 11 DOGS</td>
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<td>5:30 PM</td>
<td>NU-12</td>
<td>Rachel Lavoue</td>
<td>CHARACTERIZATION OF PROTEINURIA IN DOGUE DE BORDEAUX DOGS USING ELECTROPHORESIS AND URINARY BIOMARKERS</td>
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<td>5:45 PM</td>
<td>NU-13</td>
<td>Erika Meler</td>
<td>TREATMENT OF CONGENITAL URETROVESICULAR JUNCTION STENOSIS BY ENDOSCOPIC LASER-ABLATION IN DOGS: 10 CASES (2010-2013)</td>
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<td>6:00 PM</td>
<td>NU-14</td>
<td>Jessica Quimby</td>
<td>CERENIA® FOR THE MANAGEMENT OF VOMITING AND INAPPETENCE ASSOCIATED WITH CHRONIC KIDNEY DISEASE IN CATS</td>
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SMALL ANIMAL - NUTRITION/METABOLISM

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<tr>
<th>Time</th>
<th>Session</th>
<th>Presenters</th>
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<tr>
<td>9:00 AM</td>
<td>NM-1</td>
<td>Jeffrey Brockman</td>
<td>GENOME WIDE ANALYSIS LEADS TO A NOVEL METHOD TO INCREASE HYDRATION AND REDUCE URINE SPECIFIC GRAVITY IN THE CAT</td>
</tr>
<tr>
<td>9:15 AM</td>
<td>NM-2</td>
<td>Yuanlong Pan</td>
<td>INTERMITTENT CALORIC RESTRICTION IS MORE EFFECTIVE THAN CHRONIC CALORIC RESTRICTION IN PROMOTING WEIGHT LOSS IN OVERWEIGHT CATS</td>
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<tr>
<td>9:30 AM</td>
<td>NM-3</td>
<td>Ziad Ramadan</td>
<td>METABOLOMICS PROFILING OF AGED, MEMORY-IMPAIRED DOGS</td>
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<tr>
<td>9:45 AM</td>
<td>NM-4</td>
<td>Angela Witzel</td>
<td>SUCCESSFUL WEIGHT REDUCTION IN SEVERELY OBESE CATS</td>
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<td>10:00 AM</td>
<td>NM-5</td>
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<td>10:45 AM</td>
<td>NM-6</td>
<td>Brian Zanghi</td>
<td>PM-SUPPLEMENTATION WITH MELATONIN, ZINC, AND HAEMATOCOCCUS PLUVIALIS SELECTIVELY IMPROVES ATTENTION AND MOTOR LEARNING IN AGED, MEMORY-IMPAIRED DOGS</td>
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11:00 AM NM-7 Matthew Jackson DIETS CONTAINING BIOACTIVE FOOD FACTORS AMELIORATE AGE-RELATED SEQUELAE AND IMPROVE COAT QUALITY

11:15 AM NM-8 Myriam Hester FERMENTABLE FIBERS MODULATE AMINO-ACID METABOLISM THROUGH INTESTINAL FERMENTATION IN HEALTHY DOGS FED A LOW-PROTEIN DIET

11:30 AM NM-9 Dennis Jewell A REDUCED CALORIE, HIGH FIBER FOOD WITH ADDED COCONUT OIL, L-CARNITINE, LIPOIC ACID, LYSINE, AND LEUCINE INCREASES BASAL METABOLIC RATE IN OVERWEIGHT AND OBESE DOGS

11:45 AM NM-10 Dennis Jewell A REDUCED CALORIE, HIGH FIBER FOOD WITH ADDED COCONUT OIL, L-CARNITINE, LYSINE, AND LEUCINE INCREASES BASAL METABOLIC RATE IN OVERWEIGHT AND OBESE CATS

12:00 PM NM-11 Kiran Panickar IMPROVEMENT IN CIRCULATING MARKERS OF HEALTH IN AGING DOGS WITH DIET SUPPLEMENTED WITH ANTI-INFLAMMATORY AND ANTIOXIDANT INGREDIENTS

SMALL ANIMAL - OTHER

4:45 PM OT-1 Rory Applegate SENSITIVITY AND SPECIFICITY OF TISSUE IMPEDANCE MEASUREMENT INTERPRETATION FOR SPINAL NEEDLE PLACEMENT IN THE COXOFEMORAL JOINTS OF CADAVERIC DOGS

5:00 PM OT-2 Eileen Jenkins THE EFFECTS OF ORAL METRONIDAZOLE AND DOXYCYCLINE ADMINISTRATION ON OLFACTORY DETECTION CAPABILITIES OF EXPLOSIVE DETECTION DOGS

5:15 PM OT-3 Belle Nibblett COMPARISON OF SERUM CORTISOL IN CATS EXAMINED IN A CLINIC VERSUS A HOME SETTING

5:30 PM OT-4 Sigal Klainbartt PERIPHERAL AND CENTRAL VENOUS BLOOD GLUCOSE CONCENTRATIONS IN ACUTE ARTERIAL THROMBOEMBOLISM IN DOGS AND CATS

5:45 PM OT-5 Margaret Gruen CLINICAL TRIAL APPLICATION OF A CLINICAL PHENOMENON: DETERIORATION FOLLOWING WITHDRAWAL OF ACTIVE MEDICATION FOR THE TREATMENT OF CHRONIC PAIN IN CATS WITH DEGENERATIVE JOINT DISEASE

6:00 PM OT-6 Molly Shelton MODE OF ACTIVATION SIGNIFICANTLY IMPACTS THROMBOELASTOGRAPHIC RESULTS AND ASSAY VARIABILITY

6:15 PM OT-7 James Gaynor EFFICACY AND SAFETY OF A SUBCUTANEOUS HIGH DOSE PROPRIETARY FORMULATION OF BUPRENORPHINE FOR 72-HOUR CONTROL OF POST-OPERATIVE PAIN ASSOCIATED WITH ONYCHECTOMY IN CATS

6:30 PM OT-8 James Gaynor EFFICACY AND SAFETY OF A SUBCUTANEOUS HIGH DOSE PROPRIETARY FORMULATION OF BUPRENORPHINE FOR 72-HOUR CONTROL OF POST-OPERATIVE PAIN ASSOCIATED WITH SOFT TISSUE SURGERY IN CATS

SMALL ANIMAL - RESPIRATORY

2:15 PM R-1 Julie Trzil LONGITUDINAL EVALUATION OF EFFECTS OF INTRAVENOUS MESENCHYMYAL STEM CELLS IN A FELINE MODEL AFTER ESTABLISHMENT OF CHRONIC ASTHMA

2:30 PM R-2 Stacy Burdick EVALUATION OF SHORT AND LONG TERM OUTCOMES USING VARIOUS INTERVENTIONAL TREATMENT OPTIONS FOR NASOPHARYNGEAL STENOSIS IN DOGS AND CATS
2:45 PM  R-3  Kathleen Chow  SCINTIGRAPHIC ASSESSMENT OF DEPOSITION OF RADIOLABELLED FLUTICASONE DELIVERED FROM A METERED DOSE INHALER AND A NEBULIZER IN 10 HEALTHY DOGS: A PROSPECTIVE CROSS-OVER PILOT STUDY

3:00 PM  R-4  Nai-Chieh Liu  USING WHOLE-BODY BAROMETRIC PLETHYSMOGRAPHY TO QUANTIFY BRACHYCEPHALIC AIRWAY FUNCTION

3:15 PM  R-5  Ana Paula Sarraff-Lopes  FUNGAL PNEUMONIA BY CHAETOMIUM SP. IN TWO DOGS

3:30 PM  R-6  Vanessa Vrolyk  LUNG INFLAMMATION ASSOCIATED WITH ACUTE NECROTIZING PANCREATITIS IN DOGS

3:45 PM  R-7  Carol Reinero  SIMILARITIES IN LUNG REMODELING AS ASSESSED BY COMPUTED TOMOGRAPHY BETWEEN EXPERIMENTAL AND SPONTANEOUS FELINE ASTHMA

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4:30 PM  R-8  Mariana Petito  BLOOD GAS VALUES IN LEAN AND OBESE GERIATRIC DOGS

EQUINE

9:00 AM  E-1  Tiago Afonso  CARDIOVASCULAR EFFECTS OF ORAL AND INTRAVENOUS PIMOBENDAN IN HEALTHY ADULT HORSES

9:15 AM  E-2  Gayle Hallowell  CORRELATES BETWEEN POST-MORTEM AND ECHOCARDIOGRAPHIC MEASUREMENTS OF THE RIGHT VENTRICLE IN HORSES

9:30 AM  E-3  Gayle Hallowell  EVALUATION OF ACID-BASE AND ELECTROLYTE DISTURBANCES USING A FENCL-STEWART APPROACH AND CORRELATES TO SURVIVAL IN HORSES WITH COLIC

9:45 AM  E-4  Crystal Hoffman  GLUCOCORTICOID RECEPTOR DENSITY AND BINDING AFFINITY DETERMINATION IN EQUINE PERIPHERAL BLOOD MONONUCLEAR CELLS USING FLOW CYTOMETRY

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10:30 AM  E-5  Annie Kullmann  CHARACTERIZATION OF INSULIN RECEPTOR AND INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR IN THE DIGITAL LAMINAR TISSUE OF ADULT HORSES AND MIXED-BREED PONIES

10:45 AM  E-6  James Prutton  PRE-ANALYTICAL STABILITY OF ADRENOCORTICOTROPIC HORMONE IN BLOOD FROM HORSES WITH AND WITHOUT PITUITARY PARS INTERMEDIA DYSFUNCTION

11:00 AM  E-7  Sarah Smith  COMPARISON OF THE IN-FEED GLUCOSE TOLERANCE TEST AND THE ORAL SUGAR TEST

11:15 AM  E-8  Dianne McFarlane  ACTH RELEASE FOLLOWING TRH STIMULATION IN THRIFTY HORSES COMPARED TO METABOLICALLY NORMAL HORSES

11:30 AM  E-9  Dianne McFarlane  PHARMACOKINETICS AND PHARMACODYNAMICS OF PERGOLIDE MESYLATE AFTER CHRONIC ORAL ADMINISTRATION IN HORSES WITH PPID

11:45 AM  E-10  Sarah Smith  SUSPECTED ACORN TOXICITY IN NINE HORSES

12:00 PM  E-11  Katja Shell  EFFECTS OF METRONIDAZOLE AND FLUNIXIN MEGLUMINE ON EQUINE RIGHT DORSAL COLONIC MUCOSA
12:15 PM   E-12  Joy Tomlinson  

BREAK

2:15 PM   E-13  Nadia Saklou  
COMPARISON OF ACTIVATED HYDROGEN PEROXIDE AND PEROXYGEN DISINFECTANTS AS MISTING APPLICATIONS

2:30 PM   E-14  Marta Barba  
EXPERIMENTAL TRANSMISSION OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN HORSES BY HOUSE FLIES

2:45 PM   E-15  Siddra Hines  
VARIATION IN THEILERIA EQUI DRUG SUSCEPTIBILITY IN VITRO AND THE POTENTIAL ROLE OF ATP-BINDING CASSETTE TRANSPORTERS AS MEDIATORS OF PARASITIC DRUG RESISTANCE

3:00 PM   E-16  Kathleen Mullen  
ACUTE AND DELAYED ADVERSE REACTIONS IN HORSES THAT UNDERWENT GENERAL ANESTHESIA AND CERVICAL MYELOGRAPHY

3:15 PM   E-17  Carrie Finno  
CENTRAL NERVOUS SYSTEM TRANSCRIPTOME PROFILING IN EQUINE NEUROAXONAL DYSTROPHY

3:30 PM   E-18  Stephanie Valberg  
HISTOLOGIC AND BIOCHEMICAL ANALYSIS OF GLYCOCEN IN HORSES WITH TYPE 2 POLYSACCHARIDE STORAGE MYOPATHY

3:45 PM   E-19  Patty Weber  
A HIGH GLYCEMIC DIET ALTERS ADIPOSE TISSUE GENE EXPRESSION OF ADIPOKINES AND PRO-INFLAMMATORY MARKERS IN LEAN AND OBESE PONIES

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4:30 PM   E-20  Diego Gomez  
COMPARISON OF ANION GAP AND STRONG ION GAP AS PREDICTORS OF PLASMA L-LACTATE CONCENTRATION AND THEIR ASSOCIATION WITH OUTCOME IN 81 HOSPITALIZED FOALS

4:45 PM   E-21  Kate Hepworth-Warren  
RESPONSE TO CRYSTALLOID AND COLLOID RESUSCITATION IN HEALTHY NEONATAL FOALS

5:00 PM   E-22  Alex Bianco  
PHARMACOKINETICS OF KETOROLAC TROMETHAMINE, A POTENT NON-STEROIDAL ANTI-INFLAMMATORY DRUG, IN HEALTHY ADULT HORSES

5:15 PM   E-23  Laszlo Hunyadi  
PHARMACOKINETICS OF A SUB-THERAPEUTIC AND THERAPEUTIC DOSE OF DICLAZURIL ADMINISTERED ORALLY AS A PELLETED TOP DRESS IN ADULT HORSES

5:30 PM   E-24  Eva McElligott  
PHARMACOKINETICS OF ORAL CHLORAMPHENICOL BASE IN ADULT HORSES AT 50 MG/KG DOSAGE

5:45 PM   E-25  Marion Allano  
INFLUENCE OF SHORT TRANSPORTATION ON TRACHEAL ASPIRATES AND BRONCHOALVEOLARlavages IN HORSES

6:00 PM   E-26  Amy Stieler  
MACROLIDE INDUCED HYPERTHERMIA IN FOALS: ROLE OF IMPAIRED SWEAT RESPONSES

FOOD ANIMAL

9:00 AM   F-1  Kate O’Conor  
ARGININE STIMULATION TESTING IN LLAMAS WITH EPINEPHRINE-INDUCED ELEVATIONS IN SERUM LIPIDS

9:15 AM   F-2  Diego Gomez  
QUANTITATIVE CONTRIBUTION OF PLASMA D- AND L-LACTATE TO THE STRONG ION GAP AND THE EFFECT OF PHYSICOCHEMICAL VARIABLES ON PLASMA BICARBONATE AND pH IN 42 CALVES WITH DIARRHEA
9:30 AM  F-3 Chelsea Warren  USE OF A NOVEL COLLECTION METHOD TO MEASURE ACTIVE DRUG
CONCENTRATIONS IN THE GI TRACT OF CATTLE TO ASSESS RISK OF
ANTIMICROBIAL RESISTANCE IN ENTERIC BACTERIA

9:45 AM  F-4 Sarah Jacob  TOTAL PROTEIN AND IGG CONCENTRATION IN HOLSTEIN CALVES: A
COMPARISON OF MEASUREMENT TOOLS, BLOOD COLLECTION TUBES,
AND TIME TO ANALYSIS

BREAK

10:30 AM  F-5 Pamela Fry  GENOTYPIC IDENTIFICATION OF STAPHYLOCOCCI FROM CASES OF
SUBCLINICAL BOVINE MASTITIS PREVIOUSLY IDENTIFIED AS
STAPHYLOCOCCUS HYicus

10:45 AM  F-6 Suzanne Genova  DECLINE OF MATERNAL ANTIBODIES TO CAPRINE ARTHRITIS
ENCEPHALITIS VIRUS AS MEASURED BY COMPETITIVE ELISA

11:00 AM  F-7 Vincent Doré  HYPERKETONEMIA AS AN EARLY DIAGNOSTIC TOOL TO PREDICT
PREGNANCY TOXEMIA IN DAIRY GOATS

11:15 AM  F-8 Andrea Lear  EVALUATION OF CHOLESTEROL AND VITAMIN E CONCENTRATIONS IN
PERIPARTURIENT ALPacas AND NURSING CRIAs

11:30 AM  F-9 Andrea Lear  EVALUATION OF A MODEL DEMONSTRATING MITIGATION OF
NOCICEPTIVE RESPONSE TO OXYTETRACYCLINE INJECTION SITE
INFLAMMATION BY FLUNIXIN MEGLUMINE IN DAIRY COWs

11:45 AM  F-10 Sébastien Buczinski  THE ACCURACY OF BLOOD CARDIAC TROPONIN I AND L-LACTATE
CONCENTRATIONS TO PREDICT SURVIVAL IN DOWNER DAIRY COWs

12:00 PM  F-11 William Gilsenan  OCULAR MANIFESTATIONS OF SEPSIS AS A PROGNOSTIC INDICATOR FOR
NEONATAL CRIAs

12:15 PM  F-12 Irem Gul Sancak  CHONDROGENESIS OF EQUINE AND OVINE MESENCHYMAL STEM CELLS
IN VITRO

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4:30 PM  F-13 Joseph Smith  PHARMACOKINETICS OF FLUNIXIN MEGLUMINE IN PLASMA AND MILK OF
DOMESTIC GOATS (CAPRA AEGAGRUS HIRCUS) FOLLOWING SINGLE
SUBCUTANEOUS DOSING

4:45 PM  F-14 Kevin Washburn  PLASMA CONCENTRATIONS OF CHLortetRACYCLINE FOLLOWING ORAL
ADMINISTRATION TO SHEEP FOR EIGHT DAYS

5:00 PM  F-15 Christine Cocquyt  PHARMACOKINETICS OF MOXIDECTIN IN ALPacas FOLLOWING ORAL
AND SUBCUTANEOUS ADMINISTRATION

5:15 PM  F-16 Douglas Owens  COMPARISON OF FENTANYL PLASMA BIOAVAILABILITY BETWEEN TWO
DIFFERENT DRUG DELIVERY METHODS IN SINCLAIR MINIATURE PIGs

5:30 PM  F-17 Roland Schubotz  EVALUATION OF PROTEIN CONTENT IN EXHALED BREATH CONDENSATE
OF HOLSTEIN CALVES AND THEIR POTENTIAL USE AS A MARKER FOR
AIRWAY INFLAMMATION

5:45 PM  F-18 Ignacio Idoate  ACUTE PHASE PROTEINS IN NATURALLY OCCURRING RESPIRATORY
DISEASE OF FEEDLOT CATTLE: A NOVEL APPROACH TO DIAGNOSIS

6:00 PM  F-19 Joanne Hewson  COMPARISON OF THORACIC ULTRASONOGRAPHY AND
BRONCHIAL VEOlar LAVAGE FLUID ANALYSIS TO POST-MORTEM
EXAMINATION FOR DETECTION OF SUBCLINICAL BRONCHOPNEUMONIA
IN DAIRY CALVES

6:15 PM  F-20 Damien Achard  CEREBROSPINAL FLUID ANALYSIS IN REcUMBENT DAIRY CATTLE WITH
OR WITHOUT SPINAL CORD DAMAGE
### ORAL PRESENTATIONS – Friday, June 6

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<tbody>
<tr>
<td>8:00 AM</td>
<td>C-20</td>
<td>Steven Rosenthal</td>
<td>NT-proBNP and TROPONIN I LEVELS AS SCREENING BIOMARKERS IN GREAT DANES</td>
</tr>
<tr>
<td>8:15 AM</td>
<td>C-21</td>
<td>Hannah Hodgkiss-Geere</td>
<td>ACQUISITION AND VALIDATION OF VASOVAGAL Tonus INDEX IN A POPULATION OF HEALTHY ADULT DOGS</td>
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<tr>
<td>8:30 AM</td>
<td>C-22</td>
<td>Naris Thengchaisri</td>
<td>ABDOMINAL OBESITY IS PREDICTIVE OF HEART DISEASE IN DOGS</td>
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<tr>
<td>8:45 AM</td>
<td>C-23</td>
<td>Philip Fox</td>
<td>RELATIONSHIP OF CANINE NT-PROBNP TO HEART FAILURE CLASSIFICATION AND RESPIRATORY DISTRESS</td>
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<td>9:00 AM</td>
<td>C-24</td>
<td>Philip Fox</td>
<td>MITRAL VALVE APPARATUS MORPHOLOGY IN FELINE HYPERTROPIC AND DILATED CARDIOMYOPATHY</td>
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<td>9:15 AM</td>
<td>C-25</td>
<td>Sonja Fonfara</td>
<td>CHANGES OF HEART RATE AND RHYTHM IN HARBOR SEAL PUPS DURING REHABILITATION</td>
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<td>9:30 AM</td>
<td>C-26</td>
<td>Ioannis Giatis</td>
<td>USE OF TORSEMIDE IN 17 CATS WITH ADVANCED CONGESTIVE HEART FAILURE</td>
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<tr>
<td>9:45 AM</td>
<td>C-27</td>
<td>Chul Park</td>
<td>ALTERATIONS OF TISSUE DOPPLER AND STRAIN IMAGING IN TYPE 1 DIABETES MELLITUS DOGS WITHOUT HEART DISEASE</td>
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**SMALL ANIMAL - HEPATOLOGY**

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<td>8:00 AM</td>
<td>HP-1</td>
<td>Savannah Craig</td>
<td>SERUM C-REACTIVE PROTEIN AND S100A12 CONCENTRATIONS IN DOGS WITH HEPATIC DISEASE</td>
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<tr>
<td>8:15 AM</td>
<td>HP-2</td>
<td>Faith Buckley</td>
<td>ADRENAL FUNCTION IN CHOLESTATIC CATS</td>
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<tr>
<td>8:30 AM</td>
<td>HP-3</td>
<td>Julie Callahan Clark</td>
<td>CHOLECYSTOCENTESIS IN CATS WITH SUSPECTED HEPATOBILIARY DISEASE</td>
</tr>
<tr>
<td>8:45 AM</td>
<td>HP-4</td>
<td>Jonathan Lidbury</td>
<td>SERUM TISSUE INHIBITOR OF METALLOPROTEINASE-1 CONCENTRATIONS IN DOGS WITH HEPATIC DISEASE</td>
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<tr>
<td>9:00 AM</td>
<td>HP-5</td>
<td>Luca Malfassi</td>
<td>HEPATIC ENCEPHALOPATHY: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY (MRS)</td>
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<tr>
<td>9:15 AM</td>
<td>HP-6</td>
<td>Keitaro Morishita</td>
<td>CONTRAST-ENHANCED ULTRASONOGRAPHY OF HEPATIC VEIN CAN ASSESS THE ARTERIALIZATION OF HEPATIC VEIN FLOW IN A CANINE PORTAL HYPERTENSION MODEL</td>
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<td>9:30 AM</td>
<td>HP-7</td>
<td>Manabu Sakai</td>
<td>AZATHIOPRINE AND PREDNISOLONE TREATMENT IN LABRADOR RETRIEVERS WITH CHRONIC HEPATITIS</td>
</tr>
<tr>
<td>9:45 AM</td>
<td>HP-8</td>
<td>Keita Sato</td>
<td>MULTIDETECTOR COMPUTED TOMOGRAPHIC CHOLANGIOGRAPHY AND LAPAROSCOPY FOR THE DIAGNOSIS OF GALLBLADDER AGENESIS IN DOGS</td>
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SMALL ANIMAL - IMMUNOLOGY

8:00 AM IM-1 Carolyn Gross EXPRESSION OF HER2NEU EPIDERMAL GROWTH FACTOR RECEPTOR IN CANINE OSTEOSARCOMA: IMMUNOHISTOCHEMICAL SCORING SYSTEM AND ASSOCIATION WITH PROGNOSIS

8:15 AM IM-2 Jonathan Fogle PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF DIFFERENCES BETWEEN MONOCYTES IN DOGS WITH AND WITHOUT OSTEOSARCOMA

8:30 AM IM-3 Matthew Kornya CORRELATION BETWEEN ORAL HEALTH AND RETROVIRUS TEST RESULTS IN CATS

8:45 AM IM-4 Sidonie Lavergne IL-8 RECEPTOR EXPRESSION IN IMMORTALIZED DOG KERATINOCYTES (AND SPLEEN) AND HOW ANTIBIOTICS AFFECT IT

9:00 AM IM-5 Maciej Parys THE ROLE OF INFγ AND TNFα IN IN VITRO IMMUNOMODULATION OF FELINE MESENCHYMAL STEM CELLS

9:15 AM IM-6 Meredith Sherrill ALTERATIONS IN LYMPHOCYTE AND MONOCYTE POPULATIONS IN RESPONSE TO INFESTATION OF AMBLYOMMA AMERICANUM ON CATS

9:30 AM IM-7 Meredith Sherrill INFESTATION BY AMBLYOMMA AMERICANUM ON CATS LEADS TO INCREASED LEUKOCYTE PHAGOCYTOSIS

9:45 AM IM-8 Dae-seung Baek FLOW CYTOMETRIC ANALYSIS OF CD4+CD25+FOXP3+ REGULATORY T CELLS IN 7 DOGS WITH GENERALIZED DEMODICOSIS

SMALL ANIMAL - PHARMACOLOGY

8:00 AM P-1 Kimberly Loyd IN VITRO EFFECTS OF YUNNAN BAIYAO (YB) ON COAGULATION

8:15 AM P-2 Samantha Middleton ALTERNATE DAY DOSING OF ITRACONAZOLE IN HEALTHY CATS

8:30 AM P-3 Rikki Fitzpatrick PHARMACOKINETICS OF SUBCUTANEOUS ONDANSETRON IN HEALTHY GERIATRIC CATS, CATS WITH CHRONIC KIDNEY DISEASE AND CATS WITH LIVER DISEASE

8:45 AM P-4 Leah Ferguson MIRTAZAPINE TOXICITY IN CATS: RETROSPECTIVE STUDY OF 104 CASES (2006 - 2011)

9:00 AM P-5 Leah Cohn PHARMACOKINETICS OF MINOCYCLINE IN DOMESTIC CATS

9:15 AM P-6 Deepti Deshpande THE PHARMACOGENETIC EFFECTS OF THE MDR1-1DELTA MUTATION ON DOG SEDATION

9:30 AM P-7 Sidonie Lavergne A RETROSPECTIVE STUDY ON PHENYPROPANOLAMINE TOXICITY IN 169 LABRADOR RETRIEVER DOGS

9:45 AM P-8 Kamoltip Thungrat ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF CLINICAL ISOLATES OF E. COLI ISOLATED FROM DOGS AND CATS IN THE UNITED STATES
## POSTER PRESENTATIONS

**On Display:** Thursday, June 5, 7:00 AM – 7:00 PM  
Friday, June 6, 7:00 AM – 7:30 PM

**Attended by ALL Authors Eligible for ACVIM Resident Research Awards:**  
Thursday, June 5, 9:50 AM – 10:30 AM  
Friday, June 6, 9:50 AM – 10:30 AM

**Attended by ALL Authors – Wine & Cheese Reception:**  
Friday, June 6, 6:00 PM – 7:30 PM

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<td><strong>SMALL ANIMAL - CARDIOLOGY</strong></td>
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<tr>
<td>C-28</td>
<td>Aleksandra Domanjko Petrić</td>
<td>PLASMA VITAMIN E, PLASMA MALONDIALDEHYDE AND SERUM NT-proBNP IN DOGS WITH VARIOUS STAGES OF HEART FAILURE</td>
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<td>C-29</td>
<td>Mark Oyama</td>
<td>PROGRESSION OF “SILENT” MYXOMATOUS MITRAL VALVE DISEASE IN NORFOLK TERRIERS</td>
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<td>C-30</td>
<td>Jorge Silva Filho</td>
<td>MEASUREMENT OF LEFT ATRIAL VOLUME BY BIPLANE SIMPSON’S METHOD IN DOGS WITH AND WITHOUT DEGENERATIVE MITRAL VALVE DISEASE</td>
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<td>C-31</td>
<td>Jorge Silva Filho</td>
<td>ECHOCARDIOGRAPHIC EVALUATION IN DOGS WITH SEPSIS</td>
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<tr>
<td>C-32</td>
<td>Jorge Silva Filho</td>
<td>CORRELATION BETWEEN LEFT ATRIAL VOLUME, OBTAINED BY BIPLANE SIMPSON’S METHOD, AND LEFT VENTRICULAR DIASTOLIC VARIABLES IN DOGS WITH AND WITHOUT DEGENERATIVE MITRAL VALVE DISEASE</td>
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<tr>
<td>C-33</td>
<td>Jorge Silva Filho</td>
<td>CONTINUOUS INFUSION VERSUS BOLUS DOSING OF FUROSEMIDE FOR DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE</td>
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<tr>
<td>C-34</td>
<td>Anthony Carr</td>
<td>EFFECT OF MAINTENANCE IV FLUID THERAPY ON NT PRO-BNP CONCENTRATION IN HEALTHY DOGS</td>
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<td>C-35</td>
<td>Aparecido Camacho</td>
<td>TRANSMISSION ELECTRON MICROSCOPY AND IMMUNOHISTOCHEMISTRY DEMONSTRATE MYOCARDIAL REMODELING IN RABBITS WITH DOXORUBICIN-INDUCED DILATED CARDIOMYOPATHY</td>
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<tr>
<td>C-37</td>
<td>Fernando Rosa</td>
<td>LEFT VENTRICULAR RADIAL STRAIN, STRAIN RATE, VELOCITY AND DISPLACEMENT BY TWO-DIMENSIONAL VELOCITY VECTOR IMAGING IN NON-SEDATED HEALTHY RABBITS</td>
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<tr>
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ECHOCARDIOGRAPHIC ASSESSMENT OF RIGHT VENTRICULAR SYSTOLIC FUNCTION IN CONSCIOUS HEALTHY DOGS: REPEATABILITY AND REFERENCE INTERVALS. L.C. Visser, B.A. Scansen, N.V. Brown, K.E. Schober, J.D. Bonagura. Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

We sought to examine the feasibility and repeatability of tricuspid annular plane systolic excursion (TAPSE), RV fractional area change (FAC), pulsed wave tissue Doppler-derived lateral tricuspid annular longitudinal peak systolic velocity (S'), and speckle tracking-derived longitudinal global strain (Strain) and strain rate (SR) of the RV free-wall and to determine reference intervals for these right ventricular (RV) functional indices in healthy dogs.

Eighty dogs underwent two echocardiographic studies between 3 and 20 days apart. Repeatability was determined by comparing the coefficient of variation (CV) from the two time points. Intra-observer and inter-observer variability was determined from 6 randomly selected studies. Reference intervals were determined as the average of the two values from each time point and calculated based on mean ± SD with 90% confidence intervals around the reference limits. Associations between RV function indices and body weight, sex, age and heart rate were performed using linear regression.

Assessment of RV function was feasible in all dogs. Repeatability, intra- and inter-observer variability were considered adequate in all CVs were <10%. Proposed reference intervals were: TAPSE 13.0 ± 5.5 mm, FAC 46.5 ± 15.1%, S' 13.4 ± 9.2 cm/s, Strain -28.4 ± 8.1%, SR -3.3 ± 1.9 s⁻¹, TAPSE (R² = 0.74) and S' (R² = 0.30) were strongly-to-modestly correlated with body weight. Strain Rate exhibited a moderate negative correlation (R² = 0.37) with body weight. No other clinically significant correlations were identified.

The reported indices of RV function are feasible and warrant further study in dogs with cardiovascular disease.

C-2
ECHOCARDIOGRAPHIC ASSESSMENT OF RIGHT VENTRICULAR SYSTOLIC FUNCTION FOLLOWING A SINGLE DOSE OF PIMOBENDAN VERSUS ATENOLOL IN CONSCIOUS HEALTHY DOGS: A PROSPECTIVE, BLINDED, RANDOMIZED, CROSSOVER STUDY. L.C. Visser, B.A. Scansen, N.V. Brown, K.E. Schober, J.D. Bonagura. Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

We evaluated the effects of a single dose of pimobendan and atenolol on RV function as measured by tricuspid annular plane systolic excursion (TAPSE), RV fractional area change (FAC), pulsed-wave tissue Doppler-derived lateral tricuspid annular longitudinal peak systolic velocity (S'), and speckle tracking-derived longitudinal global strain (Strain) and strain rate (SR) of the RV free wall.

The study design was a prospective, blinded, randomized, crossover study of 80 healthy, unsedated dogs. Each dog underwent four echocardiograms - twice for baseline, once 3-hours post-pimobendan (0.25 mg/kg PO) and once 3-hours post-atenolol (1 mg/kg PO). The wash-out period between treatments ranged from 3 to 20 days. Mixed-model analysis was performed comparing relative RV functional changes among treatments along with covariates of interest (heart rate, bodyweight, sex, age, drug sequence, and time period).

Significant differences between baseline and post-drug measures were detected for all variables and treatments (p < 0.0001). Mean (% change (± SD) after pimobendan versus atenolol were: TAPSE 13.0 ± 9.6% vs. -11.5 ± 10.2%, FAC 20.7 ± 12.9% vs. -18.7 ± 8.4%, S' 32.1 ± 21.6% vs. -25.5 ± 15.0%, Strain 13.4 ± 7.9% vs. -15.7 ± 9.5%, and SR 31.5 ± 22.3% vs. -23.8 ± 17.3%; respectively. None of the tested covariates demonstrated a significant effect on the model (P > 0.05).

In conclusion, all of the RV functional indices evaluated consistently tracked changes in RV function during an induced positive- or negative-inotropic state. Further study is warranted to determine the value of these RV functional indices in dogs with cardiovascular disease.

C-3
M-MODE, 2-DIMENSIONAL, AND 3-DIMENSIONAL VOLUMETRIC ASSESSMENT OF LEFT ATRIAL SIZE AND FUNCTION IN A POPULATION OF NORMAL DOGS. N. LeBlanc, K.F. Scollan, D. Sisson. Oregon State University, College of Veterinary Medicine, Corvallis, OR.

Historically in veterinary medicine, linear M-mode and later 2-dimensional (2D) measurements of the left atrium (LA) have been used to assess LA size. Several human studies have shown that LA volume more accurately reflects LA size than linear measurements due to the complex geometry of the left atrium. The primary goal of this study was to assess LA size in a population of normal dogs using transthoracic M-mode, 2D, and 3D echocardiographic techniques.

Forty clinically normal dogs of various breeds were stratified into one of four groups (10 dogs <10 kg, 10 dogs 10-25 kg, 10 dogs >25-40 kg, and 10 dogs >40 kg). Complete echocardiographic examinations were obtained on all dogs; dogs with exceedingly poor image quality precluding measurement were excluded from analysis. All LA measurements were obtained at end-systole. Volume estimates made from the 2D left apical single plane MOD method (median LAVind = 1.000 ± 0.267) were significantly smaller than median LAVind values obtained from linear 2D measurements from the right parasternal short axis view (median LAVind = 0.878 ml/kg +/- 0.305), and median volume estimates made from the 2D left apical single plane MOD method (median LAVind = 0.880 ml/kg +/- 0.240), left apical biplane MOD method (median LAVind = 0.780 ml/kg +/- 0.249), and 3D LAVind obtained from the right parasternal (median LAVind = 0.830 ml/kg +/- 0.267) or left apical locations (median LAVind = 0.815 ml/kg +/- 0.193).

Left atrial volumes estimated by 3D echo were not significantly different from those measured by the 2D biplane area-length method or Simpson’s biplane method of disks using left apical 4- and 2-chamber views. Cubed linear M-mode measurements of the LA significantly underestimate LA volume by the cube formula. Coefficients of variation and quartile coefficients of dispersion were similar for LA volume derived from 2D and 3D data sets and lower than those obtained from M-mode or linear 2D data sets.

C-4
BIOLOGIC VARIABILITY OF N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE AND CARDIAC TROPONIN I IN HEALTHY DOGS AND DOGS WITH MITRAL VALVE DISEASE. R.L. Winter1, A.B. Saunders1, S.G. Gordon1, M.W. Miller1, G.T. Fosgate2, J.S. Sucholdolowski3, J.M. Steiner3.1 Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, USA. 2Department of Production Animal Sciences, University of Pretoria, Onderstepoort, South Africa. 3Gastrointestinal Laboratory, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, USA.

Evaluation of biologic variability (BV), the change in analyte value occurring independently of clinical status change, for N-terminal
pro-Brain Natriuretic peptide (NTproBNP) and cardiac tropo-
in I (cTnI) have proven useful in the clinical evaluation of human patients. The purpose of this study was to assess BV of NTproBNP and cTnI in healthy dogs and dogs with myxoma-
tous mitral valve disease (MMVD).

NTproBNP and cTnI were measured using commercially avail-
able current-generation and high-sensitivity assays validated in dogs. All dogs underwent comprehensive clinical evaluation, including echocardiography. Dogs were classified as healthy (n = 10) or MMVD (n = 28) staged as B1 (n = 10), B2 (n = 10), and stable C (n = 8). Healthy dogs had cardiac biomarkers eval-
uate within-day, daily, and weekly for 6 weeks. MMVD dogs had cardiac biomarkers evaluated within-day, and weekly for 6 weeks. MMVD dogs had cardiac biomarkers evaluated within-day, and weekly for 2 weeks.

Within-subject (CVi) and between-subject (Cvi) coefficients of variation were calculated. Results for healthy dogs: CVi of 46.6% for cTnI and 23.0% for NTproBNP: Cvi of 64.8% for cTnI and 57.7% for NTproBNP. Results for MMVD: CVi of 36.3% for cTnI and 17.0% for NTproBNP: Cvi of 62.0% for cTnI and 48.2% for NTproBNP. Compared with MMVD dogs, healthy dogs required smaller changes in cTnI and larger changes in NTproBNP values to indicate clinical status change (% change).

A group. MMVD dogs require a change of 106% for cTnI and 49% for NTproBNP. Within MMVD, stage B2 dogs require the greatest change in NTproBNP values to indicate clini-
cal status change. Application of BV data may facilitate accurate cardiac biomarker evaluation in future canine studies.

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<tr>
<th>Dogs (N)</th>
<th>Samples (N)</th>
<th>cTnI (ng/mL) Median (Range)</th>
<th>NTproBNP (pg/mL) Median (Range)</th>
<th>% change</th>
<th>CVi</th>
<th>Cvi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>10</td>
<td>0.000 (0.000-0.055)</td>
<td>542 (16-1483)</td>
<td>100%</td>
<td>66%</td>
<td>63%</td>
</tr>
<tr>
<td>MMVD-B1</td>
<td>10</td>
<td>0.050 (0.010-0.327)</td>
<td>676 (24-1245)</td>
<td>117%</td>
<td>53%</td>
<td>51%</td>
</tr>
<tr>
<td>MMVD-B2</td>
<td>10</td>
<td>0.064 (0.010-0.260)</td>
<td>1529 (131-3207)</td>
<td>52%</td>
<td>67%</td>
<td>64%</td>
</tr>
<tr>
<td>MMVD-C</td>
<td>10</td>
<td>0.092 (0.010-0.367)</td>
<td>1999 (48-820)</td>
<td>119%</td>
<td>41%</td>
<td>39%</td>
</tr>
<tr>
<td>A-MYX</td>
<td>28</td>
<td>0.066 (0.010-0.347)</td>
<td>991 (24-3820)</td>
<td>106%</td>
<td>49%</td>
<td>47%</td>
</tr>
</tbody>
</table>

C-6 SURVIVAL TIME WITH PACEMAKER IMPLANTATION FOR DOGS DIAGNOSED WITH PERSISTENT ATRIAL STANDSTILL, R.M. Cervence1, C.D. Stauthammer2, D. Fine2, H.B. Kellihan3, B.A. Seaman4.1University of Minnesota College of Veterinary Medicine, Saint Paul, MN, 2University of Missouri College of Veterinary Medicine, Columbia, MO, 3University of Wisconsin School of Veterinary Medicine, Madison, WI, 4The Ohio State University College of Veterinary Medicine, Columbus, OH.

Persistent atrial standstill (PAS) is an arrhythmia characteriz-
ed by the absence of atrial electrical activity with a subsequent escape rhythm arising from the atroventricular junction or sub-
sidiary ventricular pacemaker cells. The arrhythmia develops secondary to an idiopathic myopathy that leads to progressive destruction of the atrial myocardium. Pacemaker implantation is typically recommended to address the bradyarrhythmia. Prog-
nosis has historically been regarded as poor; however, no stud-
ies assessing the survival time for PAS have been performed. We hypothesized that the median survival time (MST) of dogs with PAS following pacemaker implantation is longer than pre-
viously proposed and that the presence of congestive heart fail-
uar (CHF) does not affect survival time post-pacemaker im-
plantation.

Twenty dogs diagnosed with PAS between 2002 and 2012 that underwent pacemaker implantation were included in this retro-
spetive study; 8 of which had CHF at the time of diagnosis. Survival time post-pacemaker implantation was evaluated, as well as the cause of death (cardiac versus non-cardiac).

Results indicate a MST of 489 days after pacemaker implanta-
tion for all-cause mortality. There was no significant difference (P = 0.935) in MST for cardiac (612 days) versus non-cardiac deaths (489 days). The presence of CHF at the time of diagnosis did not affect survival time (P = 0.854).

The results of this study indicate a similar MST to that previ-
ously speculated. However, this study suggests that diagnosis of CHF prior to pacemaker implantation does not affect survival, nor is the MST different for dogs that experience cardiac death versus non-cardiac related death.

C-7 INOTROPIC EFFECT OF L-LYSINE IN HEALTHY CATS. S.M. Johns, T. Moyers, S.A. Jesty. University of Tennessee Col-
lege of Veterinary Medicine, Knoxville, TN.

The efficacy of l-lysine in controlling clinical signs of feline herpes virus is controversial, but there are no reports of side effects. A recently published study identified a positive inotropic effect of l-lysine in human, rat, and mouse myocardium in vitro. No studies have been published evaluating whether this effect is also demonstrated in vivo in these or other species. Administra-
tion of positive inotropic agents to cats with hypertrophic
obstructive cardiomyopathy might worsen the hypertrophy and exacerbate the outflow obstruction, so must be undertaken with caution.

We hypothesized that l-lysine would act as a positive inotrope when administered to cats at a commonly prescribed dose of 500 mg per cat twice daily. Twelve healthy adult cats were adminis-
tered a commercially available l-lysine supplement at 500 mg per cat twice daily for 90 days. Echocardiograms, blood pres-
sures, and cardiac troponin I concentrations were evaluated before and after supplementation. No differences were detected in septal thickness (p = 0.06), left ventricular diastolic diameter (p = 0.4), left ventricular systolic diameter (p = 0.3), left ventricu-
lar free wall thickness (p = 0.8), fractional shortening (p = 0.07)

In conclusion, Vmax >2.3 m/s and EOAI < 1.46 cm²/m² during puppyhood are associated with SAS later in life, and the combination of both abnormalities results in higher sensitivity for SAS screening in golden retriever puppies.
or cTnI concentration (p = 0.6) after supplementation with l-lysine.
We found no evidence that l-lysine functions as a positive inotrope in healthy cats at a dose of 500 mg twice daily. This adds to the evidence that l-lysine is safe for use in cats.

C-8
HIGH-LEVEL SEROTONIN-BINDING IN SUBPOPULATIONS OF HIGHLY-ACTIVATED PLATELETS IN CAVALIER KING CHARLES SPANIELS WITH MYXOMATOUS MITRAL VALVE DISEASE, S.E. Cremer1, A.T. Kristensen2, J.C. Silva3, K. Filho4, G. Reimann5, N.B. Eriksen6, S.F. Petersen7, C.B. Marschner8, I. Tarnow9, M.A. Oyama2, L.H. Olsen1. 1Department of Veterinary Disease Biology, University of Copenhagen, Frederiksberg, Denmark., 2Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark., 3Actelion Pharmaceuticals, Hellerup, Denmark., 4Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA, USA.

Circulating (platelet-derived) serotonin (5HT) concentration is elevated in early stages of myxomatous mitral valve disease (MMVD) in Cavalier King Charles Spaniels (CKCS). CKCS are predisposed to early-onset MMVD and approximately 33% exhibit non-clinical macrothrombocytopenia (TCP). By means of flow cytometry, we investigated percent (%) and level (mean-fluorescence-intensity/MFI) of platelet-activation (CD62 surface expression) and surface-bound 5HT in platelet rich plasma (PRP) in CKCS with MMVD. Dogs with TCP were included but only normal-size platelets were analyzed. Effect of disease-group, age, gender, body weight, hematocrit and TCP were included in multiple linear regression analyses. Disease-groups (assessed echocardiographically) were: healthy (n = 14), mild MMVD (n = 18), moderate-severe MMVD (n = 19) and severe MMVD in treatment for heart-failure (n = 10).

There was an overall difference in level of platelet-activation (P = 0.04) with a tendency to higher activation level in moderate-severe MMVD (1661MFI (1356-2379)) (median (interquartile range)) and heart-failure (1452MFI (989-3242)) compared to healthy dogs (1325MFI (904-2505) (both P = 0.07)). In 28 dogs, a subpopulation of platelets with high 5HT-binding (5HT-P) was identified. These dogs (compared to non-5HT-P dogs) had higher percent of 5HT-positive platelets (10.4% (7.8-15.4) versus 5.7% (3.8-6.)), 5HT-binding level (3036MFI (2640-39260) versus 1230MFI (810-2830)) and mean-fluorescence level (P < 0.0001) and platelet-activation level (2360MFI (1520-2830) versus 1170MFI (890-1360), P = 0.002). 5HT-P were present in 93.8% of dogs with TCP and could not discriminate the less severe CHF animals from the healthy ones. Also, increased blood lactate carried a worse prognosis regarding the time to relapse.

C-9
BLOOD LACTATE IN DOGS WITH CONGESTIVE HEART FAILURE OWING TO MITRAL VALVE DISEASE, J.C. Silva Filho, M.G. Sousa, E. Zacchê, E.M.G. Ortiz, R.P. Franco, F.A. Regis, A.A. Camacho. São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil.

Lactate is a simple yet reliable indicator of peripheral tissue hypoperfusion, which might be useful to establish both the diagnosis and prognosis in people and animals undergoing critical conditions. The augmentation of blood lactate levels was demonstrated to correlate with a higher incidence of multiple organ dysfunction. However, little information exists regarding the accumulation of blood lactate in dogs with congestive heart failure. Therefore, this study was aimed at characterizing the levels of blood lactate in dogs with mitral valve disease (MVVD) and congestive heart failure (CHF). Also, it was aimed at determining whether lactate might be used as a diagnostic (regarding the severity of heart failure) and prognostic biomarker in these animals.

Thirty-three MVVD and 11 control healthy dogs were enrolled in the study. Exclusion criteria included any other condition other than MVVD, as well as history of any therapy being given to the animal. All MVVD dogs were classified according to the IS-AHC CHF classification. Classes Ia (n = 10), Ib (n = 10), II (n = 9), and IIIa (n = 4) were represented. Jugular vein blood was drawn to determine the concentration of lactate, which was performed immediately.

Significant differences were documented between the blood lactate of control dogs (2.50 ± 0.69 mmol/L) and CHF class II (3.99 ± 0.47 mmol/L) and IIIa (6.97 ± 1.23 mmol/L) dogs. No differences existed between control animals and CHF class Ia (3.31 ± 0.62 mmol/L) and Ib (3.32 ± 0.46 mmol/L) dogs. An area under the ROC curve of 0.8838 was calculated, showing a specificity and specificity of 73.5% and 90.0%, respectively, when using the lactate concentration of 3.25 mmol/L to differentiate between healthy and CHF animals. Significant positive correlations were found to exist between blood lactate concentration and several parameters, including left ventricular end-diastolic diameter, left-atrium-to-aorta ratio, fractional shortening, mean heart rate, P wave amplitude, and VHS. Also, a negative significant correlation was demonstrated between lactate and systolic blood pressure. Finally, the Kaplan-Meier curve showed the median time to decompensation (MTD) to differ significantly according to blood lactate concentration at time of diagnosis. In this regard, animals with blood lactate lower than 3.5 mmol/L, between 3.5 and 5.0 mmol/L and higher than 5.0 mmol/L had a MTD of 460, 153, and 57 days, respectively. No differences were documented in the median time to death ascribed to heart failure.

The results of this study suggest that dogs with MVVD and CHF develop peripheral tissue hypoperfusion, with resulting increased levels of lactate. Blood lactate may be used to distinguish between healthy and classes II and IIIa dogs, although it could not discriminate the less severe CHF animals from the healthy ones. Also, increased blood lactate carried a worse prognosis regarding the time to relapse.

C-10
ATTENUATION OF THE PRESSOR RESPONSE TO EXOGENOUS ANGIOTENSIN BY ANGIOTENSIN RECEPTOR BLOCKERS IN NORMAL DOGS, A.E. Coleman, C.W. Schmiedt, C.G. Handsford, L.R. Reno, E.D. Garber, S.A. Brown. University of Georgia College of Veterinary Medicine, Athens, GA.

Angiotensin receptor blockers (ARBs) may have an important place in canine medicine, particularly in dogs with kidney or cardiovascular disease; however, their utility has not been systematically investigated in this species.

Six healthy female dogs with telemetric arterial catheters were used to compare the efficacy of once-daily oral dosages of 2 ARBs (losartan [LOS, 2.5 mg/kg] and telmisartan [TEL, 1 mg/kg]) to that of twice-daily enalapril (EN, 0.5 mg/kg) and placebo. Following 8 doses and ~90 minutes after administration, dogs were anesthetized and arterial BP was recorded before and after the administration of IV angiotensin I (100 ng/kg). This protocol was repeated at the time of assumed trough activity for EN and TEL (12 and 24 hours post-pill, respectively). One-way repeated measures ANOVA was used to test for differences in the change in SBP (ΔSBP) among groups. Significance was defined as P < 0.05.

Compared to placebo, at 90 minutes post-pill, TEL produced significant attenuation of ΔSBP, while LOS did not. TEL attenuated ΔSBP significantly more than EN and LOS (P < 0.05), resulting in complete extinction of the pressor response in all dogs at 90 minutes post-dose. At trough for both drugs, TEL attenuated ΔSBP significantly more than EN (ΔSBP, 3.33 ± 1.14 mmHg vs. 10.33 ± 0.84 mmHg, respectively; P < 0.05). These results indicate that LOS appears to be ineffective at attenuating Ang I-induced ΔSBP at the dosage tested. Further, treatment with TEL may have advantages over EN and could prove useful in dogs with cardiovascular or renal disease.

Hypertrophic cardiomyopathy (HCM) is the most common feline heart disease and is characterized by increased cardiac mass with a hypertrophied and not dilated left ventricle. The echocardiography is the best noninvasive diagnostic tool for the differentiation of cardiomyopathies and is considered the gold standard for detection of ventricular hypertrophy present in HCM. Electrocardiographic changes are also common in animals with HCM and the electrocardiogram (ECG) is quick, easy and highly available screening test for the detection of ventricular hypertrophy in humans. In cats, few studies have been conducted regarding the sensitivity and specificity of ECG in detecting ventricular hypertrophy.

In order to assess the use of ECG as a screening tool for diagnosis of HCM in cats, we evaluated Persian cats (n = 38) by echocardiographic and electrocardiographic examinations. Animals with blocks and/or conduction disturbances were excluded from statistical analysis (n = 22). Subsequently the animals included were classified as normal (n = 38), suspicious (n = 6) and affected by HCM (n = 16).

Statistical differences were observed in the R-wave amplitude in DII, C6vLL and C6vLU, with higher values in animals with HCM. Velocities and pressure gradient of aortic flow, left atrial diameter (LA) and LA/Ao ratio were higher in cats with HCM. Among the animals with ECG changes suggestive of left atrial overload (n = 7), only two actually had LA enlargement on echocardiography, and among animals with left atrial enlargement on echocardiogram (n = 7), only two had ECG changes suggestive of LA overload (40.4% of sensitivity and 90.9% of specificity). Among the animals with ECG changes suggestive of left ventricular hypertrophy (n = 6), five actually had ventricular hypertrophy on echocardiography, and among animals with HCM by echocardiography (n = 16), only five showed electrocardiographic abnormalities suggestive of LV hypertrophy (31.25% of sensitivity and 97.72% of specificity). It was observed a positive correlation between diastolic thickness of the interventricular septum and/or left ventricular free wall and R-wave amplitude in DII and C6vLU.

The electrocardiogram is a quick method, easy to perform and has a high specificity in detecting ventricular hypertrophy in cats, however, has low sensitivity, with large numbers of false negative animals. Thus, the ECG assists in the diagnosis, but does not replace the echocardiography in confirming ventricular hypertrophy.

Metabolomic and Transcriptomic Profiling of Degenerative Mitral Valve Disease in Dogs. Q. Li1, L.M. Freeman2, J.E. Rush3, G.S. Huggins1, D.P. Lafalme2, T.A. Labuda1, S.S. Hannah1. 1Nestlé Purina Research, Saint Louis, MO., 2Tufts Cummings School of Veterinary Medicine, North Grafton, MA., 3Tufts University School of Medicine, Boston, MA.

Degenerative Mitral Valve Disease (DMVD) is the most common cardiac disease in dogs, accounting for more than two-thirds of all canine heart diseases. Many signaling pathways have been implicated in DMVD, including serotonin, TGF-β, Notch and nitric oxide (NO) signaling pathways. To further define key pathways, we performed a RNA-seq gene expression study to identify differentially expressed transcripts (DETs) between DMVD and control dogs, using tissue samples from dogs euthanized for humane reasons. Total RNA was extracted for sequencing from left ventricle (LV) samples from 2 DMVD and 4 control dogs and mitral valve (MV) samples from 3 DMVD and 3 control dogs. Approximately 25 million 50-base pair single-end reads were generated per sample. In addition, serum from 11 control and 18 DMVD dogs was collected for GC/MS metabolomic evaluation.

We identified 812 DETs in LV and 263 DETs in MV, with 114 in common. Among the DETs, 15 from LV were selected for RT-qPCR validation. 13 were confirmed, suggesting the validity of our RNA-seq results. Of 506 serum metabolites surveyed, 56 displayed differential levels. Data analysis and integration of the two studies reveal impaired fatty acid metabolism and increased glucose usage and metabolism. Endothelial nitric oxide synthase (NOS) in both LV and MV and inducible NOS in LV, but not MV, were significantly up-regulated. The level of asymmetrical dimethyl arginine, an endogenous NOS inhibitor, was significantly lower in DMVD, suggesting elevated NO signaling. These results suggest disturbances in metabolism in DMVD, some of which may benefit from nutritional management.

Prospective Evaluation of the Endothelin-1 Receptor Antagonist Bosentan in Feline Arterial Thromboembolism. E.A. Rozanski1, G.J. Buckley2, C.R. Sharp3, S.M. Cunningham1, J.E. Rush1. 1Tufts Cummings School of Veterinary Medicine, North Grafton, MA., 2University of Florida College of Veterinary Medicine, Gainesville, FL.

Feline arterial thromboembolism (FATE) is a catastrophic disease in cats, most commonly associated with cardiomyopathy. Treatment for acutely affected cats is frequently unrewarding. Endostatin, a potent vasconstrictor, is elevated in affected cats and may play a role in potentiating the disease process. Bosentan is an ET-1 receptor antagonist which is available orally. The goal of this study was to evaluate bosentan as a potential therapy for cats with FATE.

Cats presenting with bilateral pelvic limb FATE were prospectively enrolled. A clinical diagnosis of FATE was based upon lack of femoral pulses, paralysis and cold limbs. All cats were treated with clopidogrel (17.5 mg PO q24 h), dalteparin (200 IU/kg SQ q12 h), analgesics, and appropriate cardiac medications as indicated based upon echocardiographic and radiographic findings. Half of the enrolled cats were additionally assigned to receive bosentan (16 mg PO q 12 h). Limb score (pulse quality and motor function) at admit and at 24 hours were compared between groups, as was rate of survival to hospital discharge using a Fisher’s exact test with p < 0.05 considered significant.

Eighteen cats were enrolled. All cats had hypertrophic cardiomyopathy with moderate to severe left atrial enlargement on echocardiography. Eight cats received standard treatment and an additional 10 cats received bosentan. Bosentan was well-tolerated. Three cats receiving bosentan became strongly ambulatory within 6 hours, but there was no significant difference between the 2 groups in limb score at 24 hours, with 2/8 control cats having improvements in limb score and 5/10 bosentan cats improving (p = 0.37). Similarly, there was no significant difference in survival to discharge, with 5/10 bosentan cats and 2/8 control cats surviving to discharge (p = 0.37).

FATE continues to have a grim prognosis; however further investigation of bosentan may be warranted in cats.

Aldosterone Serum Concentration and Spironolactone (SP) Pharmacokinetics Following Oral Administration of SP in Cats with Heart Failure: Interim Results of the Seisicat Study. R. James1, M. Cobb1, J. Gilmour2, J. Guyonnet2, J. Elliott1. 1University of Nottingham, Loughborough, UK, 2CEVA, Av. de La Ballastière, Libourne, France, 3The Royal Veterinary College, London, UK.

Spironolactone (SP) is an aldosterone receptor antagonist, registered in Europe for the treatment of congestive heart failure.
CHF) caused by valvular regurgitation in dogs, in combination with standard therapy. Its pharmacology is well described in dogs, man and rats, while little is known in cats. In cats, cardio-myopathy (CM) is the predominant cause of heart failure. Activation of the renin-angiotensin-aldosterone system (RAAS) occurs in cats with CM and signs of CHF.

To evaluate the safety and efficacy of SP in cats with CM, a double blind, randomized placebo-controlled study is being conducted with cats receiving either SP (1.7 to 3.3 mg/kg PO once daily) or placebo for up to 15 months. The SP dose range was estimated through allometric extrapolation of the dose recommended in dogs (2 mg/kg) and with an upper limit of less than 4 mg/kg being set because ulcerative facial dermatitis was reported in 4 of 13 Main Coon cats receiving >4 mg/kg SP in a published study. Treatment compliance was assessed at each follow-up visit by counting the number of used and unused tablets. Cats were re-examined at Day 7, 28, 56, 84 then at Month 5, 7, 10 and 15 (Day 420) following initiation of treatment. Blood samples were collected at each visit at different times following dosing, for biochemistry and pharmacokinetic analysis. A validated HPLC method with UV detection, was used to assay primary metabolites of spironolactone: canrenone and 7α-thiomethyl-spirolactone (TMS). Serum aldosterone concentrations were measured by radioimmunoassay (SAM; n = 15) and cats with no echocardiographic abnormality (Normal; n = 11), and echocardiograms of each group of cats were evaluated retrospectively. The median age (5.06 yrs vs 3.24 yrs; p > 0.05) and body weight (3.98 yrs vs 5.09 yrs; p > 0.05) between groups were not significantly different.

On univariate analysis, end-diastolic interventricular septum thickness (IVSD), end-diastolic left ventricular posterior wall thickness (LVPWd) and end-diastolic left ventricular inner diameter (LVId) of two groups were not significantly different (3.60 mm vs 3.36 mm, 4.48 mm vs 3.58 mm, 15.99 mm vs 15.71 mm; p > 0.05 respectively). We also did not find any significance in the lengths of coapted anterior leaflet (AL) and posterior leaflet (PL) of the MV (7.16 mm vs 7.99 mm, 5.00 mm vs 4.48 mm; p > 0.05) and the total length of AL and the length of the residual AL (Residual AL) portion beyond coaptation point (AL-Residual AL; 10.57 mm vs 9.54 mm; p > 0.05).

However, significant correlations were observed in minimum distances between anterior PM to septum (8.01 mm vs 10.77 mm; p = 0.0010) and posterior PM to septum (8.48 mm vs 10.66 mm; p = 0.0036) of two groups. The AL/PL ratio (1.48 vs 1.81; p = 0.017), the minimum distance from echocardiographic plane to the septum (C-Sept; 7.46 mm vs 9.87 mm; p < 0.0001), the Residual AL (3.41 mm vs 1.55 mm; p < 0.0001), LVIDs (10.06 mm vs 8.45 mm; p = 0.028) were also significant factors of morbidity of SAM. Another finding in this study was that larger number of false tendons was observed in SAM group cats (1.87 vs 0.91; p = 0.005).

The results indicated that SAM is not significantly related to LVCH and is due to the displacement of papillary muscles that gives morphological abnormalities to the mitral apparatus. It involves more anterior position of coaptation point of the MV, increased slack residual AL length. The difference in the number of false tendons observed implicates that it may also contribute to the development of SAM as a congenital and a promotive factor.
C-17
SPATIAL AND TEMPORAL DISTRIBUTION OF MYOFIBROBLASTS IN CANINE MYXOMATOUS MITRAL VALVE DISEASE. C.C. Lu, M.M. Liu, G. Culshaw, D. Argyle, M. Clinton, B. Corcoran. The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Scotland, UK.

Myxomatous mitral valve disease (MMVD) is the most common cardiac disease of the dog. Histologically, the marked cellular change in MMVD is reported to be characterized by increased numbers of spindle-shaped, elongated interstitial cells (α-SMA+; activated myofibroblasts) adjacent to the sub-endothelium at the leaflet tip. It has also been reported that the numbers of myofibroblasts is associated with severity of disease. However, there is little known about the spatial and temporal distribution of myofibroblasts throughout the entire leaflet. This study was designed to investigate the distribution of myofibroblasts in normal and different graded diseased mitral valves using high resolution confocal microscopy.

Paraformaldehyde fixed mitral valves sections from normal (n = 10), mild (n = 4), and severely affected (n = 7) dogs were immunostained for α-SMA using a standard indirect immuno-fluorescence technique. To produce a detailed whole-leaflet image, tile-scanning was performed using a Zeiss LSM-710 confocal microscope.

In the normal mitral valves no α-SMA positive cells were observed at the leaflet tip. However, there was a single layer of myofibroblasts in the mid-zone and on the atrial side of the leaflet (Figure 1A). In the mildly affected valves, nodular thickening changes were noted at the distal-zone with additional linear clusters of myofibroblasts aggregating adjacent to the sub-endothelium (Figure 1B). In the severely affected valves, whorl-like aggregations of myofibroblasts were seen in the expanded distal-zone stoma (Figure 1C), in the sub-endothelium and the mid-zone, and a marked increase in cell numbers was also noted.

The location of α-SMA+ cells in the distal zone was not unexpected, but the identification of activated myofibroblasts in the mid-zone, towards the atrialis of normal valve leaflets, is a novel finding. In the absence of readily identifiable pathological changes this might suggest that initial cellular changes in MMVD originate at the mid-zone and not the valve tip. Alternatively, it might be that activated myofibroblasts are not exclusively a pathological cell phenotype in MMVD. How these changes might eventually contribute to distal valve pathology is uncertain, but requires further investigation.

C-18
ENDOTHELIAL TO MESENCHYMAL TRANS-DIFFERENTIATION IN CANINE MYXOMATOUS MITRAL VALVE DISEASE. C.C. Lu, M.M. Liu, D. Argyle, G. Culshaw, B. Corcoran. The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Scotland, UK.

Myxomatous mitral valve disease (MMVD) is one of the most common causes of heart failure in the dog. The pathology of MMVD includes initial endothelial denudation, followed by increased numbers of stromal activated myofibroblasts and excess production of disorganized extracellular matrix. The myxoid degeneration seen with MMVD is reminiscent of valvulogenesis. During embryonic valve development endothelial cells contribute to the interstitial cell population through a process of endothelial-mesenchymal trans-differentiation (EndoMT). This study was designed to investigate the potential for EndoMT in disease pathogenesis by examining expression of a variety of EndoMT related markers in native valves and cell culture.

Mitral valve endothelial cell (MVEC) clones (isolated by a limiting dilution assay) and mitral valve interstitial cells (MVIC) from primary cell cultures were investigated for the expression of the mesenchymal markers (vimentin, V), the VEC markers (CD31, CD144) and the activated VIC markers (α-SMA, SMemb) using standard immunohistochemistry. Acetylated Low Density Lipoprotein (DiI-Ac-LDL) labelling was used to further identify endothelial cells. Paraformaldehyde fixed mitral valve sections from normal and affected dogs were also examined using the same markers. Images captured from Leica-DMLB microscope were processed using ImageJ (NIH, USA) for quantitative and qualitative analysis.

MVEC clones in culture showed vessel lumen formation, mono-layered cobblestone morphology and high reactivity to DiI-Ac-LDL (Figure 1A) and expression of CD31 confirming their endothelial identity. MVICs in culture and in valve tissue did not express CD31. There was extensive expression of CD144, vimentin and SMemb in cultured MVECs and MVICs, and MVICs in all valves, but co-expression of α-SMA and CD144 was only seen in MVICs.

Clonally derived canine MVECs demonstrate typical endothelial cell traits. The majority of MVICs in culture and in normal valves are V+/CD144+/SMemb−/CD31+. MVECs and MVICs show a common mesenchymal phenotype only distin-

![Fig 1.](image_url)
guished by the expression or non-expression of CD31 (Figure 1B). In diseased valves most MVIcs are α-SMA+/CD31-, but double staining revealed the co-expression of α-SMA+/CD144+ in a sub-set of MVIcs, demonstrating a partial MVEC phenotype. The absence of α-SMA in tissue MVECs would not support the hypothesis of ongoing EndoMT, but expression of the endothelial-specific marker CD144 in MVIcs (that are CD31-) suggests a partial endothelial phenotype is present in these valves and this could partially determine the process of EndoMT.

C-19
PHARMACOKINETICS AND PHARMACODYNAMICS OF THE FACTOR XA INHIBITOR APIXABAN IN CATS: A PILOT STUDY. J.A. Myers1, L.A. Wittenburg2, C.S. Olver2, C.M. Martinez2, J.M. Bright2. 1Triangle Veterinary Referral Hospital, Durham, NC; 2Colorado State University, Fort Collins, CO.

Thromboembolism is a common complication of cardiac disease in cats. Anticoagulants are a promising strategy to treat and prevent thromboembolism in these patients, but use of this class of medications is currently limited by challenges in monitoring therapeutic drug levels and cost. Apixaban is a novel factor Xa inhibitor approved to prevent thrombus formation in human patients with atrial fibrillation. This study aimed to investigate the pharmacokinetic (PK) and pharmacodynamic (PD) properties of apixaban in healthy cats.

A single dose of apixaban (0.2 mg/kg) was orally administered to five purpose bred cats, and blood samples were obtained over 24 hours. After a one week washout period, the cats were given 0.2 mg/kg intravenously followed by repeated sampling. Apixaban concentrations in plasma were measured via liquid chromatography tandem mass spectroscopy. PD effects were determined using a commercial factor Xa assay.

Factor Xa activity decreased as a function of time following a single IV or PO dose of apixaban, and good inverse correlation with plasma apixaban concentrations was noted. PK analysis showed that apixaban has a moderate clearance rate, a short half-life and high bioavailability. A two-compartment model fit the IV PK data. Oral PK and PD data were more variable than IV data.

In conclusion, apixaban is an effective inhibitor of factor Xa in cats. Additional studies are necessary to determine multi-dose PK/PD, effect of cardiac disease on PK/PD, dosing recommendations, and efficacy of apixaban in the treatment/prevention of thromboembolic disease in this species.

C-20
NT-proBNP AND TROPONIN I LEVELS AS SCREENING BIOMARKERS IN GREAT DANES. S.L. Rosenthal1, W.D. Tyrrell1, R. Cahill2, G. Clark2, J.S. Buch2. 1Chesapeake Veterinary Cardiology Associates (CVCA); 2Towson, MD; 3Leesburg, VA; 4IDEXX Laboratories, Inc., Westbrook, ME.

Dilated cardiomyopathy (DCM) is a common cardiac disease in the Great Dane dog. We sought to evaluate normal levels of NT-proBNP and high sensitivity cTnI in the Great Dane and in the Great Dane dog. We sought to evaluate normal levels of NT-proBNP and high sensitivity cTnI in the Great Dane and whether these biomarker levels could be used as a screening tool for occult dilated cardiomyopathy.

Eighty six apparently healthy Great Dane dogs were screened at the Great Dane Club of America national breed specialty show in October 2013. Focused cardiac physical examinations, Doppler and 2D echocardiography were performed on all dogs. Medical history, family history of cardiac disease and concurrent medications were recorded on each patient. A biochemical panel, NT-proBNP level and high sensitivity cTnI was run on each patient.

Forty males and 46 females were screened. Mean age was 3.05 years (0.25-9 years) and mean body weight was 64.41 kg (24.5-83.9 kg). Each dog was classified into 5 major groups: Normal; equivocal for DCM as defined by previously reported echocardiographic normals for the Great Dane breed; DCM; Lone atrial fibrillation (AF) and Other. Of dogs classified as “Other”: 23 had minor, inconsequential valvular regurgitations; 16 had equivocal changes for aortic valve or subaortic valve stenosis (1.7-2.1 m/sec); 2 had small left to right shunting atrial septal defects; 2 had equivocal tricuspid valve dysplasia; and 1 had a small patent ductus arteriosus. NT-proBNP and cTnI levels were run on all 86 dogs and the results are summarized below.

During the study, reference interval upper limits for NT-proBNP and cTnI were calculated at 1026 pmol/L and 0.160 ng/mL, respectively. NT-proBNP appears to correlate well with cardiac disease. NT-proBNP was 10 fold higher in dogs with cardiac disease than in normal Great Danes. However, both cTnI (AUC = 0.6963 by ROC) and NT-proBNP (AUC = 0.6617 by ROC) do not appear to be adequate stand-alone screening tools for differentiating equivocal or occult dilated cardiomyopathy from normal in the Great Dane. Follow-up studies with both echocardiography and NT-proBNP on the Great Danes deemed equivocally affected for DCM will be required to determine if NT-proBNP may have a predictive factor for the development of overt cardiomyopathy.

C-21
ACQUISITION AND VALIDATION OF VASOVAGAL TONUS INDEX IN A POPULATION OF HEALTHY ADULT DOGS. H.M. Hodgkiss-Geere, Y. Martinez-Pereira, D. Shaw, G. Culshaw. Cardiopulmonary Service, Hospital for Small Animals, Royal (Dick) School of Veterinary Medicine, University of Edinburgh, EH25 9RG, UK.

Vasovagal heart rate variability (HRV) is a marker of autonomic tone, particularly vagal control of heart rate, and indicates heart health. A time-domain technique, vasovagal tonus index (VVTI), analyses HRV and is calculated by:

$$VVTI = \frac{\text{ln(standard deviation(20xR-R))}}{\text{mean R-R interval}}$$

Despite limited publications, validation of the technique in healthy dogs has not been performed. The aim of this study was to establish a standardised technique for VVTI acquisition to enable repeatable measurements to be obtained in future clinical studies.

Dogs were selected as healthy based on history and physical examination. Dogs receiving medication were excluded. ECGs were obtained in right lateral recumbency and VVTI was determined from five periods of multiple R-R intervals at time-points 2.5 minutes apart, up to 10 minutes.

Sixteen dogs were recruited. VVTI was associated with age (P = 0.019) and followed a polynomial distribution. VVTI was not associated with breed conformation (brachycephalic, mesocephalic, dolicocephalic), and females were found to have a lower VVTI at time 0 compared to males (P = 0.013). Finally, absolute VVTI increased over time-points (P = 0.017) but within each time-point, any 20 R-R intervals could be selected (P ≥ 0.12) to calculate a representative VVTI.

Based on this study, a standardised protocol for obtaining VVTI would be: right lateral recumbency, in a quiet room, with minimal stimulation, with 20 R-R intervals taken from immediate recording with no acclimatisation period. Essentially, should comparisons be made between or within dogs, the same protocol must be followed each time. This ‘standardised’ technique will be applied to dogs with non-cardiac disease to determine systemic effects on VVTI.

C-22
ABDOMINAL OBESITY IS PREDICTIVE OF HEART DISEASE IN DOGS. N. Thenganahsi1, W. Theerapan1, S. Kaewmokul1, A. Sastravaha1. 1Department of Companion Animal Medicine, Faculty of Veterinary Science, Mahidol University, Thailand; 2Medical Research Center, Department of Preventive Medicine, Faculty of Veterinary Science, Mahidol University, Thailand.

Abdominal obesity is associated with increased mortality and higher prevalence of cardiovascular diseases in human populations. Abdominal obesity has been suggested as an independent risk factor for cardiovascular disease, although the association has been inconsistent in various studies. The aim of this study was to determine the association between abdominal obesity and heart disease in dogs.
C-23 RELATIONSHIP OF CANINE NT-PROBNP TO HEART FAILURE CLASSIFICATION AND RESPIRATORY DISPROPORTION
P.R. Fox 1, M.A. Oyama 2, J.E. Rush 3, T.P. Nguyenba 4, WTLR (1.25/C6 WHSDR in the heart disease group was not different from the p with healthy canines: MBMI (65.0/C6 measurements were greater in the heart disease group compared with area under the ROC curve of 0.778 (95% CI, 0.683-0.874) and 0.727 (95% CI, 0.619-0.835), respectively.

In conclusion, our results indicate that abdominal obesity, rather than overall obesity, is a significant risk factor for heart disease in dogs.

Clinical Sciences, and Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

The relationship between overall obesity and the development of canine heart disease remains unsettled. In the present study, we evaluated the association between overall obesity and clinical heart disease in dogs by morphometric measurements.

A cross-sectional study of dimension of body part measurements was performed in dogs with and without heart diseases. In total, 87 dogs were included in the study (43 healthy and 44 adjudicated heart disease dogs). Body condition score (BCS), modified body mass index (MBMI), waist-to-hip-to-stifle distance ratio (WHSDR), waist-to-ilium wing distance ratio (WIWDR) and waist-to-truncal length ratio (WTLR) were compared between groups with (n = 44) and without (n = 43) heart disease using a Tukey’s HSD test. Receiver operating characteristic (ROC) analysis was used to compare the performance of BCS, MBMI, WHSDR, WIWDR, and WTLR as indices of central obesity by dogs, rather than the area.

BCS from healthy and heart disease groups were not different (3.3 ± 0.1 vs. 3.6 ± 0.2, p = 0.126). The following morphometric measurements were greater in the heart disease group compared with healthy canines: MBMI (65.0 ± 4.5 vs. 52.5 ± 3.7, p < 0.01 vs WHSDR (4.1 ± 0.1 vs. 3.8 ± 0.1, p < 0.05 vs. WTLR (1.25 ± 0.04 vs. 1.05 ± 0.04, p < 0.01). However, WHSDR in the heart disease group was not different from the healthy group (3.6 ± 0.1 vs. 3.7 ± 0.2, p = 0.875). Of the five morphometric indices studied, WIWDR and WTLR provided acceptable discrimination for diagnosing heart disease in dogs with area under the ROC curve of 0.778 (95% CI, 0.683-0.874) and 0.727 (95% CI, 0.619-0.835), respectively.

In conclusion, our results indicate that abdominal obesity, rather than overall obesity, is a significant risk factor for heart disease in dogs.

C-24 MITRAL VALVE APPARATUS MORPHOLOGY IN FELINE HYPTERTROPIC AND DILATED CARDIOMYOPATHY.
P.R. Fox 1, I. Cerrada 1, E. Herrold 2. 1Animal Medical Center, New York-NY, 2Cornell University, Ithaca-NY.

The mitral valve-MV apparatus (MVA) plays a key role to maintain valvular-ventricular interaction. There is little information describing feline MVA anatomy/morphology, or their features with ventricular hypertrophy (HCM) or dilatation (DCM). To characterize and evaluate these relationships, we prospectively examined 36 archived feline hearts (17 HCM; 19 DCM) sectioned in long axis (LV inflow/outflow tomographic view). Gross MVA structures were photographed using stierimicroscopy and measured with digital image software.

The MV consisted of one anterior leaflet (AMLV) that was longer than the posterior leaflet (PLMV), and a PLMV that divided into lateral, medial, and posterior cusps. Myxomatous MV degeneration affected 16/17 HCM and 6/19 DCM hearts (P < 0.001). Examination of subvalvular structures revealed each papillary muscle (PM) gave 2-5 chordal trunks to each leaflet, which then arborized into primary-( marginal) chordae tendineae (CT) attaching to leaflet edges (9-15CT/AnteriorMLV; 4-15CT/ PosteriorMLV), and secondary-(ventricular) CT to the ventricular leaflet surface (2-6CT/AMLV; 3-9CT/PLMV). All hearts had an anterior and posterior PM (APM, PPM). Morphologically, PM’s were hypertrophied (88% HCM; 5% DCM) or wide/flat (71% DCM; no HCM)(P < 0.001); There was NS difference: APM vs PPM within each HCM/DCM group (1 vs 2 heads; round vs conical morphology) (P > 0.05). HCM PM’s originated more commonly apically in HCM (47%) vs DCM (15%). HCM had smaller PPM-length ratio (apex-to-annulus:PPM-to-annulus) vs DCM (P < 0.001), suggesting longer CT or basal PPM displacement.

Knowledge of MVA morphology may help further characterize feline cardiomyopathy.

C-25 CHANGES OF HEART RATE AND RHYTHM IN HARBOR SEAL PUPS DURING REHABILITATION. S. Fonfar 1, J. Sundermeyer 2, D. Casamian-Sorrosa 1, T. Rosenberger 3. 1University of Bristol, Bristol, UK, 2Seal Centre Friedrichskoog, Fried- richskoog, Germany.

The autonomic nervous system of harbor seal pups matures during their first months of live, which is relevant for the seals’ foraging capacity. Electrocardiographic (ECG) recordings during this crucial period have not been performed previously in orphaned seal pups recovering in seal centres.

ECGs of 1 minute were recorded from 50 harbour seal pups rehabilitated in a seal centre at admission, after 2 weeks and prior to release back into the wild using an Iphone ECG adapter.

ECGs of diagnostic quality were obtained in 92% of recordings. The predominant heart rhythm at admission was sinus tachycardia (150/min, range 110-180/min), and at discharge sinus arrhythmia (127/min, range 25-180/min), with a significant reduction of heart rate (HR) during rehabilitation (p < 0.001). P-wave length and PQ distance increased (p < 0.001), and QT reduced during rehabilitation (p < 0.001), and a negative correlation of QT (r = -0.439) and QT (r = -0.389) with HR was present. First and second degree atrioventricular blocks (AVB, supra- (SVPC) and ventricular premature complexes were recorded, with second degree AVB and SVPC being more frequent after 2 weeks and first degree AVB at release.

The results of this study did show changes in the heart rhythm during rehabilitation, which will reflect potential diseases and exposure to stress, but also suggests maturation of the autonomic nervous system with a predominant vagal tone at discharge.
C-26 USE OF TORSEMIDE IN 17 CATS WITH ADVANCED CONGESTIVE HEART FAILURE. I.Z. Giatis1, T.P. Nyugenba1, M-A. Oyama2, L.B. Lehmkuhl3, D.B. Abin1, K.N. Wright3, D.M. Webber1. 1MedVet Medical and Cancer Centers for Pets, Columbus, OH. 2University of Pennsylvania College of Veterinary Medicine, Philadelphia, PA. 3MedVet Medical and Cancer Centers for Pets, Cincinnati, OH.

Torsemide is a loop diuretic that exhibits greater potency and longer duration of action than furosemide in healthy cats. Torsemide is well tolerated in dogs with advanced congestive heart failure (CHF). We hypothesized that oral torsemide, when used in place of furosemide, would be well tolerated and help extend chronic CHF control in feline patients with advanced CHF. Records from 17 client-owned cats switched from furosemide to torsemide were retrospectively evaluated. Collected data included duration of CHF therapy before torsemide, maximum furosemide dose reached before torsemide, starting torsemide dose, survival time after starting torsemide, maximum torsemide dose reached during CHF therapy, and assessment of CHF control at all visits. Medical history, physical exam, blood work, blood pressure, and diagnostic imaging findings were collected for comparison before and after starting torsemide.

All cats were initially treated with oral furosemide. Various concurrent cardiac therapy included ACE inhibitors, anti-thrombotics, pimobendan, atenolol, diltiazem, sotalol, hydrochlorothiazide, spironolactone, and potassium supplement. At initiation of torsemide, cats were either refractory to oral furosemide at ≥ 5.5 mg/kg/day (n = 11) or had 3 or more instances of CHF within the preceding 10 weeks (n = 6). Median oral furosemide dose was 6.1 mg/kg/day (range 2.9-16.6 mg/kg/day) immediately before starting torsemide, and median initial oral torsemide dose was 0.7 mg/kg/day (range 0.4-1.6 mg/kg/day). Median duration of CHF therapy before starting torsemide was 26 days (range 5-895 days). Median time to first recheck after starting torsemide was 8 days (range 2-27 days) with CHF unimproved in 2 cats, improved in 6 cats, and resolved in 9 cats. Serum creatinine was higher (p = 0.003); whereas, potassium (p = 0.01) and chloride (p = 0.002) were lower in patients at first recheck after starting torsemide. Four of 17 cats were alive at the time of writing. Median survival time for the remaining 13 cats after starting torsemide was 87 days (range 3-466 days) with a median final torsemide dose of 1.0 mg/kg/day (range 0.2-2.2 mg/kg/day). During chronic therapy, torsemide dose was reduced in 3 cats because of azotemia. Mortality was due to death or euthanasia from refractory CHF (n = 10), sudden death (n = 1), euthanasia due to pleural effusion of suspected neoplastic etiology (n = 1), and euthanasia due to azotemia 230 days after starting torsemide (n = 1).

Torsemide is tolerated per os and may help extend chronic CHF control in feline patients with advanced CHF. Additional study is needed to evaluate long-term safety and efficacy of torsemide as an initial diuretic of choice for CHF in cats.
C-29 PROGRESSION OF “SILENT” MYXOMATOUS MITRAL VALVE DISEASE IN NORFOLK TERRIERS. D.J. Trafny1, J. MacGregor2, S.M. Cunningham1, B. Bulmer3, J.E. Rush1, M-A. Oyama1. 1Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA, USA; 19104, 2Massachusetts Veterinary Referral Hospital, Woburn, MA., 3Tufts University Cummings School of Veterinary Medicine, North Grafton, MA.

The clinical diagnosis of myxomatous mitral valve disease (MMVD) is usually made in the presence of a characteristic heart murmur. The investigators previously reported on the auscultatory, clinical, and echocardiographic characteristics in a cohort of overly healthy Norfolk terriers (NFT). Based on echocardiographic changes in mitral valve leafllet thickness, area, and degree of prolapse, we postulated the presence of pre-auscultatory or “silent” MMVD (sMMVD) in a subset of NFT with valve changes but without heart murmurs. In this longitudinal follow-up study, we sought to determine the incidence of clinical diagnosis (heart murmur and echocardiographic evidence of mitral regurgitation) in dogs with sMMVD. Twenty of the original 48 NFT were available for follow-up, including 6 initially evaluated as having no evidence of MMVD (age, 10 yrs [IQR, 6.5-11]) and 14 initially suspected of having sMMVD (age, 8 yrs [IQR, P = 0.41]). Physical examination and echocardiography performed at a median of 648 days (623-690d) after first exam revealed that 1/6 healthy and 5/14 sMMVD NFT had developed a murmur and echocardiographic evidence of mitral regurgitation (x2 = 13.1, P = 0.051). NFT that were initially suspected of having sMMVD demonstrated significant increases in valve leafllet thickness (P = 0.0023), area (P = 0.0064), and prolapse (P = 0.002) from initial exam to recheck. In contrast, NFT that were previously considered healthy did not demonstrate any significant changes in thickness, area, or prolapse over the follow-up period (P = 0.39, 0.19, 0.68, respectively). In summary, identifiable echocardiographic characteristics of altered mitral leafllet morphology preceded onset of heart murmur, mitral regurgitation, and clinical diagnosis of MMVD in NFT.


Measurement of left atrial volume is considered an important prognostic marker in the evaluation of volume overload in human patients with heart disease. However, in dogs with valvular or myocardial disease and index of volume overload routinely used is left atrium diameter / aortic diameter ratio, being the left atrial volume underutilized. Thus, it can be concluded that the left atrial volume may be a useful tool in the evaluation of volume overload in patients with degenerative mitral valve disease.

Thus, it can be concluded that the left atrial volume may be an additional useful tool in the evaluation of volume overload in patients with degenerative mitral valve disease.


Sepsis is defined as a systemic inflammatory response which occurs during an infection, and myocardial dysfunction is one of its most important features. Although the cardiovascular alterations in human patients with sepsis being studied for over 50 years, data from small animals are minimal. Thus the aim of this study was to evaluate the echocardiographic alterations in bitches with sepsis due to cysic endometrial hyperplasia-pyometra complex.

For this purpose, were included in the study 17 bitches with clinical, hematological and ultrasonographic diagnosis of cystic endometrial hyperplasia-pyometra complex with scoring criteria for sepsis according to the assessment of heart rate, respiratory rate, body temperature, white blood cells and immature band forms. Prior to hysterectomy (T-0), 15 days (T-15) and 30 days (T-30) after, animals underwent a complete echocardiographic examination performed by a single operator according to standard protocol. Studied variables were diastolic left ventricular diameter corrected by body surface area (LVDLd), systolic left ventricular diameter corrected by body surface area (LVDSLd), fractional shortening (FS) and ejection fraction (EF), obtained by right parasternal short-axis view of the LV at the level of the chorda tendinea, early diastolic mitral inflow velocity/atrial peak mitral inflow velocity (E/A) and deceleration time of early diastolic mitral inflow (DTE), obtained by left parasternal long-axis four chamber view, and isovolumetric relaxation time (IVRT), obtained by left parasternal long-axis five chamber view. Data were submitted to normality test and analysis between moments was performed by using repeated measure ANOVA and Tukey’s post hoc test (P < 0.05).

Results are summarized in Table 1.

Table 1. Echocardiographic parameters (mean ± SD) of bitches with sepsis (n = 17) due to the cystic endometrial hyperplasia – pyometra complex (T-00) and 15 and 30 days after treatment (T-15 and T-30, respectively).

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>T-00</th>
<th>T-15</th>
<th>T-30</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDLd (cm)</td>
<td>5.15 ± 0.9</td>
<td>5.4 ± 1.4</td>
<td>5.2 ± 1.4</td>
<td>0.49</td>
</tr>
<tr>
<td>LVDLd (cm²/m²)</td>
<td>3.0 ± 0.6</td>
<td>3.2 ± 0.6</td>
<td>3.2 ± 0.6</td>
<td>0.52</td>
</tr>
<tr>
<td>FS (%)</td>
<td>41.3 ± 7.3</td>
<td>40.2 ± 6.2</td>
<td>36.1 ± 7.1</td>
<td>0.29</td>
</tr>
<tr>
<td>EF (%)</td>
<td>73.2 ± 8.5</td>
<td>72.1 ± 7.6</td>
<td>69.4 ± 9.2</td>
<td>0.29</td>
</tr>
<tr>
<td>E/A</td>
<td>1.9 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>0.17</td>
</tr>
<tr>
<td>DTE (ms)</td>
<td>314 ± 50</td>
<td>313 ± 42</td>
<td>301 ± 45</td>
<td>0.005</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>60 ± 15</td>
<td>60 ± 5</td>
<td>49 ± 12</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Means followed by the same letter are not significantly different.

Deceleration time of early diastolic mitral inflow (DTE) and IVRT were significant lower in patients during sepsis, suggesting that sepsis can lead to diastolic dysfunction in dogs as well as in human patients.


Left atrial volume is considered an important index to estimate diastolic function in human patients. However, in veterinary practice this measurement is not routinely used, being preferred.
Doppler flow or tissue Doppler variables to estimate diastolic function. Thus the aim of this study was to evaluate if left atrial volume is correlated with Doppler flow diastolic variables in dogs with and without degenerative mitral valve disease.

For this purpose, were included in the study 81 dogs with degenerative mitral valve disease and 107 healthy dogs. Animals underwent a complete echocardiographic examination performed by a single operator according to standard protocol. Diastolic left atrial volume corrected by body surface area (LAVds), was obtained by left parasternal long-axis four and two chamber view, by biplane Simpson’s method. Diastolic variables derived from Doppler flow evaluation were early diastolic mitral inflow velocity (E), atrial peak mitral inflow velocity (A), E/A ratio, deceleration time of early diastolic mitral inflow (DTE), isovolumetric relaxation time (IVRT) and E/IVRT ratio. The relationship between LAVds and other variables were assessed by Pearson’s correlation test. Student’s t test was used to assess difference between the diastolic parameters in dogs with normal and increased LAVds, considering increased values above the 95th confidence interval of healthy dogs. LAVds was significantly correlated with E (r = 0.50, p < 0.0001), IVRT (r = 0.25, p < 0.02) and E/IVRT ratio (r = 0.52, p < 0.0001). Dogs with increased LAVds showed lower values of E (0.81 ± 0.27 vs 0.90 ± 0.16, p < 0.0003), DTE (144 ± 48 vs 178 ± 23, p = 0.0003) and E/IVRT vs 178 ± 23, p = 0.0003) and 3.43 ± 1.65 vs 144 ± 20, p = 0.0003), and greater value of E/IVRT (1.13 ± 0.5 vs 0.95 ± 0.2, p = 0.002). Therefore, it can be concluded that LAVds correlates with diastolic variables in dogs with degenerative mitral valve disease.

Table 1. Mean ± sd for urine production for continuous rate infusion and intermittent bolus of furosemide in dogs with degenerative mitral valve disease (n = 10).

<table>
<thead>
<tr>
<th>Groups</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M7</th>
<th>M8</th>
<th>M9</th>
<th>M10</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB</td>
<td>24.2 ± 0.8</td>
<td>2.2</td>
<td>1.34</td>
<td>11.0</td>
<td>3.0</td>
<td>1.06</td>
<td>1.4</td>
<td>1.0</td>
<td>0.6</td>
<td>0.0</td>
</tr>
<tr>
<td>M</td>
<td>0.5</td>
<td>2.2</td>
<td>1.34</td>
<td>11.0</td>
<td>3.0</td>
<td>1.06</td>
<td>1.4</td>
<td>1.0</td>
<td>0.6</td>
<td>0.0</td>
</tr>
<tr>
<td>CRI</td>
<td>15.0 ± 0.5</td>
<td>12.4</td>
<td>7.8</td>
<td>7.3</td>
<td>7.5</td>
<td>6.6</td>
<td>6.5</td>
<td>5.6</td>
<td>4.6</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the line are not significantly different. *, significantly different compared to the other group at the same time.

According to the data of the present study was possible to observe that continuous rate infusion furosemide produces more consistent diuresis than intermittent bolus furosemide.

C-34 EFFECT OF MAINTENANCE IV FLUID THERAPY ON NT PRO-BNP CONCENTRATION IN HEALTHY DOGS. M.C. Gaunt, A.P. Carr, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada.

Natriuretic peptides are produced by atrial and, to a lesser extent, ventricular myocytes in response to volume or pressure overload. Several factors have been noted to elevate BNP concentration including acute or chronic kidney disease, pulmonary hypertension, and feline hyperthyroidism. N-terminal pro-B type natriuretic peptide (NT pro-BNP) is increasingly used in the diagnosis of heart disease in veterinary patients. It has also been shown in humans that BNP concentrations in some circumstances correlate to volume status in patients without heart disease and can increase with fluid therapy. In dogs acute volume overload has been shown to increase NT pro-BNP as well. In contrast, the aim of our study was to investigate the effects of a less aggressive fluid therapy over a longer period of time. Six apparently healthy dogs had central venous catheters placed in their jugular veins and were administered twice maintenance rate IV crystalloids for 24 hours. Plasma NT pro-BNP was measured at time 0, 1, 6, 12, 18 and 24 hours. Data were analyzed using a McNemar chi square test to assess for significant changes from baseline values. A value of P < 0.05 was considered to be significant. All dogs had values <50 pmol/L at baseline and no significant changes from baseline were noted in any dog at any time point during the course of fluid therapy. As such, plasma proBNP assessment is not affected by twice maintenance IV fluid therapy over a 24 hour period in healthy dogs.


Despite advances in the treatment of cardiomyopathies, intravenous loop diuretics are the most common therapy used to treat acute decompensated heart failure. Several studies in humans with heart failure have shown that continuous infusion of furosemide can produce a more consistent diuresis, optimizing patient outcome. However, data on the use of continuous infusion of furosemide in small animals are minimal. Thus, the aim of this study was to compare diuretic efficacy of furosemide administered by intermittent bolus (IB) and continuous rate infusion (CRI) in dog with mitral valve disease.

For this purpose, were included in the study 10 dogs, 7 females and 3 males, aged between 8 and 12 years, weighing between 4.8 kg and 15 kg, with degenerative mitral valve disease (n = 10).


The cardiotoxicity induced by doxorubicin generates myocardial remodeling and systolic dysfunction. The objective of this work was to evaluate the role of apoptosis and extracellular matrix components (fibronectin and myofibroblasts) in doxorubicin-induced dilated cardiomyopathy in a rabbit model.

Twenty five New Zealand rabbits were randomized in two experimental groups named G1: control group (n = 10), receiving NaCl 0.9% and G2 (n = 15), receiving DOX 1 mg/kg twice a week for 6 weeks. Echocardiographic evaluations were performed before the first and after the last administration. Assessment of myocardial remodeling was performed by transmission electron microscopy and immunodetection of apoptotic cells (caspase 3), myofibroblasts and fibronectin by immunohistochemistry. The statistical analysis was performed by analysis of variance followed by Tukey’s test and Pearson’s correlation.
Were observed significant reduction in systolic function, mitochondrial damaged, increased apoptotic fibers and myofibroblasts in treated animals. Fibronectin was not significantly increased in treated animals. A significant negative correlation between apoptotic cells and myofibroblasts on the interventricular septum and left ventricle with ejection fraction and shortening fraction was observed, revealing that as more apoptosis and myofibroblasts lower is the systolic function during treatment with doxorubicin.

The results showed that doxorubicin induces myocardial apoptosis by mitochondrial pathway and this mechanism contributes with the systolic dysfunction generated by this drug.

The case records of animals diagnosed with VSD by combined use of echocardiography and Doppler examination were reviewed (1992-2013). A dog was identified in 109 animals (56 dogs, 53 cats), and was isolated in 48.6% (53/109) of cases. Most defects were perimembranous (75.2%, 82/109). The most commonly represented canine breeds were Terrier type dogs (20/56, 35.7%) and French bulldog (8/56, 14.3%). Most animals with isolated VSD (42/53, 79.2%) were asymptomatic, with a pulmonary to systemic flow ratio (Qp/Qs)<1.5. The ratio of VSD size to aortic diameter for isolated VSD in the dog (0.26 ± 0.11 [0.08-0.57]) was significantly correlated with Qp/Qs (r = 0.689; p = 0.002). Similar results were obtained in the cat. One dog underwent surgical VSD closure, and was excluded from the survival analysis of dogs with isolated VSD. For the remaining 52 animals, 75% (39/52) had no cardiac event before 9.6 years of age. Median age at death for all causes was 12 years, and only 3/52 animals died for cardiac reason.

These results suggest that most isolated VSD in small animals are associated with a long survival time, and only minor hemodynamic and clinical consequences.

C-35
AN INVESTIGATION OF NOVEL TECHNOLOGY TO EVALUATE HEART RATE AND RESPIRATORY RATE. A. Landis-Hanna1, J. Wakshlag2, M. Kraus3, P. Tupin1, A. Goldberg2. 14C Innovations, Inc, Chantilly, VA, 2Cornell University Small Animal Teaching Hospital. Ithaca, NY., 3Blue Highway Research, Syracuse, NY.

Ultra-wideband (UWB) technology has been used to characterize complex microwave systems since 1969. In the last ten years, UWB has been applied to communications and imaging, such as human fetal monitoring and detecting stroke volume. UWB technology has recently been leveraged in a proprietary band known as "Voyce" which is worn around the canine neck. Voyce is designed to collect physiological information by utilizing variations in the dielectric properties of tissues. The band is has no wires, probes, or chest attachments making it a non-invasive device. Voyce collects data and remotely transmits it to a computer or mobile device so the information can be reviewed anytime. This investigation evaluated the accuracy of the band as compared to gold standard technology (Televet 100 EKG) and manual readings.

The study was designed in two stages. Stage 1 consisted of investigatory testing of the device using 30 canine subjects at the Cornell University Small Animal Teaching Hospital. Stage 2 consisted of testing of 10 canine subjects, in over 300 test cases, in Chantilly, VA. In both stages, a variety of ages, sizes, and breeds were tested, to provide a representative population. Subjects wore a 4-lead Televet system, monitoring R wave to R wave (R-R) intervals. The subjects also wore the Voyce band, which recorded raw UWB data. That data was then converted to heart rate (HR) and respiratory rate (RR) data using proprietary data-processing algorithms.

In both locations, significant accuracy was found for both the HR and RR. At Cornell and Chantilly, accuracy of HR and RR was found to be greater than 80%. Algorithm refinement and additional data collection is expected to allow improved accuracy throughout 2014. These tests suggest that Voyce can be utilized to obtain objective HR and RR data in a non-clinical setting.

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Congenital ventricular septal defect (VSD) consists of an opening in the interventricular septum caused by its hypoplastic development during the embryonic period. Few studies have reported the hemodynamic consequences and clinical outcome of VSD in small animals. The objective of this retrospective study was therefore to document the epidemiological, clinical, and echo-Doppler findings as well as survival, associated with VSD in a large canine and feline population.

Two-dimensional (2D) echocardiography has been widely used for years to assess left ventricle (LV) systolic function. However, there are some limitations using the conventional method of LV ejection fraction (EF). Two-dimensional strain-imaging is a simplified and angle-independent echocardiographic tool which has been recently used for the evaluation of the quantitative global and regional ventricular functions. Syngo Velocity Vector Imaging (VVI) is a speckle tracking-based method for direct analysis of myocardial motion. Since rabbits are an important model for cardiovascular research, the purpose of this study was to investigate the LV function using VVI in non-sedated healthy rabbits.

For this purpose, we evaluated 16 clinically healthy male New Zealand White rabbits weighing between 2.4 and 3.6 Kg, but only seven met the requirements for analysis and were selected. Echocardiographic evaluation was performed with a 4-9 MHz phased-array transducer with tissue harmonics imaging (Acuson X300 Premium Edition, Siemens Medical Solutions USA, Inc.). Echocardiographic clips were obtained in right parasternal short-axis left ventricle at the papillary muscles level and evaluated offline using the VVI software (Syngo® Velocity Vector Imaging™, Siemens Medical Solutions). Once a reliable endocardial tracing over a single frame was manually drawn, the endocardial borders were automatically tracked throughout the cardiac cycle. Accuracy of border tracking was visually confirmed by observing the cardiac cycle in slow-motion while only border information was displayed. Systole was manually selected by moving the left and the right cursor to the onset of the QRS complex and the end of T wave, respectively. The global means obtained for LV peak systolic strain radial was -0.29 ± 0.288% (TPK = 262 ± 75 ms), whereas the peak strain rate was -1.44 ± 0.5 cm/s (TPK = 223 ± 40 ms); the rotation velocity was 25.62 ± 8.82 deg/s (TPK = 262 ± 75 ms); the radial velocity was 0.89 ± 0.31 cm/s (TPK = 212 ± 37 ms); the rotation displacement was 1.87 ± 1.61 deg (TPK = 196 ± 39 ms); and the radial displacement was 0.01 ± 0.02 mm (TPK = 283 ± 40 ms).

This study aids in the understanding of LV systolic function in rabbits. VVI demonstrated to be a feasible tool to assess LV systolic radial function in this species. The high number of rabbits diagnosed from the study may be explained by the high heart rate observed in rabbits and the need for a higher-frequency transducer, in order to obtain a reliable endocardial tracing throughout cardiac cycle. The clinical value of LV wall motion analysis by two-dimensional strain-imaging in rabbits needs further investigation.
Quantification of left ventricle (LV) systolic function is highly important in echocardiography. Ejection fraction (EF) is an index that assesses variations in LV volume throughout the cardiac cycle and has been widely used to assess LV systolic function. However, there are important limitations in estimating LV volume based on one-dimensional measurements of LV in the short axis. Therefore, the American Society of Echocardiography recommends not to use linear measurements to calculate LV volumes and EF for clinical practice. Thus, the most commonly used two-dimensional assessment of LV volume in human beings is the modified Simpson’s method of discs. Since rabbits are an important model for cardiovascular research, the purpose of this study was to investigate the interobserver repeatability of the modified Simpson’s method to assess the LV ejection fraction (EF), end systolic volume (ESV), end diastolic volume (EDV) and systolic volume (SV) in non-sedated healthy rabbits.

For this purpose, we used 12 clinically healthy male New Zealand White rabbits, weighing between 2.4 and 3.6 kg. Animals were restrained in left lateral recumbency and echocardiographic evaluation was performed with a 4-9 MHz phased-array transducer with tissue harmonics imaging (Acuson X300 PE, Siemens Medical Solutions USA, Inc.). Echocardiographic clips were obtained in left apical four-chamber view. One clip from each rabbit was selected and two observers, with similar levels of experience, applied the modified Simpson’s method to each of these scans. The repeatability of these echocardiographic analyses was compared using a standard paired Student’s t-test to assess the interobserver repeatability.

When comparing values obtained by two observers using the modified Simpson’s method, only values of EF were equal (p = 0.83), while values of EDV, ESV and SV were significantly different (p < 0.05).

We conclude that modified Simpson’s method is a feasible tool to assess LV ejection fraction in non-sedated healthy rabbits, but not to address EDV, ESV and SV. The interobserver discrepancies may be explained by the high heart rate observed in rabbits and the need for a higher-frequency transducer, in order to obtain a reliable endocardial tracing during systole and diastole.

Degenerative mitral valve disease (MVD) is common in older small-breed dogs. Sedatives minimal hemodynamic effects are desirable for restraint in radiographic and echocardiographic procedures in dogs with MVD.

Dexmedetomidine is a reversible sedative and has dose-dependent side effects such as increased blood pressure (BP), and decreased in cardiac output (CO) and heart rate (HR) in dogs. In humans, however, low-dose dexmedetomidine (LDM) results in slight reductions in BP or afterload. We hypothesize that LDM sedation in dogs with MVD would produce negligible effects on mitral valve regurgitation (MR) and hemodynamic parameters along with adequate sedation.

This blinded crossover study included four beagles with spontaneous MVD classified as Stage B1. Dogs were allocated to receive LDM (1.0 μg/kg), acepromazine (0.02 mg/kg), butorphanol (0.4 mg/kg), midazolam (0.3 mg/kg), or saline (0.05 mL/kg) intramuscularly. Before and 20 min after the drug administration, the following measurements were obtained: systolic BP (SBP); HR; echocardiographic variables including CO, E/Ea, regurgitant jet area signal/LA area, transmitral flow velocity, and tissue Doppler imaging variables; and sedation score.

There were no significant differences among groups in the sedation scores and echocardiographic parameters after the drug administration. HR significantly increased in the midazolam group. CO and SBP significantly decreased in the acepromazine group. In the LDM group, effects on MR and hemodynamic parameters were minimal and not significant compared with the baseline values.

In conclusion, LDM sedation could be applicable in dogs with MVD, because it minimally affects echocardiographic parameters. Combinations of these sedatives should be evaluated.

Few studies report pathologic abnormalities in the feline left ventricular outflow tract (LVOT). We prospectively evaluated 36 archived, adult feline hearts with idiopathic ventricular hypertrophy (HCM; n = 17) and dilatation (DCM; n = 19) and sectioned them in long-axis to simulate the echocardiographic LV-inflow/outflow tomographic view. We defined LVOT as the muscular region delineated proximally by the area extending from chordal-anterior mitral valve leaflet (AMVL)-junction, adjacent to the basal interventricular septum (IVS), and extending distally to the AMVL-aortic root attachment. Gross examination was aided by stereo microscopy. Cardiac structures were photographed and measured with digital image software.

Gross LVOT lesions were identified in HCM (88.2%) and (52.6%) DCM hearts, comprising two categories: 1) Lesions of the basal IVS (assumed as acquired) included basal scar [11/17 HCM [adjacent to myxomatous AMVL in 10/11] and 1/19

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EFFECTS OF LOW-DOSE DEXMEDETOMIDINE ON THE CARDIOVASCULAR SYSTEM AND SEDATION SCORE IN DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE. K. Sugimoto1, H. Sano2, M. Suzuki1, H. Sunahara1, Y. Fujii1, T. Aoki1, 1Azabu University, School of Veterinary Medicine, Kanagawa, Japan. 2Massey University, Institute of Veterinary, Animal and Biomedical Science, Tennent Drive, New Zealand

Pathological features of left ventricular outflow tract lesions in feline hypertrophic and dilated cardiomyopathy. 1. Cerrada, P.R. Fox1.

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Few studies report pathologic abnormalities in the feline left ventricular outflow tract (LVOT). We prospectively evaluated 36 archived, adult feline hearts with idiopathic ventricular hypertrophy (HCM; n = 17) and dilatation (DCM; n = 19) and sectioned them in long-axis to simulate the echocardiographic LV-inflow/outflow tomographic view. We defined LVOT as the muscular region delineated proximally by the area extending from chordal-anterior mitral valve leaflet (AMVL)-junction, adjacent to the basal interventricular septum (IVS), and extending distally to the AMVL-aortic root attachment. Gross examination was aided by stereo microscopy. Cardiac structures were photographed and measured with digital image software.
and marked hypertrophy (i.e., a mound-like LVOT protuberance [15/17 HCM]). LVOT diameter (mean±SD) was narrower in HCM (4.5 ± 1.4 mm) vs DCM (6 ± 1.1 mm) (P < 0.001). The wall thickness, tapered from the chordal level to the base in all cats, most markedly in HCM. 2) Anomalous fibro-muscular structures extending into proximal LVOT [assumed congenital] included small, focal, fibrotic, ridge (1 DCM); oblique muscular ridges up to 2 mm wide (5/17 HCM; 6/19 DCM) originating from LV chamber or papillary muscle; and thickened moderator bands (3/19 DCM).

Gross LVOT lesions were prevalent in cats with ventricular hypertrophy-(HCM) and dilation-(DCM) and merit further evaluation.

C-42 MATRIX METALLOPROTEINASES 2 AND 9 IN RABBITS WITH DOXORUBICIN-INDUCED CARDIOMYOPATHY.

Doxorubicin is a chemotherapy agent frequently used in oncology patients. Its cardiotoxicity is ascribed to several cellular mechanisms, including necrosis and apoptosis. Little is known whether matrix metalloproteinases (MMP) and their tissue inhibitors play a role in the cardiotoxicity induced by doxorubicin. Therefore, this study was aimed at evaluating the plasma and myocardial activities of MMP 2 and 9 in rabbits with doxorubicin-induced cardiomyopathy.

Twenty New Zealand White rabbits were enrolled in the study. Ten rabbits were given doxorubicin intravenously at 1 mg/kg twice a week for 6 weeks to induce a dose-dependent cardiomyopathy. The remaining ten rabbits were only given saline intravenously at the same protocol. A blood sample was drawn from every animal at baseline (T0) and at 15 (T15), 30 (T30) and 45 (T45) days after either doxorubicin or saline were first given. Also, a complete echocardiogram was performed at the same moments. At the end of the study, the myocardial samples were obtained. Zimography was used to determine the activities of MMP 2 and 9.

Only the inactive MMP (pro-MMP) have been identified in the blood and myocardial samples. The plasma pro-MMP-2 was documented in the majority of control and doxorubicin animals from T0 to T45. On the contrary, pro-MMP-9 was found in just a few samples of either group, especially at T30 and T45. However, only weak bands could be demonstrated in zimography gels. A significant difference was shown to occur in pro-MMP-2 and 9 along time in both groups. While the plasma activity of pro-MMP-2 decreased, the activity of pro-MMP-9 increased from T0 to T45, although no significant differences existed between healthy and doxorubicin rabbits. Correlation tests between the plasma pro-MMP and the echocardiographic data disclosed significant results for the doxorubicin group. At T0 a correlation was found between TDI A(‘)septal and pro-MMP-2; at T15 correlations were documented between pro-MMP-2 and the parameters pre-ejection period and the Tei index; at T30 a correlation was identified between pro-MMP-9 and the left-ventricular free wall shortening; lastly, at T45 correlations have been observed between pro-MMP-2 and the parameters left-ventricular internal diameter in systole and diastole, interventricular septal thickness in systole, and the E(e)micro-to-E(‘)septal ratio, as well as between pro-MMP-9 and left-ventricular ejection time. Only the pro-MMP-2 was documented at the myocardial samples of either group, although no significant difference between healthy and doxorubicin animals could be demonstrated.

Although pro-MMP have been identified in both plasma and myocardial samples, they do not seem to be reliable indicators of the dose-dependent cardiotoxicity and myocardial remodeling in rabbits. Further studies could clarify whether the myocardial expression of these enzymes is altered in rabbits being given doxorubicin to induce a cardiomyopathy.

C-43 FREQUENCY OF HEART MURMURS IN CATS WITH AND WITHOUT CARDIAC DISEASE. G.T. Goldfelder, D.S. Schwartz, P.H. Iikawa, J.R. Castro, M.M. Mantovani, A.G. Gimenes, C.N. Duarte, M.H.M.A. Larsson, Department of Internal Medicine, School of Veterinary Medicine and Animal Science – University of São Paulo (USP), São Paulo, SP – Brazil.

In clinical cardiology, the occurrence of heart murmurs in cats can be caused by structural heart disease or physiological changes. A retrospective cross-sectional study was conducted between January 2008 and December 2012, with the purpose to determine cardiac disease frequency in the presence or absence of heart murmurs in domestic cats. From 385 cats admitted at the cardiology service of a veterinary teaching hospital 328 records were recovered, 29 were excluded for inadequate completion of clinical records. From 299 cats selected, 203 (67.89%) had murmurs and 96 (32.11%) had normal cardiac auscultation. Among the patients with murmurs, 145 (71.43%) had echocardiographic exam, where 77 (53.10%) had cardiac disease and 68 (46.90%) showed no changes. Regarding cats without murmurs on physical exam (n = 96), 86 (89.58%) were submitted to echocardiographic exam [13 had heart disease (15.12%) and 73 did not have evidence of cardiac changes (84.88%)]. In the group of patients with murmur, hypertrophic cardiomyopathy was the most frequent (23.50%), while the cats with no cardiac murmur, there was a predominance of restrictive cardiomyopathy (6.25%), followed by hypertrophic cardiomyopathy (21.5%). There was a significant association between presence of murmur and heart disease (p = 0.0001, Fisher’s Exact test). The presence of murmur had a sensitivity=85.56% (95%CI=76.56%-92.07%); specificity=51.77% (95%CI=43.21%-60.26%); PPV=53.10 (95%CI=44.65-61.43); NPV=84.88% (95%CI=75.54%-91.69%). Although the NPV is high, there is still a number of cats that can benefit from echocardiographic exam to unfold the presence of heart disease.


The unique phenotype of Persian cats has contributed to their intense breeding, and for the dissemination of diseases such as Polycystic Kidney Disease and Hypertrophic Cardiomyopathy. Thus, screening of apparently healthy animals, through accessible and low cost methods such as electrocardiography (ECG) and systemic blood pressure (SBP) measurements is very important.

Twenty-four cats, 18 month-old or older, from private catteries, were selected through history, physical examination, CBC, serum biochemistry, T4 dosage, abdominal ultrasound and echocardiography. ECG results showed that Persian cats have no particularities concerning P-wave, electric axis, PR interval, QRS complex, QT interval, R-wave on CV6LL and CV6LU, ST segment and T wave, but 8.3% of the animals (n = 2) presented sinus arrhythmia as a normal rhythm. Mean SBP and standard deviation obtained were: 135 ± 20 mmHg (n = 24); 128 ± 14 mmHg for the females (n = 14); 145 ± 24 (n = 10) for the males (p = 0,04); 126 ± 15 mmHg for cats 7 years old or younger (n = 13); and 145 ± 21 mmHg for older cats (p = 0.018).

Therefore, it was concluded that the electrocardiographic parameters described in literature are consistent with the results obtained for the Persian breed, and that sinus arrhythmia may be present on a higher frequency in healthy Persian cats than in the general cat population. As for the SBP, it was not observed breed influence on the values obtained, but it was observed that males were older cats presented higher SBP values, suggesting that age and sex might have an influence on this parameter.
C-45 CARDIAC MANIFESTATION OF FELINE INFECTIOUS PERITONITIS: COMPARISON OF TWO CLINICAL CASES. C.N. Duarte, D.S. Schwartz, P.H. Itikawa, A.S. Hora, J.R. Castro, A.M.D. Lora, P.H. Itikawa, Department of Pathology, Department of Preventive Veterinary Medicine and Animal Health.

Feline Infectious Peritonitis (FIP) myocarditis supported by molecular or histochecmy methods has not been described in domestic cats. This study aims to describe and discuss the association of FIP and cardiac manifestations in two unrelated kittens of undefined breed (cat#1: female, 2 months old; cat#2: male, 4 months old), presenting acute signs of lethargy, decreased appetite and dyspnea. Echocardiographic findings were suggestive of dilated cardiomyopathy for both. Also, they had mild pleural and pericardial effusion and the female had ascitis. Cat#1 had hypoproteinemia (5.0 mg/dL) and cat#2 had hyperglobulinemia (10.32 mg/dL) and hypoalbuminemia (0.24 mg/dL). Due to poor clinical status and suspected FIP infection, the owners decided for euthanasia. Post mortem exam was performed. On histopathology, cat #1 had deposition of fibrolipidic interstitial material in both ventricles and left atrium and cat#2 had myocarditis characterized by necrotic areas associated with inflammatory infiltrate. Both had several white dots distributed through the endocardium. Feline coronavirus (FCoV) mRNA was detected on PCR in several organs, confirming FIP diagnosis. Myocardium of cat#2 was PCR positive for FCoV mRNA, while cat#1 had only positive pericardial effusion. These findings are highly suggestive of FIP induced myocarditis in cat#2. Considering that cat#1 did not have myocardial/immunological infiltrate nor FCoV mRNA, the fibrofatty infiltrate could have replaced a previously necrotic/infamed myocardium or this could be a coincidental comorbidity of FIP and fibrofatty variety of arrhythmogenic right ventricular cardiomyopathy (ARVC), which is reported in young human patients, and may also affect left ventricle.

C-46 EFFECT OF MITRAL VALVE GEOMETRY ON PATHOLOGICAL CHANGES IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE. T. Aoki, Y. Fukuda, H. Sunahara, K. Sugimoto, Y. Fujii. Azabu University, Kanagawa, Japan.

Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs. In humans and dogs with MMVD, the caudal area of the mitral valve, particularly the septal leaflet, appeared to be the most affected. However, the causes are yet to be ascertained. We hypothesize that the caudal area of the mitral valve is more affected in dogs with MMVD due to geometrical differences of the mitral apparatus. Twenty normal heart specimens were used for morphometric investigations. Digital images of the mitral valve and mitral annulus from the surgeon's view were obtained. A digital image of the septal leaflet was also obtained after incision of the heart specimen between the papillary muscles. The images of the mitral annulus and septal leaflet were divided into 2 areas: the cranial and caudal areas. The cranial and caudal permeters of the mitral annulus were measured with a measurement software. Similarly, the lengths of the cranial and caudal edges of the septal leaflet were determined. In the mitral annulus, the caudal area was larger than that of the cranial (1.59 ± 0.13 cm vs. 1.20 ± 0.21 cm, respectively; p = 0.01). In the septal leaflet, the length of the caudal edge was longer than that of the cranial edge (1.17 ± 0.15 cm vs. 1.07 ± 0.16 cm, respectively; p = 0.01). In conclusion, the broad geometry of the caudal area may have an impact on the pathological changes and justify why the caudal area is more affected in dogs.

C-47 PROGNOSTIC VALUE OF LEFT ATRIAL FUNCTION IN DOGS WITH CHRONIC MITRAL VALVULAR HEART DISEASE. K. Nakamura, T. Osuga, S. Suzuki, K. Morishita, H. Ohta, M. Yamasaki, M. Takiguchi, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.

A strong correlation between left atrium (LA) dysfunction and the severity of cardiac disease has been described in human patients with various cardiac diseases. However, the role of LA dysfunction in dogs with chronic mitral valvular heart disease (CMVHD) has not been addressed. The purpose of this study was to investigate the correlation between LA function and prognosis of dogs with CMVHD.

Thirty-seven client-owned dogs fulfilling the following criteria were included in this study: (1) presence of echocardiographic evidence of mitral regurgitation, (2) echocardiographic examination of sufficient quality for adequate analysis, and (3) follow-up for more than one year or until the onset of cardiac related death. Dogs were divided into two groups based on the onset of cardiac-related death within one year after first echocardiographic examination. Physical examination and echocardiographic variables were compared between the two groups. For the assessment of the comparative accuracy in identifying patients with cardiac related death, receiver operating characteristic curves and multivariate logistic analysis were used.

The highest accuracy was obtained for LA active emptying fraction (%LAEFact) with area under the ROC curve (AUC) of 0.95, followed by left atrial to aortic root ratio (LA/Ao) with AUC of 0.93, the peak early diastolic mitral inflow velocity (E) with AUC of 0.85 and LA total emptying fraction (%LAEFtotal) with AUC of 0.82. At multivariable logistic regression analysis, %LAEFact emerged as the only independent correlate of cardiac related death within one year in our study population (odds ratio = 1.396, P = 0.002).

LA, in regard to both size and function, has strong correlation with prognosis of dogs with CMVHD. %LAEFact was the most significant independent predictor of mortality in the present study.

C-48 QT INTERVAL DURATION AND DISPERSION IN DOGS TREATED WITH ONDANSETRON. C.S. Freire, F.C. Teixeira, G. Nakamura, T. Osuga, S. Suzuki, K. Morishita, H. Ohta, M. Yamasaki, M. Takiguchi, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.

A prospective cross-sectional study was conducted to evaluate the duration of the corrected QT interval (QTc) and QT dispersion on the ECG of dogs treated with ondansetron. Sixty dogs were included and assigned to Control (34 healthy dogs) or Ondansetron (26 dogs with different diseases with prescription because of its anti-emetic effect, at therapeutic doses of 0.05–0.5 mg/kg). Ten-lead ECGs were recorded (50 mm/s) at three different moments for Ondansetron group: prior to the administration of the drug (T0), 10 (T10) and 20 (T20) minutes after drug administration, whilst the control group had only one recording. The QT interval was corrected with Bazett’s (QTc = QT/√RR) and Fridericia’s (QTcFridericia = QT/√RR) formulas. QT dispersion was smaller at T0 (CI95%:0.015-0.020) compared to Control (CI95%:0.020-0.030; P = 0.0227). QTcFridericia (0.2885 ± 0.0216) and QTcBazett (0.2592 ± 0.0202) were increased for sick dogs (T0) compared to Control (CI95%:0.2529 ± 0.0267; QTcFridericia = 0.2368 ± 0.0213) (P < 0.001; P = 0.004). Ondansetron subgroups were similar for QTcBazett: T0 x T10 (P = 0.408) and T0 x T20 (P = 0.461), but QTcFridericia was prolonged on T10 (0.2569 ± 0.0218) and T20 (0.2576 ± 0.0247) compared to T0 (0.2529 ± 0.0202) (P = 0.049; P = 0.034). When comparing T0 x T10 x T20, no significant difference was found for QTcBazett (P = 0.706), QTcFridericia (0.060) or dispersion (P = 0.067). The differences in QTc duration between Control and Ondansetron group at T0 make us consider that the presence of a disease by itself can already interfere with the duration of this interval. The lack of difference observed in QTcBazett between the moments
recorded for the Ondansetron group compared to a significant difference for QTc Fridericia must be reevaluated in a larger sample.

N-1 INFLAMMATORY CHEMOKINES AND CYTOKINES IN CEREBROSPINAL FLUID OF DOGS WITH ACUTE SPINAL CORD INJURY. A. Taylor\textsuperscript{1}, C.J. Welsh\textsuperscript{1}, C. Young\textsuperscript{1}, E. Spoor\textsuperscript{1}, N. Cohen\textsuperscript{1}, G. Levine\textsuperscript{1}, J. Levine\textsuperscript{1}. \textsuperscript{1}Texas A&M University College of Veterinary Medicine and Biomedical Sciences, College Station, TX.

In humans with acute spinal cord injury (SCI), cerebrospinal fluid (CSF) IL-6 and IL-8 concentrations are elevated. These cytokines are closely correlated with neutrophilic inflammatory responses after SCI and IL-8 serves as a predictive biomarker for SCI severity and recovery. This study measured inflammatory cytokines in the CSF of dogs with acute SCI due to thoracolumbar disk herniation (IVDH). The hypothesis was that CSF IL-6 and IL-8 would be comparable to normal controls and would correlate with SCI severity at admission as measured by a modified Frankel score.

CSF from dogs with surgically confirmed thoracolumbar IVDH (n = 39) and control dogs (n = 21) was used to measure inflammatory cytokines. Serum concentrations of IL-8 were 10.1 ng/ml (range 0.5-53.2 ng/ml) versus 2.6 ng/ml (range 0.1-9.0 ng/ml). Concentrations of IP10 and IL-18 were significantly higher in controls than cases (P < 0.0001). Concentrations of IP10 and IL-18 were significantly higher in controls than cases (P < 0.0006). Neither cytokines nor chemokines were correlated with SCI severity.

Innate inflammatory responses mediated by IL-8 are prominent in acute IVDH-associated SCI. Reasons for down-regulation of IP10 and IL-18 were inconclusive compared to normal controls and would not correlate with SCI severity as admitted by a modified Frankel score.


The use of mycophenolate mofetil (MMF) for a variety of immune-mediated diseases in veterinary medicine has been described. However, there are no studies documenting its use in dogs with meningoencephalomyelitis of unknown etiology (MUE). We hypothesized that the use of MMF in dogs with MUE would be comparable to normal controls and treatment protocols and is associated with limited adverse effects.

A retrospective study of medical case records of dogs clinically diagnosed with MUE recorded signalment, magnetic resonance imaging findings, cerebrospinal fluid analysis results, medications administered, follow-up neurologic examinations at 1, 2, and 6 months, survival and adverse events. Variables were compared between dogs in which MMF was adjunctive to corticosteroids (immediate group) vs. dogs in which MMF was started >30 days after diagnosis (delayed group).

Twenty-five cases of MUE were identified. The overall median survival time from diagnosis was 731 days (range 43-1672 days). There was no significant effect of any recorded parameter on survival. Dogs with delayed treatment (n = 14) had a significantly lower rate of clinical remission at their 6-month follow-up than dogs treated immediately (p = 0.043). Using multiple logistic regression, delayed initiation of MMF therapy was the only recorded parameter found to significantly affect the possibility of clinical remission at the six-month follow up. Adverse events were identified in two cases (8%) and were characterized by mild gastrointestinal signs (vomiting and decreased appetite).

Administration of MMF appears safe in dogs with MUE. The use of MMF results in comparable survival times to alternate immunosuppressive protocols. Initiation of mycophenolate mofetil therapy within thirty days of suspected MUE diagnosis may improve long-term clinical response.

N-3 IDENTIFICATION OF GENETIC RISK LOCI IN MALTESE DOGS WITH NECROTIZING MENINGOENCEPHALITIS. R.M. Barber\textsuperscript{1}, S.J. Schatzberg\textsuperscript{2}, I. Schrauwen\textsuperscript{3}, A. Sinaird\textsuperscript{4}, J.J. Corneveaux\textsuperscript{5}, B.F. Porter\textsuperscript{6}, K.M. Vernau\textsuperscript{7}, R.I. Keesler\textsuperscript{8}, K. Matias\textsuperscript{1}, T. Fiegel\textsuperscript{7}, A.D. Miller\textsuperscript{9}, T. Southard\textsuperscript{9}, C.L. Mariam\textsuperscript{10}, G.C. Johnson\textsuperscript{11}, M.J. Huentelaman\textsuperscript{12}. \textsuperscript{1}University of Georgia, Athens, GA., \textsuperscript{2}The Animal Neurology and Imaging Center, Algo- dones, NM., \textsuperscript{3}Translational Genomics Research Institute, Phoenix, AZ., \textsuperscript{4}Texas A&M University, College Station, TX., \textsuperscript{5}University of California, Davis, CA., \textsuperscript{6}Utrecht University, Neth- erlands., \textsuperscript{7}Ludwig-Maximilians University, Germany., \textsuperscript{8}University of Leipzig, Germany., \textsuperscript{9}Cornell University, Ithaca, NY., \textsuperscript{10}North Carolina State University, Raleigh, NC., \textsuperscript{11}University of Mis- souri, Columbia, MO.

Necrotizing meningoencephalitis (NME) has been reported in numerous toy dog breeds, including the pug and Maltese. Genetic risk for NME development has been associated with dog leukocyte antigen (DLA) class II in pug dogs. This investigation sought to identify genetic risk factors in Maltese dogs with NME with the hypothesis that pug and Maltese dogs would share similar risk loci for NME development. Eleven affected and 38 unaffected Maltese were genotyped across 173662 single nucleotide polymorphisms (SNPs). Affected dogs included 8 with histopathologically confirmed disease and 3 with a presumptive diagnosis based on clinical presentation, magnetic resonance imaging, and cerebrospinal fluid analysis. PLINK was used for statistical analysis. All samples had a call rate of > 80% and were included in analysis. SNPs on the X and Y chromosomes and those with call rates < 97%, minor allele frequency < 0.10, or deviation from Hardy-Weinberg equilibrium were excluded, resulting in final evaluation of 57222 SNPs. Evaluation of population structure by pairwise identity by descent estimation, multidimensional scaling analysis, and a QQ plot of observed against expected p-values resulted in exclusion of 3 controls from further analysis. Fisher’s exact tests were performed to compare SNP allele frequencies and Bonferroni correction was applied with a resulting p-value of 8.7 x 10-7 for genome-wide significance. To further confirm genome-wide significance, MaxT permutation testing of 10,000 permutations and a false discovery rate correction were applied.

Two regions reached genome-wide significance. The first is an approximately 10 Mb region on chromosome 4 (p = 8.07 x 10-7). Among the genes in this region is IL7R, which codes for part of the interleukin 7 receptor expressed on lymphocytes and plays a crucial role in immune system function. The second is an approximately 1.9 Mb region located on chromosome 15 (p = 1.55 x 10-7). Among the genes in this region is FBXW7, which codes for 3 isoforms. The most abundant alpha isoform has been shown to be important in control of inflammatory signaling. There was no compelling association of the DLA II locus and NME in the Maltese.

In conclusion, we identified two risk loci for the development of NME in the Maltese dog breed both of which contain potential genes of interest. Based on this data, the pug and Maltese breeds appear to have different risk loci for the development of NME.
N-4 EVALUATION OF TRANSDERMAL ADMINISTRATION OF PHENOVARBITAL IN HEALTHY CATS. D.P. Krull1, S.A. Thomovsky2, A.V. Chen-Alien1, K.A. Mealey1, M.G. Papich1.

Three healthy adult cats, with normal physical and neurologic examination and normal hematologic and serum biochemical analyses including bile acids testing, were included in the study. The transdermal phenobarbital was obtained from a nationally advertised compounding pharmacy as two separate concentrations (120 and 180 mg/mL) based on body weight in a pleuronic lecithin organogel-based vehicle. 0.1 mL was applied to the inner pinnae of both ears delivering a dosage of 3.0-3.1 mg/kg per ear (total of 6.0-6.2 mg/kg) every 12 hours for 14 days. The dose used in this study was based on a pilot study. Serum was obtained to determine phenobarbital concentrations 3-6 hours after dosing on days 3, 5, 7, 9, 11, 13 and 15. Neurologic and physical examinations were performed prior to each blood draw. Repeat hematologic and serum biochemical analyses including bile acids testing were performed at the completion of the study to screen for adverse effects. Descriptive statistics were used to analyze the data. The mean, median, and standard deviation of phenobarbital serum concentrations were determined at day 15, as well as means and standard deviations for peak concentration and time to peak concentration. Adverse effects were reported.

N-5 DYNAMIC LUMBOSACRAL INSTABILITY: CLINICAL EVALUATION AND OWNER PERCEIVED OUTCOME OF A NOVEL APPROACH FOR SURGICAL DISTRACTION-STABILIZATION OF L7-S1: 51 CASES (2008-2013). R. Beam1, D. Powers1, J. Hauptman2, S. Sanders1. 1Seattle Veterinary Specialists, Kirkland, WA., 2Michigan State University College of Veterinary Medicine, East Lansing, MI.

Fifty-one dogs admitted to a private practice veterinary hospital were treated for lumbosacral instability with a novel implant to stabilize the L7–S1 intervertebral disk space. Determination of post-operative success was evaluated with client questionnaire and through comparison of pre and post-operative neurological examinations. Patients were evaluated with magnetic resonance imaging for suspected spinal disease and all subjects were diagnosed with one or more of the following: lumbosacral spinal canal stenosis (dynamic or static), intervertebral foraminal stenosis (dynamic or static), vertebral instability, or degenerative intervertebral disk disease. Treatments consisted of dorsal laminectomy at L7-S1, +/- disk fenestration, followed by implant placement using screws, pins and polymethylmethacrylate, in distraction to stabilize the lumbosacral space in a neutral position. Follow-up questionnaires were evaluated to obtain owner information regarding perceived outcome. Follow-up exams were performed several months to years following surgery.

Results of the questionnaire regarding client perceived outcome was deemed good to excellent in 81% of cases available for evaluation. There was a significant association with improvement of pre and post-operative pain (p < 0.0001), return of withdrawal reflexes (p < 0.02) and the presence or absence of pre and post-operative neurologic deficits (p < 0.02). This technique provides a robust implant, with minimal incidence of post-operative complications [major (2%); minor (13%)]. This technique allows creation of a customized, enginnered implant which can be created on-site to best fit the patient or accommodate for surgical complications.

Treatment for lumbosacral instability with described implant was successful and well tolerated in patients, including functional outcome and perceived improvement by owners.
Leishmania spp, FIV, FeLV, FIP. CSF analysis included also differential cell count. Cytological and histological specimens were examined after routine staining.

Data considered were: localisation of lesions determining the symptomatic type, type of pathology responsible for neck pain (malformative, traumatic, vascular, inflammatory, degenerative, neoplastic), primary or indirect involvement of Nervous System, any neurological or systemic signs.

95% of patients suffered from neurological pathology whereas 5% were non-neurological. Lesions were in the neck in 72.5% of cases, in 17.4% both head and neck were involved. In 9% of cases the lesions were only in the head, in 1.1% in the chest.

52.5% of dogs suffered from intervertebral degenerative disc disease (IVDD), 19.5% were neoplastic, 14.5% inflammatory, 8.5% traumatic, 2-5% malformation, 2-5% vascular. 66.6% of cats were neoplastic, 22.2% traumatic, 11.2% inflammatory.

78% of cases showing a cervical spinal lesion had IVDD. 77% of cases with brain and spinal lesions had an inflammatory disease, whereas in all cases of neck pain with a lesion located in the head, the pathology is neoplastic.

The findings of this study indicate that lesions responsible for neck pain can localise in the neck, head or chest. Pain and lesion localisation does not always correlate. There are differences in the prevalence and location of diseases in dogs and cats.

Based on the results of this study, a complete diagnostic work for animals with neck pain must include clinical examination, advanced imaging of the head, neck and chest. If appropriate, CSF analysis must be performed.

N-8
ATLANTO-OCCIPITAL DISLOCATION IN 4 DOGS: MRI FINDINGS AND SURGICAL TREATMENT. M. Dolera, L. Mailfarsi, La Cittadina Fondazione Studi e Ricerche Veterinarie, Romanengo (CR), ITALY.

Atlanto-occipital dislocation can occur in dogs following vehicle trauma. Few reports of this pathology have been described in veterinary medicine. Non-surgical approach may not adequately stabilize the extensive joint instability. Various surgical techniques have been proposed, sometimes with disappointing results.

The purpose of this study was to present MRI findings and a new technique of surgical stabilization of atlanto-occipital dislocation in the dog. Four dogs suffering from atlanto-occipital dislocation were considered. In cases 1 and 3, a car run over the patient in a caudocranial direction in the neck region. In cases 2 and 4, while the patient was sleeping a car ran slowly over it, crashing it between the ground and the vehicle floor. All dogs exhibit varying degrees of quadriparesis and dyspnea. Head and neck 1.5T MRI showed atlanto-occipital dislocation, with disruption of atlanto-occipital and occipitoaxial ligaments and various degrees of spinal cord compression. Surgery was performed immediately. A peri-operative antibiotic prophylaxis with cefazolin (30 mg/Kg i.v.) was started. Each patient, anesthetized and mechanically ventilated, was placed in sternal recumbency. With the head kept horizontal, four surgical accesses were carried out at the zygomatic processes and at the atlas wings on each side respectively. Once these structures had been exposed, a 2-mm diameter hole was drilled in each atlas wing 5 mm caudal to the cranial margin and 10 mm medially to the lateral margin. A nylon monofilament (φ 1 mm) was inserted in the hole and an O-shaped ligature was carried out externally to the skin through the ipsilateral zygomatic arch. Once the anatomical reduction of the dislocation was controlled through a fluoroscopy image, the surgical openings were sutured in planes. On the four skin emergences of the two ligatures an antiseptic ointment was applied on a daily basis. Two weeks after the surgical treatment the patients had regained the ability to walk. Ligatures were removed after 2 months. Gentle manipulations aimed to verify the atlanto-occipital stability revealed a good result in the absence of rotational movements against a slight reduction in the physiological excursion in flexion and extension. At that time, as at follow up examinations (average 24 months) all dogs were normal. MRI is useful to confirm atlanto-occipital dislocation in dogs. The surgical technique that we developed is simple and safe to perform compared to other described in veterinary literature. However, the limited number of cases considered requires further clinical confirmations.

N-9
DYNAMIC SUSCEPTIBILITY CONTRAST IMAGING OF NORMAL CANINE BRAIN. N.K. Lee1, D.G. An1, J. Park2, C. Lee1, D.I. Jung1, J.H. Kang1, D. Chang3, M.P. Yang1, B.T. Romanengo (CR), ITALY.

The cerebral hemodynamics is one of the most important parameters related to brain physiology and function. Several magnetic resonance imaging (MRI) methods have been proposed to measure cerebral perfusion. Dynamic susceptibility contrast (DSC)-MRI is currently the most commonly used method to yield measures of cerebral blood volume (CBV), cerebral blood flow (CBF), and mean transit time (MTT) of the paramagnetic contrast agent. Although DSC-MRI is highly popular perfusion imaging method, its application to veterinary medicine has been restricted. Therefore, the purpose of this study was to assess cerebral perfusion of normal canine brain using a DSC-MRI.

DSC-MR scans of the brain were acquired from 5 healthy laboratory beagle dogs to calculate functional maps of CBF, CBV, MTT, and time to peak enhancement (TTP). On T2*-weighted fast gradient-echo MR images, regions of interests (ROIs) were manually drawn over intracranial structures, including cerebral cortex (frontal, parietal, temporal and occipital lobes), cerebral white matter, caudate nucleus, hippocampus, diencephalon, midbrain, pons, medulla oblongata, and cerebellum. Relative CBV and CBF ratios (rCBV = CBV/ROICBF of cerebellum; rCBF = CBF/ROICBF of cerebellum) as well as MTT and TTP were calculated for each ROI. Friedman test revealed significant differences of rCBV, rCBF, and TTP among examined structures (P < 0.05). Regional CBV and CBF in the cerebellum were significantly greater than those in the cerebral white matter (rCBV = 0.56 ± 0.07, rCBF = 0.54 ± 0.06), diencephalon (rCBV = 0.71 ± 0.09, rCBF = 0.77 ± 0.10), and midbrain (rCBV = 0.74 ± 0.11, rCBF = 0.77 ± 0.09). However, CBF of the parietal (rCBF = 1.18 ± 0.13) and occipital (rCBF = 1.0 ± 0.11) lobes was significantly higher than those in the midbrain. The examined structures, except for the pons and medulla oblongata, had a significant delayed TTP in comparison with the cerebellum.

The cerebral perfusion of normal dogs was different according to intracranial structures. The data acquired from the present study may be complementary for investigating canine cerebrovascular accidents such as ischemic stroke and intracranial hemorrhage.

N-10
COMPARATIVE GENOMIC AND HISTOLOGIC ANALYSIS OF CANINE AND HUMAN MENINGIOMAS. B. Choe1, D.G. Patel1, G.Y. Gillespie2, D. Chen3, T.R. Schoeb4, R. Hackney2, M. Bray3, C. Johnson2, A. Shores1, D. Sorjonen2, M.R. Chambers5, 1College of Veterinary Medicine, Mississippi State University, Mississippi State, MS., 2Auburn University College of Veterinary Medicine, Auburn, AL, 3Department of Neurosurgery, 4Department of Pathology, and, 5Department of Genetics, The University of Alabama at Birmingham School of Medicine, Birmingham, AL.

Meningiomas are the most common primary intracranial neoplasms in humans and dogs. Some meningiomas are histologically more malignant, and have an increased tendency to recur. Human and canine meningiomas share striking similarities in location, imaging characteristics, gross and histological appear-
ance, biological behavior and presenting clinical signs, suggesting a common molecular basis in both species. In dogs, as in humans, molecular classification and genetic characterization of meningiomas are poorly understood, although several genes have been identified as candidates for playing a role in tumor development and progression.

We performed genotyping on 48 naturally occurring canine meningiomas using the Scan™ system and the CanineHD BeadChip™ to identify cytogenetic abnormalities conserved in human neoplasms. Tumors were categorized by molecular signatures and by histology using the WHO classification for human meningiomas. DNA segmental alterations, which could be amplifications (chromosome 37) and deletions (chromosomes 1, 6, 9, 14, 31, 37), were identified when histologically aggressive tumors (n = 14) were compared with benign tumors (n = 34). We identified 32 genes in the altered regions correlating to proteins in five signaling pathways - including MSP-RON, interferon gamma and cytokine signaling, which control cell movement, morphology, interaction, and survival. We genotyped canine DNA and identified candidate genes involved in tumorogenesis and/or malignant progression (aggressive vs. benign) of canine meningiomas. Our results indicate that the canine meningioma provides a promising model of the human counterpart for development and evaluation of potential therapies based on molecular targets.

N-11 DESIGN, DEVELOPMENT, AND TESTING OF AN INTRACRANIAL PRESSURE BOLT FOR USE IN VETERINARY PATIENTS. M. Perez', A. Shores', L. Williams'. 1College of Veterinary Medicine and, 2College of Agricultural and Bioengineering, Mississippi State University, Mississippi State, MS.

Therapeutic management of traumatic brain injury (TBI) includes measures to reduce intracranial pressure (ICP); however, in veterinary medicine, this therapy is empirical because no system for this measurement is easily adaptable to the veterinary patient. In humans, continuous ICP monitoring is used for severe TBI patients (Glasgow Coma Scale ≤ 8) and the measurements are used to guide therapeutic management. The currently available systems for humans require placement of an intracranial bolt that relies on engaging the bolt threads in a screw-like fashion within the skull bone. Veterinary patients have thinner bone and often it is not possible to engage enough threads of the bolt to hold it securely. In addition, the bolts extend for a distance of at least 3 cm above the skull, so they can be easily dislodged by the patient or by patient movement such as occurs with cage confinement.

We designed and tested an intracranial bolt for use in dogs and cats that can be used with the Integra® Camino® Parenchymal Intracranial Pressure Monitoring Kit. The designed system is adaptable to any thickness of skull and relies on a toggle bolt principle for securing in place rather than a screw-in bolt. In addition, this bolt lies flat against the skull so it cannot be dislodged by the patient or by patient movement. The material used in construction of this bolt is a high-impact, biocompatible plastic.

In our project testing, the bolt was placed in cadaver skulls of 6 dogs and 3 cats. Pull out strength was compared to the ICP screw-type bolt used in humans and was significantly higher for the veterinary bolt (p < 0.001) in all cadaver skulls. Additional testing established this material, unlike some commercially available human ICP bolts, is 3.0T MRI safe.

We conclude this product is an easily applicable clinical device for the canine or feline skulls of any thickness, produces a secure attachment for continuous or long-term ICP monitoring in veterinary patients, is biocompatible, and 3.0T MRI compatible. Development of this product will assist in facilitating the use of ICP monitoring as a more practical tool in veterinary medicine. IACUC approval is pending for testing in clinical patients with Small Animal Coma Scale values of ≤ 9.

N-12 THE EFFECT OF ANGLE SLICE ACQUISITION ON COMPUTED TOMOGRAPHIC CERVICAL VERTEBRAL COLUMN MORPHOMETRY IN GREAT DANES. A.M. Jurkoshek, R.C. da Costa, P. Martin-Vaquero. Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

Computed tomography (CT) is a routinely used diagnostic modality for evaluation of dogs with neurologic conditions, including cervical spondylomyelopathy (CSM). CT scans can be acquired with the transverse images aligned either parallel to the endplates or perpendicular to the vertebral canal. The purpose of this study was to determine the effect of angle acquisition on morphometric evaluation of the cervical vertebral column of Great Danes with and without CSM.

Twenty-eight Great Danes, 15 CSM-affected and 13 control dogs, underwent CT imaging. A set of images was obtained with the transverse slices aligned parallel to the endplates and another one with the transverse images aligned perpendicular to the vertebral canal. For each different set, transverse slices from the cranial, middle, and caudal aspect of the vertebral body from C2-C7 were evaluated. At each location the following measurements of the vertebral canal were made: height, width, transverse area, left dorsal to right ventral height (LDRV), and right dorsal to left ventral height (RDVL). Measurements were analyzed using random-effects linear regression models.

Significant differences between the measurements obtained from the two sets of transverse images acquired at different angles were found only at the cranial locations (P < 0.05). No differences were seen at middle and caudal vertebral locations.

The funnel-shape morphology of the vertebral canal with stenosis of its cranial aspect may be responsible for the significant differences found. Considering that the morphometric parameters showed significant differences, it is important to define and follow a standardized scanning protocol when morphometric evaluations are planned.


Cognition in dogs, like in humans, is not a unitary process. Some functions, such as simple discrimination learning, are relatively insensitive to age; others, such as visuospatial learning can provide behavioral biomarkers of age. The present experiment sought to further establish the relationship between various cognitive domains, namely visuospatial memory, object discrimination learning (ODL) and selective attention (SA). In addition, we also set up a task to assess motor learning (ML). Thirty-six beagles (9-16 yrs) performed a variable Delay-Non-Matching-to-Position (vDNMP) task using 2 objects with 20-sec and 90-sec delay and were divided into 3 groups based on a combined score (HMP=88-93% accuracy [N = 12]; MMP=79-86% accuracy [N = 12]; LMP=61-78% accuracy [N = 12]). Variable object oddity task was used to measure ODL (correct or incorrect object) and SA (0-3 incorrect distractor objects with same [SA-same] or different [SA-diff] correct object as ODL). ML involved reaching various distances (0 to 15 cm). Age did not differ between memory groups (mean: 11.6 yrs). ODL (ANOVA P = 0.43), or SA-same and SA-diff (ANOVA P = 0.96), performance did not differ between the 3 vDNMP groups, although mean errors during ODL was numerically higher for LMP dogs. Errors increased (P < 0.001) for all dogs with increasing number of distractor objects during both SA tasks. vDNMP groups remained different (ANOVA P < 0.001) when re-tested with vDNMP task 42-days later. Maximum ML distance did not differ between vDNMP groups (ANOVA P = 0.96). Impaired short-term memory performance in aged
dogs does not appear to predict performance of cognitive domains associated with object learning, SA, or maximum ML distance.

N-14 CERVICAL VERTEBRAL TRABECULAR BONE MINERAL DENSITY IN GREAT DANES WITH AND WITHOUT CERVICAL SPONDYLOMYEOLOPATHY. J. Armstrong, R.C. da Costa, P. Martin-Vaquero. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Although cervical spondylomyelopathy (CSM) is the most common disease of the cervical spine in large and giant breed dogs, a definitive understanding of the underlying disease mechanisms remains elusive. Previous studies have described that the osseous associated form of CSM seen in Great Danes (GDs) is a result of developmental vertebral malformations and osteoarthritic changes of the articular facets. This study aimed at characterizing and comparing vertebral trabecular bone density at the articular processes, vertebral bodies, and pedicles in GDs with and without CSM.

Twelve control and 10 CSM-affected client-owned GDs were enrolled. Non-contrast computed tomography (GE Lightspeed Ultra 8-slice helical scanner) scans of the cervical vertebral column (C2-C3 to T1-T2) were obtained for each dog under sedation in sternal recumbency. A calibration phantom (Model 3T, Mindways Software) was scanned alongside each dog. A single investigator measured the density of the trabecular bone in Hounsfield units (HUs) by placing a circular region of interest (ROI) at the level of the articular processes, vertebral body, and pedicles using ClearCanvas Workstation software. Hounsfield units were also measured for ROIs placed within each rod of the calibration phantom. Using previously developed and utilized software, HUs for each ROI were converted to diphosphate equivalent densities (PPEDs). Calibrations were completed on a slice-by-slice basis so that the attenuation of each voxel within a given ROI could be converted to a PPED. Comparisons of PPEDs were made between males and females within the control group, and between CSM-affected and control GDs using random-effects linear regression models.

Although males had greater PPEDs across all ROIs, no significant differences were identified based on gender when the model was adjusted for age and region. The PPEDs for CSM-affected dogs were consistently lower than the equivalent values for the control group, however these differences were not significant when adjusted by age and gender.

These results indicate that vertebral trabecular bone mineral densities at the level of the articular processes, vertebral body, and pedicles are not significantly different between CSM-affected and control GDs.

N-15 BODY CONFORMATION IN GREAT DANES WITH AND WITHOUT CLINICAL SIGNS OF CERVICAL SPONDYLOMYEOLOPATHY. P. Martin-Vaquero, R.C. da Costa. College of Veterinary Medicine, The Ohio State University, Columbus, OH.

A combination of large head and long neck has been suggested to cause abnormal forces on the cervical vertebral column and be involved in the pathogenesis of cervical spondylomyelopathy (CSM). The purpose of this study was to compare the body conformation of Great Danes (GDs) with and without clinical signs of CSM by use of six body measurements, and to investigate if body measurements had any correlation with the severity of neurological signs and the cervical vertebral body height and length as measured on magnetic resonance imaging (MRI) in the CSM-affected GDs.

Thirty client-owned GDs were prospectively enrolled. 15 clinically normal and 15 CSM-affected. Six body measurements (head length, head circumference, neck length, base width, withers height, and back and loin length) were obtained for each dog. A video of the gait of all CSM-affected GDs was obtained at the time of enrollment. Gaits were assigned a grade from 1-6 and grouped as mild, moderate, or severe. All dogs underwent MRI of the cervical vertebral column. Vertebral body length and height were obtained from C3 through C7.

Base width was the only statistically different body measurement between groups (P < 0.046). No differences were identified for the remainder body measurements. No significant correlations were identified between the degree of neurological signs or the vertebral body heights and lengths and the body measurements in the CSM-affected GDs.

The results of this study do not support the hypothesis that differences in body conformation play a role in the pathogenesis of CSM in Great Danes.

N-16 ENTROPY INDICES ARE PREDICTIVE OF AN AWAKE RESPONSE ELICITED DURING SEVOFLURANE ANESTHESIA IN DOGS. C. Mahidol1, S. Niyom1, K. Wasanasuk2, N. Koatsang2, N. Thengchaisri1. 1Department of Companion Animal Clinical Sciences, and 2Kasetsart Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

Subjective measurements of minimal alveolar concentration (MAC) for volatile anesthetic agents in response to tail clamping may vary widely. Entropy indices provide objective measures of the electroencephalographic signals corresponding to the awake and sleep stages. The use of entropy indices (SE and RE values with the highest Youden’s indexes were 75% and 65%, respectively, which corresponded to odds ratios of 2.52 and 1.3, respectively). RE and SE values served as independent predictors of an awake response elicited during tail clamping while under sevoflurane anesthesia was analyzed using multivariate logistic regression analysis.

The MAC baseline for sevoflurane ranged from 1.8% to 2.6% (mean MAC baseline for sevoflurane ranged from 2.2% to 2.3%). RE and SE values during positive responses to tail clamping were significantly higher than during negative responses (RE: 88 ± 2 vs. 63 ± 3, p < 0.001; SE: 76 ± 2 vs. 52 ± 3, p < 0.001). The RE-SE difference was not significant when positive and negative responses to tail clamping were compared (11 ± 1 vs. 13 ± 1, p > 0.178). SE and RE values served as independent predictors of an awake response with the area under Receiver Operator Characteristic (ROC) curve of 0.810 (95% CI: 0.716-0.903) and 0.828 (95% CI: 0.741-0.916), respectively. RE-SE difference was not an independent predictor with the area under the ROC curve of 0.588 (95% CI: 0.468-0.708). The cutoff points for SE and RE values with the highest Youden’s indexes were 75% and 65%, respectively, which corresponded to odds ratios of 2.52 ± 15.6 (for RE) and 14.9 ± 7.9 (for SE) to predict an awake response elicited during tail clamping.

In conclusion, SE and RE values are good predictors of an awake response elicited by tail clamping during determination of MAC for sevoflurane. Spectral indices are possible objective measures for MAC determination for volatile anesthetic agents.

N-17 CONGENITAL SPONGIFORM LEUKODYSTROPHY IN TWO FEMALE LITTERMATE GERMAN SHEPHERD PUPPIES. Z. Demeter1, D.W. Hague, D.A. Coleman2, H.M. Randol1, J.P. Hekman1, T. Johnson3, A.V. Kukekova1, S. Lezmi1. 1University of Illinois Department of Pathobiology, Urbana, IL, 2University of Illinois Department of Veterinary Clinical Medicine, Urbana, IL, 3University of Illinois Department of Animal Sciences, Urbana, IL.

Two 9-week-old female littermate German shepherd puppies were presented with progressive whole body tremors present...
since birth. Neurologic examination revealed normal mentation, wide based stance when standing followed shortly by collapse, and high frequency and low amplitude tremors of the entire body that worsened with movement. The puppies came from a litter of six: four unaffected males, and the examined two female puppies. These affected females were about one half the sizes of their male littermates. Due to poor prognosis and worsening tremors, both examined animals were humanely euthanized.

Necropsy revealed no gross abnormalities. Histology revealed vacuolation of the central nervous system white matter with decreased myelin staining, evidence of cell necrosis, apoptosis, reactive gliosis, and mild secondary lymphohistiocytic perivascular cuffing. The changes were consistent with leukoencephalomalacia with spongy degeneration (leukodystrophy). The most severe changes were observed in the white matter of the cerebellum, and the large white matter bundles of the brainstem, optic tracts, and spinal cord. The white matter of the corona radiata and the peripheral segments of the optic nerves were less severely affected.

Congenital spongiform leukodystrophies have been previously described in several canine breeds and humans, in which aspartoacylase (ASPA) deficiency is described as Canavan disease. In Australian cattle dogs and Shetland sheepdogs the disease has been associated with a missense mutation in cytochrome b. Genetic testing has ruled out relevant mutations in the ASPA and cytochrome b regions in the case of the examined puppies. To the authors’ knowledge, this is the first description of a congenital spongiform leukodystrophy in the German shepherd breed.

N-18 TEMPOROSPATIAL AND KINETIC VARIABLES OF GAIT IN DOBERMAN PINSCHERS WITH AND WITHOUT CEREBRAL SPONDYLOMELAPATHY. C.G.D. Lima1, R.C. da Costa1, K. Foss1, M. Allen1. 1Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, USA.

By utilizing temporospatial and kinetic gait analysis, an unabashed comparison of treatment and outcome in the assessment of neurologic status can be obtained. This is particularly important in diseases with multiple treatments, such as cerebral spongylomalacia (CSM). The aim of this study was to characterize and compare the gait variables of Doberman Pinchers with and without CSM using a pressure-sensitive walkway (PSW)

Eighteen Doberman Pinschers were prospectively studied, 9 clinically normal and 9 CSM-affected dogs. Neurologic examination was performed in all dogs and MRI was used to confirm CSM. The temporospatial and kinetic variables of gait were measured with a PSW (High Resolution floor mat HR Mat®; Tekscan). Variables evaluated included stance phase duration (ST), swing phase duration (SW), gait cycle duration (GC), stride length (SL), peak vertical force (PVF) and the vertical impulse (VI). Random-effects linear regression was used to compare differences between groups.

Due to space constraints only a portion of results are reported. In regards to temporospatial variables, the mean values of 4 parameters (ST, SW, GC, and SL) were significantly (P < 0.05) shorter in the thoracic limbs (TLs) of CSM-affected dogs when compared to TLs of normal dogs. The PVF mean value was significantly higher in all 4 limbs of the CSM-affected dogs when compared to the normal dogs (P < 0.05).

In conclusion, significant differences in the temporospatial and PVF values between normal and CSM-affected dogs were found. This suggests that the PSW is a valid option for objective assessment of the gait of CSM-affected dogs.

N-19 PHARMACOKINETIC EVALUATION OF GENERIC EXTENDED RELEASE FORMULATIONS OF LEVETIRACETAM IN DOGS. L. Boozer1, S.R. Platt1, A. Haley1, A. Linville1, M. Kent1, B. Nie2, R. Arnold2. 1College of Veterinary Medicine, University of Georgia, Athens, GA., 2Harrison School of Pharmacy, University of Auburn, AL.

An extended release (XR) formulation of levetiracetam is available for use in dogs and is often prescribed as a generic. The potential benefits of the XR formulation include reduced daily dosing leading to improved compliance and relatively constant plasma concentrations. The aim of this study was to compare the pharmacokinetics of two generic levetiracetam XR formulations with Keppra XR® and Keppra® immediate release (IR) tablets following single oral dosing in dogs.

Six clinically and neurologically normal mixed breed dogs were used in a cross-over design. Following a 12 hour fast, each dog was administered oral IR levetiracetam. Serum for drug analysis was taken from each dog prior to and at 0.25, 0.5, 0.75, 1, 2, 4, 8, 24 and 36 hours after administration. The study was repeated three times, each after seven day wash-out periods, with each dog administered 500 mg (20/mg/kg) oral Keppra®, Keppra XR® and two different generic preparations. Serum samples were analyzed by ultra high-pressure liquid chromatography - tandem mass spectrometry (Agilent, UHPLC-MS/MS). Concentrations of levetiracetam were determined by peak area comparison to the internal standard. Pharmacokinetic (PK) parameters were estimated for each animal and averaged to examine differences, a p-value <0.05 were deemed significant.

No adverse clinical effects were noted in any of the dogs. A significant decrease in maximum plasma concentration (C max) and increase time to C max (T max) were observed for Keppra XR® and both generic XR formulations compared to the Keppra® IR formulation. The XR formulations had a 5.5 to 13x lower C max and 3 to 5.7 hour longer T max. The area under the drug concentration time curve (AUC), clearance normalized to bioavailability (CL/F), volume normalized (V/F), half-life (t 1/2) and elimination rate (k) were similar in all XR formulations.

The findings suggest that the generic formulations are well absorbed and bioavailable with similar overall pharmacokinetic properties to the branded version. Specific dosing recommendations cannot be made; however the favorable pharmacokinetic data for all XR formulations suggests that single, daily administration could be efficacious relative to IR BID dosing.

N-20 PREDICTION OF FUNCTIONAL OUTCOME IN DOGS WITH CEREBELLAR ISCHEMIC INFARCTION: A RETROSPECTIVE, MULTICENTER STUDY OF 91 DOGS. L. Boozer1, S.R. Platt1, A.C. Freeman1, M. Higginbotham2, J.F. McConnell3, R. Goncalves4, M. Kent1, A. Haley1, T. White1, M. Beasley1, L. De Risio1. 1University of Georgia, Athens GA., 2Animal Health Trust, Newmarket, United Kingdom., 3Mississippi State University, Starkville, MS.

The cerebellum is the most common site of acute brain infarction in dogs. In general, the prognosis for patients with these lesions is favorable, but a specific outcome assessment has not been possible in a large population of patients due to a small number of cases available in past studies. The aim of this multicenter study was to retrospectively evaluate the ability to predict functional outcome using presentation, clinical signs, neurologic exam findings, and diagnostics in a large cohort of dogs.

Medical records from dogs with lesions consistent with cerebellar ischemic infarction were included in the study. Lesions anywhere within the cerebellum were diagnosed via magnetic resonance imaging (MRI) according to previously published criteria. Data including signalment, clinical signs, treatments, concurrent diseases and imaging findings were analyzed for significance. Specific data points were analyzed alongside clinical signs to attempt to correlate historical or neurological exam findings to neurologic status and outcome score after discharge from the hospital. Outcome was scored 0-4 based on progression as well as owner perception of the impact of neurological dysfunction on quality of life.

Ninety-one dogs of 39 breeds from five institutions were included in the study (15-192 month old; mean 105 months). Forty-four percent were nonambulatory at presentation, and...
there was overall improvement in 72% of dogs. There was a significant association of MRI based lesion location and improvement and ambulatory status at discharge; 65% of dogs with infarcts restricted to the cerebellar hemisphere were ambulatory at presentation while only 30% were ambulatory with lesions restricted to the vermis. Neurological exam findings including mentation, ambulation, postural reaction deficiencies or cranial nerve abnormalities were not associated with an improved outcome score although ambulatory status was significantly correlated to improvement of function on discharge. Elevated protein concentration on cerebrospinal fluid analysis was significantly correlated to a worsened outcome score. MRI lesion intensity did not correlate to outcome or improvement. Forty-two patients had documented concurrent disease, but this was not significantly related to outcome score.

The findings in this group of dogs with cerebellar ischemic infarctions document a range of presentations. Location of cerebellar infarcts and ambulatory status was useful in predicting outcome while other clinical findings were not. MRI volumetric analysis is currently underway to further assess this large population for markers of improved prognosis.


Castration resistant prostate cancer (CRPC) is the second most common cause of cancer death in men in the United States. Bombesin receptor subtype two (BBr2) has been shown to be overexpressed in human prostate cancer. The objective is to evaluate the efficacy of 177Lu-BBr2 agonist versus 177Lu-BBr2 antagonist targeted radiotherapy in a xenografted mouse model of CRPC.

Male SCID mice were bilaterally flank inoculated with 5X10⁶ PC-3 cells for generation of human xenografts positive for the bombesin receptor subtype 2 (BBr2). Commercially synthesized BBr2 agonist and antagonist were labeled with 177Lu. Eighty mice were randomized into 8 groups for either 177Lu-BBr2 agonist or antagonista n. Treatment began at 8 days after tumor bearing control, radiotherapy (doxetaxel; 8 mg/kg IP weekly), single agent radiotherapy (high dose 177Lu-BBr2 antagonist peptide IV; administered weekly), or combination therapy (177Lu-BBr2 antagonist, high dose 177Lu-BBr2 antagonist and low dose 177Lu-BBr2 antagonist plus doxetaxel). Treatment was delivered for six consecutive weeks. Tumor volume, body condition score, and weight were measured weekly to assess efficacy, and mice were euthanized when end-point criteria were met.

Single agent 177Lu-BBr2 antagonist resulted in superior tumor volume control (p < 0.0001) and survival (p < 0.0001) when compared to the 177Lu-BBr2 agonist administered in combination with chemotherapy. No toxicity was observed in mice receiving 177Lu-BBr2 antagonist alone, and the overall response rate was 100%.

177Lu-BBr2 antagonist may have therapeutic efficacy against CRPC, and application for canine prostate cancer should be explored.

(VCs Award Winner)

O-2 PHARMACOKINETICS AND TOXICITY OF THE NOVEL ORAL DEMETHYLATING AGENT ZEBULARINE IN LABORATORY DOGS AND DOGS WITH TRANSITIONAL CELL CARCINOMA. C.M. Fulkerson, J. P. Bonney, D.W. Knap, J. Balkman. Purdue University College of Veterinary Medicine, West Lafayette, IN.

DNA hypermethylation in the promoter region is a common epigenetic change resulting in silencing of tumor suppressor genes. Zebularine (zeb) is an oral cytidine analog that acts as a demethylating agent. The purpose of this study was to determine zeb plasma pharmacokinetics and toxicity, and describe antitumor activity in dogs with transitional cell carcinoma (TCC).

Plasma was collected from laboratory (n = 3) and tumor-bearing dogs (n = 6) over 24 hours following doses of 8 (n = 3), 4 (n = 3) and 0.2 (n = 3) mg/kg of zeb. Toxicity was assessed in laboratory dogs treated with 4 mg/kg q24 hours (n = 3) and tumor-bearing dogs treated with 0.2 (n = 6), 0.3 (n = 7) and 0.4 (n = 3) mg/kg q24 hours. Toxicity was graded using veterinary cooperative oncology group common terminology criteria for adverse events. Tumor response was evaluated using ultrasound and radiographs.

Constant plasma clearance was detected in dogs treated with zeb. Mean Cmax for treatment with 8, 4 and 0.2 mg/kg was 23 ± 4.8, 8.6 ± 1.4 and 0.16 ± 0.06 µM, respectively. Dogs treated with 4 mg/kg developed grade 4/4 neutropenia (n = 3) with one febrile neutropenia. Two dogs developed grade 1/4 anorexia. All adverse events resolved with supportive care. No remarkable adverse events occurred in dogs treated with 0.2 mg/kg (n = 6). Grade 1/4 neutropenia developed in 1/3 dogs treated with 0.3 mg/kg. Grade 2/4 anorexia developed in 1/3 dogs treated with 0.4 mg/kg. Stable disease of at least 8 weeks with a median progression free interval of >80 days (range 47 to 265 + ) was detected in 10/11 tumor-bearing dogs.

Treatment with 4 mg/kg zeb resulted in unacceptable but reversible hematologic adverse events. Treatment 0.2, 0.3 or 0.4 mg/kg daily was well-tolerated in dogs with TCC. Initial response data warrant further investigation of zeb's potential as a single agent and in combination with other cytotoxic drugs.

(VCs Award Winner)

O-3 THE EFFECT OF PALLADIA ON THYROID FUNCTION. V. Bizzozero, K.R. Hume, M. Thompson, C. Balkman. Cornell University College of Veterinary Medicine, Ithaca, NY.

The purpose of this study was to determine whether or not the tyrosine kinase inhibitor (TKI) Palladia (toceranib) will induce hypothyroidism in tumor bearing dogs. Although TKIs are generally well tolerated, adverse events include anorexia, vomiting and diarrhea. An additional, relatively common adverse event seen in humans receiving TKIs is thyroid dysfunction with an estimated incidence of 36-71%. Whether or not TKIs induce hypothyroidism in dogs is currently unknown.

We conducted a prospective, pilot study to evaluate pre- and post-Palladia thyroid hormone levels in dogs with various tumor types, excluding thyroid tumors. Thyroid hormone levels were tested on days 0, 30 and 90. Hypothyroidism was defined as having low total T4, low or normal total T3, low free T4, and normal or increased TSH.

A total of 15 dogs have been enrolled in this study. On d0, 1 was hypothyroid, 6 were sick euthyroid and 1 was not evaluable. Evaluable data on d30 was available in 12 dogs. One dog that was not hypothyroid on d0 became hypothyroid. Two were sick euthyroid and 3 withdrew from the study. By d90, 2 dogs were sick euthyroid, 3 were euthyroid, 6 dogs withdrew from the study and 4 have not reached this time point.

Interim data evaluation reveals that Palladia did not induce hypothyroidism in this population of dogs. We plan to accrue data until a total of 21 dogs are evaluable at d90.

O-4 A RETROSPECTIVE ANALYSIS OF HYPOFRACTIONATED RADIOOTHERAPY FOR TREATMENT OF SOLID TUMORS IN DOGS. M. Tollett, L. Duda, D. Brown, E. Krick. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Although hypofractionated radiotherapy (RT) is a palliative treatment option for a variety of solid tumor types, response and outcome data is limited. The study purpose was to evaluate outcome and toxicity according to tumor type, location, and radia-
tion protocol in a large population of dogs treated with hypo-
fractionated RT at the same institution.

This retrospective study evaluated 103 dogs with sarcoma, car-
cinoma, melanoma, primary bone tumor, mast cell tumor (MCT), or odontogenic tumors. The majority of dogs were treated with one of two protocols: (1) 4 x 8 Gy, applied every one to two weeks; (2) 6 x 6 Gy, applied once or twice weekly.

The median survival time (MST) for all dogs was 146 days (95% CI: [111-195] days). The median progression-free survival (MPFS) was 106 days (95% CI: [69-163] days). Overall response rate (ORR) was 75%. Dogs with MCT experienced a shorter MPFS compared to dogs with sarcoma (31 days vs. 163 days, p < 0.005). Tumor location and radiation protocol did not have a significant effect on MST or MPFS. Acute and late radiation side effects were documented in 55% and 4% of dogs, respecti-
vably.

This study supports the use of hypofractionated RT for a variety of solid tumor types. Tumor location and radiation protocol did not significantly affect survival or tumor progression in this patient population. However, tumor type did have a significant effect on tumor progression as dogs with mast cell tumor had a significantly shorter progression free survival than dogs with sarcoma.

O-5 DNA DAMAGE IS A FEATURE OF FELINE INJECTION SITE SARCOMA.

Feline injection site sarcoma (FISS) is commonly treated with surgery and radiation therapy. Despite aggressive therapy, FISS has a high recurrence rate. The true benefit of adjuvant chemo-
therapy is not known. Multiple mechanisms cause resistance to chemotherapeutics, including enhanced DNA damage repair or inactivation of cell death pathways. Therefore, alterations in DNA damage response (DDR) mechanisms may help predict chemosensitivity. The presence of DNA damage in tumor tissue can be evaluated by γH2AX immunohistochemistry. H2AX is phosphorylated to form γH2AX following DNA double strand breaks. Given that DDR mechanisms may affect chemosensitivity of FISS, the objective of our study was to determine if DNA damage is a feature of FISS.

11 tumor samples were prospectively collected via incisional biopsy (n = 6), post amputation (n = 3), or post mortem (n = 2). Samples were fixed for definitive histological diagnosis and γH2AX immunohistochemistry. For γH2AX quantification, 9 random sites were evaluated on 3 independently stained tissue sections from each tumor. Mean percent positive cells per tumor was calculated.

DNA damage in FISS ranged from 5-33%, with a mean of 18%. To evaluate whether differences between cats existed, a mixed effect model was used to analyze the mean percent positive cells. Individual cat was used as a fixed effect in the model and tissue sections within cat as a random effect. The individual cat was used as a fixed effect in the model and mixed effect model was used to analyze the mean percent positive cells per tumor section from each tumor. Mean percent positive cells per tumor.

O-6 INVESTIGATING THE PRO-TUMORIGENIC EFFECTS OF TRANSFORMING GROWTH FACTOR BETA 1 (TGFβ1) IN CANINE OSTEOSARCOMA.

Transforming growth factor beta (TGFβ1) is a cytokine princi-

was to determine if TGFβ1 signaling is subverted by osteosar-
coma (OS) cells via paracrine/autocrine mechanisms to promote tumorigenesis.

Five canine OS cell lines (D17, Abrams, HMPOS, POS and COS1) were used for experimentation. Western blot (WB) and immunohistochemistry (IHC) were used to detect TGFβRI/TGFβRII expressions, and TGFβ1 secretion was quantified by ELISA. The effect of receptor signaling inhibition by the small molecule inhibitor LY2109671 (300-5000 nM) on SMAD2 phos-
phorylation, cell proliferation (colony-forming assay), survival (WB phospho-Akt), angiogenesis (VEGF ELISA), and migration (scratch assay) were assessed. Plasma levels of TGFβ1 in dogs with OS were measured via ELISA and correlated with urine N-Telopeptide (NTX) levels. In spontaneous OS samples, expres-
sions of TGFβRI/TGFβRII were characterized.

Our results showed that both canine OS cell lines and sponta-
neous tumors express TGFβRI/TGFβRII, and cell lines actively secrete TGFβ1. Inhibition of TGFβ1 signaling by receptor block-
ade attenuated SMAD2 phosphorylation, with subsequent reduc-
tions in OS cell proliferation, VEGF secretion and cell migration. Circulating TGFβ1 positively correlates with the degree of patho-
logic bone resorption in dogs with spontaneous OS.

In summary, canine OS cells possess the requisite cellular ma-
terity to exploit the transforming activities of TGFβ1, and therefore blocking TGFβ1 signaling might be a rationale strategy for the management of canine OS.

O-7 COMPARISON OF HEMOSTATIC AND CLINICAL FINDINGS IN DOGS WITH SPLENIC HEMATOMA VERSUS HEMANGIOSARCOMA: 71 CASES (2006-2013).
A.S. Bishop, M.A. Bishop, M.B. Pashmakova. 'Best Friends Veterinary Hospital, Spring, TX, 2Texas A&M University, College of Veteri-

nary Medicine, College Station, TX.

Currently, minimal data exists comparing hemostatic charac-
teristics of dogs with splenic hemangiosarcoma (HSA) versus benign splenic hematoma (SH). Similarly, the presence of he-
moabdomen and number of splenic masses has not been com-
pared between the two diagnoses. The purpose of this study was to compare the hemostatic characteristics, presence of hemoabdo-
men, and number of splenic masses between dogs with SH versus HSA.

Medical records were reviewed from 2006-2013. Dogs were included if they underwent a splenectomy, had hemostatic evalu-
ation on admission, and a histopathological diagnosis. Addition-
ally, signalment, presence of hemoabdomen and any blood products transfused were recorded. Coagulation parameters were compared between groups using a Mann-Whitney test A Fisher’s exact test and odds ratios were calculated to compare numbers of dogs presenting with a hemoabdomen and the number of splenic masses. All data was checked for normality and alpha was set at 0.05.

In total, 71 cases (30 SH, 41 HSA) were identified. Dogs with HSA had a significantly increased median prothrombin time (8.5 sec vs. 7.8 sec; P = 0.018), decreased fibrinogen (Fib-Clauss) (196 mg/dL vs. 541.5 mg/dL; P = 0.001), increased d-dimers (3308 ng/mL vs. 1565 ng/mL; P = 0.002), and decreased manual platelet counts (95,000 K/μL vs. 148,000 K/μL; P = 0.041) when compared to dogs with SH. There were no differences in the median partial thromboplastin time and antithrombin III concent-
trations. Dogs presenting with a hemoabdomen were 3.83 (95% CI: 1.28-11.46) times more likely to have HSA and dogs with more than one splenic mass were 6.67 (95% CI: 2.22-20.03) more likely to have HSA then SH. There were no differences between groups in the need for transfusion and blood product received.

These parameters may be useful in predicting the diagnosis pre-operatively in patients presenting with a splenic mass. Further studies are warranted.

The T-cell lymphoma phenotype is an established negative prognostic indicator for achieving complete remission (CR), remission length and length of survival. Few studies however, have looked at outcomes for a purely T-cell population of dogs.

The purpose of this retrospective study was to evaluate factors that may influence the likelihood of achieving CR and length of survival in 75 dogs diagnosed with multicentric, non-indolent, T-cell lymphoma treated with vincristine, L-asparaginase, cyclophosphamide, lomustine (CCNU), doxorubicin, prednisolone, procarbazine and actinomycin-D (VELCAP-TSC).

All cases were confirmed as T-cell lymphoma using immunocytochemistry, immunohistochemistry or PCR antigen receptor rearrangement assay (PARR). Pathology was reviewed by two ACVP certified pathologists. Chi-squared test was used to determine factors affecting the likelihood of achieving CR. Kaplan-Meier survival statistics and Cox regression analysis were used to investigate the relationship between independent variables and the length of survival, with the end point being death due to any cause.

Independent variables investigated for their effect on achieving CR and length of survival included tumor stage and subtype (a or b), boxier breed, inappetence, hematology and biochemistry parameters at the time of diagnosis as well as histopathological factors including mitotic index, grade and cell size.

54.7% of dogs achieved CR. Neutropenia at presentation negatively affected the likelihood of achieving CR while elevated serum ALP activity increased chances for CR.

The overall median survival was 214 days. Two factors independently affected survival: achieving CR and inappetence at diagnosis. Thirty-five inappetent dogs lived a median of 133 days whilst 40 normally eating dogs lived a median of 327 days. Thirty-four dogs that did not achieve CR had a median survival of 92 days, with 10.2% alive 1 year, and 6.8% alive 2 years after commencing chemotherapy. Forty-one dogs that did achieve CR had a median survival of 342 days, with 47.8% alive 1 year and 30.3% alive 2 years later.

O-9 IGHV USAGE AND SOMATIC HYPERMUTATION ANALYSIS IN CANINE B CELL CHRONIC LYMPHOCYTIC LEUKEMIA. S. George, S. Mancha, C. Abbott, R. Burnett, A. Avery. Colorado State University, Fort Collins, CO.

Somatic hypermutation of the immunoglobulin heavy chain variable region (IGHV) gene is prognostic in human B-cell chronic lymphocytic leukemia (B-CLL). B-CLL clones that have undergone somatic hypermutation (“mutated”) are associated with an indolent course, whereas “unmutated” clones tend to yield a greater incidence of clinical signs, a resistance to chemotherapy, and an overall poorer prognosis. There are roughly 74 IGHV genes identified in humans. Despite this potential for diversity, a subset of IGHV genes is preferentially used in human B-CLL. This bias implies recognition of a common antigen across tumors, suggesting antigenic stimulation as a possible etiology. This study aimed to determine whether canine B-CLL has similar characteristics such that it could become a model for the human disease.

DNA was extracted from convenience blood samples from 63 dogs with B-CLL. Immunoglobulin genes were amplified with PCR using a primer panel for the conserved portion of the leader sequence located upstream of each variable (V) region and 3′ primers specific for unique intron sequences located downstream of junctional (J) segments 2, 4, and 6. PCR amplicons were sequenced directly as well as cloned and sequenced. Acquired sequences were compared to germline IGHV using NCBI BLAST to determine IGHV usage. In accordance with human studies, sequences were considered mutated if they differed more than 2% from the published germline sequence.

In 15 cases we were unable to detect the neoplastic clone. In the remaining 48, we could unequivocally identify the clone and determine IGHV usage. Of 85 possible IGHV genes in the dog, 22.9% (11/48) of B-CLLs utilized VH41. In particular, VH41 was found in 75% (6/8) of boxers, a breed thought to be predisposed to the disease. VH41 does not appear to be common in dogs without B-CLL. A subset of clones (21/48, 43.8%) featured unmutated IGHV. Notably all of the VH41 clones identified were either unmutated or highly similar to the germline sequence.

Canine B-CLL shares some features with human B-CLL, including biased IGHV gene usage and populations of mutated and unmutated B-CLLs. Usage of VH41 predominates in B-CLL and does not appear to be common in dogs without B-CLL. Biased VH41 usage supports an antigenic-stimulation model for oncogenesis; thus naturally-occurring canine B-CLL has potential as a model for human B-CLL.

O-10 BONE TUMORS IN DOGS AND CATS: A PERSPECTIVE FROM ANKARA UNIVERSITY (2001-2010). O. Kutsal1, M. Saglam2, A.S. Tunç3, S. Cagatay, I.G. Sancak3, Department of Pathology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey. Department of Surgery, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey.

The purpose of present study was to evaluate samples of bone tumors submitted to department of pathology. A total of 81 cases obtained from department of clinical sciences and private practices between 2001 and 2010 were clinically and pathologically assessed.

During the study period, a total of 20 cases of bone tumors were diagnosed in dogs and cats. Of 20 cases, 8 (40%) were in the age range of 4 to 9 years old and 12 (60%) were in the age range of 10 to 15 years old. Of 20 cases, 14 (70%) were in dogs and 6 (30%) were in cats. Of 14 dogs with bone tumors, 8 (57.1%) were males and 6 (42.9%) were females. Of 6 cats with bone tumors, 2 (33.3%) were males and 4 (66.6%) were females. Of 20 cases, 14 (70%) were located in legs of dogs and cats. Of 14 bone tumors diagnosed in dogs, 11 (78.6%) were malignant and 3 (21.4%) were benign. Of 6 bone tumors diagnosed in cats, 5 (83.3%) were malignant and 1 (16.7%) was benign. Majority of bone tumors of dogs (9 cases; 64.2%) and cats (3 cases; 90%) were classified as osteosarcomas. Furthermore, lung metastasis was determined in only 1 dog among the 20 bone tumor cases.


Under the normal immune homeostatic state, there exists a protective mechanism, called cancer immunosurveillance, by which cytotoxic T lymphocytes recognize tumor-associated antigens in autologous tumor cells, although they are not sufficiently strong to eradicate tumors in the majority of patients. However, there are growing evidences which implies that malignant tumors may evade the immunosurveillance by promoting the expansion, recruitment and conversion of CD4+CD25+FOXP3+ regulatory T cells (Treg cells) resulting in subsequent suppression of anti-tumor immunity performed by CD4+, CD8+, and NK cells, and this could be a key to answer why the immunosurveillance against cancer is not fully effective in preventing cancer.

Bloods from 8 dogs diagnosed with canine lymphoma were collected to evaluate the percentages of Treg cells in total lymphocytes using flow cytometer. Lymphocytes having surface markers CD4 and CD25, and intracellular marker FOXP3 were
defined as Treg cells. Interestingly, the average percentages of Treg cells were significantly higher (15.44%) in the peripheral blood of dogs with lymphoma than that of healthy dogs (4.3%) (Table 1).

Although this study has a limitation on the fact that 8 patients analyzed in this study were all in the same clinical TNM stage III, limiting this study to find how proportionally is the percentage of Treg cells related to the tumor behavior or prognosis, we could possibly assume that the increased percentages of Treg cells compared to those in healthy dogs could be an evidence of immune evasion process implemented by tumor cells to escape the cancer immunosurveillance of the host.

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<th>Table 1. Treg cell levels in 8 dogs with Lymphoma</th>
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The novel retinoid derived excipient XR-17 is composed by a 1:1 mixture of N-(all-trans-retinyl)-L-cysteic acid methyl ester (XMeNa) and N-(cis-retinoyl)-L-cysteic acid methyl ester (13XMeNa). XR-17 increases the solubility of compounds sparingly soluble in aqueous solutions by forming nanosized micelles (20-40 nm). This has successfully been applied in animal and human products where XR-17 has replaced the hypersensitivity causing excipient Cremophor EL® for dissolution of paclitaxel, producing micellar injectable formulations (e.g. Paccal Vet®) with a high drug-loading capacity of 1:1.3 (paclitaxel:XR-17).

Different concentrations/doses of XR-17 have been studied: in vitro, toxicology in dogs and rats, and excretion and distribution studies in the rat. Pharmacokinetics was investigated following a single intravenous administration of [14C]-XMeNa and [14C]-13XMeNa in rats.

Genotoxicity studies (Ames Test) showed no mutagenicity at clinically relevant concentrations. No proliferation inhibition was attributed to XR-17 alone in tumor cell cultures. Clearance of total radioactivity in plasma was low with moderate volumes of distribution (Vds =1.7 L/kg). The elimination half-life was 6.7 hours. Biodistribution showed XR-17 concentration in the liver, stomach-wall and minimally in the kidney. Elimination occurred primarily via liver/feaces (70%) and faintly via kidney/urine (2%). Maximal tolerable dose (MTD) in dog was 108.8 mg/kg. High dose toxicity mainly occurred as elevation of liver enzymes and hemolysis.

The tolerability in dogs is very good as demonstrated by the XR-17 dose in Paccal Vet of 5 mg/kg, which is more than 20 times below the MTD.

O-13 EVALUATION OF FACTORS INFLUENCING SURVIVAL TIME IN 77 DOGS WITH LYMPHOMA. S.Y. Jeong, H.M. Park. Department of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul, South Korea.

Canine lymphoma is the most commonly reported hematopoietic tumors. A few retrospective studies have performed complex evaluations, but these studies seldom demonstrated variable factors that affected survival time. The purpose of this study was to identify variable prognostic factors, which can be simply detected, such as abnormalities of physical examinations, hematologic examinations, and treatment protocols in dogs with lymphoma.

Clinical records were reviewed in 77 dogs diagnosed with lymphoma from 2006 to 2013. All dogs were classified into the treatment (T) and no-treatment (NT) groups for survival analysis.

Lymphoma affected most frequently middle-aged small breed dogs. The hematologic examinations showed that thrombocytopenia and anemia represented common abnormalities observed in lymphoma. Muticentric type and late stage according to WHO classification were predominant in dogs with lymphoma. Moreover, incidence of B-cell type is higher than T-cell type.

As results of survival analysis, the high performance status evaluation (PSE) contributed to shorter survival time in both T and NT groups. According to diagnostic features, there were significant differences in survival time among anatomic types and immunologic types in the T group, and WHO subtype in the NT group. At hematologic examinations, leukocyte and platelet abnormalities, imbalances of potassium and chloride also affected survival time in the T group. The improvements of anemia, thrombocytopenia, and lymphoblast detected at peripheral blood after chemotherapy were also presented positive prognostic factors. During chemotherapy, there was a significant difference in survival time according to gastrointestinal toxicity grade, also, delayed chemotherapeutic schedule or reduced dosage chemotherapeutic agents contributed to shorter survival time. For dogs with relapsed or failed to achieve CR, dogs administered rescue protocol of multiple agents based protocol revealed longer survival time.

In conclusion, in this study newly identified high PSE, leukocyte and platelet abnormalities, and imbalances of electrolytes as negative prognostic factors. Also, lower gastrointestinal toxicity and improvements of hematologic abnormalities during chemotherapy act as positive prognostic factors. Finally, strict following of protocol and selecting multiple agents as rescue protocol are important to prolong survival time.

O-14 VALIDATION OF AN INDEXED RADIOTHERAPY HEAD POSITIONING DEVICE FOR USE IN DOGS. K.S. Hansen1, A.P. Theon2, S. Dietrich3, M.S. Kent4. 1WR Pritchard Veterinary Medical Teaching Hospital, Department of Surgery and Radiological Sciences, University of California-Davis; Davis, California. 2University of California-Davis Medical Center, Department of Radiation Oncology; Sacramento, California.

The purpose of this prospective study was to determine the accuracy of an indexed board immobilization device for positioning of radiation patients. Twenty-four dogs were enrolled for treatment with the positioning device. Table index numbers were defined at the first treatment based on portal films. At subsequent treatments, patients were moved to the table index numbers, orthogonal films were acquired, table position was modified and the displacement was recorded. The mean, systematic, random and overall displacement (SD, RD, and OD), and the mean displacement of the three dimensional (3D) vector were calculated. These values were compared to historical values for an immobilization device. A total of 332 pairs of orthogonal portal films were analyzed for displacement in the cranial-caudal, lateral, and dorsal-ventral directions. The SD was 0.52 mm, 0.8 mm, and 0.9 mm, respectively. The OD was 1.09 mm, 1.39 mm, and 0.87 mm, respectively. The mean displacement value of the 3D vector was 1.59 mm with a standard deviation of 1.17 mm. Ninety-five percent of all vectors were 

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Introduction: The beta-adrenergic (β-AR) signaling pathway mediates physiologic stress responses and is implicated in tumor growth and metastasis. Propranolol, a clinically useful non-selective β-AR antagonist, is reported to exert anti-angiogenic effects on endothelial and carcinoma cells and has clinical efficacy for human infantile hemangioma. Our goal was to characterize β2-AR expression and effects β2-AR antagonism in canine hemangiosarcoma cell lines.

Methods: Two canine hemangiosarcoma cell lines (Fitz and Den) were assessed. Protein expression of β2-AR was evaluated in cell lysates via Western Blot and in paraffin-embedded cell pellets and spontaneous tumors via immunohistochemistry. Polyclonal β2-AR antibodies were used with positive and negative controls. Cells were incubated with β2-AR agonists and propranolol (β-AR antagonist) at 0-100 μM for up to 48 hrs. Protein expression of downstream effectors in the β2-AR cascade (Src and phosphorylated Src) was evaluated via Western Blot. Cell proliferation was assessed with a commercial assay (Cell Titer AquousOne, Promega). Soluble angiogenic proteins were assessed via commercial VEGF ELISA and angiogenic protein array (R&D systems).

Results: Den and Fitz express β2-AR via WB and IHC. Propranolol exerts a modest effect on cell proliferation and angiogenic cytokine secretion, including VEGF, FGF-1, and IGFBPs. FITZ and Den express Src and baseline phospho-Src.

Conclusion: Canine hemangiosarcoma cell lines express β2-AR and propranolol exerts downstream effects. Further investigation into the effector pathway and biologic activity of this receptor is warranted.

(VCS Award Winner)

EN-1 COMPARISON OF TWO DOSES FOR ACTH STIMULATION TESTING IN DOGS SUSPECTED OF OR TREATED FOR HYPERADRENOCORTICISM, C. Aldridge1, E. Behrend1, R. Kemppainen1, T. Lee-Fowler1, L. Martin1, and W. Ward1. 1Auburn University, College of Veterinary Medicine, Auburn, AL., 2Washington State University, College of Veterinary Medicine, Pullman, WA., 3University of Georgia, College of Veterinary Medicine, Athens, GA.

The ACTH stimulation test, using cosyntropin at 5 mcg/kg IV, is the preferred method for monitoring medical management of hyperadrenocorticism (HAC) and is a screening test for diagnosing HAC. Previous studies have shown maximal stimulation of the adrenal glands using 1 mcg/kg cosyntropin in normal dogs. No studies have evaluated the efficacy of the lower dose in dogs suspected of or being treated for HAC. Our objective was to compare 1 mcg/kg to 5 mcg/kg cosyntropin IV to determine if both doses result in a similar adrenocortical response.

Testing was prospectively performed in dogs suspected of and being treated for pituitary-dependent HAC (PDH) with mitotane or trilostane. Dogs suspected of having HAC or being treated with mitotane received 1 mcg/kg cosyntropin IV followed four hours later by 5 mcg/kg cosyntropin IV. Blood samples were obtained pre- and one hour post-ACTH for each dose (4 measurements total). Preliminary studies were conducted to confirm the validity of performing two ACTH stimulation tests using this timing on the same day. Dogs receiving trilostane therapy were tested on consecutive days at the same time post-pill (4-6 hours post). Cortisol was measured using a previously validated radioimmunoassay. To detect differences in cortisol concentration between cosyntropin doses (1 and 5 mcg/kg) and between time points (baseline and 60-min), data were analyzed using a repeated-measures ANOVA by a commercial statistical computer program. Data for each group of dogs (suspect HAC, mitotane-treated and trilostane-treated) were evaluated separately. Significance was set at p ≤ 0.05 level.

Overall, 46 dogs were included, with 26 suspected of HAC, 12 being treated for PDH with mitotane and 8 being treated for PDH with trilostane. No significant difference was detected between the post-ACTH cortisol concentrations within each group, comparing responses to both doses. For the suspect dogs and dogs treated with mitotane, the pre- and post-ACTH cortisol concentrations were significantly different with both doses ( p ≤ 0.001 and p = 0.001 respectively). For dogs treated with trilostane, no difference was detected between pre-ACTH and post-ACTH cortisol concentrations for either dose.

The 1 mcg/kg IV dose of cosyntropin causes maximal adrenal response as does the standard 5 mcg/kg IV dose. The lower dose is sufficient for ACTH stimulation testing in those patients suspected of HAC or diagnosed with PDH and being treated with mitotane or trilostane. A lower dose of cortroyn may be used to help lower cost of diagnosing and monitoring this disease.

EN-2 HYPOCOBALAMINEMIA AND COBALAMIN DEFICIENCY IN CATS WITH HYPERTHYROIDISM, B. Geersman, W. Whitehouse, K. Viviano. Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI.

Hypocobalaminemia is primarily associated with small intestinal disease and exocrine pancreatic insufficiency (EPI). Recently, hypocobalaminemia has been reported in hyperthyroid cats. The purpose of this study was to determine in hyperthyroid cats whether the hypocobalaminemia reflects a functional cobalamin deficiency and resolves once euthyroid following radiiodine therapy.

A prospective, observational study design was used to enroll client-owned hyperthyroid cats presenting for radiiodine therapy. Cats with confirmed small intestinal disease or EPI were excluded. Prior to radiiodine therapy all cats had their hyperthyroidism evaluated using history/clinical signs, physical exam, CBC, biochemistry profile, urinalysis, total T4, blood pressure, and thoracic radiographs. In addition, serum cobalamin, folate, tryptophan-like immunoreactivity, and methylmalonic acid (MMA) were determined. Once euthyroid, 2 months following radiiodine, blood and urine were collected for biochemistry profile, USG, T4, cobalamin, folate, and MMA concentrations.

Seventeen hyperthyroid cats have been evaluated, including 16 DSH and 1 purebred, 8 spayed females and 9 neutered males, with a median age of 14 years (9-17.5). The median cobalamin concentration in this well-characterized population of hyperthyroid cats was 913 ng/dL (159-2156) with a cobalamin below the reference interval in 5/17 cats (median 293 ng/dL (159-346)). Of these 5 cats with hypocobalaminemia, none had a high MMA concentration (median 276 nmol/L (140-509)) and following radiiodine 2/3 cats with follow-up available had resolution of their hypocobalaminemia once euthyroid.

The prevalence of hypocobalaminemia in this group of hyperthyroid cats was 29%; however a functional cobalamin deficiency was not documented. In most cats the hypocobalaminemia resolved once euthyroid.

EN-3 PHARMACOLOGY OF THE GLP-1 ANALOG LIRAGLUTIDE IN HEALTHY CATS, M.J. Hall1, C.A. Adin1, S. Borin-Crivellenti1,2, A.J. Rudinsky1, C.G. Gior1. 1The Ohio State University, Columbus, OH, USA., 2FCAV/Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil.

GLP-1 is an intestinal hormone that induces glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion and increasing beta cell mass, satiety and gastric-emptying time. Liraglutide is a fatty-acid derivative of GLP-1 with a protracted pharmacokinetic profile that is used in people for treatment of type II diabetes mellitus and obesity. The aim of this study was to determine the pharmacodynamics of liraglutide in healthy cats.
A hyperglycemic clamp was performed on day -1 (Clamp-I) and 13 (Clamp-II) in seven healthy cats. Liraglutide was administered subcutaneously (0.6 mg/cat) once daily on days 7 through 13. During the clamp, blood glucose concentrations were measured every 5 minutes and 20% dextrose infusion was adjusted to achieve hyperglycemia (225 mg/dl) at 30 min and to maintain that level of glycemia for 60 min. Plasma insulin and glucagon concentrations were measured at 15, 0, 30, 45, 60, 75, and 90 min.

Weight loss was recorded in all cats at day 13 (9% ± 3; P = 0.006). Appetite was subjectively decreased in all cats and one cat was withdrawn on day 10 because of 48 hrs of anorexia. Compared to Clamp-I, there was a trend during Clamp-II towards increased 60 min total glucose infused (median [range] 29% [11 – 178%], P = 0.087) and insulin concentrations (47% [-11 – 234%], P = 0.084). Glucagon concentrations (P = 0.67) and baseline glucose concentrations (P = 0.66) did not differ significantly between clamps.

Liraglutide may aid in weight loss in overweight cats but further evaluation is needed to determine its efficacy on improving glycemic control in diabetic cats.

EN-4

THE PHARMACOLOGY OF EXENATIDE EXTENDED-RELEASE IN HEALTHY CATS. A.J. Rudinsky 1, C.A. Adin 1, A.V. Bertalan, K.J. Drobatz, S. Borin-Crivellenti 1, M.J. Hall 1, C. Giler 2. The Ohio State University, Columbus, OH, USA. 2Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil.

GLP-1 is an intestinal hormone that induces glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion and increasing beta cell mass, satiety and gastric-emptying time. Exenatide extended-release (ER) is a microencapsulated formulation of the GLP-1-receptor agonist exenatide. It has a protracted pharmacokinetic profile that allows a once-weekly injection to replace insulin therapy safely and effectively in type-II diabetic people.

Here we studied the pharmacology of Exenatide-ER in six healthy cats. A single subcutaneous injection of Exenatide-ER (0.13 mg/kg) was administered on day 0. A hyperglycemic clamp was performed on days -7 (Clamp-I) and 21 (Clamp-II). During the clamp, blood glucose concentrations (BG) were measured every 5 minutes and 20% dextrose infusion was adjusted to achieve hyperglycemia (225 mg/dl) at 30 min and to maintain that level of glycemia for the subsequent 60 min. Plasma insulin and glucagon concentrations were measured at -15, 0, 30, 45, 60, 75, and 90 min. Glucose tolerance was defined as the amount of glucose required to maintain hyperglycemia during the 60 minutes of the clamp.

Comparing Clamp-1 to Clamp-2 using paired t-tests, fasting BG decreased (mean ±SD) = -11 ± 8 mg/dl, P = 0.02), glucose tolerance improved (median [range] +33% [-4-138%], P = 0.04) and median glucagon concentrations decreased (-4.7% [0-12.1%], P = 0.04). Insulin concentrations did not differ significantly. No side effects were observed throughout the study.

Exenatide-ER was safe and effective in improving glucose tolerance 3 weeks after a single injection. Further evaluation is needed to determine its efficacy on improving glycemic control in diabetic cats.

EN-5


The study goal was to determine the degree of correlation between blood glucose concentrations (BG) measured by an automated chemistry analyzer and BG measured using whole blood, serum, and plasma with an Accu-Chek Aviva point-of-care glucometer (POCG).

Ninety-six blood samples from 80 dogs and 90 blood samples from 65 cats were analyzed. Data were compared using Lin’s concordance correlation. In cats, chemistry analyzer BG correlation with plasma and serum POCG BG (Rho 0.99, standard deviation 0.002, and P < 0.001 for each, 95% confidence interval 0.989-0.995 and 0.986-0.994, respectively) was superior to the correlation with whole blood POCG BG (Rho 0.90, standard deviation 0.01, P < 0.001, 95% confidence interval 0.907-0.912). In dogs, mean differences in chemistry analyzer BG and whole blood, plasma, or serum POCG BG were 32 mg/dl, 6.1 mg/dl, and 6.9 mg/dl, respectively. In dogs, chemistry analyzer BG correlation with plasma and serum POCG BG (Rho 0.98, standard deviation 0.003, and P < 0.001 for each, 95% confidence interval 0.977-0.989 and 0.976-0.987, respectively) was superior to the correlation with whole blood POCG BG (Rho 0.62, standard deviation 0.04, P < 0.001, 95% confidence interval 0.542-0.706). In dogs, mean differences in chemistry analyzer BG and whole blood, plasma, or serum POCG BG were 31 mg/dl, 0.4 mg/dl, and 0.3 mg/dl, respectively.

Correlation between POCG BG and an automated chemistry analyzer improves with use of serum or plasma, rather than whole blood.

EN-6

NPH AND LISPRO INSULIN FOR TREATMENT OF DOGS WITH DIABETES MELLITUS. A.V. Bertalan, K.J. Drobatz, R.S. Hess. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Some dogs, treated with twice-daily NPH insulin and Hill’s W/D diet, have postprandial hyperglycemia despite having clinically well-regulated diabetes mellitus (DM). The goal of this study was to determine whether postprandial hyperglycemia and fructosamine concentration can be decreased by adding lispro insulin to the treatment protocol.

Six dogs were enrolled into this ongoing prospective study. Dogs were enrolled if they had clinically well-regulated DM while treated with NPH insulin and W/D q12h and if they had postprandial hyperglycemia defined as an increase in blood glucose concentration (BG) within two hours of NPH insulin administration and feeding. Fructosamine was quantified and BG was measured just before feeding and NPH insulin administration (T0), every 30 minutes for the first 2 hours (T30, T60, T90, T120), and every two hours thereafter for eight additional hours. Dogs were then treated at home with the same NPH insulin dose and W/D, but a separate lispro insulin injection of 0.1 Units/Kg SC was added to the NPH insulin and W/D protocol. Serial BG and fructosamine were measured two weeks later and compared to the original values using the Wilcoxon Signed Rank Test.

Median [range] fructosamine (400 μmol/l [289-624 μmol/l]), and BG at T30 (313 mg/dl [187-376 mg/dl]) and T90 (239 mg/dl [166-332 mg/dl]) were significantly higher before lispro insulin was introduced compared to two weeks later (390 μmol/l [253-486 μmol/l]), p = 0.046, 117 mg/dl [42-307 mg/dl], p = 0.028, and 94 mg/dl [48-197 mg/dl], p = 0.028, respectively.

It is concluded that addition of lispro insulin to an NPH and W/D treatment protocol may significantly decrease fructosamine and postprandial hyperglycemia.

EN-7

MICRONRNA BIOMARKERS OF CANINE DIABETES MELLITUS. E. Bell 1, A. Hardikar 1, A. Jenkins 1, E. Bell 1, A. Hardikar 1, A. Jenkins 1. 1Facility of Veterinary Science, University of Melbourne, Werribee, Victoria, Australia. 2NHMRC Clinical Trials Centre, University of Sydney, Camperdown, New South Wales, Australia.

MicroRNAs are small molecules present within the genome that regulate the expression of genes, and thus a variety of cellular processes. Dysregulation of microRNAs has been associated with various diseases, including diabetes in rodents and humans. MicroRNAs are attractive targets for consideration as biomarkers, as they are measurable in many body fluids, are highly conserved between species, are resistant to degradation by enzymes, freezing, thawing and changes in environmental pH, and have a long half-life in serum. The purpose of this study was to identify microRNAs that are markers of canine diabetes mellitus, specifically pancreatic beta cell dysfunction in dogs. A biomarker of beta cell dysfunction would be useful to assess response to novel therapies aimed at preserving beta cell mass.

Immune-mediated destruction of adrenocortical tissue causes the majority of cases of hypoadrenocorticism in both dogs and humans (Addison’s disease). Approximately 55-70% of humans with Addison’s disease as part of autoimmune polyglandular syndrome type 1 (APS1) and those with APS2 with premature ovarian failure (POF) have autoantibodies against P450 side-chain cleavage enzyme (P450scc). The aim of the current study was to investigate P450scc autoantibodies in a large population of dogs with hypoadrenocorticism.

The coding region of canine P450scc was amplified, cloned and 35S-radiolabelled recombinant protein expressed in an in vitro transcription and translation reaction. Gel electrophoresis and autoradiography demonstrated a translated protein of expected size (60 kDa). A radioimmunoassay was validated using human sera of known P450scc autoantibody status. Serum samples were collected from 213 dogs diagnosed with hypoadrenocorticism and 110 dogs from a hospital control population with a range of non-endocrine conditions. Inter-assay variability was controlled by indexing radioactivity for each sample against previously identified positive and negative standards. Thirty randomly selected control dogs were used to establish a threshold for antibody positivity (mean control antibody index + 3 standard deviations).

A higher proportion of dogs with hypoadrenocorticism were P450scc autoantibody positive than hospital controls, 51/213 (23.9%) vs. 1/80 (1.2%) respectively (p = 0.0016). Of the samples taken within one month of diagnosis, dogs diagnosed in first-opinion practice were more likely to be P450scc autoantibody positive than dogs in referral practice (39% vs. 12% respectively; p = 0.005). Sex was significantly associated with the presence of p450scc autoantibodies in the case population, with 30% of females testing positive compared to 17% of males (p = 0.037).

Significant differences in CTLA4 promoter haplotypes were present comparing cases and controls (p = 0.002); haplotypes 3 (OR = 4.43, p = 0.006) and 12 (OR = 7.77, p = 0.042) were associated with an increased risk of hypoadrenocorticism and haplotype 8 (OR = 0.22) associated with a decreased risk. Three individual polymorphisms, making up these haplotypes, segregated with hypoadrenocorticism; namely SNP9 (OR = 7.77, p = 0.042), SNP10 (OR = 5.56, p = 0.048) and C INDEL (OR = 4.43, p = 0.006). The minor allele was associated with an increased risk of hypoadrenocorticism in each case.

This case-control study demonstrates an association between CTLA4 promoter polymorphisms and hypoadrenocorticism in Cockerspaniels, indicating that CTLA4 might have an important role in the immune-mediated pathogenesis of canine hypoadrenocorticism.

EN-9 HYPOADRENOCORTICIS IN COCKER SPANIELS IS ASSOCIATED WITH POLYMORPHISMS IN THE CYTOTOXIC T-LYMPHOCYTE-ANTIGEN 4 PROMOTER. A.M. Boag1, A. Short7, A. Threlfall1, L. Kennedy, W. Otter7, P. Graham1, H. Syme1, B. Catchpole1. 1Royal Veterinary College, London, UK., 2Centre for Integrated Genomic Medical Research, University of Manchester, UK., 3Nation Wide Laboratories, Poulton-Le-Fylde, UK.

Hypoadrenocorticism in dogs and humans (Addison’s disease) is characterised by corticosteroid deficiency requiring replacement hormone therapy. The underlying genetic susceptibility to this immune-mediated disease is incompletely understood. Cytotoxic T-Lymphocyte antigen 4 (CTLA4) is a critical regulator of immune function and has been associated with Addison’s disease in humans and implicated in genome-wide association studies and a recent candidate gene analysis in dogs. Variation in the CTLA4 promoter has also been associated with diabetes mellitus in six dog breeds. This study investigated whether CTLA4 promoter variability is associated with hypoadrenocorticism in Cocker Spaniels.

Nineteen dogs with hypoadrenocorticism and 67 dogs ≥ 9 years old with no history of endocrine or immune-mediated disease were recruited. Genomic DNA was extracted from EDTA blood samples and CTLA4 promoter polymorphisms assessed using PCR to amplify the 1.6 Kb region upstream of the start codon, followed by sequencing. Categorical data were analysed using the Fisher’s exact test, with permutation analysis used to correct for multiple testing when individual markers were assessed. The cut off for significance was set at p < 0.05.

Significant differences in CTLA4 promoter haplotypes were present comparing cases and controls (p = 0.002); haplotypes 3 (OR = 4.00) and 12 (OR = 7.76) were associated with an increased risk of hypoadrenocorticism and haplotype 8 (OR = 0.22) associated with a decreased risk. Three individual polymorphisms, making up these haplotypes, segregated with hypoadrenocorticism; namely SNP9 (OR = 7.77, p = 0.042), SNP10 (OR = 5.56, p = 0.048) and C INDEL (OR = 4.43, p = 0.006). The minor allele was associated with an increased risk of hypoadrenocorticism in each case.

This case-control study demonstrates an association between CTLA4 promoter polymorphisms and hypoadrenocorticism in Cocker Spaniels, indicating that CTLA4 might have an important role in the immune-mediated pathogenesis of canine hypoadrenocorticism.

Thyrotoxicosis refers to the biochemical and physiological manifestations of excessive thyroid hormone. Hyperthyroidism, the most common cause of thyrotoxicosis, is a term reserved for disorders that result in the overproduction of hormone by the thyroid gland. Hyperthyroidism is uncommon in the dog, and is most commonly caused by hyperfunctional thyroid carcinomas. Exogenous thyrotoxicosis is the term used to describe thyrotoxicosis caused by ingestion of excessive amounts of thyroid hormone. In man, numerous reports of thyrotoxicosis secondary to consumption of ground beef contaminated with thyroid tissue, so called “hamburger thyrotoxicosis,” are available (N Engl J Med 1987;316:993; Am J Med 1988;84:10; CMAJ 2003;169:415). Similarly, 2 European reports describe dogs that developed thyrotoxicosis secondary to consumption of diets containing raw meat or dried gullets contaminated with thyroid tissue (J Small Anim Pract 2012; 53:182; Schweiz Arch Tierheilkd 2013;155:149).

The purpose of this report is to describe the clinical, laboratory, and scintigraphic finding in 11 dogs with exogenous thyro-
toxicosis secondary to dietary consumption of excessive thyroid hormone, diagnosed between 2008-2013. All dogs were being fed one of various "real meat" varieties of commercially available, air- or freeze-dried, beef, lamb, or venison dog food or treats.

At presentation, 8 of 11 dogs had clinical signs consistent with thyrotoxicosis, including PU/PD and weight loss (n = 2), PU/PD alone (n = 4), or weight loss alone (n = 2). One dog had a decreased appetite, and 2 had no clinical signs. Serum concentrations of total T₄ (n = 11) and free T₃/E₃ (n = 9) were high in all cases measured. T₃/T₄ autoantibodies (n = 5) and thyroglobulin antibody levels (n = 7) were negative in all cases measured. TSH levels were low in 7 of 8 cases measured. In all 11 dogs, thyroid scintigraphy demonstrated a diffuse, bilateral, symmetric reduction of the thyroid uptake of ¹²³Iedaric acid, without areas of abnormal extrathyroidal uptake.

Following discontinuation of the suspect offending foods, recheck serum concentrations of total T₄ (n = 8), free T₃/E₃ (n = 5), and TSH (n = 3) returned to normal in all dogs in which it was measured. Clinical signs also resolved in all dogs following discontinuation of the offending food.

Analysis of 8 samples of meat-based food/treats fed to 6 of the 11 dogs revealed a significantly high T₄ content (mean T₄, 1.42 μg/g food), compared with 16 other randomly chosen dog food samples or samples of beef muscle/liver purchased at a grocery store (mean T₄, 0.37 μg/g food).

Overall, the reversible thyrotoxicosis in these dogs was related to consumption of commercially available "real meat" food or treats, which appear to have been contaminated by thyroid tissue (e.g., beef gullets and gross thyroid tissue). Given the numerous varieties of diets implicated in this report, this process appears to be affecting the pet food chain at the wholesale level.

**EN-11 CONCENTRATIONS OF POLYBROMINATED DIPHENYL ETHERS (PBDES) IN MATCHED SAMPLES OF SERUM AND HOUSE-DUST OF HYPERTHYROID CATS.** K. Chow¹, L.K. Hearn², M. Zuber³, J.A. Beatty⁴, J.F. Mueller², V.R. Barnes¹. ¹Faculty of Veterinary Science, University of Sydney, NSW Australia., ²National Research Centre for Environmental Toxicology, The University of Queensland, QLD Australia., ³Gladesville Veterinary Hospital, NSW Australia.

The aetiology of feline hyperthyroidism, a common endocrinopathy of older cats worldwide, is poorly understood. Polybrominated diphenyl ethers (PBDES), used as flame retardants in the manufacture of many consumer products, have been identified as goitrogenic environmental toxins, contaminating indoor air and dust. The objective of this study was to compare PBDE levels and congener profiles in the serum and environmental house dust of hyperthyroid cats with those of age-matched, euthyroid control cats. Inclusion criteria for hyperthyroid cats were elevated total T₄ and consistent clinical signs; and, for control cats, were euthyroid levels of total T₄ in the reference range. Serum samples were recruited prospectively from hyperthyroid cats and euthyroid cats (n = 68) together with a subset of matched house dust samples (n = 33).

All extracts were analyzed for PBDEs using a gas chromatograph (HP 5890 II) coupled to a mass spectrometer (VG AutoSpec) in electron ionization mode. Prior to extraction and clean-up, samples were weighed and spiked with a known amount of ¹³C₁₂ surrogate PBDE standard. A 1 mL aliquot of serum was collected from each original sample for lipid determination with the current standard of care for diabetic patients is to initiate a fixed dose of insulin between 0.25 and 0.5 U/kg SC BID (NPH, Glargine, protamine zinc recombinant human insulin, porcine insulin zinc suspension) and 0.1-0.2 U/kg SC BID (Detemir). The day to day variability of blood glucose (BG) has been well established and results in variable response to a fixed insulin dose. Historically, modifying insulin dose based on Home Blood Glucose Monitoring (HBGM) has been met with resistance as the dose was adjusted by the owner rather than directed by a veterinarian. The purpose of this study is to evaluate the value of using a veterinarian-directed insulin dosage chart (VIDC) compared to a fixed insulin dosage (FID) with HBGM.

Fifteen dogs and five cats diagnosed with diabetes mellitus were included in the study, which was not controlled for insulin or diet. Insulin was chosen based on the individual need of the patient, client and availability, and included NPH, NPH 70/30, Detemir, Glargine, protamine zinc recombinant human insulin, porcine insulin zinc suspension. The first 2 weeks, a FID was administered BID and a consistent diet was fed. HBGM was performed an average of 2 - 6 times per day using a hand held glucose monitoring device calibrated for the species of the animal. Owners supplied documentation of daily insulin dosages, glucagon concentrations and clinical data. After 1 week the patient's FID was increased by 20-25%, if the mean BG was ≤300 mg/dL and not ≤80 mg/dL. After 2 weeks, the patient's BG data was evaluated and a VIDC was generated using a consistent algorithm. HBGM continued twice daily and BG data was evaluated every 1-2 weeks by a veterinarian for 4 weeks. The VIDC was adjusted based on a consistent algorithm.

All patients responded to the VIDC with improved glycemic control (mean BG <300). 4/18 patients had a ≥50% decrease in mean BG compared with a fixed dose, 9/18 had 30-49% decrease in mean BG, and 5/18 had a <30% decrease in mean BG compared with mean BG on a fixed insulin dose. All patients had an average decrease of at least 15% in mean BG compared with fixed dose, and the average decrease in mean BG was 39.8% (p <0.01). Biochemical hypoglycemia (<60 mg/dL) was detected in 1.4% of FID and 0.06% of all VIDC BG measurements and occurred on average of 0.7 times/patient during the study period. Use of a VIDC did not statistically reduce the mean insulin dose.

In conclusion, implementation of a VIDC in conjunction with BID HBGM, significantly improved overall glycemic control and reduced the incidence of biochemical hypoglycemia in this small pilot study irrespective of species, insulin or diet. These findings support the use of VIDC as a first line therapy for diabetic patients, and may provide an improved means of establishing glycemic control to avoid short & long-term complications.

**EN-12 USE OF VETERINARIAN-DIRECTED VARIABLE INSULIN DOSAGE CHART RESULTS IN IMPROVED GLYCEMIC CONTROL WHEN COMPARED WITH FIXED INSULIN DOSE.** S.L. Ford¹, D.D. Roce², H.M. Lynch³, ¹VCA Emergency Animal Hospital & Referral Center, San Diego, CA., ²Ta-Pum Animal Hospital, Phoenix, AZ.

The current standard of care for diabetic patients is to initiate a fixed dose of insulin between 0.25 and 0.5 U/kg SC BID (NPH, Glargine, protamine zinc recombinant human insulin, porcine insulin zinc suspension) and 0.1-0.2 U/kg SC BID (Detemir). The day to day variability of blood glucose (BG) has been well established and results in variable response to a fixed insulin dose. Historically, modifying insulin dose based on Home Blood Glucose Monitoring (HBGM) has been met with resistance as the dose was adjusted by the owner rather than directed by a veterinarian. The purpose of this study is to evaluate the value of using a veterinarian-directed insulin dosage chart (VIDC) compared to a fixed insulin dosage (FID) with HBGM.

Fifteen dogs and five cats diagnosed with diabetes mellitus were included in the study, which was not controlled for insulin or diet. Insulin was chosen based on the individual need of the patient, client and availability, and included NPH, NPH 70/30, Detemir, Glargine, protamine zinc recombinant human insulin, porcine insulin zinc suspension. The first 2 weeks, a FID was administered BID and a consistent diet was fed. HBGM was performed an average of 2 - 6 times per day using a hand held glucose monitoring device calibrated for the species of the animal. Owners supplied documentation of daily insulin dosages, glucagon concentrations and clinical data. After 1 week the patient’s FID was increased by 20-25% if the mean BG was ≤300 mg/dL and not ≤80 mg/dL. After 2 weeks, the patient’s BG data was evaluated and a VIDC was generated using a consistent algorithm. HBGM continued twice daily and BG data was evaluated every 1-2 weeks by a veterinarian for 4 weeks. The VIDC was adjusted based on a consistent algorithm.

All patients responded to the VIDC with improved glycemic control (mean BG <300). 4/18 patients had a ≥50% decrease in mean BG compared with a fixed dose, 9/18 had 30-49% decrease in mean BG, and 5/18 had a <30% decrease in mean BG compared with mean BG on a fixed insulin dose. All patients had an average decrease of at least 15% in mean BG compared with fixed dose, and the average decrease in mean BG was 39.8% (p <0.01). Biochemical hypoglycemia (<60 mg/dL) was detected in 1.4% of FID and 0.06% of all VIDC BG measurements and occurred on average of 0.7 times/patient during the study period. Use of a VIDC did not statistically reduce the mean insulin dose.

In conclusion, implementation of a VIDC in conjunction with BID HBGM, significantly improved overall glycemic control and reduced the incidence of biochemical hypoglycemia in this small pilot study irrespective of species, insulin or diet. These findings support the use of VIDC as a first line therapy for diabetic patients, and may provide an improved means of establishing glycemic control to avoid short & long-term complications.
EN-13 FALCIFORM FAT DEPTH MEASURED BY X-RAY IS A MODERATELY PRECISE PREDICTOR OF TOTAL BODY FAT MASS AND ABDOMINAL FAT MASS AS MEASURED BY DUAL-ENERGY X-RAY ABSORPTIOMETRY IN CATS. M. Coradini1, J.S. Rand1, J.M. Morton2, G. Covin2, J.M. Rawlings3, 1School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia., 2Jemora Pty Ltd, Geelong, VIC, Australia., 3Centre for Advanced Imaging, The University of Queensland, Brisbane, QLD, Australia., 4WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicester LE14 4RT, UK.

Obesity is common among cats, and is associated with metabolic and hormonal changes that predispose cats to diabetes, among other diseases. “Gold standard” methods for quantitative measurement of fat mass are typically expensive and often unavailable for clinical research investigating weight loss strategies in obese client-owned cats. The aim of this study was to determine whether regional fat measured by magnetic resonance imaging, ultrasound and X-ray predict total body fat and abdominal fat masses measured by dual-energy X-ray absorptiometry (DEXA); to develop regression-based predictive equations utilizing the regional fat measures to estimate total and abdominal fat masses as measured by DEXA, and to estimate the intra-individual variability of these measures. Eight adult, healthy cats, four lean and four obese, were used. Each measurement was taken twice in the same cat, 6 to 11 days apart. X-ray measurement of falciform fat depth was the most precise predictor of total (R² = 95.1%) and abdominal fat (R² = 96.2%), followed by the ultrasound measurement of falciform fat depth (R² = 90.4 and 89.3%, respectively). Apart from DEXA (mean coefficient of variation [CV] 3 to 4%), all measures had high intra-individual variability (mean CVs 15 to 50%) From our equations, X-ray measures of falciform fat depth, based on the mean of 2 values from separate days, have moderate precision for predicting total and abdominal fat masses as measured by DEXA. X-ray and ultrasound could be useful methods for determining fat mass in clinical research, provided fat mass is expected to vary markedly amongst study cats or within cats over time, and sufficient animals are included in the study.

EN-14 METABOLIC DETERMINANTS OF BODY WEIGHT AFTER CATS WERE FED A LOW-CARBOHYDRATE, HIGH-PROTEIN OR A HIGH-CARBOHYDRATE, LOW-PROTEIN DIET AD LIBITUM FOR 8 WEEKS. M. Coradini1, J.S. Rand1, J.M. Morton2, J.M. Rawlings3, 1School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia., 2Jemora Pty Ltd, Geelong, VIC, Australia., 3Centre for Advanced Imaging, The University of Queensland, Brisbane, QLD, Australia., 4WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicester LE14 4RT, UK.

The overweight and obese conditions are common in cats, and associated with the development of a number of diseases. Knowledge of metabolic determinants and predictors of weight gain may enable better preventative strategies for obesity in cats. Lean, healthy cats were fed either a low-carbohydrate, high-protein (n = 16), or a high-carbohydrate, low-protein (n = 16) diet ad libitum for 8 weeks. Potential determinants and predictors of final body weight assessed were body fat and lean masses, energy required for maintenance, energy requirements above maintenance for each kg of weight gain, insulin sensitivity index, fasting, mean 24-hour and peak plasma glucose, insulin and leptin concentrations, and fasting and mean 24-hour serum adiponectin concentrations. In cats fed the low-carbohydrate, high-protein diet, after adjusting for initial body weight, those with higher energy requirements for weight gain and higher fasting glucose concentration had higher final body weights (P ≤ 0.01). The predicted final body weights using initial body weight, fasting glucose and mean 24-hour insulin concentrations were imprecise. An equation using just initial body weight and fasting glucose concentration would be of more practical value, but was marginally less precise. In cats fed the high-carbohydrate, low-protein diet, those with lower fasting leptin concentration initially had higher final body weights (P = 0.01). The predicted final body weights using initial body weight, energy requirements for maintenance, total body fat percentage and fasting leptin concentration were reasonably precise. Further studies are warranted to confirm these findings and to improve the precision of predicted final body weights.

EN-15 COMPARISON OF PRECISION AND ACCURACY OF U100 AND U40 INSULIN SYRINGES. S. Borin-Crivellenti1, 2, J.D. Foggia1, C. Gilor1, 1College of Veterinary Medicine, The Ohio State University, Columbus, OH., 2FCAV/Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil.

Day-to-day variability of insulin action is an important factor in attaining glycemic control in diabetes. In part, this variability is caused by imprecise dosing of insulin.

We hypothesized that a U40 insulin syringe (U40) would be more precise than a U100 insulin syringe (U100). We dispensed 1, 2.5 and 4 international units (IU) of insulin using 24 syringes for each dose from a BD Ultra-Fine 0.3 cc U100 (½ Unit Markings) and a VetOne 0.3 cc U40. Each dose was weighed on an analytical scale, and accuracy (mean ±SD of [actual dose – target dose] * 100/target dose) and precision (the coefficient of variation [SD/mean] of the actual dose) were calculated. The proportions of CID (clinically-important-deviation: ≥20% off target) outcomes were compared between syringe types.

U40 was more accurate for 1, 2.5 and 4 IU (13.2 ± 8.7%, 6.0 ± 7.26%, 3.2 ± 1.6%, respectively) than U100 (28.2 ± 15.4%, 10.7 ± 8.0%, 4.6 ± 2.9%, respectively) (p < 0.05). Precision was lowest for 1 IU but improved with increasing dose (U40: 1 IU = 15.8%, 2.5 IU = 6.4%, 4 IU = 3.5%; U100: 1 IU = 15.1%, 2.5 IU = 8.1%, 4 IU = 3.3%). U40 was more precise than U100 for dosing of 2.5 IU (p < 0.05) despite the 1/2-unit markings on U100. CID outcomes were more frequent in U100 vs. U40 in 1 IU (16/24 vs. 8/24 respectively, P = 0.02) and 2.5 IU (13/24 vs. 0.24 respectively, P = 0.07) but did not occur in 4 IU.

For administration of small insulin doses, U40 are more accurate and precise than U100 and are less likely to result in clinically important over- or under-dosing. These results favor the use of U40 for administration of small doses of insulin.

EN-16 CORTICOSTEROID-INDUCED ALKALINE PHOSPHATASE ACTIVITY IN DOGS WITH ADDISON’S DISEASE. S. Borin-Crivellenti1, 2, M.L. Wellman1, C. Gilor1, 1College of Veterinary Medicine, The Ohio State University, Columbus, OH., 2FCAV/Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil.

In dogs, corticosteroid-induced alkaline phosphatase (CiALP) is an isoenzyme induced by corticosteroids. CiALP activity is measured routinely in our laboratory using an automated chemistry analyzer with a reference interval (RI) in healthy dogs of 0 – 6 IU/L. Our objective was to determine whether CiALP activity can be used as a screening test for typical and atypical hypoadrenocorticism (HA) in dogs.

This was a retrospective study of 50 dogs with clinical findings suggestive of HA but a final diagnosis of some other disease, and 50 dogs with final diagnosis of typical (Na < 27, n = 34) or atypical HA (Na ≥ 27, n = 16). We assessed the sensitivity and specificity of either ≤12 IU/L or ≤65 IU/L CiALP to detect dogs with HA, ACTH stimulation testing and basal cortisol concentrations (<4mcg/dl) were used as reference tests for the diagnosis or exclusion of HA respectively.

CiALP ≤12 IU/L had a sensitivity of 51% and a specificity of 38%. CiALP ≤65 IU/L had a sensitivity of 98% and a specificity of 13%. The area under the receiver operating characteristic (ROC) curve for CiALP was low (0.71) for dogs with typical HA and even lower (0.66) for dogs with atypical HA. No cutoff yielded a clinically useful combination of sensitivity and specificity.

Based on our data, CiALP can be used to rule out HA only if activity levels are ≥ 11 times the upper end of the RI and cannot
EN-17
PASIREOTIDE (SOM230) LONG-ActING RELIEF TREATMENT FOR FELINE HYPERSOMATOMATROPISM: A PROOF OF CONCEPT TRIAL. R. Gostelow1, C. Scudder1, S. Keyte1, Y. Forcada1, R.C. Fowkes1, H.A. Schmidt2, D.B. Church3, S.J.M. Niessen3,1. 1The Royal Veterinary College, London, UK. 2Novartis Institutes for BioMedical Research, Oncology Research, Novartis Pharma AG, Basel, Switzerland. 3Newcastle Medical School, Newcastle, UK.

Hypersomatotropism (HS) is a relatively common cause of feline diabetes mellitus. Attempts at its long-term medical management with somatostatin (sst) analogues have previously proven unrewarding. However, Pasireotide (SOM230, Novartis, Basel, Switzerland), a novel sst-analogue with binding affinity for sst-receptor subtypes 1, 2, 3 and 5, was recently shown capable of decreasing serum insulin-like growth factor 1 (IGF-1) and improving insulin sensitivity in cats with HS when administered for 3 days as a short-acting, BID subcutaneous (SC) preparation. A long-acting release formulation (LAR) has been developed to allow convenient, monthly dosing and has led to successful biochemical control of human HS. The current study aimed to assess the potential of once monthly Pasireotide LAR as a treat-ment for feline HS.

Feline HS was diagnosed in 12 diabetic cats based on increased serum IGF-1 (>1000 ng/ml) and pituitary enlargement on computed tomography. Cats received 8 mg/kg SC Pasireotide LAR once monthly for 6 months. Fructosamine concentration, IGF-1 concentration and a 12-hour blood glucose curve (BGC) were performed at baseline and once monthly thereafter to monitor treatment response. A repeat CT-scan was performed at the end of the trial. A mixed effects model was used to assess signifi-cance of changes in fructosamine, IGF-1 concentration, mean blood glucose (MBG) of BGCs, and insulin dose (U/kg).

Seven of 12 cats completed the trial; 3 of 12 cats entered diab-etic remission. Trial withdrawal occurred after a median of 2 months (range 1-4.5 months) due to persistence of uncontrolled diabetes mellitus (n = 1), diarrhoea (n = 2), a hypoglycemic event (n = 1) and an episode of diabetic ketoacidosis (n = 1). A signifi-cant decrease in IGF-1 (p < 0.001), insulin dose (p < 0.001), Fruc-tosamine (p = 0.04), though not MBG (p = 0.71) was documented. Adverse events included soft stools (9/12), worsen-ing polyphagia (3/12), hypoglycaemia (4/12) and delayed hair re-growth (1/12). Maximum pituitary mass height had increased in 2/7, decreased in 4/7 and remained the same in 1/7 cats.

In summary, Pasireotide LAR is the first drug that shows potential to cause long-term biochemical and clinical improvement in cats with HS. In a proportion of cases, diabetic remission can even be achieved. Further work should focus on dose optimisation to enable higher success and lower withdrawal rates, specifically by trying to reduce adverse gastrointestinal events. The observed decrease in pituitary tumor size in some cats further establishes this as a useful primary long-term treatment modality, although its preoperative use, enabling glycemic stabilization and tumor shrinkage before hypophysectomy, may also be of benefit.

EN-18
TARGETING PHOSPHATIDYLYSINOYL-3-KINASE SIGNALING IN CANINE CORTISOL-SECRETING ADRENO-CORTICAL TUMORS: A NOVEL THERAPEUTIC PROSPECT? M.M.J. Koole1, S. Galea2, N.van der Helm3, S. Corradi4, H.S. Koostra5, J.A. Mol6, 1Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands. 2University of Bologna, Ozzano dell’Emilia, Italy.

Hypercortisolism is one of the most common endocrinopathies in dogs, and is caused by cortisol-secreting adrenocortical adeno-

mas or carcinomas in 15% of cases. The aim of this study was to investigate involvement of the insulin-like growth factor (IGF) – phosphatidylinositol-3-kinase (PI3K) signaling pathway in the pathogenesis of adrenocortical tumors (ATs), in order to identify components of this pathway that may hold promise as future therapeutic targets, prognostic and/or diagnostic markers.

The tumor group consisted of histologically confirmed cortisol-secreting adenomas (n = 14) and carcinomas (n = 30). Whole tissue explants of normal adrenal glands (n = 10) were used as controls. Quantitative RT-PCR was used to assess the relative mRNA expression levels of IGF1 and 2, IGF- and EGF-recep-tors, IGF-binding proteins, PI3K inhibitor PTEN and down-stream target genes of the PI3K signaling pathway. Localization of PTEN was immunohistochemically evaluated. Additionally, mutation analysis was performed on the full coding region of PTEN and the PI3K catalytic subunit, on mRNA level.

When compared to normal adenals, in carcinomas the differ-ential expression of PI3K target genes indicated activation of the pathway. Also, carcinomas showed a decreased expression of PI3K inhibitor PTEN and an increased expression of the EGFR receptor ErbB2. Gene expression levels in adenomas were mostly unchanged. Immunohistochemical staining of PTEN was predominantly negative in both ATs and normal adrenals. No sen-sible mutations of PTEN and the PI3K catalytic subunit were detected.

Based on gene function and reports in human ATs, the low expression of PTEN in carcinomas is of particular interest with regard to tumor pathogenesis. Target gene expression suggests PI3K activation in carcinomas, possibly due to decreased PTEN and increased ErbB2 expression. Based on these results, targeting of ErbB2, PI3K or its downstream effectors may have potential as a therapeutic option in canine cortisol-secreting adre-

nocortical carcinomas.
Radioactive iodine ($^{131}$I) provides a simple, effective, and safe treatment for cats with hyperthyroidism. Ideally, treatment with a single dose of $^{131}$I restores euthyroidism without inducing hypothyroidism (i.e., goal is to irradiate and destroy all adenomatous thyroid tissue but to leave enough normal thyroid tissue to prevent hypothyroidism). The protocol used to determine the $^{131}$I dose differs widely among treatment facilities, which may influence the prevalence of persistent hyperthyroidism ($^{131}$I dose too low) and iatrogenic hypothyroidism ($^{131}$I dose too high). The methods for $^{131}$I dose determination can be generally divided into either fixed $^{131}$I dosing (usually administration of 4 mCi to all cats) or an individualized or “patient-specific” $^{131}$I dose scoring system based primarily on the pretreatment serum $T_4$ concentration ($^{131}$I doses generally range from 3-5 mCi; Peterson et al. J Am Vet Med Assoc 1995;207:1422). In our clinics, we have developed a graded dose scoring system that also takes into account the serum $T_4$ concentration; however, with our method, thyroid volume is measured using data derived from thyroid scintigraphy and the $^{131}$I dose calculated primarily on the basis of each cat’s thyroid tumor volume.

During a 1-year period (Sept 2012 to Sept 2013) in which we treated approximately 900 cats with hyperthyroidism, an ultra-low ($^{131}$I) dose ($\leq 2$ mCi; $\leq 75$ mBq) was calculated for 225 of these cats (25%). The purpose of this report is to review the prevalence of persistent hyperthyroidism and iatrogenic hypothyroidism in $^{131}$I cats in which 1- and 3-month follow-up data was available.

In the $^{131}$I cats, the low doses of $^{131}$I administered ranged from 1.0-2.0 mCi (median, 1.85 mCi; 68 mBq). Before treatment, the serum $T_4$ concentrations ranged from 3.5-20.8 g/dl (median, 6.2 g/dl, reference range, 0.8-4.0 g/dl); the 5 cats with high-normal $T_4$ values all had high free $T_4$ concentrations. After treatment, median $T_4$ values fell significantly ($P < 0.001$) to 1.7 g/dl at both 1- and 3-month recheck periods. At 1 month, $T_4$ values were normal in 119 cats, low in 8, and persistently high in 4. At 3 months, $T_4$ values were within normal limits in 125 cats (95%), low in 4 (3%), and persistently high in only 2 (1.5%). In 3 of the 4 cats with low $T_4$ values, hypothyroidism was confirmed via measuring a high cTSH concentration (0.7-7.5 ng/mL; normal < 0.3 ng/mL).

Results of this study indicate that very low doses of radioactive iodine (e.g., $\leq 2$ mCi; $\leq 75$ mBq) result in cure of most cats with milder forms of hyperthyroidism that have small thyroid tumors on scintigraphy. These radioiodine doses are much lower than the lowest dose given with our original scoring system (3 mCi) or with the fixed-dose methods (4-5 mCi). Despite our ultra-low doses, 3% still developed hypothyroidism, suggesting that many more would become hypothyroid after larger doses commonly used. Overall, we believe that determination of thyroid tumor volume plays a key role in calculating the best $^{131}$I dose needed to ablate all tumor tissue (resulting in cure) while preserving enough normal thyroid tissue to prevent iatrogenic hypothyroidism.
Obese senior cats had a 1.8-fold increase in fatty acids (9-z-hexadecenoic acid, hexadecenoic acid and tetradecenoic acid), a 1.4-fold increase glycerol and glycolic acid, and a 0.7-fold decrease in the amino acid alanine. Increased free fatty acid and accompanying glycerol levels in obese cats agrees with earlier reports in humans, rodents and dogs. Such results reflect the enlarged mass of adipose tissue. In humans, insulin sensitivity is improved by reducing fatty acid levels. Increased glycolic acid levels in obese cats could be indicative of increased gluconeogenesis (alanine to pyruvate to glucose), or related to increased glycolic acid detoxification in hepatocytes (glycolic acid being oxidised to glyoxylic acid before combining with alanine to produce pyruvate). However neither pyruvate nor glucose were significantly increased in obese cats. This study is the first to report metabolite markers of obesity in healthy senior cats, and could provide useful markers of metabolic dysfunction in obesity.

EN-23
CAPROMORELIN, AN ORALLY ACTIVE GHRELIN AGONIST, STIMULATES APPETITE AND WEIGHT GAIN IN INAPPETENT DOGS IN A MULTI-SITE FIELD STUDY. B. Zollers, L. Rhodes, Aratana Therapeutics, Inc., Kansas City, KS.

Capromorelin (AT-002) is a small molecule mimic of the ghrelin hormone, the hormone associated with appetite and food intake. A randomized, masked, placebo controlled multi-site field study was conducted to measure the effects of capromorelin on appetite and body weight in inappetent client owned dogs. A questionnaire was developed which consisted of 5 questions, each of which could be scored from 1-5, allowing owners to evaluate their dog’s appetite. The five question scores were added to give a total appetite score with the lowest total score (worst appetite) being 5 points.

Of the 36 dogs enrolled, 7 cases were considered unevaluable, leaving a population for analysis of 29 dogs. Of the 29 dogs, 5 (17.2%) dogs treated with capromorelin and 2 (6.9%) dogs treated with placebo (p = 0.037) showed an increase in total appetite score of ≥ 5 points from Day 0 to Day 6.

EN-24

Maximal adrenal width (AW) of 0.74 cm for healthy dogs has recently been challenged by studies demonstrating increased adrenal size with age and bodyweight in healthy dogs or dogs with non-adrenal disease. However, reference intervals specific for age and bodyweights remain ill-defined. This study examined canine AW spanning a continuum of ages and bodyweights. Of 1,981 dogs undergoing abdominal ultrasonography (2007-2013), 276 with non-adrenal diagnoses without glucocorticoid exposure were selected from 12 breeds (3-small, 6-medium, 3-large). Clinicopathologic variables were transcribed. Maximal left and right AW (LAW, RAW) were assessed in static-longitudinal images (board-certified radiologist). Dogs were stratified into 3-age and 5-weight categories (Table). Significant differences between age- and weight-categories (all dogs, by gender) in clinicopathologic variables and AW (Wilcoxon rank-sum test, P = 0.05 with Bonferroni corrections), and relationship of AW with age in weight-categories (linear regression) were determined. Young dogs had higher lymphocyte counts than dogs ≥ 4 yrs. AW were smaller in ≤ 6 kg and ≥ 12 kg vs > 12 kg dogs (P < 0.0001 LAW, P = 0.02 RAW). Because AW between ≤ 6 kg and > 6 ≤ 12 kg dogs and between > 12 ≤ 20, > 20 ≤ 30, and > 30 kg dogs were not significantly different, combined weight groups (≤ 12 kg, > 12 kg) were examined by linear regression. LAW increased with age (all dogs [P < 0.0001, F-statistic=29.8]; ≤ 12 kg [P = 0.02, F-statistic=5.6]; > 12 kg [P < 0.0001, F-statistic=39.8, Figure]); RAW increased with age (all dogs [P = 0.02, F-statistic=5.82]; > 12 kg [P = 0.0006, F-statistic=12.3]). There were no gender differences in AW between age groups, but males > 12 ≤ 20 kg and > 20 ≤ 30 kg kg had larger AW and LAW, respectively (P < 0.03). Findings suggest ranges to better guide detection of adrenomegaly in dogs with non-adrenal disease.

EN-25
LOW CORTISOL CONCENTRATIONS IN WELL-REGULATED TRILOSTANE TREATED DOGS WITH HYPERADRENOCORTICISM. J.N. Midence, K.J. Drobatz, R.S. Hess. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Currently there are no clear treatment guidelines for dogs with clinically well-regulated hyperadrenocorticism in which cortisol concentration before and after ACTH stimulation test performed 3-6 hours after trilostane (Vetoryl®) administration is <2.0 µg/dL. The goal of this study was to determine if an ACTH stimulation test performed 9-12 hours after trilostane administration may clarify treatment guidelines.

Ten client-owned dogs were enrolled into this ongoing prospective study if they had clinically well-regulated hyperadrenocorticism and had serum cortisol concentrations <2.0 µg/dL before (Pre1) and after (Post1) ACTH stimulation test performed 3-6 hours following trilostane administration. Dogs then had a second ACTH stimulation test (Pre2 and Post2) performed 9-12 hours after trilostane administration, on the same day they had the first ACTH stimulation test. Mean (+standard deviation)
EN-26

Obesity is a chronic disease in canine population that is characterized by excessive accumulation of adipose tissue and it has become increasingly common in veterinary medicine. This paper aims at identifying, through a survey, the perception of the owner regarding dog obesity, verify nourishment quality and determine regular exercise habits in the canine population of Curitiba and metropolitan area.

In this study, a hundred and fifteen animals of different genders, ages, breeds and body condition score (obese, overweight and lean) were selected. Clinical data of those animals revealed prevalence of the female sex (76.5%). Around 38% of the dogs were neutered and the most predominant breeds were Poodle (15.6%) and Cocker spaniel (11.3%). Additionally, the ages ranged from 1 to 15 years. Owners of obese or overweight dogs (75.65%) were asked about their pets’ life habits. Around 77% of these owners agreed with the body condition classification of their dogs and 89.6% of them were concerned about their animal’s weight. While the majority (57.4%) of the obese or overweight dogs were fed with both homemade food and industrialized dog food, 47.1% were fed only with industrialized dog food and very few dogs (1.1%) were fed only with homemade food. Besides, 63.2% of these animals did not exercise regularly.

In conclusion, this study confirms the importance of the role of the veterinarian to inform the owners the cares with the animal’s weight, diet and regular exercise, reducing the number of future obesity in dog population.

EN-27
RENAL PATHOLOGICAL PROTEINURIA IN OBESE AND OVERWEIGHT DOGS, S.B. Lucina1, C.Z. Cavalcante, C. Domingues1, B. Cottar2, G. Delinski3, J. Posebom4, G. Dittrich1, 1Pontifical Catholic University of Paraná (PUCPR), São José dos Pinhais, PR, Brazil, 2Institute of Technology of Paraná (TEC-PAR), Curitiba, PR, Brazil.

Obesity has systemic and renal implications that promotes or aggravates renal proteinuria. This study aims to determine the frequency of albuminuria, in a sequentially and quantitatively way, and the influence of systolic blood pressure (SBP) in the quantification of urinary albumin in overweight and obese dogs. The hypothesis is that obesity/overweight worsens or promotes renal pathological proteinuria. There were selected sixteen obese and overweight dogs, among males and females of varying ages and breeds. Patients were submitted under the classification of body condition score, lumbaroscal ultrasonography, complete blood count, serum biochemistry, measurement of SBP, urinalysis, determination of urinary protein/creatinine ratio (UPC-Coomassie Blue), quantification of albuminuria (Dog Albumin ELISA Kit®) and determination of urinary albumin/creatinine ratio (UAC) sequentially. Animals with pre-renal, post-renal and functional renal proteinuria, or which were taking medications that interfere in this determination, were not considered on the statistical sampling. Among the total selected dogs, 25% (n = 4) were overweight and 75% (n = 12) obese, and 75% (n = 12) of them showed alteration in the amount of UPC and/or albuminuria. Regarding the results of UPC, 18.75% (n = 3) of the dogs had renal pathological proteinuria, although by measuring urinary albumin, 50% (n = 8) of patients had persistent albuminuria. Considering the UAC, 25% (n = 4) of the selected animals showed changes in only one of the samples and 31.25% (n = 5) presented a persistent change. Since the correlation between albuminuria and SBP was low, it can be concluded there was no influence between hypertension and the quantification of urinary albumin. Despite the fact that obese and overweight dogs showed renal pathological proteinuria, in veterinary medicine additional studies need to be held to evaluate the frequency of renal pathological albuminuria.

EN-28
PERFORMANCE OF A POINT-OF-CARE FRUCTOSAMINE TEST FOR DOGS AND CATS, B. Eldridge, P. Marietta, G. Pangakos, T. McFadd, P. Kintzer; IDEXX Laboratories, Westbrook, ME.

The incidence of diabetes in both dogs and cats is increasing. In addition to blood glucose testing, fructosamine tests are an effective component in diabetes diagnosis and regulation. Fructosamines are stable complexes of glycated proteins and carbohydrates that relate to average circulating glucose concentrations over the preceding 2-3 weeks in patients.

Typically, samples are sent to outside laboratories for fructosamine testing. The IDEXX Catalyst Dx™ platform is an automated system that incorporates dry-slide technology for in-clinic, diagnostic results. The new Catalyst fructosamine test provides veterinarians with rapid results to aid in the real-time diagnosis and management of diabetic patients. The test requires 6 µL of serum or plasma and reports results within 9 minutes. The purpose of this study was to assess patient correlation between the Catalyst fructosamine test and the IDEXX Reference Laboratory fructosamine test.

Seventy-five feline and seventy-nine canine samples were collected. Fructosamine levels were analyzed using three Catalyst Dx Analyzers and one Olympus AU400 Chemistry Analyzer (Catachem reagent). Analyzers were maintained and assays performed according to manufacturer’s specifications.

Least squares linear regression and calculation of Pearson’s coefficient of regression $R^2$ were used to compare the overall agreement of fructosamine concentrations between the two methods (feline: $m = 1.00$, $y_a = 1.42$, $R^2 = 0.93$, canine: $m = 1.02$, $y_a = 0.98$, $R^2 = 0.90$).

Results suggest that the Catalyst fructosamine test produces accurate results when used to quantify fructosamine in cats and dogs. The new IDEXX Catalyst Dx fructosamine test provides a viable point of care option for diabetes management in dogs and cats.

EN-29

Dysregulation of adipokine has been reported to be associated with the pathogenesis of diabetes mellitus (DM) in humans; however, there is a lack of data available on the role of adipokine in dogs with DM. The objective of this study was to examine whether serum adipokine concentrations were different between healthy dogs and dogs with (DM). Nineteen dogs with newly diagnosed, untreated DM were enrolled in this case-controlled study; a control group of 20 healthy dogs was also included.

The serum concentrations of visfatin, leptin, interleukin (IL)1β, IL-6, IL-18, and tumor necrosis factor-α in the DM
Capromorelin treated dogs increased food consumption and body weight when compared to controls. The percent increase in body weight (treatment vs. placebo) was Day 1 (-1% to Day 7) was 4.52%, 3.78% and 4.17% for Groups 2, 3, and 4 respectively, while the placebo group (Group 1) lost 1.17% (p < 0.001 for all treatment groups). Comparing food consumption during the baseline period (mean of Days -3, -2 and -1) to the treatment period (mean of Days 1-7), Groups 2, 3, and 4 had an increase of 57.7%, 37.9% and 36.4% respectively (p < 0.005 for all groups). Group 1 showed a decrease of 13.5%.

Treatment with capromorelin caused increased serum GH, and levels remained elevated through 4-6 hrs post-dose on Day 1 and through 2-4 hrs on Days 4 and 7 before returning to baseline by 8 hrs post-dose. The magnitude of the increase was significantly less on Days 4 and 7 compared to Day 1. The pre-dose levels on Days 4 and 7 indicated no sustained effect on GH from the capromorelin dose of the previous day. IGF-1 levels gradually increased over 8 hrs in all capromorelin treated dogs beginning ~2 hrs post-dose, reaching maximum levels at 8 hrs and remaining sustained for 24 hrs. Serum cortisol was increased at 30 minutes post-dosing, returning to baseline by 8 hrs. The magnitude of the cortisol increase was less on Day 7 compared to Day 1.

Capromorelin has been shown to increase body weight and owner assessment of appetite in a clinical study of dogs with inappetence (Zollers and Rhodes, abstract submitted 2013). We hypothesize that the accompanying increase in GH and IGF-1 demonstrated in this study may also result in positive effects on lean muscle mass. This study has been conducted in dogs treated with GH (Molon-Noblot et al., Toxicol Pathol 26:207-212, 1998).

EN-32 FRUCTOSAMINE LEVELS DO NOT AGREE WITH CLINICAL CLASSIFICATION REGARDING DIABETIC COMPENSATION IN DIABETIC DOGS UNDER TREATMENT. P. Claus, A.M. Gimmens, J.R. Castro, D.S. Schwartz, School of Veterinary Medicine and Animal Science, University of São Paulo (USP), São Paulo, SP – Brazil, Department of Internal Medicine.

Fructosamine levels are measured for diabetes management in veterinary medicine, but are rarely used in human clinical practice. A prospective cross-sectional study was conducted between January/2010 and August/2012, to assess serum fructosamine levels of diabetic dogs under treatment, in order to determine glycemic control, compared to clinical classification of “compensated” versus “non-compensated”, based on clinical signs and owner evaluation of the animal clinical status.

The study population included 86 dogs: 25 were healthy, non-diabetic dogs (controls), 14 were diabetic dogs at diagnosis, 24 were diabetic under treatment (at least 30 days), and 23 had diabetic ketoacidosis (DKA). Compared to controls, serum fructosamine levels were significantly higher for all the diabetic groups, which were similar between each other. Considering all dogs, 8.3% were within the
lower level (300–350 mg/dL), 11.9% had excellent glycemic control (350–400 mg/dL), 14.3% had good glycemic control (400–450 mg/dL), 14.3% had regular glycemic control (450–500 mg/dL) and 51.2% had poor control (>500 mg/dL). Considering dogs under treatment, 95.8% were classified as having poor glycemic control and only 4.2% had a good control. Although 17/24 (70.8%) were clinically classified as “compensated”, they all had fructosamine levels >500 μmol/L, therefore, a poor glycemic control. Only one dog in this group had fructosamine levels indicating good glycemic control, but in this case, the owner had reported polyuria, polydipsia, polyphagia and therefore, had been classified as non-compensated. Further studies must assess if insulin therapy adjustment based on fructosamine levels, and not only on clinical status would lead to hypoglycemia episodes.

EN-33

The aim of this study was the identification of comorbidities and main laboratorial findings in dogs with diabetic ketoacidosis (DKA), considered the main endocrine emergency in dogs and cats. A retrospective study was conducted, based on information gathered from the clinical records, considering history, physical exam data and laboratorial findings from dogs diagnosed with DKA during the period of January 2007 and June 2013. Among 195 dogs diagnosed with DKA, 143 (73.33%) had comorbidities. Pancreatitis (26.66%), renal disease (20%), hyperadrenocorticism (19.48%), pyometra (4.10%) and urinary tract infection (3.60%) were the most prevalent diseases. Besides hyperglycemia, metabolic acidosis and ketonuria, the main laboratorial findings were azotemia (47.70%), hypokalemia (37.80%), hyperkalemia (3.00%), increased activity of ALT (53.84%) and alkaline phosphatase (82.05%). Mean age was 10.38 ± 2.08 years, with a predominance of females (77.95%). These findings are similar to those reported in another study that described values of azotemia (46%), hypokalemia (45%) and increase activity of ALT (57%). Mortality rate was 41.5%, for the population studied, compared to 20% reported in literature.

Most dogs in DKA presented comorbidities, enhancing the importance of investigating the presence of concurrent diseases which might have contributed to diabetes decompensation. The high mortality rate indicates the severity of the disease and the need for intensive care, as well as the preventive evaluation of diabetic dogs in order to access health status and decrease possibility of decompensation.

EN-34

In human diabetics oxidative stress is known to increase production of advanced glycation end-products (AGE). Increased production of AGE contributes to the adverse clinical consequences associated with Type 2 diabetes. The hypothesis of this study is that oxidative stress increases AGE production in the serum of diabetic (DM) cats.

Feline-specific assays for AGE quantification were developed and applied to serum samples from cats with and without DM. The methylglyoxal (AGE precursor) reaction with diaminobenzene to form 2,4-dinitrophenylhydrazine to form bis(2,4)-dinitrophenyldiazine is the bases for the alternative methylglyoxal (2,4-dinitrophenyldiazine (DNPH MEG) assay. Both protocols were adapted to run as spectrophotometric microplate assays for free serum feline methylglyoxal. D-sorbitol was quantified indirectly using a modified R-bioPharm® (Boehringer Mannheim) kit to quantify formazan. The CUPRAC (cupric ion reducing antioxidant capacity) assay utilized a copper-neocuprine reagent reacting with an antioxidant to form a chelated copper florescent product. This assay of antioxidant capacity was measured in trolox units (soluble vitamin E). A standard bicinechonic acid copper reduction assay was used to determine protein concentration.

Serum from 21 DM and 26 non-DM cats were compared using these assays. Sample BCA total protein was not significantly different between groups. The CUPRAC mean antioxidant capacity for the DM and non-DM cats, 1.20 mM (±0.15) and 1.17 mM (±0.18) respectively, was not significantly different between groups. Mean serum sorbitol concentration tended to be higher in DM cats, but this was not a statistically significant difference between DM cats (6.39 ± 7.42 mg/dL) and non-DM cats (1.66 ± 1.09 mg/dL). This appeared even smaller using the DNPH MEG methodology.

Although no statistical differences were seen between DM and non-DM groups for antioxidant capacity or AGE and AGE precursors, the DM cats in this study were deemed clinically well controlled and stable, with a mean serum fructosamine of 478 μmol/L (±127; range 200-360 μmol/L). Future work will compare well controlled to poorly controlled DM cats, and newly diagnosed diabetic cats over time, to determine if insulin therapy decreases AGE production in this population of Type 2 diabetic cats.

EN-35
IATROGENIC HYPOADRENOCORTICISM FOLLOWING TRILOSTANE THERAPY FOR PITUITARY-DEPENDENT HYPERADRENOCORTICISM IN DOGS. A. Schrage, E. Applemain, C. Langston. The Animal Medical Center, New York, NY.

This retrospective case series identified 13 dogs that developed iatrogenic hypoadrenocorticism (iHAC) following administration of trilostane for treatment of pituitary-dependent hyperadrenocorticism (PDH). Inclusion criteria required a previous diagnosis of PDH, monotherapy with trilostane (i.e. no other medications used for treatment of PDH), and a post-ACTH stimulated cortisol concentration of <1 ug/dL while receiving trilostane. Clinical signs of PDH resolved in 92% (12/13) of dogs prior to development of iHAC. At the time of diagnosis, 7/13 (53%) dogs had clinical signs consistent with iHAC. Lethargy and inappetence were the most common signs. Median age of dogs was 12 years with a median weight of 10 kilograms. No single breed was overrepresented. Dogs were treated with trilostane for a median of 8.5 months at a median dosage of 4.75 mg/kg/day prior to development of iHAC. Mineralocorticoid deficiency (hypokalemia +/- hyponatremia) was identified in 3/13 (23%) dogs. Trilostane was discontinued in all 7 dogs displaying clinical signs and later restarted at a lower dose in 2 dogs. Permanent hypoadrenocorticism developed in 4 dogs. No dog died or was euthanized as a result of iHAC.

This report illustrates that, while trilostane is an effective treatment for PDH, transient or permanent iatrogenic hypoadrenocorticism may occur. Development of mineralocorticoid deficiency is less common in comparison to glucocorticoid deficiency. These dogs were being closely evaluated and had received manufacturer-recommended doses of trilostane prior to development of iHAC. Close monitoring of dogs on trilostane therapy is warranted, with special emphasis on clinical signs, electrolyte levels, and cortisol concentrations.
A commercial cat food that is indicated for the management of hyperthyroidism is effective only if fed as a sole source of nutrition to cats with the disease. Approximately 55% of cat owners in North America have multiple cats. Feeding each cat separately so that they do not share foods is difficult; therefore, this study was designed to show that the food is safe for prolonged feeding (~1 yr) for healthy adult cats.

Two groups of healthy adult cats (n = 15) were fed one of two foods – test, the commercially available food limited in iodine to manage hyperthyroidism, or control, a formulation that was identical to the test food, except that it contained 2 ppm iodine. Cats were group-housed to allow for ample exercise and social interaction in spacious rooms with natural light. All procedures were approved by an ACUC committee. At baseline thyroid ultrasonography (US), serum thyroid stimulating hormone (TSH), total thyroxine (TT4), free thyroxine (fT4), total tri-iodothyronine (TT3) and free tri-iodothyronine (fT3), as well as complete blood count (CBC), serum biochemistry profile, urinalysis and urine iodine measurements were compared. Thyroid US was repeated at months (m) 6, 12, and 18. All other measurements were repeated monthly for 4 m, then at 6 m and every 6 m thereafter.

After the 1st m, three cats had been dismissed from the study. Two due to low food intake and one due to sudden death prior to the 6-m time point because of an underlying hypertrophic cardiomyopathy. After 18 m on the foods there were no biologically significant differences in any measures between treatment groups at any time point. Body weight did not change over the course of the study. There was no difference in food intake between the two treatment groups or from baseline within the groups. All thyroid hormones stayed within the normal reference range for cats. The concentrations of TT3 were significantly higher at 6 and 12 m, and not different at 18 m compared to baseline. Total T3 was significantly lower after 18 m on the food in both treatment groups. Free T4 was significantly higher at 6 m compared to baseline. At 18 m fT4 was not different and significantly lower than the baseline in the control and test group, respectively. Thyroid stimulating hormone was significantly lower in both treatment groups at 12 m compared to baseline.

No changes in the size of the thyroid glands were observed. As expected, urinary iodine concentration decreased significantly with the test food.

After 18 m on the food there is no indication that limiting iodine in healthy cats has any detrimental effect on their health.

## EN-37

**USE OF THE IMPLANTABLE PUMP ITHETIS™ TO DELIVER SUBCUTANEOUS INSULIN IN HEALTHY CATS. E. Zini¹, I. Pgdru1, K. Macha², N. Stergiopulos², O. Vavasseur³, T.A. Lutz4, C.E. Reusch5. ¹Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland, ²Antila SA, Lausanne, Switzerland, ³Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zurich, Switzerland.**

The cornerstone of treatment in cats with diabetes mellitus is insulin which is typically provided once or twice daily, in many cases for the rest of the cat's life. Implantable insulin delivery pumps have been developed and successfully used in diabetic humans. In cats, the use of insulin pumps has not been described so far. The purpose of this study was to test the applicability, safety and reliability of the pump ITHETIS™ (Antila SA, Lausanne) to deliver insulin in healthy cats.

ITHETIS™ is a small drug delivery device (42 x 16 x 7 mm) developed for veterinary applications that has a refillable reservoir and that is telemetrically controlled. The pump was surgically implanted in the subcutaneous tissue of the dorsal neck in 10 healthy cats, under general anaesthesia. The reservoir was filled with insulin glargine (Lantus) and was programmed to deliver 4 insulin boluses of 0.3 U/kg each, 2 to 3 weeks apart. For comparison, insulin glargine (0.3 U/kg) was injected subcutaneously one week before or after each pump bolus. Glucose levels were measured with a portable glucose meter using capillary blood when the bolus was delivered, and every 2 hours thereafter, for 8 hours. Plasma insulin glargine concentrations were measured with a validated ELISA. Physical examinations were performed twice daily until removal of the device.

The pumps were implanted without procedural difficulties and were well tolerated without obvious signs of discomfort in all cats. The experiment was completed with all 4 boluses in 5 of the 10 cats (i.e., 5 first boluses and 15 successive boluses). In 4 cats the pumps failed after 0-2 insulin boluses and were removed (i.e., 3 first boluses and 1 successive bolus). In one cat severe hypoglycaemia developed before administration of the first bolus and the pump was removed; leakage from the puncture site of the reservoir was documented. Overall, in 7 of the 8 (87.5%) first pump boluses and in 3 of the 16 (18.8%) successive boluses plasma insulin glargine increased and capillary glucose decreased. With the 14 remaining boluses (i.e., 1 first bolus and 13 successive boluses) insulin and glucose did not change. Subcutaneous insulin injections were followed by an increase of plasma insulin glargine and a decrease of capillary glucose 18 of 20 (90.0%) times; with 2 injections insulin glargine and glucose did not change. The median% difference between insulin peak and nadir after insulin bolus was 39% (range: 10-447) and 52% (range: 7-511) after subcutaneous injections.

For the first time an implantable pump was used in cats. The pump did not affect wellbeing but life threatening hypoglycaemia occurred in one cat due to pump leakage. Most intact pumps were well tolerated without obvious signs of discomfort in all cats. The experiment was completed with all 4 boluses in 5 of the 10 cats (i.e., 5 first boluses and 15 successive boluses). In 4 cats the pumps failed after 0-2 insulin boluses and were removed (i.e., 3 first boluses and 1 successive bolus). In one cat severe hypoglycaemia developed before administration of the first bolus and the pump was removed; leakage from the puncture site of the reservoir was documented. Overall, in 7 of the 8 (87.5%) first pump boluses and in 3 of the 16 (18.8%) successive boluses plasma insulin glargine increased and capillary glucose decreased. With the 14 remaining boluses (i.e., 1 first bolus and 13 successive boluses) insulin and glucose did not change. Subcutaneous insulin injections were followed by an increase of plasma insulin glargine and a decrease of capillary glucose 18 of 20 (90.0%) times; with 2 injections insulin glargine and glucose did not change. The median% difference between insulin peak and nadir after insulin bolus was 39% (range: 10-447) and 52% (range: 7-511) after subcutaneous injections.

## GI-I

**DETERMINATION OF THE SENSITIVITY AND SPECIFICITY OF A RAPID UREASE TEST FOR THE DETECTION OF GASTRIC HELICOBACTER SPECIES IN DOGS WITH CLINICAL SIGNS OF UPPER GASTROINTESTINAL DISEASE. B.A. Hoffmann, D.P. Spornenberg, M.S. Leib, D.C. Grant. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg VA.**

Gastric Helicobacter spp. are an etiology of chronic gastritis in dogs. However, the sensitivity and specificity of many diagnostic tests for these organisms have not been established in a large group of dogs with clinical signs of upper gastrointestinal disease. Rapid urease tests, such as the CLOtest®, offer a rapid and inexpensive means of detecting Helicobacter spp. in humans. The purpose of this study was to establish the sensitivity and specificity of the CLOtest for detection of Helicobacter spp. in the gastric biopsy samples of dogs evaluated for upper gastrointestinal disease.

Two-hundred and eighty-four dogs that underwent upper gastrointestinal endoscopy and had a full set of gastric biopsies collected (pylorus, angularis incisura, cardia, and fundus) and a CLOtest performed were identified from retrospective review of endoscopy records. The CLOtest was performed on biopsy samples obtained from the angularis incisura. The previously prepared H&E stained histopathology slides from each biopsy section from each dog were retrieved and prospectively classified according to the presence or absence of Helicobacter species in the gastric biopsy sections. The CLOtest was performed on biopsy samples obtained from the angularis incisura. The previously prepared H&E stained histopathology slides from each biopsy section from each dog were retrieved and prospectively classified according to the presence or absence of Helicobacter species in the gastric biopsy sections. The CLOtest was performed on biopsy samples obtained from the angularis incisura. The previously prepared H&E stained histopathology slides from each biopsy section from each dog were retrieved and prospectively classified according to the presence or absence of Helicobacter species in the gastric biopsy sections.
GI-2

SAFETY OF ADMINISTRATION OF 25% HUMAN ALBUMIN TO DOGS DIAGNOSED WITH A PROTEIN-LOSING ENTEROPATHY. K.A. Loyd1, C.G. Cocayne1, J.M. Cridland2, R.M. Mack, L.T. Moon, M.C. Fritz, C.A. Bolin, S.A. W.R. Hause1. 1Associated Veterinary Specialists, Bridgeton, MO., 2University of California-Davis, Davis, CA.

The purpose of this retrospective study was to determine the safety of 25% human serum albumin (HSA) in dogs diagnosed with idiopathic inflammatory bowel disease (IBD) that have a protein-losing enteropathy (PLE) with a serum albumin < 2.0 g/dL. It was hypothesized that the administration of acute transfusions of 25% HSA in dogs with moderate to severe hypoalbuminemia from PLE would not be statistically different to the reported rate of acute reactions to red blood cell transfusion in dogs (3.3%). In addition, the development of delayed reactions was investigated.

Medical records were reviewed for canine patients that met the inclusion criteria and received a 25% HSA transfusion. Patients were included in the study if they had biochemistry results consistent with PLE. IBD confirmed on histopathology, a serum albumin <2.0 g/dL and complete medical records.

Twenty-one dogs met the criteria for inclusion in the study for a total of 54 transfusions administered. Each patient received between 1 and 10 transfusions. Three (5.7%) patients developed signs consistent with an acute reaction, which was not statistically different from the reported rate of acute reactions to red blood cell transfusions. Two (3.7%) patients developed signs consistent with a delayed reaction.

In conclusion, 25% human albumin transfusions appear to be safe in dogs with IBD and PLE with moderate to severe hypoalbuminemia since acute and delayed reactions are uncommon.

GI-3

ROLE OF OROPHARYNGEAL BACTERIA IN ESOPHAGEAL FEEDING TUBE PERISTOMAL INFECTIONS IN CATS; R.M. Mack, L.T. Moon, M.C. Fritz, C.A. Bolin, S.A. Carey. Michigan State University College of Veterinary Medicine, East Lansing, MI.

Esophageal feeding tubes (e-tubes) offer a minimally-invasive means of providing enteral nutrition for critically ill cats. Peristomal inflammation and infection of the esophagostomy site is a frequent and potentially serious complication that can result in loss of the feeding tube as well as patient morbidity. Translocation of oropharyngeal bacteria during tube placement may be implicated in this process. We hypothesized that pre-surgical topical decolonization of the oropharynx will reduce the severity and frequency of e-tube peristomal infections in cats. The aims of this study were to determine the normal flora of the feline oropharynx, to develop a safe and effective method of oropharyngeal decolonization in cats, and to determine whether pre-surgical oropharyngeal decolonization was associated with a decrease in indicators of e-tube peristomal inflammation. Thirty healthy cats that were hospitalized for elective anesthetic procedures were used to determine the normal oropharyngeal flora and to determine the efficacy of topical chlorhexidine as a method of pre-surgical decolonization. Bacterial swabs were collected from the buccal mucosa prior to (pre) and five minutes after (post) application of 0.12% chlorhexidine to all mucosal surfaces of the oral cavity and oropharynx. Swabs were submitted for aerobic bacterial culture, and isolates were identified and assessed on a semi-quantitative growth scale. Eight cats receiving e-tubes were randomized to receive either topical chlorhexidine or sham decolonization (saline) prior to e-tube placement. Peristomal inflammation was assessed using a modified Jain scoring system, which includes semi-quantitative assessments of peristomal erythema, induration, and exudation. Bacterial swabs were collected from e-tube peristomal skin either 2 days following tube placement or at the onset of tube infection.

Polymicrobial cultures were isolated from all (30/30) pre-decolonization samples. The most common isolates in the pre-decolonization samples were Pasteurella multocida (18/30), Actinomyces spp. (18/30), Neisseria spp. (9/30), Bergeyella spp. (8/30), non-hemolytic Streptococcus spp. (6/30), and Corynebacterium spp. (5/30). Chlorhexidine decolonization was effective in significantly reducing the median growth of several oropharyngeal bacterial species, including Neisseria spp., Bergeyella spp., and Actinomyces spp. However, growth reduction of P. multocida, the most frequently isolated bacterial species, was not statistically significant. The most common bacteria isolated from e-tube peristomal skin were Enterococcus spp (6/8), Pasteurella multocida (3/8), Bacillus spp. (2/8), and Escherichia coli (2/8). Pre-surgical decolonization did not significantly decrease Jain peristomal inflammation scores. No adverse reactions were observed following topical chlorhexidine application. These preliminary data suggest that topical oropharyngeal chlorhexidine treatment is a safe and effective procedure prior to the placement of e-tubes, and that oropharyngeal, enteric, and environmental bacteria should all be considered as potential contamination sources of esophageal feeding tube sites.

GI-4

NO CORRELATION BETWEEN MUCOSAL IMMUNOGLOBULIN A POSITIVE PLASMA CELL NUMBERS AND TLR5 GENOTYPES IN GERMAN SHEPHERD DOGS. A. Lee1, S. Priestnail1, K. Smith2, D. Werling2, K. Allenbach1. 1Department of Clinical Sciences and Services, and 2Department of Pathology and Pathogen Biology, Royal Veterinary College, University of London, UK.

It has previously been suggested that a deficiency in mucosal Immunoglobulin A (IgA) production could be involved in the pathogenesis of Inflammatory Bowel Disease (IBD) in German Shepherd Dogs (GSD). Recent research has shown that mutations in Toll-like receptor 5 (TLR5) are associated with an increased risk of development of IBD in this breed. IgA is essential for mucosal immunity and studies in mice have linked the interaction of TLR5 with its ligand flagellin to class-switching of B-cells into IgA-producing plasma cells. The hypothesis of this study was that dogs carrying the risk-associated (RA) genotypes for G22A and C100T genes of TLR5 will have a decreased number of mucosal IgA+ plasma cells in the duodenum and colon compared to dogs carrying the risk-protective (RP) genotypes.

Thirty-one GSD were diagnosed with IBD at the Royal Veterinary College (n=31 sections from the duodenum and n=17 sections from the colon). Immunohistochemistry was performed using a goat anti-dog primary antibody at 1:400 dilution (ABD Serotec, AA131), a secondary antibody at 1:200 dilution and peroxidase as supplied by Vectastain Elite ABC kit. Slides were viewed at x40 magnification using an Olympus BX60 microscope. Two sections of duodenum, and colon if available, were examined from each animal. Twelve images were captured of each section. IgA+ cells manually counted and expressed per 10’000um². TLR5 genotypes for the G22A and C100T genes were determined by PCR on residual EDTA blood samples using previously published primer sequences.

Number of IgA+ cells in the duodenum and colon were higher than those previously published for GSD with or without IBD (mean duodenum crypt: 52.6 SD 16.2; mean duodenum villous tip 51.12 SD 3.83; mean duodenum villous base 55.02 SD 3.3; mean colon crypt 67.4 SD 4.3; Kruskal Wallis p < 0.02). There was an increased number of IgA+ cells in the colon compared to the duodenum (post hoc t test, colon crypt vs duodenal villous tip). There was no correlation between numbers of IgA+ cells in the duodenum or colon and dogs carrying the RA versus the RP alleles of TLR5 gens (t tests comparing duodenum: G22A: RA n = 5 vs RP n = 26; C100T: RA n = 23 vs RP n = 8; colon: G22A: RA n = 4 vs RP n = 13; C100T: RA n = 12 vs RP n = 5).
GI-5

Chronic enteropathies in dogs are often empirically treated with dietary trials, antimicrobials or immunosuppressive agents. However, there are few reports in the literature reporting short- and long-term outcome in dogs with these disorders. The goal of this study was to compare clinical activity index (CCECAI), Allenspach et al (2007), age at diagnosis, serum albumin concentrations, as well as outcome at various time points after diagnosis between a group of dogs treated with dietary management alone (food-responsive diarrhea = FRD), diet and antimicrobials (antibiotic-responsive diarrhea =ARD) or diet and immunosuppressive agents (steroid-responsive diarrhea = SRD; prednisolone, cyclosporine and/or azathioprine).

The electronic medical records at the Royal Veterinary College, London, were searched for dogs diagnosed with chronic enteropathies. 203 dogs were included in the study (2005-2012). FRD was defined as dogs that responded to elimination diet alone within 2 weeks after initiating therapy, whereas ARD dogs were had an unsuccessful dietary trial before and responded to metronidazole within 2 weeks after initiation of therapy, and SRD dogs had an unsuccessful dietary and antimicrobial trial before, and required immunosuppressive therapy to control their clinical signs. Outcome data was extracted from the medical record database, when available, at 2-4 weeks, 4-8 weeks, 8-12 weeks and at 12 weeks-6 months after diagnosis. Outcome data at 6-12 months was collected by telephone interviews and was defined as improved or not improved as compared to at time of diagnosis.

The FRD group consisted of 131 dogs (64%), whereas the ARD and SRD groups consisted of 33 (16.2%) and 39 dogs (19.2%), respectively. Median age at diagnosis was significantly different between FRD and SRD dogs, and between FRD and SRD groups (FRD median 3 yrs, range 0-12, ARD median 2 years, range 0-11, SRD median 6 years, range 1-13, p < 0.001) Serum albumin concentration was significantly different between FRD and SRD groups (mean FRD 3.2 g/dL SD 5.8; SRD 2.6 g/dL SD 0.4, p = <0.001). CCECAI at diagnosis was significantly different between FRD and ARD groups, and between FRD and SRD groups (median FRD 6, range 2-12; median ARD 8, range 0-14; median SRD 9, range 5-14, p < <0.001). Outcome was significantly better for FRD vs ARD and for FRD vs SRD at 2-4 weeks after discharge from the hospital (Chi2 p < 0.001, and p = 0.002). At 4-8 weeks after discharge, outcome was significantly better in FRD vs ARD (Chi2 p < 0.001), and at 6 months-1 year, outcome was also significantly better for FRD vs ARD and for FRD vs SRD (Chi2 p < 0.001, and p = 0.002).

GI-6
LIPASE, CPLI, AND CTLI IN THE DOGUE DE BORDEAUX. IS THERE A NEED FOR BREED-SPECIFIC REFERENCE INTERVALS? R. Lavoue1, C. Rocher1, C. Trumel1, D. Coutand1, C. Suchodolski1, J.M. Steiner1, D. Dossin1. 1Department of Clinical Sciences and Clinical Research Unit, 2Department of Biological Sciences, National Veterinary School - INP, University of Toulouse, France, 3Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA.

It has recently been reported that the upper limit of the reference interval (RI) for plasma lipase activity in the Dogue de Bordeaux (DDB) is 3 times higher than the manufacturer’s RI (Lavoue et al, 2013). The goal of this study was to document canine pancreatic lipase immunoreactivity (cPLI) and trypsin-like immunoreactivity (cTLI) concentrations in the same group of dogs.

Ninety four clinically healthy adult DDB dogs aged 1 to 10 years were included. Blood was sampled after withholding food for 12 hours. Lipase activity and cPLI and cTLI concentrations were measured in plasma and serum, respectively. Correlations between lipase and cPLI or cTLI were assessed with the Spearman’s rank correlation coefficient. RI were determined using a non parametric method (Reference Value Advisor, V 2.1).

Median (minimum to maximum) lipase activity, cPLI and cTLI concentrations were 2.709 U/L (535-15,348), 83.5 μg/L (29-649) and 15.5 μg/L (8.3-127.5), respectively. There was no significant correlation between plasma lipase activity and neither serum cPLI nor serum cTLI concentrations. Specific RIs for cPLI and cTLI concentrations in DDB were 29-571 μg/L and 9-113 μg/L, respectively.

This study is the first to report breed-specific RIs for canine pancreatic markers and our findings suggest that the high values of lipase activity observed in healthy DDB dogs are due to a non-pancreatic enzyme.

GI-7
SERUM AND URINE METABOLITES IN DOGS WITH ACUTE DIARRHEA. B.C. Guard1, J.W. Barr2, L. Reddivari3, J.M. Steiner4, J. Vannaman4, T.S. Suchodolski3, 1Gastrointestinal Laboratory and Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX, 2Department of Plant Science and, 3Food Science, Penn State University, University Park, PA.

Recent molecular studies have greatly increased our knowledge concerning the microbiota harbored in the gastrointestinal (GI) tract of dogs and the dysbiosis that occurs in patients with GI disease. However, little information is available from these studies pertaining to the functional (metabolic) potential of the microbiome. Metabolomics is a branch of research that focuses on the identification and quantification of small molecule metabolites in the metabolome. Profiling metabolites of the host and the microbiota may help to understand disease processes and test the hypothesis that different metabolic profiles are reflected in biofluids. Therefore, the aim of this study was to compare concentrations of serum and urine metabolites between healthy dogs and dogs with acute diarrhea using an untargeted metabolomics approach.

Serum samples were collected by venipuncture from healthy dogs (n = 10) and dogs with acute diarrhea (n = 13).

Urine samples were collected by cystocentesis from healthy dogs (n = 10) and dogs with acute diarrhea (n = 7). Serum and urine samples were subjected to ultra-performance liquid chromatography (Waters ACQUITY UPLC® System) followed by mass spectrometry (Waters Xevo® G2-S QTof). Feature detection and peak alignment were performed using the XCMS package (Scripps Center for Metabolomics, La Jolla, CA). Analysis of differentially abundant metabolites and implicated pathways (an impact value ≥ 0.10 indicates a potential target pathway of interest) was performed using the web-based metabolomic data processing tool MetaboAnalyst 2.0 (http://www.metaboanalyst.ca). Statistical significance was set at p < 0.05.

Potential pathways of interest in the urine of both groups were, D-glutamine and D-glutamate metabolism; phenylalanine, tyrosine, and tryptophan biosynthesis; nicotinate and nicotinamide metabolism; vitamin B6 metabolism; phenylalanine metabolism; thiamine metabolism; alanine, aspartate, and glutamate metabolism; tryptophan metabolism; and tyrosine metabolism (impact value > 0.3 for all). In urine, univariate analysis showed that acetylcarnitine was significantly decreased in dogs with acute diarrhea compared to healthy dogs (p < 0.0001). Phenylalanine, tyrosine, and tryptophan biosynthesis were also potential pathways of interest (impact value = 1.0 for all). In these pathways, L-tyrosine was significantly decreased in dogs with acute diarrhea compared to healthy dogs (p < 0.05). In serum, univariate analy-
sis showed that kynurenic acid was significantly decreased in dogs with acute diarrhea compared to healthy dogs (p < 0.01).

In conclusion, this study revealed altered metabolic activity between healthy dogs and dogs with acute diarrhea as well as prominent pathways of interest in the serum and urine. Further studies are warranted to discover diagnostic and therapeutic biomarkers for GI disease based on metabolic profiles.

GI-8
THE Fecal microbiome IN DOGS WITH ACUTE DIARRHEA. B.C. Guard, J.W. Barr, J.M. Steiner, J.S. Suchodolski. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Recent molecular studies have revealed that the canine gastrointestinal tract (GIT) harbors a highly complex microbial ecosystem. Gut microbes play a very important role in the development and regulation of the host immune system, which is believed to be mediated in part through the production of immunomodulatory metabolites, such as short-chain fatty acids (e.g., butyrate, propionate, or indole). Limited information is available concerning potential changes in predominant bacterial groups in dogs with acute diarrhea and the functional gene content of the microbiome. Therefore, the aim of this study was to characterize the fecal microbiome in healthy dogs, dogs with acute non-hemorrhagic diarrhea (NHD), and dogs with acute hemorrhagic diarrhea (AHD) using 16S rRNA gene sequencing and to describe the functional gene content of their fecal microbiome.

Fecal samples were collected from healthy dogs (n = 8), dogs with NHD (n = 5), and dogs with AHD (n = 6). DNA was extracted using the ZR Fecal DNA Kit™ (Zymo Research Corporation, Irvine, CA). The fecal microbiota were analyzed by 454-pyrosequencing of the 16S rRNA gene. Functional genes were predicted from the 16S rRNA gene data using the bioinformatics software package PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). Differences in sequence percentages and functional content between the groups were evaluated using a Mann-Whitney test or Kruskal-Wallis test. To determine differences in the composition of the microbiota between groups, the analysis of similarities (ANOSIM) function in the statistical software package PRIMER 6 (PRIMER-E Ltd., Plymouth, UK) was used based on the unweighted UniFrac distance matrices. Statistical significance was set at p < 0.05.

The Shannon Index for bacterial diversity was significantly decreased in dogs with acute diarrhea (AD: both groups combined) compared to healthy controls (p < 0.01). Abundance of sequences belonging to Bacteroidetes, Ruminococcaceae, Faecalibacterium, and Blautia was significantly decreased in dogs with AD compared to healthy controls (p < 0.05 for all). Microbial communities of dogs with both NHD or AHD differed significantly from healthy controls (ANOSIM; p < 0.01 for both). Also, the functional gene content differed significantly between dogs with AD and healthy controls and the following pathways were implicated: xenobiotics biodegradation and metabolism; restriction enzymes, sulfur relay, and glycosaminoglycan degradation; biosynthesis of siderophore group nonribosomal peptides; drug metabolism; and transporters (p < 0.05 for all).

In conclusion, this study revealed a bacterial dysbiosis and altered functional gene content between dogs with AD and healthy controls. Further studies are warranted to evaluate the resulting functional effects and implicated metabolites in affected dogs.

GI-9
FLOW CYTOMETRY AS A DIAGNOSTIC TOOL IN GASTROINTESTINAL ENDOSCOPIC BIOPSIES IN DOGS WITH CHRONIC ENTEROPATHY. E. Haas1, B.C. Ruetgen2, W. Blau3, B. Richter4, C. Tilley3, A. Bilek1, J.G. Thallhammer1, A. Saalmueller1, N. Luckschander1. 1Internal Medicine, Small Animal Clinic, Department for Companion Animals and Horses, University of Veterinary Medicine, Vienna, Austria, 2Clinical Pathology Platform, Department for Pathobiology, University of Veterinary Medicine, Vienna, Austria, 3Institute of Immunology, Department for Pathobiology, University of Veterinary Medicine, Vienna, Austria, 4Institute of Pathology and Forensic Veterinary Medicine, Department for Pathobiology, University of Veterinary Medicine, Vienna, Austria, 5Bioinformatics and Biostatistics Platform, Department of Biomedical Sciences, University of Veterinary Medicine, Vienna.

Canine chronic enteropathies (CE) are a heterogeneous group of gastrointestinal dysfunctions caused by inflammation of the intestinal tract. Intestinal intraepithelial lymphocytes (IEL) are known to play an important role in gut homeostasis and development of CE.

For a better phenotypical characterization of canine IEL subsets, 3-color Flow Cytometry (FCM) was performed. IEL were isolated from healthy Beagles (HB; n = 6), healthy non Beagles (HD; n = 10) and CE dogs of different breeds (n = 10). Additionally, samples from full thickness (FT) and endoscopic biopsies (EB) taken from the same HD (n = 5) were compared. All dogs were clinically evaluated using the CIBDAI-system. Duodenal endoscopic biopsies were histologically scored according to WSAVA guidelines. After IEL-isolation 3-color FCM using anti-canine T-cells specific and anti-human cross-reactive antibodies against CD45, CD3-12, CD4, CD8α, CD8β, TCR-αβ and TCR-γδ was performed. Differences in sequence percentages and functional content between the groups were evaluated using a Mann-Whitney test or Kruskal-Wallis test. To determine differences in the composition of the microbiota between groups, the analysis of similarities (ANOSIM) function in the statistical software package PRIMER 6 (PRIMER-E Ltd., Plymouth, UK) was used based on the unweighted UniFrac distance matrices. Statistical significance was set at p < 0.05.

The Shannon Index for bacterial diversity was significantly decreased in dogs with acute diarrhea (AD: both groups combined) compared to healthy controls (p < 0.01). Abundance of sequences belonging to Bacteroidetes, Ruminococcaceae, Faecalibacterium, and Blautia was significantly decreased in dogs with AD compared to healthy controls (p < 0.05 for all). Microbial communities of dogs with both NHD or AHD differed significantly from healthy controls (ANOSIM; p < 0.01 for both). Also, the functional gene content differed significantly between dogs with AD and healthy controls and the following pathways were implicated: xenobiotics biodegradation and metabolism; restriction enzymes, sulfur relay, and glycosaminoglycan degradation; biosynthesis of siderophore group nonribosomal peptides; drug metabolism; and transporters (p < 0.05 for all).

In conclusion, this study revealed a bacterial dysbiosis and altered functional gene content between dogs with AD and healthy controls. Further studies are warranted to evaluate the resulting functional effects and implicated metabolites in affected dogs.

GI-10
PREVALENCE OF ACUTE PANCREATITIS IN CANINE BABESIOSIS CAUSED BY BABESIA ROSSI - A MODEL FOR THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS). L.S. Köster1, J.M. Steiner2, J.S. Suchodolski, J.P. Schoeman3. 1Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa, 2Gastrointestinal Laboratory, Texas A&M University, College Station, Texas, USA.

Pancreatitis has been suspected to be a complication in canine patients with babesiosis, but only a few case reports have been published. In a previous retrospective study of canine babesiosis, 24% of dogs were thought to have concurrent acute pancreatitis based on elevated serum amylase and lipase activities. Previous reports of canine complicated babesiosis found that 8/16 fulfilled the requirements for systemic inflammatory response syndrome (SIRS).

A total of 87 dogs diagnosed with an acute Babesia rossi infection were recruited for this investigation. There were 54 male and 33 female dogs enrolled. The data was examined for normality using the One-Sample Kolmogorov-Smirnov test. The Mann-Whitney U-test was used to examine differences between groups (p ≤ 0.05) and the Spearman’s Rank order correlation (R_s) was used to determine correlation between parameters (p ≥ 0.05). The median body mass was 16.4 kg (range: 1.9 to 54.3 kg) and age was 14 months (range: 2 to 156 months). A total of six dogs
GI-11 IDENTIFICATION OF ASTROVIRUSES ASSOCIATED WITH CANINE GASTROENTERITIS IN THE UK. S. Caddy 1,2, I. Goodfellow 1. 1Department of Pathology, University of Cambridge, UK, 2Department of Medicine, Imperial College London, UK.

A number of novel viruses have been associated with canine gastroenteritis in recent years, including canine astroviruses (CAstV) and canine norovirus (CNV). Viral gastroenteritis in dogs worldwide is acknowledged as being predominantly caused by canine parvovirus (CPV) and canine enteric coronavirus (CECoV), with the contribution of recently identified viruses associated with gastroenteritis uncertain. Norovirus and Astrovirus strains that infect man cause significant levels of disease in humans and yet canine strains of these viruses have not previously been recognized in any dogs in the UK. This study sought to investigate the prevalence and potential significance of both CAstV and CNV in the UK dog population.

Forty-eight dogs with severe gastroenteritis requiring treatment as in-patients were enrolled in the study. An additional 180 dogs were recruited as healthy controls. With owner consent, stool samples were collected and nucleic acid was extracted. Quantitative PCR targeting a range of canine viruses associated with gastroenteritis was used to screen all the stool samples.

CAstV was detected in two dogs suffering from gastroenteritis, whereas CAstV was not identified in any healthy control dogs. No CNV cases were identified in any of the dogs screened, though CPV and CECoV positive samples were identified in all dogs as anticipated. The first CAstV positive case was that of a 7 week old puppy co-infected with CPV, whereas the second was a 7 year old dog with hemorrhagic gastroenteritis, and with no other viral aetiology identified. Sequencing of the viral polymerase of the two CAstV isolates showed significant similarity with strains from Italy (Martella et al, 2011), and ongoing work is focused on the sequencing of the full viral genome to gain a better insight into the genetic heterogeneity of astroviruses.

This report suggests that CAstV may be an important and hitherto unrecognized cause of canine gastroenteritis in the UK. It would be prudent to consider testing for CAstV in future suspect cases of viral gastroenteritis.

GI-12 QUANTITATIVE CONTRAST-ENHANCED ULTRASONOGRAPHIC ASSESSMENT OF NATURALLY OCCURRING PANCREATITIS IN DOGS. S.Y. Lim 1, K. Nakamura 2, K. Murishita 3, S. Suzuki 2, H. Ohba 2, M. Yamasaki 1, M. Takiguchi 1. 1Laboratory of Veterinary Internal Medicine, Graduate School of Veterinary Medicine, Hokkaido University, Hokkaido, Japan., 2Hokkaido University Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Hokkaido, Japan.

Accurate noninvasive diagnosis of pancreatitis in dogs can be challenging. Contrast-enhanced ultrasonography (CEUS) is a major breakthrough for ultrasound imaging and is able to detect perfusion changes in experimentally induced pancreatitis in dogs. However, its usefulness in detecting naturally occurring pancreatitis is still unclear. This study aimed to investigate the applicability of CEUS to detect naturally occurring pancreatitis in dogs based on changes in pancreatic perfusion.

31 client-owned dogs diagnosed with pancreatitis were prospectively enrolled. Nine healthy laboratory-owned dogs were used as controls. CEUS was performed on the pancreas using continuous infusion of contrast agent. Time-intensity curve was then obtained from regions of interest placed within the pancreas. Five perfusion parameters were obtained for statistical analyses: Time to upslope (TTU), peak time (Tp), time to wash-out (TTW), peak intensity (PI) and area under the curve (AUC).

All perfusion parameters except for TTU differed significantly between dogs with pancreatitis and controls. Time-dependent parameters, Tp and TTW (60 ± 11 versus 36 ± 15 s; P < 0.001 and 277 ± 68 versus 228 ± 27 s; P = 0.0028, respectively) were longer in dogs with pancreatitis when compared with controls. Intensity-dependent parameters, PI and AUC (94.2 ± 14.4 versus 79.0 ± 14.3 MPV; P = 0.0082 and 151.1 ± 3.4 versus 10.9 ± 3.2 x 10^3 MPV s; P = 0.0025, respectively) were also increased when compared to controls.

Results suggested that CEUS is able to detect pancreatic perfusion changes in naturally occurring pancreatitis. CEUS may be useful as an additional, noninvasive method in the diagnosis of pancreatitis in dogs.

GI-13 ANALYTICAL VALIDATION OF TARGETED AMINO ACID ANALYSIS IN DOG SERUM BY GAS-CHROMATOGRAPHY/ MASS SPECTROMETRY. R. Lopes, N. Grützner, N. Berghoff, J.A. Lidbury, J.S. Suchodolski, J.M. Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Amino acids play a key role in many physiologic pathways. The intestinal mucosa can utilize many dietary amino acids directly for metabolic energy, protein synthesis, nitric oxide, and modulation of signaling processes. Altered amino acid metabolism based on changes of various amino acids (e.g., glucose, glutamic acid, methionine, lysine, tyrosine, and tryptophan) has been described in humans with various gastrointestinal diseases. Electron impact gas-chromatography (GC) coupled with mass spectrometry (MS) is a sensitive analytical technique that has shown good resolution and reproducibility for identification of small molecules such as amino acids. The aim of this study was to analytically validate a GC/MS method for the measurement of
glycine, glutamic acid, methionine, tyrosine, tryptophan, and lysine in canine serum and to assess the stability of each amino acid in serum samples.

Surplus canine serum samples were used for method validation. Sample extraction and derivatization followed the technique described by Dunn et al. 2011 (Nature Protocols, vol.6, no.7:1060-1083). Validation variables included precision, spiking recovery, linearity, and lower limit of detection. All samples were run in triplicates. To evaluate stability of amino acids in serum samples during storage, sera were obtained from 12 healthy dogs and serum amino acids concentrations were evaluated after 1 day, 7 days, and 60 days of storage at each of the following temperature conditions: 4°C, -20°C, and -80°C.

Intra- and inter-assays coefficients of variation (%CVs) were 13.4% and 16.6% for glycine, 9.3% and 12.4% for glutamic acid, 5.1% and 6.3% for methionine, 14.0% and 15.1% for tryptophan, 6.2% and 11.0% for tyrosine, and 7.4% and 12.4% for lysine, respectively. Observed to expected ratios for dilutional parallelism (n = 6) were 79.5% - 111.5% for glycine, 80.9% - 123% for glutamic acid, 77.8% - 111% for methionine, 85.2% - 98% for tryptophan, 79.4% - 115% for tyrosine, and 79% - 110% for lysine. Glycine displayed the lowest (2.6 μmol/L) and lysine the highest (69 μmol/L) limit of detection. Recoveries for all six amino acid spiked serum samples showed coefficients of variation <15% and spiking recoveries between 79.9 to 112.7%.

The stability study showed no significant differences for glycine (p = 0.0781), methionine (p = 0.0781), or tyrosine (p = 0.6013) concentrations when stored at 4°C for 7 days. In contrast, significant increases of glutamic acid and lysine (p < 0.01) concentrations were found at 4°C for 7 days, lysine (p < 0.05) at -80°C for 7 days, and glutamic acid at -80°C for 19 days (p < 0.01).

These assays evaluated here were shown to be precise, accurate, and reproducible. In this study, changes in amino acid concentrations found at different storage conditions showed that most amino acid concentrations are not stable during even short-term storage.

GI-14
EVALUATION OF SIX DIFFERENT AMINO ACID CONCENTRATIONS IN SERUM OF HEALTHY DOGS AND DOGS WITH HYPOCOBALAMINEMIA.

Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Recently, it has been shown that hypcobalaminemia in dogs is associated with methylmalonic acidemia. Methylmalonic acid (MMA) is a competitive inhibitor of pyruvate carboxylase. Also, MMA has shown to inhibit alpha-ketoglutarate dehydrogenase and succinate dehydrogenase enzyme activities in the citric acid cycle. Ketotic hyperglycinemia has been reported in humans with gastrointestinal (i.e., glycine, glutamic acid, tyrosine, methionine, lysine, and tryptophan) has been described in humans with gastrointestinal diseases (e.g., ulcerative colitis and colorectal cancer). Methionine, lysine, and tryptophan have been classified as indispensable.

The aim of this study was to investigate changes in amino acid concentrations between normocobalaminemic healthy dogs and dogs with hypcobalaminemia.

Fresh serum samples from healthy dogs (n = 10) were collected after physical examination and were submitted for chemical profile and cobalamin analysis. Healthy dogs included 5 female and 5 male dogs (median age [range]: 7.5 [4.2-10] years) and were normocobalaminemic (median [range] serum cobalamin concentrations: 479 [400-666] ng/L). Surplus serum samples from 15 hypcobalaminemic (undetectable serum cobalamin concentrations < 150 ng/L) dogs were prospectively selected. Hypocobalaminemic dogs included 7 females and 8 males (median age [range]: 8 [4-12] years). A previously validated gas-chromatography/mass spectrometry method was used to quantify amino acid concentrations (i.e., Dunn et al. 2011 (Nature Protocols, vol.6, no.7:1060-1083) in the serum of the dogs enrolled. Unpaired t-tests or Mann-Whitney U tests were performed to compare the six different amino acid concentrations between healthy dogs and dogs with hypcobalaminemia.

Compared to normocobalaminemic dogs, hypcobalaminemic dogs had significantly lower glycine (median [range]: 3 [3-10] vs.16 [11-21] μmol/L), tyrosine (mean ±[SD]: 2.5 ±[0.74] vs. 7.4 ±[1.5] μmol/L), and tryptophan (median [range]: 23 [13-68] vs. 116 [78-191] μmol/L) concentrations (p < 0.0001 for all). In contrast, hypcobalaminemic dogs had significantly higher concentrations of glutamic acid (median [range]: 69 [9-214] μmol/L vs. 4 [3-6] μmol/L) and lysine (74 [47-170] μmol/L vs. 25 [16-40] μmol/L; (p < 0.0001 for both)). No significant changes in methionine (mean ±[SD]: 6.1 ±[2.5] μmol/L vs. 6.6 ±[1.4] μmol/L) concentrations were observed between healthy and hypcobalaminemic dogs (p = 0.6293).

In conclusion, changes in amino acid concentrations were observed between healthy dogs and dogs with hypcobalaminemia. The exact cause of these changes requires further investigation.

GI-15
PANCREATIC ULTRASOUND IN 54 DOGS WITH ACUTE PANCREATITIS: DIFFERENT CLINICAL PRESENTATION WITH LEFT OR RIGHT LIMB INVOLVEMENT OF THE PANCREAS. R. Lobetti, E. Lindquist, J. Frank, Bryantwon Veterinary Hospital, Box 67092, Bryanston, South Africa, Sonopath, New Jersey, USA, Sound Eklin New Jersey Mobile Associates, New Jersey.

The diagnosis of acute pancreatitis can be difficult because of anatomic inaccessibility of the pancreas, vague clinical signs and physical examination findings, and inconsistent laboratory results. Common, yet non-specific, clinical signs include abdomi- nal pain, anorexia, vomiting, and diarrhea. Ultrasonography is the imaging modality of choice to evaluate the pancreas and to differentiate from other intra-abdominal pathology that may mimic acute pancreatitis.

The purpose of this study was to correlate clinical signs with the region of the pancreas affected as based on ultrasound findings with the hypothesis that left-sided pancreatitis would result in a greater percentage of anorexia and right-sided pancreatitis would result in a greater percentage of vomiting.

The records of 54 privately owned dogs that were diagnosed with acute pancreatitis based on history, clinical signs, laboratory testing, and abdominal ultrasonography were retrospectively evaluated. Based on the ultrasound examination, the dogs were divided into two groups: Group 1 consisted of 24 dogs diagnosed with pathology within the left limb of the pancreas and group 2 consisted of 30 dogs that were diagnosed with pathology within the right limb of the pancreas. The presence of abdominal pain, anorexia, vomiting, and diarrhea was correlated between the two groups.

There was no difference between age, breed and sex of dogs in each group, and in both groups, small breeds were over-repre- sented. In group 1, pain in 36%, anorexia in 48%, vomiting in 17%, and diarrhea in 20% of dogs was recorded. In group 2, pain in 37%, anorexia in 30%, vomiting in 73%, and diarrhea in 8% of dogs was recorded. A statistical difference between the two groups was present with vomiting and diarrhea.

These findings indicate that there is a clinical difference between right- and left-sided pancreatitis, with diarrhea statistical more significant in left-sided pancreatitis and vomiting statistical more significant in right-sided pancreatitis. These differences between the two groups can possibly be ascribed to duodenal and upper gastro-intestinal tract involvement when the right side of the pancreas is affected.
GI-16

COMPARISON OF GASTROSCOPY AND ULTRASOUND FINDINGS IN CATS AND DOGS WITH HISTOLOGICALLY CONFIRMED GASTRIC NEOPLASIA. A. Maroli, J. Sharber, A. Bachand, H. Twedt. Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO.

Ultrasound of the stomach is a common imaging procedure performed in veterinary patients with gastrointestinal clinical signs. However, this imaging modality may be limited by the presence of gas or food in the stomach hindering complete evaluation. Gastroscopy often follows ultrasound for further evaluation and for biopsy. The purpose of this study was to compare sonographic and gastroscopic findings in a group of dogs and cats with known gastrointestinal neoplasia.

A retrospective study including 21 dogs and cats with histologically proven gastric neoplasia was performed. Patients with sonography and gastroscopy were included. The sonographic and gastroscopic images were reviewed for presence of wall abnormalities, size, and location. Both data sets were evaluated descriptively and by utilizing Cohen's kappa and Fisher's exact tests for comparison.

Five cats and 16 dogs were included. Five cats and one dog had T cell/ B cell lymphoma. Ten dogs were diagnosed with adenocarcinoma/carcinoma and 5 with leiomyosarcoma/leiomyoma. Ultrasound identified 10 of 21 tumors (47.6%) and failed to find abnormalities in cases of lymphoma (5), adenocarcinoma (5), and leiomyosarcoma (1). Four sonographic studies that failed to identify a wall lesion included images of large gas and fluid distended stomaches. One gastroscopy exam diagnosed a normal appearing stomach but biopsy indicated lymphoma. Gastroscopy correctly identified tumors in 20 of 21 animals (95.2%). All sonographically identified tumors demonstrated the following changes: wall thickening/ mass, loss of wall layering, and altered echogenicity. Gastroscopic findings of neoplasia included: mass lesion/thickening (11), ulcerated mass/thickening (7), ulceration only (2). One stomach was identified as normal. Of the 20 animals with sonographically identified tumors, ultrasound identified 10 with a Cohen's kappa = 0.09, indicating only slight agreement between the two tests. There was sonographic and gastroscopic tumor localization agreement in 33% of cases with a Cohen's kappa of 0.22, indicating only slight agreement. There was not a statistically significant stomach location that was able to predict ultrasound's ability to find gastric tumors based on gastrointestinal location data (gastroscopy Fisher's exact p-value = 1.00 and ultrasound Fisher's exact p-value = 0.6156). However, 5 sonographically identified tumors were localized to the antrum/pylorus.

In conclusion, ultrasound identified stomach abnormalities in nearly half of the animals with confirmed gastrointestinal changes. There is only slight agreement between these two tests, and tumor location does not increase or decrease the predictive value of ultrasound to identify gastric neoplasia. Ultrasound can raise the clinical suspicion of gastric wall abnormalities and may provide useful information prior to gastroscopy but may be limited due to gas or fluid distention of the stomach.

GI-17

INTESTINAL DYSBIOSIS AND PRESENCE OF ENTEROTOXIGENIC CLOSTRIDIUM PERFRINGENS IN DOGS WITH DIARRHEA. Y. Minamoto, N. Dhanani, M.E. Markel, J.M. Steiner, J.S. Súchodziński. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Intestinal dysbiosis, which describes an alteration of the gastrointestinal (GI) microbiota, is thought to play a pivotal role in the pathogenesis of GI disease. While GI inflammation can be associated with an altered microbiota in the GI tract, it is also thought that the prolonged perturbation of the GI microbiota can aggravate inflammation, thus potentially exacerbating GI disease. Enteric pathogens have been implicated in the pathogenesis of diarrhea. However, in most cases, their role in dogs with diarrhea remains unknown. Also, there are no studies evaluating the relationship between diarrhea, dysbiosis, and/or the presence of a potential enteric pathogen. The aims of this study were to characterize the fecal microbiota in dogs with diarrhea and to evaluate the relationship between bacterial dysbiosis and the presence of C. perfringens and its virulent factor.

Fecal samples were collected from dogs with diarrhea (n = 80: acute diarrhea [ACT] n = 22, chronic diarrhea [CHR] n = 58) and healthy control dogs (n = 95). Fecal DNA was evaluated using a quantitative PCR (qPCR) for 7 bacterial groups that have previously been shown to be altered in dogs with GI disease. A qPCR was also used for the quantification of both non-toxigenic and toxigenic strains of C. perfringens, while its enterotoxin (CPE) was detected using a commercially available ELISA kit. The differences in bacterial abundance among dogs with diarrhea and healthy dogs were evaluated using a Mann Whitney test. The Benjamini & Hochberg's False Discovery Rate was used to correct for multiple comparisons. Significance was set at an adjusted p < 0.05.

The abundances of Fusobacteria, Ruminococcaceae, Blautia spp., and Faecalibacterium spp. were significantly lower in dogs with diarrhea (p < 0.001), while the abundances of Bifidobacterium spp., E. coli, and Lactobacillus spp. were significantly higher in dogs with diarrhea than in healthy dogs (p < 0.001). No significant differences in abundances were observed between dogs with ACT and CHR. C. perfringens was detected in all dogs. Of those, 50.0% (40/80) of dogs with diarrhea and 33.7% (32/95) of healthy dogs harbored toxigenic strains. The prevalence of toxigenic strains was significantly higher in dogs with ACT (16/22) than in dogs with CHR (24/58, p = 0.023). CPE was detected in only 16.4% (12/73) of dogs positive for the toxigenic strains. No significant difference in the prevalence of CPE was observed between dogs with ACT and dogs with CHR. There were no significant differences in abundances of the selected bacterial groups between dogs that did or did not harbor toxigenic strains or CPE.

In conclusion, significant alterations of the fecal microbiota were observed in dogs with diarrhea. Only a small proportion of dogs harboring toxigenic strains were also positive for CPE. The presence of an intestinal dysbiosis needs to be considered in dogs with diarrhea regardless of the presence of C. perfringens and/or its virulence factor.

GI-18

MAJOR HISTOCOMPATIBILITY CLASS II HAPLOTYPES ARE ASSOCIATED WITH INFLAMMATORY BOWEL DISEASE IN GERMAN SHEPHERD DOGS. F. Procoli,1 A.M. Boog,1 F. Šoutter1, A. Holder1, B. Catchpole2, D. Werling1, K. Allenspach1. 1Department of Clinical Sciences and Services, Royal Veterinary College, University of London, North Mymms, Hertfordshire, UK. 2Department of Pathology and Pathogen Biology, Royal Veterinary College, University of London, North Mymms, Hertfordshire, UK.

Canine inflammatory bowel disease (IBD) represents a group of chronic enteropathies characterized by persistent or recurrent gastrointestinal (GI) signs with histological evidence of inflammatory cell infiltration in the intestinal lamina propria. Although IBD can affect any breed, German shepherd dogs (GSDs) in the UK are at increased risk of developing IBD.

Susceptibility to several canine immune-mediated diseases is associated with the major histocompatibility complex (MHC) class II locus (Dog Leukocyte Antigen, DLA), including anal furunculosis and chronic superficial keratitis in GSDs. Moreover, specific MHC class II variants are strongly associated with susceptibility to human IBD.

The aim of the current study was to investigate whether MHC class II genes (DLA-DRB1, -DQA1 and -DQB1) are associated with IBD in GSDs.

Genomic DNA was extracted from residual diagnostic EDTA blood samples of 56 GSDs affected with IBD and 50 breed-matched controls, over eight years old without any history of chronic GI signs. Dogs with a history of immune-mediated or dermatological diseases were excluded from the study. DLA genotypes were determined by PCR and sequence-based typing using SBTengine® software (GenDx, Utrecht). Fisher’s exact test was used to compare haplotype frequencies between groups; odds ratios (OR) with 95% confidence intervals (CI) were calculated. Statistical significance was set at p < 0.05.
The haplotype DLA-DRB1*015:01/DQA1*006:01/DQB1*003:01 was significantly associated with IBD (OR = 2.85, CI = 1.2-6.7, p = 0.014). Homozygosity for this risk haplotype further increased the risk of IBD (OR = 4.0, CI = 1.7-9.0, p = 0.001), suggesting a "gene-dosing" effect. The haplotype DLA-DRB1*015:02/DQA1*006:01/DQB1*023:01 was associated with a reduced risk of IBD (OR = 0.05, CI = 0.06-0.4, p < 0.001).

This study has identified an association between DLA-type and canine IBD, suggesting a potential role for MHC class II molecules in disease pathogenesis and further supporting the immunogenetic aetiology of this disease.

GI-19 CORRELATION OF FECAL MICROBIOTA TO FECAL SCORES OF CATS FED WITH CANNED THERAPEUTIC DIETS FOR THE MANAGEMENT OF NATURALLY OCCURRING CHRONIC DIARRHEA. Z. Ramadan, H. Xu, D.P. Laffamme, G. Czarnecki-Maulden, Q. Li, J.A. Labuda, B. Bourqi. Nestlé Purina Research, St. Louis, MO.

Disturbances among the gut microbiota are common in companion animals with diarrhea. The objective of this study was to determine associations between specific bacteria and fecal quality in cats receiving dietary management for chronic diarrhea. Adult cats (n = 15) with naturally occurring chronic diarrhea completed a cross-over trial to evaluate 2 therapeutic diets intended for the management of cats with diarrhea. At baseline and after 3 weeks of adaptation and recovery, fecal scores (FS: 1 = extremely dry and firm, 2 to 3 = normal stools, and 7 = very watery) were recorded during the final week of each dietary period and freshly voided fecal samples were collected. Fecal DNA was extracted and the V1-V2 hypervariable regions of the microbial 16S rRNA gene were amplified for high-throughput pyrosequencing. The resulting reads were denoised to eliminate sequencing errors for homopolymers and variable selection methods were applied to improve prediction of FS using orthogonal partial least square method (OPLS). Average FS improved in cats fed the therapeutic diets, with differences among diets. Significant correlations were found between the microbiome and FS (Q2 = 0.4) irrespective of which diet was fed. Bacteria with the strongest negative correlation (R > -0.35) with FS, i.e., better fecal quality, included Prevotella spp, Alistipes putredinis, Bacteroides fragilis, and Bacteroides coprocola. Bacteria with the strongest positive correlation (R > 0.35) with FS included Fusobacterium spp, Ruminococcus gnavus, Eubacterium dolichum, and bacteria of unidentified genera within the Ruminococcaceae family. These data provide potential targets for enhancing gut health in cats.

GI-20 EVALUATION OF SERUM 3-BROMOTYROSINE CONCENTRATIONS IN DOGS WITH EOSINOPHILIA, HYPOCHOBALAMINEMIA, OR PANCREATITIS. P. Sattasatthananan1, N. Gritzer2, N. Thengchaisri2, L. Lopes3, B.C. Guird4, J.S. Suchodolski5, J.M. Steiner6, 1Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA., 2Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

In patients with eosinophilic gastrointestinal (GI) disease, a large number of eosinophils reside in the GI tract. However, this infiltration of the GI tract with eosinophils may not be reflected by the peripheral eosinophilia. 3-Bromotyrosine (3-BrY) is a product of eosinophil peroxidase, which is released during eosinophil activation. A method to measure 3-BrY concentrations in the serum of dogs has recently been established and analytically validated. However, it was not determined if the clinical usefulness of measurement of 3-BrY in dogs has not yet been determined. The aims of this study were to determine the relationship between peripheral eosinophil counts and serum 3-BrY concentrations and to compare serum 3-BrY concentrations in healthy dogs and dogs with various GI diseases.

Serum samples were collected from healthy dogs (n = 41), dogs with eosinophilia (n = 21), hypochobalaminemia (HypoCBL; n = 16), and pancreatitis (n = 16). 3-BrY concentrations were measured by gas chromatography/mass spectrometry. The Mann-Whitney U test was used to compare concentrations of 3-BrY in healthy dogs and dogs with eosinophilia. A Spearman rank sum correlation coefficient (r) was used to test the relationship between serum 3-BrY concentrations and the numbers of eosinophils in the peripheral blood in healthy dogs and dogs with eosinophilia. Kruskal-Wallis and Dunn’s post tests were applied to evaluate the differences of serum 3-BrY concentrations between healthy dogs and dogs with HypoCBL, EPI, or pancreatitis.

Serum 3-BrY concentrations were significantly higher in dogs with eosinophilia (median [range]: 1.53 μmol/L [0.63-10.95]) than in healthy dogs (median [range]: <0.63 μmol/L [0.63-1.13], p < 0.001). Serum 3-BrY concentrations and peripheral eosinophil counts showed a significant correlation (r=0.59, p < 0.0001). Serum 3-BrY concentrations among groups were significantly different (p = 0.0006). The post test showed that serum 3-BrY concentrations were significantly higher in dogs with pancreatitis (median [range]: 1.25 μmol/L [0.63-3.15]) than in healthy dogs (p = 0.0003). However, serum 3-BrY concentrations were not different between healthy dogs and dogs with HypoCBL (median [range]: <0.63 μmol/L [0.63-3.15], p = 1.0000).

In conclusion, serum 3-BrY concentrations were increased in dogs with eosinophilia when compared to healthy dogs and the degree of eosinophilia was correlated with serum 3-BrY concentrations. Also, dogs with pancreatitis had higher serum 3-BrY concentrations than healthy dogs. However, the clinical relevance of this finding requires further investigation.


Lymphocytic-plasmacytic enteritis (LPE) is the most common form of canine inflammatory bowel disease (IBD). Studies in human IBD demonstrated that disruptions of amino acid metabolism are related to the pathophysiology of IBD and that analysis of plasma amino acid profiles is a novel marker of human IBD. Therefore, the aim of this study was to investigate the changes in plasma amino acid concentrations in dogs with LPE.

Heparinized blood samples were collected from dogs with LPE (n = 10) and healthy dogs (n = 12). Amino acids concentrations in the plasma were determined using ninhydrin method. Correlation between plasma amino acid concentrations and both plasma cytokine concentrations were investigated. Plasma amino acid concentrations in 6 out of 10 LPE dogs were also measured after treatment of LPE.

In LPE dogs, 10 amino acid concentrations (asparagine, glutamine, glycine, histidine, methionine, proline, serine, threonine, tryptophan, and tyrosine) were significantly lower than those in controls. In contrast, glutamic acid concentration in LPE dogs was significantly higher than that of controls. Inverse correlation was detected between CCECAI and both concentrations of plasma proline and serine. There was no correlation between plasma amino acid concentrations and CCECAI. There was no significant difference in plasma amino acid concentrations between pre- and post-treatment in dogs with LPE.

These results revealed that plasma amino acid concentrations were disturbed in dogs with LPE. Further study using a large number of cases is necessary to determine whether treatment can ameliorate amino acid disturbances in dog with LPE.
GI-22
ORAL COBALAMIN SUPPLEMENTATION IN DOGS WITH CHRONIC ENTEROPATHIES AND HYPOCOBALAMINEMIA. L. Toresson1, J-M. Steiner1, J-S. Suchodolski1, T. Spillmann2,1, Z. Neiger2, N. Torsvik2, N. K. Parnell2, J. Mansell1, 1Gastrointestinal Laboratory, Texas A&M University, College Station, TX., 2Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland.

Chronic enteropathies (CE) and exocrine pancreatic insufficiency (EPI) can both cause hypocobalaminemia in dogs. Current supplementation protocols for cobalamin (cbl) in dogs call for repeated parenteral injections. In humans, several studies have reported equal efficacy of oral administration of cbl. Thus, the purpose of this retrospective study was to evaluate whether oral cbl supplementation can restore normocobalaminemia in dogs with CE and hypocobalaminemia.

A computerized database search for dogs treated at Evidensia Specialist Animal Hospital, Helsingborg, Sweden during 2011-2013 was performed. Inclusion criteria were dogs with symptoms of CE, an initial serum cbl below 200 pmol/L (reference interval: 173-599 pmol/L) and treatment with cbl tablets (cyanocobalamin 1 mg/tablet; dogs < 20 kg ¼ tablet/10 kg dog, dogs ≥ 20 kg 1 tablet/day). Serum cbl was analyzed using an automated chemiluminescence immunoassay (ADVIA Centaur, Siemens). 39 dogs of 27 breeds met the inclusion criteria, of which 18 (46%) had a history of suffering from PLE (n = 7) to investigate the feasibility of oral cbl supplementation and 12 (31%) had cbl supplementation for CE and hypocobalaminemia.

GI-23
EVALUATION OF (99 M) TC-LABELLED HUMAN SERUM ALBUMIN (HSA) SCINTIGRAPHY TO DIAGNOSE PROTEIN- LOSING ENTEROPATHY (PLE) IN DOGS: A PILOT STUDY. N. Engelmann, R. Neiger. Small Animal Clinic, Internal Medicine, Justus-Liebig University of Giessen, Germany.

In this pilot study scintigraphy was performed in a group of clinically healthy beagles (n = 8) and a group of dogs suspected of suffering from PLE (n = 7) to investigate the feasibility of (99mTc) HSA scintigraphy in diagnosing PLE. In the healthy group scintigraphy was performed to prove that there was no tracer exudation in the intestinal tract. In the PLE group dogs were excluded if pretreated with steroids 30 days prior to presentation or other causes for hypoalbuminemia were diagnosed. A scan was considered positive in PLE if visible tracer was detectable in the intestinal tract.

GI-24
DEVELOPMENT AND VALIDATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR MEASUREMENT OF SERUM AND FECAL CANINE S100A12. R.M. Heilmann1, R.M. Hennig1, N. Grützner1, S. Sattasathuchana1, N. G. Kook2, S.M. Cranford1, J.S. Suchodolski1, J.M. Steiner1. 1Gastrointestinal Laboratory, Texas A&M University, College Station, TX., 2Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland.

The endogenous damage-associated molecular pattern molecule S100A12 plays an important role in innate immunity. Preliminary data show that canine S100A12 (cS100A12) is a useful marker for detecting inflammation in dogs. A radioimmunoassay (RIA) for the measurement of cS100A12 was recently developed and validated, but this assay requires use of a radioactive tracer. Also, fecal cS100A12 levels were below the assay detection limit in 79% of healthy dogs. An analytically more sensitive enzyme-linked immunosorbent assay (ELISA) may be useful to study fecal cS100A12 concentrations in dogs with gastrointestinal inflammatory diseases. The aim of this study was to develop and validate an ELISA for measuring cS100A12 in serum and feces.

Canine S100A12 was purified, anti-cS100A12 antibodies were raised in rabbits, monospecific antibodies were purified by affinity chromatography, and a sandwich-ELISA was developed and analytically validated. Control intervals for serum (n = 101) and fecal cS100A12 (n = 53) were established. The stability of cS100A12 in fecal samples, the effect of hydrocortisone treatment on serum cS100A12 concentrations, and the agreement of the ELISA with the RIA were determined.

GI-25
SENSITIVITY OF A RIA AND ELISA MARKERS OF INFLAMMATION IN DOGS. N. G. Kook1,2, N. Berghoff1, T. Minamoto1, P. Sattasathuchana1, N. Grützner1, J.S. Suchodolski1, J.M. Steiner1. 1Gastrointestinal Laboratory, Texas A&M University, College Station, TX., 2Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland.

Canine S100A12 (cS100A12) is a useful marker for detecting inflammation in dogs. A radioimmunoassay (RIA) may be useful to study fecal cS100A12 concentrations in dogs with gastrointestinal inflammatory diseases. The aim of this study was to develop and validate an ELISA for measuring cS100A12 in serum and feces.

In this study, sensitivity of a RIA and an analytically more sensitive enzyme-linked immunosorbent assay (ELISA) was studied in healthy dogs.

GI-26
LINKED IMMUNOSORBENT ASSAY FOR MEASUREMENT AND VALIDATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR MEASUREMENT OF SERUM AND FECAL CANINE S100A12. N. G. Kook1,2, N. Berghoff1, T. Minamoto1, P. Sattasathuchana1, N. Grützner1, J.S. Suchodolski1, J.M. Steiner1. 1Gastrointestinal Laboratory, Texas A&M University, College Station, TX., 2Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland.

Canine S100A12 (cS100A12) is a useful marker for detecting inflammation in dogs. A radioimmunoassay (RIA) may be useful to study fecal cS100A12 concentrations in dogs with gastrointestinal inflammatory diseases. The aim of this study was to develop and validate an ELISA for measuring cS100A12 in serum and feces.

In this study, sensitivity of a RIA and an analytically more sensitive enzyme-linked immunosorbent assay (ELISA) was studied in healthy dogs.
inflammatory bowel disease (IBD) or lymphoma. Alpha-1-protease inhibitor (α1PI) is a protease-resistant protein that can be measured in serum and fecal samples. The concentration of fecal canine α1PI (α1PI) has been shown to be increased in dogs with severe IBD or lymphangiectasia. This study aimed to evaluate serum and fecal α1PI concentrations in relation to the severity of histologic findings in dogs with IBD.

Serum and fecal samples were collected from 98 dogs with IBD at the time of histologic diagnosis. Serum and fecal α1PI concentrations were measured, and were compared between IBD dogs with vs. without crypt abscesses and/or lacteal dilation, and with the severity of histologic disease severity (based on the WSAVA GI histopathology grading system) using a Wilcoxon rank sum test. Receiver-operating characteristic (ROC) curve analysis was performed for sensitivity and specificity calculations. A Spearman rank sum correlation coefficient was used to test the relationship between serum and fecal α1PI concentrations with serum concentrations of albumin.

Serum α1PI and albumin concentrations were significantly lower in IBD dogs with crypt abscesses and/or lacteal dilation (n = 43) than in those without (n = 55; both p < 0.0001). The severity of lacteal dilation was significantly associated with the concentration of α1PI in serum (p < 0.0001) and fecal samples (p = 0.0136 for the mean and p = 0.0149 for the maximum α1PI concentration in samples collected from 3 consecutive days), and also with serum albumin concentrations (p < 0.0001). Serum albumin and histologic disease severity were negatively correlated (r = -0.29, p = 0.0033), but neither serum nor fecal α1PI concentrations were associated with the severity of histologic lesions. A serum α1PI of 1.16 mg/mL, a 3-day mean fecal α1PI of 11.0 μg/g, and a 3-day maximum fecal α1PI of 25.5 μg/g distinguished dogs with moderate/severe GI crypt abscesses and/or lacteal dilation (n = 17) from those without crypt abscesses and only mild/without lacteal dilation (n = 81) with a sensitivity of 79%, 80%, and 67%, respectively, and a specificity of 70%, 65%, and 73%, respectively. Serum albumin and serum α1PI were moderately correlated (r = 0.67, p < 0.0001), especially if lacteal dilation was moderate to severe (r = 0.76, p = 0.0016).

This study showed that serum and fecal α1PI concentrations are associated with the severity of GI crypt abscesses and/or lacteal dilation in dogs with IBD, suggesting that both are valuable non-invasive markers of GI protein loss in dogs. Due to its specificity for the GI tract, measurement of fecal α1PI appears to be superior to that in serum for the diagnosis of GI protein loss in dogs.

GI-26
Evaluation of Two Dry Therapeutic Diets for Dogs with Acute Diarrhea. S.A. Wennogle1, L.E.R. Martin1, H. Xu2, C. Jean-Phillip2, M.R. Lappin2,3 Colorado State University, Fort Collins, CO. 2Nestle Purina Research, St. Louis, MO.

Acute diarrhea is a common cause of morbidity in both client-owned dogs and those housed in animal shelters. Additionally, the development of acute diarrhea may delay the time to adoption in shelter dogs, which negatively impacts both the individual dog and shelter resources. The purpose of this study was to compare the use of two diets, Purina EN® and Hills Science Diet i/d®, in a population of young, otherwise healthy shelter dogs with acute diarrhea.

Dogs estimated to be 3 months to 3 years of age and heavier than 10 pounds were eligible for inclusion in the study if diarrhea (7 = watery stools; 6 = texture but no shape; 5 = moist piles; 4 = moist log shape) without blood or tenesmus had been noted for at least 2 days. In addition, there could be no evidence of systemic illness including fever or inappetance and the dog had to be deemed behaviorally suitable for adoption once diarrhea was resolved. All dogs were evaluated for gastrointestinal parasites using fecal flotation by zinc sulfate centrifugation and fecal immunofluorescence assay for Giardia and Cryptosporidium (Meridian Diagnostics) on entry to the study and all were administered Droncit Plus® (Bayer Animal Health) for the first 3 days of the study, starting on Day -2 or Day -1. Qualifying dogs were randomly assigned to be fed EN or i/d beginning on Day 0 with fecal scores, appetite, and overall health status monitored daily for the next 14 days.

To date, 13 dogs fed EN (n = 13) and 9 dogs fed i/d (n = 9) met the entry criteria and completed the study through at least Day 11. No dog was pulled from the study for refusing to eat a diet and complete portions were ingested by all dogs. Dogs fed EN had a mean fecal score of less than 3 by Day 2 and dogs fed i/d had a mean fecal score less than 3 by Day 5, but these findings were not statistically different. Of the dogs fed i/d, 44.4% had a recurrence of a fecal score greater than 3 after Day 7 versus 15.4% of the dogs fed EN (p = 0.18). The proportions of fecal samples with a score greater than 3 after the dogs were fed the respective diet were stratified into Days 1 – 7, Days 8 - 14, and Days 1 - 14. Dogs fed EN had significantly greater (p = 0.02) proportion of normal stools between Days 1 – 7 (81.7%) than dogs fed i/d (63.2%). Parasites were detected in the feces of 38% of dogs fed EN and 0% of dogs fed i/d while DGGR-lipase results were not significantly different. Giardia (8 dogs) or Anyclostoma caninum (2 dogs) were most common and both respond to Droncit Plus®.

Both diets were well tolerated and apparently effective in this study design, with dogs fed Purina EN® having a greater proportion of normal stools than those fed Hills Science Diet i/d®.

GI-27

Pancreatic lipase immunoreactivity (PLI) and abdominal ultrasound are considered the best tests for noninvasive diagnosis of pancreatitis in dogs. Lipase activity may be considered weaker than PLI, but methods vary. A catalytic lipase assay using the substrate 1,2-diacyl-sn-glycero-3-phosphoethanolamine (DGGR-lipase) [DGGR-lipase] has reportedly substantial agreement and good correlation with PLI in cats (JFIM 2013:27:708,1077-1082). To examine if DGGR-lipase has a similar relationship to PLI in dogs, all records from 2007-2013 were retrieved where: 1) Spec cPL® had been ordered (IDEXX Laboratories, Meridian Diagnostics, Inc); and 2) DGGR-lipase had been measured (Animal Health Laboratory, Guelph), on the same sample, using the LIPC lipase assay (Roche Diagnostics), with reference interval (RI) of 25-350 U/L based on 86 mature dogs. Ultrasound exams performed by board-certified radiologists within 24 hr of lipase tests were reviewed for findings supportive of pancreatitis as previously described (JFIM 2013:27:707-708).

There were 177 dogs (median age 8 yrs, range 0.5-16, various breeds) with 205 evaluations. All dogs presented for variable lethargy, vomiting and diarrhea. Median (range) for Spec cPL® was 221 (17 to 1443) U/L, and for DGGR-lipase were 296 (17 to 78520) U/L. Comparisons of DGGR-lipase and Spec cPL® (overall and stratified by ultrasound) are in Table 1. The two were strongly correlated within Spec cPL® analysis. Agreement between DGGR-lipase >RI and Spec cPL® > 200 or >400μg/L were examined with kappa - agreements were moderate to very good. Elevated DGGR-lipase strongly predicted elevated Spec cPL®. DGGR-lipase value < 52U/L ruled-out Spec cPL® > 200μg/L; DGGR-lipase < 113U/L ruled-out Spec cPL® > 400μg/L. Values were trended in 25 dogs - DGGR-lipase and Spec cPL® always changed in the same direction. Agreements between ultrasound findings and Spec cPL® > 200μg/L, Spec cPL® > 400μg/L, and DGGR-lipase >RI were fair (kappa ± SE: 0.44 ± 0.05, 0.36 ± 0.06, 0.31 ± 0.07, respectively).

If DGGR-lipase is below or above the RI, measuring PLI has limited diagnostic utility. For DGGR-lipase values within the RI, PLI may be elevated; it is not known if this represents increased sensitivity and/or reduced specificity of PLI for pancreatitis compared to DGGR-lipase.
GI-28

Inflammatory bowel disease (IBD) is a common cause of persistent or recurrent gastrointestinal signs in dogs and is associated with histological evidence of intestinal inflammation. Inflammation is the basis for the clinical signs of IBD, making its detection and monitoring crucial to clinical management. Several indices are used to evaluate the severity of the disease, all of which differ in terms of their objectivity. Despite the different indices available, there is no consensus as to which index is the most valid.

The aim of the study was to evaluate serum C-reactive protein (CRP) and fecal inflammatory markers (fecal S100A12 and calprotectin) as indicators of intestinal inflammation in dogs.

Twenty dogs referred to the Small Animal Teaching Hospital at the School of Veterinary Medicine and Animal Science, University of São Paulo, SP, Brazil; with a diagnosis of IBD based on histopathologic evaluation of gastrointestinal tissue biopsies were included in this prospective study. Blood and fecal samples were collected from these animals and from healthy control dogs (n=20). Dogs with IBD were separated into 2 subgroups according to the affected site (small bowel, n=9 and large bowel, n=11). Serum CRP and fecal S100A12 and calprotectin were measured using validated in-house immunoassays. Data were expressed as medians and ranges. Statistical significance was set as P < 0.05 for all analyses.

Dogs in the IBD group had significantly higher serum CRP concentrations than dogs in the control group (medians – IBD group: 1.85 mg/L, control group: 0.10 mg/L; P = 0.024; Mann-Whitney U test). Fecal calprotectin concentrations were also significantly higher in the IBD group (median: 411.4 μg/g) compared to the control group (39.6 μg/g; P = 0.026; Mann-Whitney U test). Although there was no significant difference in fecal S100A12 concentrations between both groups, IBD dogs had higher fecal S100A12 concentrations compared to control dogs (25.1 ng/g and 12.3 ng/g, respectively; P = 0.091). A direct strong positive correlation was found between fecal parameters (r = 0.810; P < 0.001; Spearman correlation coefficient). All 3 biomarkers were not significantly different among the subgroups large and small bowel (CRP: 4.10 mg/L versus 1.30 mg/L; P = 0.16; calprotectin: 299.60 μg/g versus 411.60 μg/g; S100A12: 17.15 ng/g versus 41.16 ng/g).

Our results suggest that fecal markers, especially fecal calprotectin, can differentiate dogs with IBD from healthy control dogs but it was not found useful to identify the site of intestinal inflammation (small vs. large bowel). A combination of these novel fecal biomarkers and serum CRP may increase the diagnostic accuracy in dogs with IBD.

<table>
<thead>
<tr>
<th>N</th>
<th>S100A12 Median (μg/g)</th>
<th>Calprotectin Median (μg/g)</th>
<th>CRP Median (mg/L)</th>
<th>CRP SD</th>
<th>Calprotectin SD</th>
<th>S100A12 SD</th>
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<tbody>
<tr>
<td>All (205)</td>
<td>39.6 (1 to 92)</td>
<td>411.4 (32 to 258)</td>
<td>0.10 (0.05 to 0.14)</td>
<td>0.024</td>
<td>0.026</td>
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<tr>
<td>Un (115)</td>
<td>39.6 (1 to 92)</td>
<td>411.4 (32 to 258)</td>
<td>0.10 (0.05 to 0.14)</td>
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<tr>
<td>US N (44)</td>
<td>41.16 (32 to 258)</td>
<td>411.4 (32 to 258)</td>
<td>0.58 (0.57 to 0.61)</td>
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</tbody>
</table>

GI-29
ANALYTICAL VALIDATION OF AN ENZYMELINKED IMMUNOSORBENT ASSAY FOR THE QUANTIFICATION OF S100A12 IN SERUM AND FECES FROM CATS. C.S. Bridges, R.M. Heilmann, C. Neudecker, J.S. Suchodolski, J.M. Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

S100A12 is a Ca²⁺ binding protein that plays an important role in both the innate and the acquired immune response. S100A12 has been reported to be a sensitive and specific marker for inflammatory disease in humans, including gastrointestinal inflammation, leading to increased serum and fecal S100A12 concentrations. Recently, an enzyme-linked immunosorbent assay (ELISA) for the quantification of S100A12 in canine serum and feces has been established. Canine S100A12 is currently being investigated as a marker for gastrointestinal inflammation in dogs, and fecal S100A12 concentrations have been shown to be increased in dogs with inflammatory bowel disease. Purified antibodies against canine S100A12 cross-immunoreact with feline S100A12. Therefore, the aim of this study was to analytically validate the canine S100A12-ELISA for the quantification of S100A12 in serum and fecal specimens from cats.

Serum and fecal samples from cats were collected and were evaluated using a recently developed and analytically validated in-house canine S100A12-ELISA. The assay was analytically validated for use with feline samples by determination of the lower detection limit, dilutional parallelism, spiking recovery, and intra- and inter-assay variability. Control intervals for serum (n=46) and fecal (single sample): n=53; 3-day study mean: n=53. S100A12 was established from healthy cats using the upper 97.5th percentile.

Analytical sensitivity of the assay for serum and fecal samples assayed in a 1:100 dilution were 1 μg/L and 1 ng/g, respectively. Observed-to-expected ratios (O/E) for serial dilutions ranged from 70-159% (mean ± standard deviation [SD]: 116 ± 23%) for seven different serum samples, and from 100-128% (mean±SD: 114 ± 8%) for five different fecal samples, O/E for spiking recovery ranged from 83-171% (mean ± sd: 103 ± 17%) for four different serum samples and six different spiking concentrations, and from 69-164% (mean ± sd: 105 ± 25%) for four different fecal extracts and six different spiking concentrations. Intra-assay coefficients of variation (CV) for four different serum samples were 4.6, 1.2, 5.6, and 2.6% and 3.8, 2.2, 3.7, and 2.8% for four different fecal samples. Inter-assay CV for four different serum samples were 10.3, 12.2, 8.9, and 14.0% and 10.5, 12.3, 19.1, and 11.2% for four different fecal extracts. In healthy cats, serum and fecal S100A12 concentrations ranged from <1 to 45 μg/L and <1 to 258 ng/g, respectively. The control intervals for serum and fecal S100A12 in cats were established as <32 μg/L and <88 ng/g (single fecal sample) or <75 ng/g (3-day fecal sample mean), respectively.

We conclude that the canine S100A12-ELISA is analytically sensitive, accurate, precise, and reproducible, and sufficiently linear for the measurement of S100A12 in serum and fecal samples from cats. Further studies evaluating the clinical usefulness of measuring serum and/or fecal S100A12 concentrations in cats with gastrointestinal disease are currently underway.

GI-30
CHARACTERIZATION OF FUNCTIONAL PATHWAYS OF THE FECAL MICROBIOTA IN DOGS DURING POSTNATAL DEVELOPMENT. A. Buono1, M.R. Horton1, B.C. Guard1, J.M. Steiner1, J.S. Suchodolski1. Gastrointestinal Laboratory, 1Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX.

Recent 16S rRNA gene sequencing studies have furthered our knowledge of the microbial complexity in the gastrointestinal (GI) tract. The GI microbiota is believed to play a crucial role in the evolution of gut function and the promotion of host health. At parturition, the gut is nearly sterile but a rapid colonization with microbes occurs within the first few hours of life. The intestinal microbiota undergoes profound changes during the postnatal period. The initial bacterial colonization is crucial for the
GI-32

EPIDEMIOLOGICAL DATA IN DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY- A RETROSPECTIVE STUDY (2003-2012).
J.C. Parambeth, J.S. Suchodolski, J.M. Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Exocrine pancreatic insufficiency (EPI) in dogs is a syndrome of inadequate synthesis and secretion of pancreatic enzymes, leading to clinical signs of malabsorption. A strong breed association for German Shepherd dogs has been reported in earlier studies. The canine trypsin-like immunoreactivity (cTLI) assay has a high sensitivity and specificity for the diagnosis of EPI. The aim of this retrospective study was to determine the number of dogs diagnosed with EPI annually over a 10-year-period (2003-2012) and to assess the breed, age, and gender distribution of these dogs.

Data from the Gastrointestinal Laboratory at Texas A&M University database were reviewed for all submissions that were diagnosed with EPI (cTLI ≤ 2.5 μg/L) during this period. Repeated submissions from the same dog were excluded from the analysis. Breed representation was analyzed using two control populations: (a) all submissions for the cTLI test during this period, and (b) a microchip database registry (American Kennel Club Companion Animal Rescue) for the year 2011. The Chi-square test was used to test the associations between breed, age, sex, and a diagnosis EPI. The resulting P-values were adjusted for multiple comparisons using Bonferroni’s correction. Statistical significance was set at P < 0.05.

A total of 8,027 dogs had serum samples submitted for the cTLI assay from 2003-2012. Of these, 5,626 dogs (7.6%; 95% CI: 7.5-7.8%) had a cTLI concentration diagnostic for EPI, with a mean of 802 dogs/year (range: 523 - 940 dogs/year). EPI was diagnosed in 125 different breeds. Breeds were reported for 5,626 submissions and we noted a significantly increased prevalence in Cairn terriers, German Shepherd dogs, Akitas, Westland High White terriers, Welsh Corgis (Cardigan), Border collies, Australian heellers, Australian shepherds, mixed breeds, and Shetland sheepdogs, in comparison to both control populations. In contrast, a decreased prevalence was observed for Bichon Frises, Great Danes, Yorkshire terriers, Greyhounds, Boxers, and Soft Coated Wheatens terriers. The median age at diagnosis for 5,236 dogs was 4 years (range: <1 year – 21 years) and was significantly different between the different breeds (P < 0.001). Female dogs with EPI were overrepresented (60.0%; 3255/5425 P < 0.001). Neutered animals made up 77.6% of the sample population and 75.4% of dogs with EPI.

Limitations of this retrospective analysis include possible inconsistencies with report breed and age during sample submission. We conclude that exocrine pancreatic insufficiency is more prevalent in certain breeds of dogs and spayed female dogs.

GI-31

USE OF FECAL TRANSPLANT IN EIGHT DOGS WITH REFRACTORY CLOSTRIDIUM PERFRINGENS ASSOCIATED DIARRHEA. T. Murphy, J. Chaitman, E. Han. Veterinary Internal Medicine and Allergy Specialists, New York City, NY.

Clostridium perfringens is commonly recognized as a cause of diarrhea with mucus and blood in the dog. The purpose of this study was to determine if fecal transplantation could be used to cure Clostridium perfringens infections that were not cured by treatment with metronidazole and amoxicillin trihydrate/clavulanate potassium.

Eight dogs who tested positive for Clostridium perfringens on RealPCR™ panels by IDEXX Laboratories and whose infections were not cured with antimicrobial therapy alone underwent fecal transplantation from an infection-free donor dog.

Donor stool was mixed with saline in a blender and given as an enema on an outpatient basis in an unsedated patient. Eight out of eight dogs had immediate resolution of their diarrhea and six out of eight dogs were negative on follow-up PCR panels for Clostridium perfringens alpha toxin gene expression. Dogs had between one and three fecal transplants.

To the authors’ knowledge, this is the first report of fecal flora transplantation in the dog for refractory Clostridium perfringens infections. This treatment plan is an option for dogs failing antibiotic therapy and also reduces antibiotic use.

GI-33

ASSOCIATION OF EXOCRINE PANCREATIC INSUFFICIENCY OR HYPOFOLEATEMIA WITH HYPOCOBALAMINEMIA IN DOGS. N. Grützner, J.S. Suchodolski, J.M. Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Exocrine pancreatic insufficiency (EPI) has been shown to be an important cause of cobalamin (vitamin B12) deficiency in dogs. Also, Border Collies and German Shepherd dogs (GSD) with an unalterable serum cobalamin concentration (<150 ng/mL) were more likely to have a serum canine trypsin-like immunoreactivity (cTLI) concentration diagnostic for EPI (≤ 2.5 μg/L) than dogs of other breeds. However, such an association has not been shown for any other dog breed. The majority of cobalamin and folate (vitamin B9) is absorbed in the distal and proximal small intestine, respectively. An investigation of low serum folate concentrations in dogs with hypobetalaminemia could help identify breeds in which gastrointestinal disease affects both the proximal and distal small intestine. Therefore, this study was conducted to identify breeds where a higher proportion of hypo-
cobalaminemic dogs have a) a serum cTLI concentration diagnostic for EPI or b) a decreased serum folate concentration. Canine submissions for analysis of serum cobalamin, folate, and cTLI concentrations between 2008 and 2010 were analyzed, with re-check samples from the same dog were excluded. The proportion of hypocobalaminemic dogs (undetectable serum cobalamin concentration <150 ng/L) with a) a serum cTLI concentration ≥2.5 μg/L diagnostic for EPI or b) with a serum folate concentration <7.7 μg/L (below the lower limit of the reference interval) from dog breeds with a minimum of 30 submissions were compared by calculating the odds ratio (OR) and the 95% confidence interval (CI). Because of multiple comparisons between the 164 dog breeds evaluated in this study the statistical significance level for a difference was adjusted from \( p < 0.05 \) to \( p < 0.0003 \) using a Bonferroni correction for multiple statistical comparisons.

A total of 164 dog breeds (28,675 submissions) were reviewed. Pugs (OR [CI]: 28.5 [5.8-139.9], \( p = 0.0001 \)), Shih Tzus (OR [CI]: 27.6 [7.1-107.0], \( p = 0.0001 \)), Miniature Schnauzers (OR [CI]: 19.9 [5.3-75.5], \( p = 0.0001 \)), Chinese Shar-Peis (OR [CI]: 18.2 [4.7-71.2], \( p = 0.0002 \)), Golden Retrievers (OR [CI]: 13.8 [4.4-43.0], \( p = 0.0002 \)), and Labradors (OR [CI]: 12.7 [7.1-22.7], \( p < 0.0001 \)) with hypocobalaminemia were more likely to have a serum cTLI concentration close to or above the upper limit of the RI. Boxers (OR [CI]: 3.8 [1.9-7.8], \( p = 0.0002 \)), Yorkshire Terriers (OR [CI]: 3.1 [2.0-4.8], \( p < 0.0001 \)), and Labradors (OR [CI]: 3.0 [2.1-4.1], \( p < 0.0001 \)) with hypocobalaminemia were more likely to also have a decreased serum folate concentration, suggesting diffuse small intestinal disease.

In addition to GSDs and Border Collies, six other breeds were identified as having an association between hypocobalaminemia and EPI. Interestingly, all newly identified breeds had higher odds ratios than the two breeds previously identified as having such association. Also, hypocobalaminemia was associated with hypofolatemia in three breeds, which would suggest that diffuse small intestinal disease in these breeds is more common than in the other breeds evaluated.

GI-34

**SERUM ALPHA1-PROTEASE INHIBITOR CONCENTRATIONS IN DOGS WITH EXOCRINE PANCREATIC DISEASE, CHRONIC HEPATITIS, OR CHRONIC KIDNEY DISEASE.** N. Grützner 1, R.M. Heilmann 1, J.A. Hokamp 1, D. Lundby 1, M. Efferth 1, J.S. Suchodolski 1, J.M. Steiner 1. 1Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences and, 2Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

In humans, alpha1-protease inhibitor (alpha1-PI), also referred to alpha1-antitrypsin, is mostly synthesized by hepatocytes, although it can also be synthesized by macrophages, alveolar cells, and intestinal epithelial cells. Decreased serum alpha1-PI concentrations have been reported in human patients with inflammatory bowel disease (IBD), pancreatitis, chronic liver disease, or renal disease. Recently, decreased serum canine alpha1-PI (calpha1-PI) concentrations have been reported in a subset of dogs with IBD, protein-losing enteropathy, and/or cobalamin deficiency. Theoretically, a decreased serum alpha1-PI concentration could also be found in dogs with chronic hepatitis (CH) or chronic kidney disease (CKD), potentially altering the systemic proteinase/protease-inhibitor balance. Furthermore, the proteinase/protease-inhibitor balance could be affected by other diseases, such as pancreatitis or exocrine pancreatic insufficiency (EPI). Serum calpha1-PI concentrations have not previously been evaluated in dogs with gastrointestinal diseases other than IBD, or in dogs with CH or CKD. The aim of this study was to evaluate serum calpha1-PI concentrations in dogs with pancreatitis, EPI, CH, or CKD.

The present understanding of canine idiopathic inflammatory bowel disease (IBD) assigns a significant role to the interaction between the gastrointestinal microbiota, the immune system, and host genetic factors. Sequencing of the 16S rRNA gene has shown significant alterations in microbial groups among dogs with IBD compared to healthy control dogs, but the functional effect of the resident microbiota in health and disease has not been fully elucidated. The gastrointestinal microbiota is considered one of the causes of canine chronic enteropathy (CE). In this study, fecal microbiota in canine CE was evaluated by terminal restriction fragment length polymorphism (T-RFLP) profiling.

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This study targeted 42 cases diagnosed as CE by clinical examination and by histopathological examination using endoscopy. In addition, in all CE cases, gastrointestinal symptoms occurred for more than three weeks. CE cases were classified using the following therapeutic responses: food responsive enteropathy (FRE; seven cases), antibiotic responsive enteropathy (ARE; 13 cases), steroid responsive enteropathy (SRE; 17 cases) and mortality cases with non-responsive enteropathy (NRE; five cases).

The T-RFLP analysis classified CE into 28 operational taxonomic units (OTUs). The major bacterial species were Lactobacillus (657 OTU), Bacteroides (366, 469, 853 OTU), Clostridiales (1,10, 338, 754, 912, 955 OTU). In addition, 940 OTU was also observed in many cases, but Fusobacteriaceae and Clostridiales could not be classified by using this method. Furthermore, the percentage of Clostridiales was significantly increased in the ARE compared to the NRE and SRE (\( P < 0.05 \)). Results calculating the distance for building a phylogenetic tree, tended to classify NRE as different from the other clusters.

This study suggests that Clostridiales are involved in the pathogenesis of CE. Therefore, it is suggested that the evaluation of fecal microbiota by T-RFLP profiling is useful in characterizing the pathogenesis in canine CE.

GI-36

**BIOMICROBIAL PATHWAYS OF BACTERIA IN THE DUODENUM OF DOGS WITH IDIOPATHIC INFLAMMATORY BOWEL DISEASE.** J.B. Honneffer 1, B.C. Guard 1, J.M. Steiner 1, A.E. Jergens 1, J.S. Suchodolski 1, “Gastrointestinal Laboratory, Texas A&M University, College Station, TX., 1College of Veterinary Medicine, Iowa State University, Ames, IA.

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This study suggests that Clostridiales are involved in the pathogenesis of CE. Therefore, it is suggested that the evaluation of fecal microbiota by T-RFLP profiling is useful in characterizing the pathogenesis in canine CE.
been elucidated. The aim of this study was to utilize a computational approach to explore possible alterations in microbial biochemical pathways between dogs with idiopathic IBD and healthy controls.

Previously published 16S rRNA gene sequencing data describing alterations in the microbiota of duodenal biopsies from dogs with moderate to severe IBD (n = 14) and healthy controls (n = 6) were used for this study. The data were analyzed using the bioinformatics software PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) to predict the functional capabilities of bacteria within each sample. The relative abundance of genes associated with a given pathway may indicate an increased metabolic capacity of the enteric microbiota with regard to that pathway. Inferences of the functional gene content were grouped by function according to the three-level KO (Kyoto Encyclopedia of Genes and Genomes Ortholog) hierarchy and compared using Linear Discriminant Analysis (LDA) through LEfSe (LDA Effect Size) with the significance threshold set to an LDA score of log 2.5.

Among pathway categories, lipid metabolism was increased in dogs with IBD (LDA = 3.4), while pathways associated with carbohydrate, nucleotide, and energy metabolism were relatively decreased when compared to healthy dogs (LDA = 3.7, 3.5, and 3.5, respectively). Pathways categorized into translation or replication or repair, which are subcategories of genetic information processing, were decreased in abundance among dogs with IBD (LDA = 3.6 and 3.8, respectively) while the category of environmental information processing was more abundant in the dogs with IBD compared to healthy dogs (LDA = 4.0). At the individual KO level, the iron complex outer membrane receptor protein was significantly increased in dogs with IBD (LDA = 3.2), along with two KOs associated with the NiT/TauT family transport system (LDA = 2.7, 2.5), which is involved in osmoprotection. The abundances of three KOs associated with the multiple sugar transport system were decreased in dogs with IBD (LDA = 3.0, 2.9, and 2.7). The abundance of the KO corresponding to 6-phosphofructokinase 1 was also decreased relative to the healthy dogs (LDA = 2.5).

These predictions of the functional metagenomic milieu provide insight into the changes occurring in the duodenum in dogs with idiopathic IBD. Future studies are warranted to evaluate these pathways and related metabolites for potential diagnostic and/or therapeutic targets.

GI-38

Hematemesis denotes the presence of blood in the vomit and can result from various causes. Although it is generally agreed amongst veterinarians that small amounts of blood in the vomit can be seen with any vigorous vomiting, hematemesis is regarded as an alarming sign by most pet owners; often resulting in cost-intensive emergency consultations. Because no information has been published on the clinical problem of hematemesis in dogs and cats, the purpose of this retrospective study was to better characterize the clinicopathologic factors and outcome of dogs and cats presenting for hematemesis.

Medical records of patients presenting between February 2004 and July 2012 were reviewed. For inclusion “hematemesis”, “bloody vomiting”, or “blood in vomit” had to be noted at least once as a presenting complaint in the medical files. Chi-square analysis was performed to compare clinicopathologic findings between survivors and non-survivors. Chi-square analysis also was used to evaluate the relation between clinicopathologic variables and length of hospitalization. An association was considered significant when the $x^2$ value was $> 3.84$ (df = 1, $p = 0.05$).

A total of 148 dogs (median age 6 y) and 58 cats (median age 8 y) were included. Emergency consultations comprised 131 (88.5%) of the canine and 50 (86.2%) of the feline patients. Concurrent diarrhea was present in 91 (61.5%) dogs and 17 (29.3%) cats. The use of NSAIDs was reported in 23 (15.5%) dogs. The general clinical condition at presentation (dogs/cats in %) was normal (23.6/17.2), mildly (24.3/19), moderately (37.2/51.7), or severely (10.8/6.6) reduced. An ICU admission was deemed necessary in 31 (20.9%) dogs and 16 (27.6%) cats. The mean/median length of hospitalization was 3.48/3 d for dogs (range, 0-14d) and 3.47/3d for cats (range, 0-11d). While 130 (87.7%) dogs and 47 (81%) cats could be discharged, 18 (12.2%) dogs and 11 (18.9%) cats died or were euthanized. In dogs there were significant associations for clinical pathology results obtained at admission and survival for anemia, microcytosis, thrombocytopenia, increased serum creatinine concentrations, and elevated alkaline phosphatase activity. In cats, significant associations were only found for anemia, a left shift, and hyperglycemia. In dogs, hospitalization was significantly associated with an emergency consultation, referral status, diarrhea, hematocrit, melena, and hypoprothrominemia. Whereas in cats, length of hospitalization was significantly associated with a history of repetitive hematemesis, hypoprothrominemia, and elevated alkaline phosphatase activity. Cats could be allocated to the following diagnosis groups (dogs/cats in %): gastrointestinal disease (60.9/60.4), intoxication (17.2/10.4), pancreatic disease (4.7/12.5), non-gastrointestinal neoplastic disease...
In the present study data provide the clinician for the first time with etiologic and prognostic information on the presenting clinical sign hematemesis in dogs and cats.

GI-39
ANALYTICAL VALIDATION OF A COMMERCIALLY AVAILABLE IMMUNOASSAY FOR THE MEASUREMENT OF MOTILIN CONCENTRATIONS IN DOG SERUM. T. Minamoto, J.S. Suchodolski, J.M. Steiner. Gastrointestinal Laboratory, Veterinary Small Animal Clinical Sciences, Texas A&M University, College Station, TX.

Motilin is a small peptide (22 amino acids) secreted by M-cells of the small intestine. Its primary function is to stimulate the migrating motor complex (MMC) and cause intestinal contractions during the interdigestive fasting period. It is well known that dogs experiencing acute diarrhea can show a decrease in MMCs phase III contractions of the MMC during the interdigestive period. Serum motilin concentrations may serve as a potential biomarker for assessing gastrointestinal motility in dogs. Thus, the aim of this study was to analytically validate an immunoassay for the measurement of motilin in canine serum samples.

For the analytical validation of the assay, serum motilin concentrations were measured using a commercially available canine motilin radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals, INC, Burlingame, CA, USA). Surplus canine serum samples and serum samples from healthy dogs obtained for this study were utilized for validation. Validation of the assay consisted of determination of dilutional parallelism, spiking recovery, and intra-and inter-assay variability. Observed to expected ratios (O/E) for serial dilutions ranged from 0.95 to 1.15 (mean ± SD: 1.03 ± 11.2%) for three different serum samples at dilutions of 1:1, 1:2, 1:4, and 1:8. O/E for spiking recovery ranged from 66.9% to 94.1% (mean ± sd: 80.2 ± 8.0%) for five different canine serum samples that had been spiked with the standards. Intra-assay coefficients of variation (%CV) for five different serum samples were 4.8, 5.4, 5.5, 6.2, and 8.8%. Inter-assay%CVs for five different serum samples were 3.0, 3.6, 4.7, 4.7, and 6.1%.

In conclusion, the RIA for the measurement of canine motilin concentrations evaluated for this study is linear, accurate, precise, and reproducible for measurement of serum motilin concentrations in dogs. Further studies will be needed to evaluate the clinical utility of the measurement of serum motilin concentrations for assessment of gastrointestinal motility and/or disease in dogs.

GI-40
VIRAL DIVERSITY IN THE INTESTINE OF HEALTHY DOGS AND DOGS WITH ACUTE DIARRHEA. P.S. Moreno-1,2, J. Wagner, M. Stevens, J.R. Gilkerson, C. Kirkwood, C.S. Mansfield, 1 Faculty of Veterinary Science, The University of Melbourne, VIC, Australia. 2Murdoch Children Research Institute, VIC, Australia. 3The Australian Genome Research Facility.

Little is known about the population of viruses present in the intestine of dogs, the virome, despite advances in molecular technology. The viral flora in the intestine of dogs has been mostly studied in disease settings, and there is a gap in our knowledge of the virome present in healthy conditions. This study aimed to identify and characterize the virome present in feces of healthy dogs compared to the virome present in dogs with acute diarrhea. Fecal samples were collected from 8 healthy dogs and 8 dogs with acute diarrhea. A viral enrichment protocol, using a mixture of endonucleases and bacterial filtration was used to enrich viral DNA and RNA. The total DNA and RNA isolated from the stool samples were amplified using a sequence-independent single-primer amplification protocol and subsequently sequenced by next generation sequencing using the Illumina MiSeq platform at the Australian Genome Research Facility. A bioinformatic pipeline was used to analyze the viral population present in each sample; selecting high quality reads (HQs) and removing dog and bacterial sequences, before comparing sequence information against the CAMBase viral reference database. An average of 1.2 million HQS were obtained per sample (422577 to 1885864) of which 22% and 9.3% were nonDog/nonBacteria sequences from healthy and diarrheic samples, respectively. The largest proportion of the viral contigs identified in both the healthy and diarrheic sample sets were classified as bacteriophages, mainly from the order Caudovirales (63.9% and 79.7% respectively). Eukaryotic viruses were identified in both healthy and diarrheic sample sets. The most prevalent families in both sets were Herpesviridae and Retroviridae, present in all samples. Other eukaryotic viral families identified were from the Papillomaviridae and the giant virus family Mimiviridae, detected in the majority of samples. Sequences similar to viruses in the algae virus family Phycodnaviridae were also identified. Interestingly, sequences with high similarity to viruses within the Astroviridae and Caliciviridae families were identified only in samples from the dogs with acute diarrhea (5/8 dogs and 2/8 dogs, respectively).

In conclusion, this metagenomic analysis of the dog virome revealed that largest proportion of viruses detected in fecal samples from both the diarrheic and healthy samples were bacteriophages. The virome contained viral sequences from a range of different virus families, including known pathogens.

GI-41
ANALYTICAL VALIDATION OF A GAS CHROMATOGRAPHY/MASS SPECTROMETRY METHOD FOR THE QUANTIFICATION OF 3-BROMOTYROSINE IN DOG SERUM. P. Sattasathuchana1, N. Grützner2, V.R. Rangachari3, N. Berghoff4, N. Thengchaisri5, B.C. Guard6, J.S. Suchodolski7, J.M. Steiner. 1Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA, 2Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

Peripheral eosinophil counts do not always correlate with the presence of eosinophilic infiltration in the gastrointestinal tract. 3-Bromotyrosine (3-BrY) is a stable product of oxidation formed in eosinophils by eosinophil peroxidase. In humans, 3-BrY has been described as a non-invasive biomarker of diseases associated with eosinophilic activation, such as asthma. However, serum 3-BrY concentrations have not previously been evaluated in dogs. Therefore, the objectives of this study were to develop and analytically validate an Electron Impact Ionization Gas Chromatography/Mass Spectrometry assay for the measurement of 3-BrY concentrations in canine serum and to establish a reference interval for serum 3-BrY concentrations in healthy pet dogs.

To analytically validate the method, excess serum samples from dogs were utilized. Limit of blank (LOB), limit of detection (LOD), linearity, accuracy, precision, and reproducibility were determined. To establish a reference interval for serum 3-BrY concentration a nonparametric method was used. Serum samples were collected from 34 healthy pet dogs (median age: 4.0 years [range: 1-10]; sex: female [n = 15] and male [n = 19]; dog breeds: pure breed [n = 27] and mixed breed [n = 7]). The LOD and LOD of the assay were 0.33 and 0.63 μmol/L, respectively. The observed signal-to-expected (O/E) ratios for dilutional parallelism (4 different samples diluted at 1:2, 1:4, and 1:8) ranged from 84.0% to 134.4% (mean ± standard deviation (SD): 109.6 ± 17.2%). The O/E for spiking recovery ranged from 79.1% to 126.7% (mean ± sd: 98.7 ± 11.2%) for three different serum samples that were spiked with 3-BrY standards (2.5, 5, 10, and 20 μmol/L). Intra-assay coefficients of variation (%CV) for four different serum samples were 7.3, 13.9, 4.7, and 13.8%. Inter-assay%CVs for four different serum samples were 5.7, 5.6, 8.1, and 11.0%. The reference interval for serum 3-BrY concentrations in healthy pet dogs was established as <1.13 μmol/L (median: <0.63 μmol/L [range: <0.63 to 1.13]).
In conclusion, the Electron Impact Ionization Gas Chromatography/Mass Spectrometry method for measurement of 3-BrY described here is sensitive, linear, accurate, precise, and reproducible for the quantification of 3-BrY in dog serum. Further studies to validate the clinical utility of the measurement of 3-BrY concentrations in serum samples from dogs with various cosinophilic diseases, including cosinophilic gastroenteritis, are warranted.

GI-42
EFFECT OF BODY CONDITION SCORE AND BODY MASS INDEX ON SERUM LIPIDS AND PANCREATIC LIPASE IN CATS. M.S.M. Castelo, A.B. Vieira, A.S. Mattos, M.C.N. Castro, F. Mendes-de-Almeida, N.F. Almosny, N.X. de Alencar. College of Veterinary Medicine, Universidade Federal Fluminense, Rio de Janeiro, Brazil.

Obesity is an important disorder in cats that might be associated with changes in lipid metabolism, which could have a role in the development of obesity-associated diseases. The effect of obesity and dyslipidemia on serum concentrations of feline pancreatic lipase (fPLI) is unknown. The purpose of the study was to determine the influence of body condition score (BCS) and body mass index (BMI) on serum triglycerides (TG), total cholesterol (TC), and fPLI concentrations in healthy cats.

Serum samples from 86 clinically healthy, lean and overweight, pet cats (12 h fasted) were used to measure serum lipids (colorimetric assay, Labtest®) or stored at-20°C until ILIPI (Snap ILIPI Test®) determination. All BCS and BMI determinations were performed by 2 of the authors (MSMC & ASM). Using the BCS system, 33 cats were classified as lean (BCS 3/5), 32 as overweight (BCS 4/5), and 21 as obese (BCS 5/5). Based on BMI calculation as previously described (Burrutwick, 2000), 17 cats were considered lean (≤ 30% estimated body fat) and 69 were overweight (> 30% estimated body fat). Data are presented as mean ± sd.

Overweight and obese cats had significantly higher serum TG concentrations than lean cats regardless of the body composition method used. Using ANOVA, mean serum TG concentration increased in cats with BCS 4/5 (100.3 ± 74.02 mg/dL; p = 0.0006) and BCS 5/5 (123.0 ± 64.54 mg/dL; p < 0.0001) compared with BCS 3/5 (45.8 ± 19.52 mg/dL). Using Student t test, cats with BMI > 30% had higher TG concentration (94.3 ± 68.27 mg/dL; p = 0.006) than cats with BMI < 30% (47.1 ± 20.35 mg/dL). No changes were seen in serum TC concentration between lean and overweight cats (p = 0.60-1.00).

Although 10 cats had abnormal Snap ILIPI results (BCS 3/5 = 5, BCS 4/5 = 4, BCS 5/5 = 1; BMI ≤ 30% = 4, BMI > 30% = 6) no differences on this qualitative determination were seen between lean and overweight cats when analyzed by Fisher’s exact test (p = 0.10-0.50).

We conclude that overweight and obese cats is associated with increased serum concentrations of TG but not TC, and that an abnormal ILIPI result occurs in approximately 12% of healthy cats regardless of body composition.

HM-2
REFERENCE INTERVAL GENERATION OF THREE PLATELET FUNCTION TESTS IN HEALTHY CATS. K. Ho, A. Abrams-Ogg, D. Wood, L. O’Sullivan, G. Kirby, S. Blois. Comparative Hemostasis Laboratory, University of Guelph, Guelph, Ontario, Canada.

Traditional optical aggregometry is operator intensive and dependent, making it impractical for clinical use. In this study, platelet function of 55 healthy client-owned cats was assessed using user-friendly tests that assessed agonist-induced aggregation by different methods: Multiplate Analyzer® (MP, by impedance), PFA-100® (by mechanical aperture closure), and Plateletworks® (PW, by platelet enumeration), to develop institutional reference intervals. PFA-100 and PW reference intervals have been previously reported (“Vet Clin Path 2008;37:385-388, “Can J Vet Res 2009;73:73-76). Cats were healthy based on history, physical examination, CBC, serum biochemistry, and FELV/FIV tests. After sedation with ketamine (2 mg/kg) and butorphanol (0.2 mg/kg) IV, atraumatic jugular venipuncture was performed using a 21ga butterfly and Vacutainer® system to draw blood sequentially into serum, citrate, and EDTA tubes, followed by syringe draw for PW tubes. Platelet function testing was performed for MP and PFA-100 in replicates using citrated whole blood as per manufacturers’ instructions. PW platelet counts were obtained by impedance (Vetscan HM®5, in duplicate) and flow cytometry (Advia 2120®, single value); the EDTA sample was used for baseline platelet count. Agonists were ADP, colla-

induced inhibition of platelet function; then determine if aggregometry analysis after the incubation of platelets with aspirin, a methodology established in human medicine, could predict those individual dogs that were previously shown to be resistant to aspirin-induced platelet dysfunction.

Platelet function was evaluated in 16 healthy dogs prior to (Day 0) and during (Days 3 and 7) low-dose aspirin therapy (1 mg/kg Q24 hrs PO, 7 days) using turbidimetric (platelet-rich plasma) and impedance (whole blood) aggregometry with collagen as an agonist and PFA-100® closure time (collagen/epinephrine cartridge). Aggregometry was tested in quadruplicate, and PFA-100® tested in duplicate, and averaged. Following a 2 week washout period, platelet-rich plasma collected from each of the previously evaluated dogs was incubated with aspirin (100 μM) for 15 minutes and was assessed via turbidimetric aggregometry with collagen as an agonist.

Low-dose aspirin decreased mean maximal amplitude on turbidimetric aggregometry to a more than 25% reduction from baseline Day 0 value (an established human criterion) in 56% of dogs by Day 3 and in 75% by Day 7. Mean maximal amplitude on impedance aggregometry was decreased to a more than 25% reduction from baseline in 81% of dogs on Day 3 and in 69% on Day 7. Only 25% of dogs had mean PFA-100® closure times of more than 300 seconds (established human cut-off) on Days 3 & 7 of aspirin therapy. In contrast, turbidimetric platelet aggregometry performed after in vitro exposure of platelets to aspirin using platelets collected from all 16 previously-studied dogs demonstrated marked and consistent suppression of platelet aggregation.

Based on turbidimetric aggregometry results after low-dose aspirin, the majority of dogs studied were classified as ‘aspirin responders’. Only half responded after 3 days of aspirin, while 75% responded after 7 days, indicating progressive inhibition of platelet function. Platelet dysfunction was not consistent in all dogs at all times, and some dogs had retained platelet function despite aspirin therapy. Compared to turbidimetric, impedance aggregometry and PFA-100® results were inconsistent from sample to sample even when run concurrently, suggesting that turbidimetric aggregometry is the preferred technique for assessing aspirin-induced platelet dysfunction.

Poor agreement was seen between in vitro aspirin incubation test results and all prior tests of platelet function in dogs receiving aspirin. Platelet function in dogs, unlike people, is consistently inhibited by aspirin incubation, making this a poor technique for predicting aspirin resistance.

HM-1
IN VITRO AND IN VIVO ASSESSMENT OF PLATELET FUNCTION IN HEALTHY DOGS DURING EXPOSURE TO LOW-DOSE ASPIRIN. A. Haines, J. Thomason, C. Bulla, E. Seage, R. Wills, K. Lunsford, A. Mackin. Mississippi State University, Starkville, MS.

Low-dose aspirin is a common anti-platelet therapy used to prevent thromboembolism in dogs. However, despite low-dose aspirin, some dogs still experience thromboembolic complications, a phenomenon that in human medicine is termed aspirin resistance. There are no established diagnostic tests that can predict aspirin resistance in dogs prior to therapy. The purposes of our study were to use platelet aggregometry and point-of-care platelet function analysis (PFA-100®) to evaluate dogs receiving low-dose aspirin and identify individuals with resistance to drug-induced inhibition of platelet function; then determine if aggregometry analysis after the incubation of platelets with aspirin, a methodology established in human medicine, could predict those individual dogs that were previously shown to be resistant to aspirin-induced platelet dysfunction.

Platelet function was evaluated in 16 healthy dogs prior to (Day 0) and during (Days 3 and 7) low-dose aspirin therapy (1 mg/kg Q24 hrs PO, 7 days) using turbidimetric (platelet-rich plasma) and impedance (whole blood) aggregometry with collagen as an agonist and PFA-100® closure time (collagen/epinephrine cartridge). Aggregometry was tested in quadruplicate, and PFA-100® tested in duplicate, and averaged. Following a 2 week washout period, platelet-rich plasma collected from each of the previously evaluated dogs was incubated with aspirin (100 μM) for 15 minutes and was assessed via turbidimetric aggregometry with collagen as an agonist.

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HM-2
REFERENCE INTERVAL GENERATION OF THREE PLATELET FUNCTION TESTS IN HEALTHY CATS. K. Ho, A. Abrams-Ogg, D. Wood, L. O’Sullivan, G. Kirby, S. Blois. Comparative Hemostasis Laboratory, University of Guelph, Guelph, Ontario, Canada.
gen (COL), and arachidonic acid (AA) for MP; ADP/COL for PFA-100; and ADP and COL for PW.

Reference intervals for the three tests and their respective agonists were calculated using EP Evaluator and Analyze-it (Table 1). Strong aggregation responses occurred with MP, however the 95% CIs were wide. PW-ADP and PFA-100 results were similar to previous reports in healthy cats1,2. For PW, % aggregation based on impedance and flow cytometric platelet counts were significantly correlated (rho = 0.66 for ADP, rho = 0.72 for COL), with impedance-based % aggregation significantly lower than that of flow cytometry. To assess for analytic variation of PFA-100 and PW, intra and interindividual coefficients of variation (CVa and CVg respectively) and intraclass correlation coefficients (ICC) were calculated (Table 1). Analytic variation of MP could not be calculated as the instrument reports mean values. Correlations between the results of the three tests for any agonist were weak (all rho < 0.5). In conclusion, these platelet function tests are feasible for use in cats. Because of the wide CIs, single test results may be difficult to interpret. Both the HM-5 and Advia methods were used for PW platelet counts, but results are not inter-changeable.

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HM-4 ASSESSMENT OF PLATELET FUNCTION IN HEALTHY CATS IN RESPONSE TO COMMONLY PRESCRIBED ANTI-PLATELET DRUGS USING THREE POINT-OF-CARE PLATELET FUNCTION TESTS. Scuderi, E. Snead, S. Mehain, C. Waldner, T. Epp. Western College of Veterinary Medicine. University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Feline hypertrophic cardiomyopathy (HCM) increases the risk of thromboembolism. Cats with HCM are commonly prescribed anti-platelet drugs with limited proven efficacy of their use in this species. This study aimed to evaluate the effect of commonly prescribed anti-platelet drugs on feline platelets using three analyzers which evaluate platelet function by different means: Multiplate® (MP, by impedance), PFA-100® (by mechanical aperture closure), and Plateletworks® (PW, by platelet enumeration).

Thirty-six healthy laboratory-owned cats were randomly assigned to receive one of 3 treatments during an 8-day period: 1) aspirin 5 mg PO q72 h (ASA5); 2) aspirin 20.25 mg PO q72 h (ASA20), or 3) clopoidogrel 18.75 mg PO q24 h. Additionally, cats in groups 1 and 2 also received dual-agent therapy with the addition of clopoidogrel PO q 24 h on days 4-8. On days 1 (baseline), 4, and 8, cats were sedated with ketamine (2 mg/kg) and butorphanol (0.2 mg/kg) IV for jugular venipuncture using a 21ga butterfly catheter and Vacutainer® system to sequentially draw blood into serum or heparin, citrate and EDTA tubes, followed by syringe draw for PW. Platelet function testing was performed for MP and PFA-100 using citrated whole blood as per manufacturers’ instructions. PW platelet counts were obtained using a 21ga butterfly catheter and Vacutainer® system to sequentially draw blood into serum or heparin, citrate and EDTA tubes. The EDTA samples were used for baseline platelet counts. Data were analyzed by ANCOVA including treatment, age, sex, platelet count and Hct. In comparison to baseline, PW revealed platelet inhibition with both clopoidogrel alone and in conjunction with ASA at either dose in response to either ADP or collagen (COL) (p < 0.0001 for all), whereas platelet inhibition was demonstrated with ASA5 only with ADP (p < 0.0008), and ASA20 only with COL (p = 0.014). For PFA-100 using ADP/COL cartridges, platelet inhibition was seen with clopoidogrel alone on day 4 (p = 0.021) and day 8 (p = 0.0001), and with ASA5 (p = 0.0002), but not with ASA20 (p = 0.06); no inhibition was seen with either ASA5 (p = 1.0) or ASA20 (p = 1.0) alone. Platelet inhibition was not demonstrated for any treatment when assessed with MP using ADP or COL (p = 0.13-1.0), however inhibition was seen with clopoidogrel+ASA20 using arachidonic acid (p = 0.0009). Regardless of treatment group, platelet function assay, or agonist used, median results of all tests remained within institutional reference intervals, emphasizing the importance of comparing a post-treatment value to individual baseline value.

PW and PFA-100 may be clinically useful in assessing platelet inhibition in response to clopoidogrel, and PW may also be useful for monitoring response to ASA. Clopoidogrel resulted in more in vitro platelet inhibition than ASA, but the clinical relevance of this finding is not known. The MP system was of limited utility in this study, and further studies are required to optimize this assay for cats.
immunosuppressive drugs, intravenous immunoglobulins and vincristine can be given in combination with corticosteroids. The purpose of this retrospective study was to investigate the relationship between treatment protocol, outcome, and risk of relapse in 58 dogs diagnosed with primary ITP at the Western College of Veterinary Medicine between April 2000 and October 2013. Dogs were included in the study if they had been diagnosed with primary ITP after ruling out secondary causes. Data collected from the medical records included signalment, presenting complaint, physical exam, laboratory and imaging findings, treatment protocol, and outcome.

Of the 46 dogs whose outcomes (i.e. survived vs. died) were known and who received treatment, 37 (80.4%) were treated in our hospital and 9 (19.6%) were treated as outpatients. Twenty-seven (69.2%) of the inpatients survived to discharge; 10 (27.0%) either died or were euthanized in the hospital. Of all dogs treated, 13 (28.2%) received only a corticosteroid, 14 (30.4%) received a corticosteroid in combination with another immunosuppressant medication, 13 (28.2%) received a corticosteroid with a second immunosuppressant and vincristine, 4 (8.7%) received a corticosteroid and vincristine and 2 (4.3%) received other treatments. The average in-hospital stay was 5.1 days. The average time for all dogs in this study to develop a platelet count of >100,000/µL was 10.1 days. Of the 36 surviving dogs, 14 (38.9%) experienced a relapse of their ITP at some point during their lives. There was no association between survival to discharge for inpatients and treatment protocol (corticosteroid alone versus a corticosteroid in combination with another treatment (P=0.19)). There was also no significant association between outcome (i.e. alive vs. dead) and treatment protocol (P=0.34) for all dogs (inpatients and outpatients). However, dogs that received a corticosteroid alone were only half as likely to be confirmed as surviving compared to those treated with a corticosteroid in combination with another drug (relative risk 0.56, 95%CI 0.19 to 1.62).

Our study failed to show a significant difference in outcome for dogs that received only a corticosteroid versus a corticosteroid plus an additional medication(s). However, there was some evidence suggesting that the addition of a secondary treatment could improve the likelihood of survival in dogs with ITP.

HM-6
EICOSANOID LEVELS IN STORED UNITS OF CANINE PACKED RED BLOOD CELLS. R. Blake, J. Lee, M.K. Ross, T. Archer, A. Mackin, J. Thomason. Mississippi State University College of Veterinary Medicine, Starkville, MS.

Packed red blood cell transfusions are commonly used to treat anemia in dogs. However, despite transfusions being potentially life-saving, transfusion reactions can occur in over 10% of all dogs receiving blood products. The duration and environment in which packed cells are stored and handled can have an impact on the accumulation of vasoactive and pro-inflammatory molecules such as eicosanoids, and the presence of such molecules in transfused blood may contribute to transfusion reactions. Our study measured the levels of eicosanoids that accumulated in units of canine packed red blood cells during standard clinical storage and transfusion procedures.

Twenty-five units of packed cells were collected from 14 healthy donor greyhounds and stored at 4°C until needed for clinical use (storage duration ranged from 1 to 23 days, median 16 days). Initial baseline plasma samples were collected from each unit following processing and before storage. Additional samples were collected from the same unit immediately prior to transfusion, and at the completion of the transfusion. Concentrations (ng/mL) of arachidonic acid, PGF₂α, PGE₂, PGD₂, thromboxane B₂ (a stable metabolite of thromboxane A₂), 6-keto PGF₁α (a stable metabolite of prostacyclin, PGI₂), leukotriene B₄, and the endocannabinoid 2-arachidonoylglycerol (2-AG) were quantified by liquid chromatography-mass spectrometry.

Mean arachidonic acid concentration decreased by 47% during storage, with a 71% decrease from baseline after transfusion. During storage, there was a 3.1 and 2.9-fold increase in thromboxane B₂ and 6-keto-PGF₁α concentrations, respectively, while, after transfusion, there was a 7.4 and 5.3-fold increase above baseline in thromboxane B₂ and 6-keto-PGF₁α concentrations, respectively. There was a 3.4 and 16.5-fold increase above baseline in PGF₂α concentrations after storage and transfusion, respectively. There was a 19.4 and 9.4-fold increase above baseline in the leukotriene B₄ concentration during storage and after transfusion, respectively. Concentrations of the endocannabinoid 2-AG decreased by 56% and 35% below baseline during storage and after transfusion respectively.

Our study suggests that both during storage of packed red blood cells and during subsequent transfusion, arachidonic acid is continually being utilized through either the leukotriene or the prostaglandin pathways. During storage and transfusion, concentrations of many vasoactive eicosanoids within units of stored packed cells increase markedly. Further exploration of collection, storage and transfusion methods that may reduce levels of eicosanoids in canine packed cells is warranted, as is investigation of the relationship between increased eicosanoid concentrations in stored blood products and the subsequent incidence of transfusion reactions in recipient dogs.

HM-7
STERNAL BONE MARROW ASPIRATION IN 69 DOGS WITH CLINICAL DISEASE (2008-2013). C.J. Greenwood, A. Marges, A. Abraham-Ogburn, D. Bienne. Ontario Veterinary College (OVC), University of Guelph, Guelph, ON, Canada.

Bone marrow aspirates from patients with hematological abnormalities have historically been acquired from the proximal humerus, iliac crest or femur. Use of these sites may be associated with lameness and anesthetic risk. In 2008, clinicians at OVC began routinely acquiring bone marrow aspirates from an alternative site (the cranial sternum) in patients under light or no sedation using 20-23 gauge hypodermic needles rather than surgical aspiration needles. Sternal aspirates are of equivalent quality to humeral and iliac samples in healthy Beagles (Vet Clin Pathol 2013;42:170-176).

The purpose of this retrospective case series was to characterize the diagnostic value, quality, and safety of sternal aspirates (SA) for cases with hematological abnormalities. Anemia was defined as hemoglobin <10 g/dL, hematocrit <33%, and red blood cell count <4.5 million/µL. Neutropenia was defined as neutrophil count <1.8 million/µL, and lymphocytosis was defined as lymphocyte count >2.0 million/µL. Pet owners were asked to consent to an alternative site being used for bone marrow aspirates. Bone marrow aspirates could be used to diagnose hematological or cancer-related disease.

Sixty-nine dogs underwent sternal marrow aspirates from healthy Beagles (n = 54) and macrocytosis (n = 42) were the most frequent hematological abnormalities. Patient weight (range: 2.97 - 54.5 kg), breed, and age (range: 0.65 - 13.11 years) were highly variable. Thirty-three clinicians in training programs obtained SA assisted by 9 faculty. Sedation was reported in 46 cases, and specified in 38 cases. Butorphanol by itself was most frequently used (n = 26). Complications reported included vocalization (1 case), mild agitation (2 cases), and bruising (1 case). Pneumothorax did not occur. In 82.6% (57/69) of cases a morphological diagnosis was initially reported, and included 73.8% (31/42) “excellent”, “remarkable” or “good” quality assessments, while 10.5% (5/47) of smears were considered of insufficient quality. Forty-six slides were available for review. Single pathologist re-evaluation of smears revealed (mean, SD, median) appropriate quality (3.41/5, 1.81, 5/5), cellularity (2.65/3, 0.60, 3/3), and particle number (1.83/3, 1.35, 3/3). A cytologic diagnosis was established in 76.1% of re-graded cases (35/46). Acute myeloid leukemia and granulocytic hyperplasia were most frequently diagnosed. Post-mortem reports concurred with SA findings in 8/8 cases.

Sternal bone marrow aspiration is a safe procedure suitable for application in non-sedated or lightly sedated patients, which yields smears of high quality, cellularity, and particle number. Sternal bone marrow aspiration is a valuable and safe clinical test for diagnosing most common canine bone marrow diseases.
**HM-8**

**PREVALENCE OF THE DAL BLOOD TYPE IN DOBERMAN PINCERS AND IN CANINE BLOOD DONORS.** S. Maruyama, H. Hosoe, K. Nagamatsu, R. Nakine v using the gel column technology (DiaMed). Blood banks were enrolled in the study. Dogs were blood typed for Dal transfusions or in hemolytic transfusion reactions. Its high frequency of anti-Dal phenotype did not vary significantly by gender (p = 0.0001). DEA 1.1 blood type was 37.4% among Doberman Pinchers (95% C.I.: 32.0-42.8%). The prevalence of Dal-negative blood type and its mode of inheritance in Doberman Pinchers. The secondary objective was to identify Dal-negative healthy blood donors.

From February 2008 to November 2013, 308 Doberman Pinchers and 112 blood donors from Tufts University, the University of Guelph and the University of Montreal client population and their blood donor programs, along with some private blood banks were enrolled in the study. Dogs were blood typed for Dal using the gel column technology (DiaMed).

The prevalence of Dal-negative blood type was 37.4% among Doberman Pinchers (95% C.I.: 32.0-42.8%). The Dal-negative phenotype did not vary significantly by gender (p = 0.75, male: 60/157; female: 55/151) or by age (p = 0.14); however, it varies significantly between breeders (p < 0.0001). DEA 1.1 blood type did not vary significantly between Dal-negative (34/106) and Dal-positive (34/87) Doberman Pinchers (p = 0.31). Pedigree analysis suggests an autosomal dominant mode of inheritance of the Dal blood type among Doberman Pinchers. Only 5 Dal-negative dogs enrolled in blood donor program were identified, but all were Doberman Pinchers (17 out of 132 being Doberman Pinchers).

In this study, more than a third of Doberman Pinchers tested were Dal-negative. Given the prevalence of von Willebrand’s disease among the breed and the scarcity of Dal-negative canine blood donor, Doberman Pinchers have a significant risk of facing transfusion incompatibilities when in need of blood.

**HM-9**

**EFFECTS OF IN VITRO EXPOSURE OF CANINE PLATELETS TO PENTOXIFYLLINE ON PLATELET AGGREGOMETRY.** J. Thomason, T. Archer, C. Bulla, A. Mackin. Mississippi State University College of Veterinary Medicine, Starkville, MS.

In humans, platelet aggregation is significantly decreased by exposure to pentoxifylline. However, there is greater inhibition of platelet function when measured by impedance aggregometry using whole blood than when measured by turbidimetric aggregometry using platelet-rich plasma, suggesting that the presence of red and/or white blood cells enhances the anti-platelet effects of pentoxifylline. In dogs, a recent study using impedance aggregometry showed that a single dose of pentoxifylline did not inhibit platelet aggregation. However, that study also utilized ChronoLume, a commercial agent containing luciferin, luciferase, glucose sulphate, human serum albumin, stabilizers and buffer that is used during aggregometry to study concurrent APTT release as an indicator of platelet activation. Unlike other species, the addition of ChronoLume to canine platelets can induce spontaneous irreversible platelet aggregation, potentially negating and concealing drug-induced platelet inhibition. The aim of our study was to evaluate the effects of in vitro exposure of canine platelets to pentoxifylline on platelet function as measured via both turbidimetric and impedance aggregometry, with and without ChronoLume.

Blood was collected from seven healthy dogs, and platelet function was determined both without exposure to pentoxifylline (baseline) and after a 30 minute in vitro exposure to two clinically relevant concentrations of pentoxifylline (1 µg/ml & 2 µg/ml). Platelet function was assessed via turbidimetric and impedance aggregometry, with collagen as an agonist, both with and without ChronoLume.

Based on impedance and turbidimetric aggregometry, with and without ChronoLume, there was no significant change in platelet function after exposure to two concentrations of pentoxifylline. With impedance aggregometry, however, there was significantly greater platelet aggregation with ChronoLume when compared to no ChronoLume (Figure 1). Our study confirms that canine platelet aggregation, unlike in people, is not inhibited by exposure to pentoxifylline. Our study also demonstrates that the apparent lack of inhibition reported in previous studies is not due to the effects of ChronoLume. Our study has revealed that, when using impedance aggregometry, the addition of ChronoLume can markedly enhance platelet aggregation in normal canine platelets.

**HM-10**

**IDENTIFICATION OF A MISSENSE MUTATION IN THE FACTOR XII GENE IN SIBLING CATS WITH FACTOR XII DEFICIENCY.** H. Maruyama, H. Hosoe, K. Nagamatsu, R. Nagamatsu, R. Kano, T. Watari, H. Kamata. Nihon University, School of Veterinary Medicine, Kanagawa, Japan.

Factor XII (FXII) deficiency is a common hereditary condition in cats. This deficiency presents as prolonged activated partial thromboplastin time (APTT). Disseminated intravascular coagulation (DIC) also results in prolonged APTT. In FXII deficiency cats with disease that can cause DIC, differentiating between DIC and non-DIC is difficult, and a genetic test of FXII gene might be useful to distinguish these conditions. The aim of this study was to identify mutations responsible for FXII deficiency in cat.

FXII activity (FXIIa) and the FXII gene were examined in six clinically healthy sibling cats; two had with prolonged APTT, and four had normal APTT. Prothrombin time (PT) was within normal range in all cats. FXIIa in the two cats with prolonged APTT was severely reduced (7.1% and 9.3%, respectively), but FXIIa in the four cats with normal APTT was only moderately reduced (36.0 to 46.3%). A missense FXII gene mutation (G554A) was identified in each cat; this mutation was homozygous in both cats with severely reduced FXIIa, and it was heterozygous in the four cats with moderately reduced FXIIa.

HEK293 cells and western blot analysis were used to assess the effect of this mutation on the expression of feline recombinant FXII protein. Wild-type feline FXII recombinant protein was secreted into supernatant; however, the G554A mutant feline FXII recombinant protein remained within cells. These results may be useful, not only to establish genetic test of FXII deficiency, but also to facilitate research on pathology of FXII deficiency in cats.
Hematopoietic Stem and Progenitor Cells Mobilization after Administration of Recombinant Human Granulocyte-Colony Stimulating Factor in Dogs. E.P. Son, J.I. Han, M.H. Kang, H.M. Park. KonKuk University College of Veterinary Medicine, Seoul, South Korea.

Dose-dependent effects of granulocyte colony stimulating factor (G-CSF) on canine hematopoietic stem and progenitor cells (HSPCs) are unknown. The study was conducted to evaluate the dose-dependent effect of recombinant human G-CSF (rhG-CSF) on the intravascular mobilization of HSPCs in dogs.

A total of 12 healthy dogs were included in the study. All dogs were randomly divided into 4 groups: control and G-CSF group receiving different dosage of G-CSF (5, 10 and 50 μg/kg, respectively). Each group was administrated subcutaneous saline or G-CSF for 5 consecutive days (from day 0 to day 4). During the experiment, complete blood count and serum biochemistry profiles were performed every day. On day 0, 3, 5 and 7, the peripheral blood or bone marrow was collected and each sample was double-immunostained with CD34 and CD45 or IgG1 and CD45, respectively. Flow cytometric analysis was performed by using the immunostained samples. To assess the changes in WBC, neutrophil, lymphocyte, monocyte, eosinophil, RBC, hemoglobin, MCH, MCHC, platelet, and HSPCs, repeated measures analysis of variance (RM ANOVA) was used. Differences for all analysis were considered significant at P < 0.05.

In the peripheral blood, flow cytometry showed that the numbers of HSPCs in the group 3 and 4 were increased 3 days after the rhG-CSF administration in parallel with the dosage of the rhG-CSF. In the bone marrow, the numbers of HSPCs in the group 2 and 3 were decreased after rhG-CSF administration 3 days after the rhG-CSF administration. However, the level of decrease of HSPCs was not proportional to the dosage of rhG-CSF.

Taken together, the study demonstrated that the short-term G-CSF administration accelerates the mobilization of HSPCs to the peripheral blood in dogs by a dose-dependent manner.

Investigation of the Effects of Anemia on Thromboelastography. J.A. Vacca1, E.A. Spangler2. 1Department of Clinical Sciences and, 2Department of Pathobiology, College of Veterinary Medicine, Auburn University, Alabama.

The goal of this study was to determine if hematocrit has a predictable effect on the results of thromboelastography (TEG). TEG uses a sample of whole blood to assess coagulation based on viscoelastic changes and provides information regarding the time for initiation of clot formation, the rate of clot development, maximum clot strength and fibrinolysis. TEG has been used to identify hypercoagulable states in dogs with a variety of underlying diseases. The clinical significance of this finding is uncertain, but it may have prognostic value and/or help to guide anticoagulant therapy in these patients. Whole blood assays of coagulation are affected by all elements of blood, including RBCs, platelets and plasma. Studies in people show that clot strength measured by TEG increases as hematocrit decreases. Limited reports suggest that the same is true in dogs. Because many diseases that have been associated with hypercoagulable states are also characterized by anemia of varying severity, it is crucial to understand the magnitude of effect that anemia has on clot strength as an independent factor.

Blood samples from 16 healthy adult dogs were evaluated. Dogs were determined to be healthy based on history and normal laboratory findings including a complete blood count, chemistry panel and standard coagulation tests (PT, aPTT, AT, fibrinogen, D-dimer). Kaolin-activated TEG was performed 30 minutes after blood collection, before and after dilution of blood with autologous platelet-poor plasma to a hematocrit of 15% (n = 5), 20% (n = 5) or 25% (n = 6). To determine if there was also an effect associated with dilution of platelet number, blood samples from an additional 10 dogs were evaluated by TEG after dilution to a hematocrit of 20% using autologous platelet-poor and platelet-rich plasma.

Clot strength (MA) was significantly increased relative to baseline when the hematocrit was decreased, but the magnitude of change could not be predicted based on the severity of anemia and nearly all measured values remained within the reference interval. No significant difference in clot strength was found when the dilution groups were compared to one another. No significant difference in clot strength was seen following dilution of blood with platelet-poor vs platelet-rich plasma, although the mean platelet count in these groups was significantly different (platelet-poor 110,000/μL; platelet-rich 165,000/μL).

Anemia is associated with a significant increase in the clot strength measured by TEG, but most values remain within the reference interval and would not result in dogs being classified as hypercoagulable. These results indicate that it is important to recognize the effect of anemia when evaluating TEG results from sick dogs, although additional factors are likely contributing when dogs are found to be hypercoagulable. No effect associated with a moderate decrease in platelet number was found in our study.


The aims of the current study were to determine the effect of treatment with intravenous administration of human immunoglobulin G (hIgVgG) on outcome in dogs with idiopathic immune-mediated hemolytic anemia (IMHA), and to identify parameter variables that may be used as prognostic factors for affected dogs. Data were collected from medical records of dogs diagnosed with idiopathic IMHA. Thirty-seven dogs who met the inclusion criteria were enrolled in this retrospective study. Survival analysis was performed with the Kaplan-Meier curve. Using univariate and multivariate Cox proportional hazard model, variables recorded at the time of diagnosis were examined as possible prognostic factors.

The median survival time of dogs with IMHA who did not receive hIgVgG was 285 days (range: 2 to 1,526 days); for those who received hIgVgG, the median survival time was 225 days (range: 7 to 1,039 days). Survival rates in dogs with IMHA who did not receive hIgVgG were 72% at 3 months, 64% at 6 months, 47% at 1 year, and 25% at 2 years. In dogs with IMHA who received hIgVgG, the survival rates were 78% at 3 months, 67% at 6 months, 39% at 1 year, and 22% at 2 years. Age predicted the likelihood of survival among dogs with IMHA; however, no specific variable acted as a negative prognostic factor for the outcome of dogs with IMHA who were treated with hIgVgG.

This study showed that there was insufficient evidence to determine the effectiveness of hIgVgG in dogs with idiopathic IMHA. In addition, no associations between any variable and overall survival were identified in dogs who received hIgVgG.

Serum C-Reactive Protein and S100A12 Concentrations in Dogs with Hepatic Disease. S.M. Craig1, P. Manino1, R.M. Heilmann1, J.S. Suchodolski1, J.M. Stenér1, H.A. Hottinger2, S.L. Hunter1, J.A. Lidbury2. 1Gulf Coast Veterinary Specialists, Houston, TX, 2Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

C-reactive protein (CRP) is an acute-phase protein that is used in human medicine to assess inflammation. S100A12 is a marker of activated phagocytic cells and inflammation in humans. Serum concentrations of CRP and S100A12 have not previously been reported in dogs with hepatic disease. The objectives of this study
were to measure serum CRP and S100A12 concentrations in dogs with hepatic disease and to determine if there is a correlation between the concentration of either marker and the severity of clinical signs in dogs with inflammatory hepatic disease.

Serum samples from 63 dogs undergoing hepatic biopsy at Gulf Coast Veterinary Specialists and Texas A&M University Veterinary Medical Teaching Hospital, between 3/1/12 and 5/1/13, were collected. Dogs were divided into groups: inflammatory hepatic disease; 30 dogs (48%), hepatic neoplasia; 21 (33%), congenital portosystemic shunts; 6 (10%), and other hepatic disease; 6 (10%). The severity of clinical signs was assessed using a scoring scheme that included: activity, weight loss, GI signs, icterus, ascites, and hepatic encephalopathy (max. total score 18). Serum CRP concentrations were measured using a commercially available ELISA (Tridelta Development). Serum S100A12 concentrations were measured using an in-house ELISA. The association between disease group and increased serum CRP or S100A12 concentrations was assessed using the χ² test. For dogs with inflammatory hepatic disease, the correlation between the clinical score and serum inflammatory marker concentrations was evaluated using Spearman’s rank correlation coefficient (rₛ). Statistical significance was set at P < 0.05.

Twenty-eight out of 61 dogs (46%) had CRP concentrations greater than the upper limit of the reference interval (7.6 mg/L). Nineteen dogs out of 61 dogs (31%) had S100A12 concentrations greater than the upper limit of the RI (49-319 μg/L). The median (interquartile range) serum CRP and S100A12 concentrations were 6.4 (2.3-19.5) mg/L and 192 (113-417) μg/L, respectively. There was no association between disease group and concentrations was assessed using the 2 test. For dogs with inflammatory hepatic disease, the correlation between the clinical score and serum inflammatory marker concentrations was evaluated using Spearman’s rank correlation coefficient (rₛ). Statistical significance was set at P < 0.05.

Increased serum CRP and S100A12 concentrations were common in dogs with various types of hepatic disease. The dogs with inflammatory hepatic disease that had higher serum CRP or S100A12 concentrations had higher clinical scores, suggesting more severe clinical signs.

HP-2
ADRENAL FUNCTION IN CHOLESTATIC CATS. F. Buckley, J. A. Lilly, B. de Laforcade, C.R.L. Webster. Tufts Cummings School of Veterinary Medicine, Grafton, MA.

Cats with cholestatic liver disease that undergo invasive procedures under anesthesia, experience significant morbidity and mortality. One complication is peri-procedural refractory hypotension, a hallmark of critical illness associated adrenal insufficiency (CIRCI). We hypothesized that hyperbilirubinemic cats have relative adrenal impairment putting them at risk of such complications. Cats with hyperbilirubinemic disease that underwent cholecystocentesis between January 2003 and September 2012 were retrospectively reviewed (83 PUC, 9 during laparoscopy and 10 during laparotomy).

Complications and follow-up information were only reported for the PUC group. Additional procedures were performed concurrently with PUC in 79 of 83 (95%) cats. These included hepatic aspiration (65/83), esophagostomy tube placement (20/83), hepatic tru-cut biopsy (19/83) upper gastrointestinal endoscopy (18/83), and splenic aspiration (15/83). Complications were noted in 14 of 83 (17%). Eleven cats increased abdominal fluid, however all 11 had concurrent hepatic or splenic aspiration. In one cat a second attempt was required to penetrate the gallbladder wall, one cat developed a pneumoperitoneum, and in one cat the aspiration needle became occluded. There were no reports of gallbladder rupture or bile peritonitis. Hypotension requiring vasopressor therapy was not encountered in any cat. Seventy-two cats (87%) survived to discharge and 61 (74%) had a follow-up visit at the median 11 days. Of the cats that were euthanized (9/83, 11%) or died (2/83, 2%), none were reported as a consequence of the PUC. Amongst all cases, 78 had bile cytology performed and 98 had bile culture performed. Bacteria were identified cytologically in 10 of 78 (13%) samples, and 9 of these 10 had a positive bacterial culture. Overall, bile culture was positive in 15 of 98 (16%) samples, with the most common isolate being Escherichia coli (7). Of all cases, the most common cytologic diagnosis was hepatic lipidosis (51/67, 76%). The most common histopathologic diagnosis was inflammation (22/38, 58%).

In conclusion, PUC was safe in this group of cats with hepatic disease. Complications were likely associated with ancillary procedures performed at the time of PUC. Bile cytology was abnormal and/or bile culture was positive in 20/101 (20%) cats with suspected hepatic disease.

HP-3

While the etiology of feline cholangitis is unknown, an association with bacterial infection of the bile has been demonstrated. Cholecystocentesis has been utilized for bile collection in several species. Percutaneous ultrasound guided cholecystocentesis (PUC) has been shown to be safe in a small group of healthy cats. We aimed to evaluate the diagnostic utility of cholecystocentesis and the safety of PUC in cats with suspected hepatic disease.

One hundred and two records of cats with suspected hepatic disease that underwent cholecystocentesis between January 2003 and September 2012 were retrospectively reviewed (83 PUC, 9 during laparoscopy and 10 during laparotomy).

HP-4
SERUM TISSUE INHIBITOR OF METALLOPROTEINASE-1 CONCENTRATIONS IN DOGS WITH HEPATIC DISEASE. J.A. Lidbury, A. Rodrigues Hoffmann, J.K. Fry, J.S. Suchodolski, J.M. Steiner. Gastrointestinal Laboratory and, Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, J.M. Steiner. Nashville Veterinary Specialists, Nashville, TN.

Serum biomarkers for hepatic fibrosis have been developed for use in human patients. Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a component of the extracellular matrix. In humans with hepatic fibrosis increased amounts of this substance are released into the bloodstream due to an increased rate of extracellular matrix turnover. The aim of this study was to assess the utility of TIMP-1 as a biomarker for canine hepatic fibrosis.

Serum samples from 53 dogs with hepatic disease were collected: 18 with chronic hepatitis, 17 with hepatic neoplasia, 5 with congenital portosystemic shunts, and 13 with other hepatic diseases. For a control group serum samples from 24 healthy dogs were collected. Hematologic and biochemical data were assessed in these dogs.

Serum TIMP-1 concentrations were measured using a commercially available ELISA. All hepatic samples were evaluated histologically to determine the degree of fibrosis. Fibrosis was staged using a 5-point scale: no fibrosis, minimal fibrosis, mild fibrosis, moderate fibrosis, or severe fibrosis. Serum TIMP-1 concentrations were measured using a commercially available ELISA for use in dogs (USCN Life Science).
Differences in serum TIMP-1 concentrations between groups were analyzed using a Kruskal-Wallis or a Mann-Whitney test as appropriate. Post-hoc testing was performed with a Dunn’s test. Area under the receiver operating characteristic curve (AUROC) was used to assess diagnostic accuracy. Significance was set as $P < 0.05$.

The median (min-max) serum TIMP-1 concentrations for all dogs with liver disease and healthy dogs were 24 (5-100) and 20 (5-100) ng/mL, respectively ($P = 0.43$). The median serum TIMP-1 concentrations for dogs with none-to-mild (n = 30) hepatic fibrosis and dogs with moderate-to-severe hepatic fibrosis (n = 12) were 25 (5-100) and 22 (7-63) ng/mL, respectively ($P = 0.69$). The median serum TIMP-1 concentrations for dogs with hepatic neoplasia (n = 17) and dogs with non-neoplastic hepatic disease (n = 36) were 45 (6-100) and 21 (5-63) ng/mL, respectively ($P = 0.002$). Dogs with hepatic neoplasia had higher serum TIMP-1 concentrations than healthy dogs, but this difference did not reach statistical significance ($P < 0.1$). The AUROC for discriminating between dogs with hepatic neoplasia and healthy dogs was 0.75 (95% CI; 0.58-0.91). The AUROC for discriminating between dogs with hepatic neoplasia and dogs with non-neoplastic hepatic disease was 0.76 (95% CI; 0.60-0.93).

The results of this study do not support the clinical utility of serum TIMP-1 as a biomarker for canine hepatic fibrosis. However, in this group of dogs, measurement of serum TIMP-1 concentration had fair diagnostic accuracy for discriminating between dogs with hepatic neoplasia and healthy dogs or dogs with non-neoplastic hepatic disease. Further studies are needed to confirm this finding and to evaluate the diagnostic utility in a larger number of clinical patients.

**HP-5**

HEPATIC ENCEPHALOPATHY: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY (MRS) IN THE DOG. L. Malfassi, M. Dolera. La Ciudadana Fondazione Studi e Ricerche Veterinarie, Romanengo (CR), Italy.

Hepatic encephalopathy is a neuropsychiatric syndrome observed in patients with severe hepatic dysfunction. An abnormal serum levels of substances normally metabolized by the liver normal functioning (ammonia, mercaptans, phenols, short-chain fatty acids) together with glucose and electrolyte imbalances can cause an alteration of brain neurons and glia. In Human Medicine, this syndrome is demonstrable in vivo by magnetic resonance spectroscopy (MRS) of hydrogen. The purpose of this study was to evaluate the cerebral biological compounds using MRS in canine patients with hepatic encephalopathy. 20 dogs with clinical signs of hepatic encephalopathy, were enrolled in this study. The investigation included 1.5T H-MRS with a STEAM single-voxel sequence, TR 35-144-288 ms at level of piriform lobe. 10 normal dogs were studied as a control. The spectroscopic findings were compared to the duration and gravity of clinical symptoms, the morphological MRI findings, the blood ammonia level detected.

16 dogs presented congenital portosystemic shunt, 2 microepatitis, 1 microangiodysplasy and 1 cirrhosis. The values of ammonia detected ranged between 180 and 340 mcg/dl. At MRT examination, all acute patients showed hyperintensity of brain gray and white matter in T2-weighted images, whereas in chronic cases various degrees of brain atrophy were found. The most commonly MRS results encountered were: increased glutamine-glutamate complex (Gln/GLX/Cr: mean 0.40, SD 0.13), decreased choline (CHO/Cr: mean 1.14, SD 0.34), decreased myo-inositol (mI/Cr: mean 0.15, SD 0.08), reduction of n-acetyl-aspartate (NAA: 1.02 average, standard deviation 0.35). These findings in the spectroscopic variations compared to the normal spectrum are greater in patients with high levels of ammonia.

The increase in Gln/GLX, the reduction of CHO, NAA and ml may be suggestive of hepatic encephalopathy in dogs. These findings are similar to what is known in human medicine. The safe attribution of a general or neurological symptoms to liver dysfunction associated with hepatic encephalopathy may be difficult to implement by the clinician. The authors believe that the arguments deserve further study to evaluate the quantitative analysis of changes in metabolites as a function of duration of the symptoms.

**HP-6**


Contrast-enhanced ultrasonography (CEUS) of hepatic vein can assess the intrahepatic hemodynamic changes, and it has been studied as a noninvasive method assessing the severity of portal hypertension. However, there is no study about its usefulness in veterinary medicine. The purpose of this study was to clarify the utility of CEUS to evaluate the hemodynamic changes in a canine portal hypertension model.

Firstly, repeatability of CEUS of hepatic vein variables was investigated in six normal dogs. The time-intensity curve was analyzed for four parameters; hepatic vein arrival time (HVAT), time-to-peak (TTP), time-to-peak ratio (TTPR), area under the curve (AUC). The coefficients of variation of these parameters were 11.3%-31.9%, which were similar to those of human studies.

Secondly, we established a canine portal hypertension model and investigated the correlation between portal vein pressure and CEUS parameters. Catherization of the portal vein was performed by laparotomy and the outer end of the catheter was fixed subcutaneously in the abdominal wall. Intra-portal injections of 15 mg/kg microspheres at five-day intervals induced stable portal hypertension models within two months. TTPH showed a time-dependent reduction that was significantly correlated with increase of portal vein pressure ($P < 0.05$).

In conclusion, these results showed that CEUS of hepatic vein in dogs has similar repeatability as CEUS studies in people, which were clinically acceptable. Furthermore, the measurement of TTPH may be valuable to evaluate the arterialized hepatic blood flow and can be a noninvasive method to predict portal hypertension.

**HP-7**

AZATHIOPRINE AND PREDNISOLONE TREATMENT IN LABRADOR RETRIEVERS WITH CHRONIC HEPATITIS. M. Sakai, H. Tateishi, M. Koide, S. Hayakawa, Y. Sakamoto, T. Watai. Nihon University, Department of Veterinary Medicine, Kanagawa, Japan.

Similar to the USA and EU, Labrador retrievers in Japan have an increased risk of developing chronic hepatitis (CH). Prednisolone alone is a common therapy for canine CH; however, this drug is associated with side effects in large breeds. The medical records of eight Labrador retrievers with CH treated using a combination of azathioprine and prednisolone were retrospectively reviewed. CH was diagnosed by laparoscopic and histopathological examination in all dogs. The median age was 10 years (5.2-12.7 years), and included seven spayed females and one castrated male. Three dogs were asymptomatic upon presentation and were referred for increases in liver enzymes. In the other cases, clinical signs included icterus and/or ascites. Serum alanine aminotransferase was increased in all dogs, and hyperbilirubinemia (4), hypoalbuminemia (2), and abnormal results of coagulation tests (6) were also recorded. On laparoscopic findings, the liver had an irregular surface in seven dogs and acquired portosystemic collaterals were verified in four dogs with ascites. After azathioprine and prednisolone treatment, clinical symptoms and/or blood tests improved in seven dogs. There were no adverse effects in association with azathioprine but portal vein thrombosis (PVT) was found in two dogs. At the follow-up, one dog was euthanized and seven dogs had died of a hepatic insuffi-
ciency-related cause. The median survival time of dogs was 630 days (range, 21–2336 days).

The azathioprine-prednisolone combination may be a useful therapy for Labrador retrievers with CH. However, portal hypertension is a negative prognostic factor and we should be attentive to PVT.

IM-1

EXPRESSION OF HER2NEU EPIDERMAL GROWTH FACTOR RECEPTOR IN CANINE OSTEOSARCOMA: IMMUNOHISTOCHEMICAL SCORING SYSTEM AND ASSOCIATION WITH PROGNOSIS. C.E. Gross1, A. Ahuja, G. Habjaneza-N'dikuye1, A. Gaurnier-Hausser1, J. Engiles1, C. Bradley1, N.J. Mason1. 1University of Pennsylvania, Philadelphia, PA. 2Drexel University, Philadelphia, PA. 3Domino Veterinary Hospital, Concord, MA.

Osteosarcoma (OSA) is a highly aggressive mesenchymal tumor accounting for ~85% of canine bone tumors. Epidermal Growth Factor Receptor-2 (Her2/neu), is a membrane-bound receptor tyrosine kinase that plays an oncogenic role in different tumor types and is a potential immunotherapeutic target.

Approximately 50% of human primary OSA lesions express Her2/neu and high expression correlates with reduced survival. Studies evaluating Her2/neu expression in canine OSA are limited to one small retrospective study that identified over-expression in 4/10 primary tumors and suggested a correlation with reduced survival.

Medical records were reviewed from dogs diagnosed with appendicular OSA between 2005 and 2013. Primary and metastatic lesions were evaluated for Her2/neu expression by IHC. Signalment, tumor types and is a potential immunotherapeutic target.

The purpose of this study was to determine whether oral health status correlates with seropositivity for feline immunodeficiency virus (FIV) or feline leukemia virus (FeLV) in cats. Veterinarians at veterinary clinics, humane societies and shelters completed on-line training on oral conditions in cats, and then scored the oral health status of cats not previously vaccinated for FIV. Age, sex and results of retroviral ELISA testing were collected on-line training on oral conditions in cats, and then scored the oral health status of cats not previously vaccinated for FIV. Age, sex and results of retroviral ELISA testing were collected.

Results were analyzed by a standard logistic regression with binary outcome. Among all cats, 20.6% had gingivitis, 11.1% had periodontitis, 3.9% had stomatitis and 4.6% had other oral disease for an overall oral disease prevalence of 40.2%. Seropositivity was 4.6% and 3.7% for FIV and FeLV, respectively. Cats with inflammatory oral disease were older than cats without oral disease. Across all age categories, inflammatory oral disease was associated with a significantly higher risk of testing FIV positive than other oral disease or no oral disease. Stomatitis was most highly associated with risk of FIV

IM-2

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF DIFFERENCES BETWEEN MONOCYTES IN DOGS WITH AND WITHOUT OSTEOSARCOMA. J. Tuohy, B.D.X. Lascelles, J. Fogle. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

The purpose of this study was to identify phenotypic and functional differences between peripheral monocytes from untreated dogs with osteosarcoma (OSA) and age-matched controls.

Ficoll centrifugation was used to isolate peripheral blood mononuclear cells (PBMCs). The PBMCs were stained with antibodies against CD14, CD32, CD62L, CD11c, CCR2, CCR7, CD43, CX3CR1, and CXCR2 and analyzed using flow cytometry. In OSA dogs (n = 18), surface expression of CD16 was increased, and expression of CD62L, CCR2, CCR7, CD43, CX3CR1 and CXCR2 was significantly decreased, compared to control (n = 13) dogs, (p < 0.01 for all receptors, Mann-Whitney U). There was a trend towards higher peripheral blood monocyte counts in OSA (n = 18) compared to control (n = 10) dogs.

For functional studies, monocytes were then isolated from PBMCs using high speed cell sorting and half of the cells were stimulated with LPS. Following a six-hour LPS stimulation, reverse transcription rtPCR was used to assess TNFα, IL-10, IL-12, and COX-2 mRNA expression, and culture supernatant PGE2 levels were assessed by ELISA. No significant difference was noted in relative mRNA expression. PGE2 secretion was higher in OSA (n = 8) compared to control (n = 8) dogs (p = 0.04, Mann-Whitney U).

These results indicate a down-regulation of monocyte chemoattractant receptors in OSA-bearing dogs. Based upon these results, we postulate that monocytes are sequestered in the peripheral blood of OSA dogs and are unable to migrate to the primary tumor or sites of metastasis. Further work should evaluate chemotaxis in monocytes from control and untreated OSA dogs.
seropositivity. Cats with any type of oral inflammatory disease were more likely to test FeLV positive than oral healthy cats. The presence of inflammatory oral disease was associated with increased risk of retroviral seropositivity in infected cats. The retroviral status of cats with oral inflammatory disease should be determined, and appropriate management should be initiated.

**IM-4**

**IL-8 RECEPTOR EXPRESSION IN IMMORTALIZED DOG KERATINOCYTES (AND SPLEEN) AND HOW ANTIBIOTICS AFFECT IT. C. Wollic, C.R. Rinker, F.T.J. Fosset, S.N. Lavergne. University of Illinois, Urbana-Champaign, Department of Comparative Biosciences, Urbana, IL.**

Skin keratinocytes are more than the structural basis of the epidermis; they also play a role in skin immunity. For instance, they can release various cytokines and chemokines, which are mediators of inflammation and immune activation, in response to microbial challenges. In a previous study, we found that a commercial line of immortalized canine keratinocytes (CPEK cells) expressed an elevated amount of interleukin-8 (IL-8), and that its levels increased in presence of certain antibiotics.

Our aim in this project, therefore, was to investigate if CPEK cells also expressed the interleukin-8 receptor (CXCR1) and how exposure to antibiotics affected this expression.

CPEK cells were cultured in the presence or absence of several antibiotics (amoxicillin 2 mM; cephalixin 2 mM; sulfadimethoxine 2 mM; sulfamethoxazole 2 mM; amikacin 2 mM; enrofloxacin 0.5 mM) for a 24 h. The cells were collected and stored at -80°C. The lysate protein concentrations were quantified using a Bradford assay. Dog spleen lysates were used as positive controls. A commercial ELISA kit was used to measure IL-8 receptor protein levels.

We detected CXCR1 in both canine spleen and CPEK lysates, but at significantly higher levels in CPEK cells (102.5 +/-43 vs 24.5 +/-14.3 pg/mg of protein). All the antibiotics tested significantly decreased CXCR1 expression in CPEK cells (by 22-52%), but the cephalixin effect was significantly lower than with the other drugs.

This study shows that IL-8 can act in an autocrine and paracrine mode in CPEK cells, and that antibiotics can decrease its receptor’s expression. Further work will be required to confirm the presence of the IL-8 receptor in primary dog keratinocytes.

**IM-5**

**THE ROLE OF INFγ AND TNFα IN IN VITRO IMMUNOMODULATION OF FELINE MENCESHYMAL STEM CELLS. M. Parys, K. Anderson, J. Kruger, V. Yuzbashyan-Gurkan. Michigan State University, College of Veterinary Medicine, East Lansing, MI.**

Inflammation is a frequent component of many chronic diseases encountered in feline medicine. Cell-based therapy using mesenchymal stem cells (MSC) has been suggested as an alternative modality for treatment of inflammatory diseases. MSC express a variety of factors such as TGFβ, PGE2, iNOS, PD-L1 and IDO, which are capable of inducing long-term immunotolerance through induction of T-regulatory cells. The purpose of this study was to characterize changes in mRNA expression of genes known to play important roles in immunomodulation of MSC. Fully characterized MSC (n = 3) shown to have trilinage differentiation ability and to express CD90, CD105 and CD44, were exposed to varying amounts (0.1-100 ng) of feline recombinant INFγ, TNFα or both for 24 hours. Total RNA was isolated and quantitative RT-PCR was performed using custom designed primers targeting multiple genes with known involvement in immunomodulation of MSC. Our data indicate that stimulation with INFγ leads to strong upregulation of IDO (14 fold) and PD-L1 (4 fold) mRNA levels at even very low (1 ng/ml) cytokine concentration when compared to unstimulated cells. Stimulation with TNFα leads to strong (5 fold) upregulation of IL6. Expression of genes encoding TGFβ, HGF, PGES, and HMOX1 did not significantly differ from unstimulated cells. Upregulation of IDO after INFγ exposure highlights its important role in immunomodulatory properties of feline MSCs, while IL6 upregulation suggests that TNFα exposure may cause a proinflammatory response. In conclusion, immunomodulatory cytokines INFγ and TNFα differentially modulate the immunomodulatory properties of feline MSCs. Understanding these responses is crucial for future clinical applications of MSCs.

**IM-6**

**ALTERATIONS IN LYMPHOCYTE AND MONOCYTE POPULATIONS IN RESPONSE TO INFESTATION OF AMBLYOMMA AMERICANUM ON CATS. M.K. Sherrill¹, C.R. Reiner², M.V. Reichard³, J.E. Thomas⁴, A.J. Birkenheuer², H.K. Outi⁵, L.A. Cohn⁴.¹University of Missouri College of Veterinary Medicine, Columbia, MO., ²Oklahoma State University College of Veterinary Medicine, Stillwater, OK., ³North Carolina State University College of Veterinary Medicine, Raleigh, NC.**

*Amblyomma americanum* serves as a vector for multiple pathogens including *Cyttauxzoon felis* and *Francisella tularensis*, both important and potentially fatal infections of cats. Tick feeding is known to modulate the immune response, including depletion of T-cells populations, in humans, dogs, ruminants, and other species. The immune response to tick feeding on cats has not been described. The aim of this study was to characterize changes in populations of lymphocytes and monocytes following infestation by *A. americanum* on healthy cats.

Ten healthy purpose-bred cats were experimentally infested with 25 pairs of laboratory raised *A. americanum* ticks that were allowed to feed for 9 days. A complete blood count and whole blood flow cytometric assay were performed at baseline, 3, 6, 12, 15, 18, and 21 days post infestation (dpi). White blood cell populations assessed included percentage of T-cells (CD3+), T-helper (CD3+CD4+), T-cytotoxic (CD3+CD8+), B-cells (CD21+), regulatory cells (CD14+CD25+FoxP3+) and monocytes (CD14+). Numbers of each type of white blood cell on the CBC and percentages of aforementioned populations assessed by flow cytometry were compared to baseline using a repeated measures ANOVA; log transformations were used when residuals were not normally distributed. In three cases, Friedman’s test was used when nonparametric tests were more appropriate. Dunnett’s method was used to adjust for multiple comparisons. If the main effect of time was significantly different than zero, pair-wise comparisons to baseline were made.

Unlike previous reports of T-cell depletion in tick-infested animals, we found no significant change in lymphocyte numbers or mean percentage of circulating CD3+ T-lymphocytes, T-regulatory, or T-helper cells. There was an increase in% T-cytotoxic cells from days 3 to 21 dpi compared to baseline. From 3 to 12 dpi, the% B-lymphocytes were significantly decreased and the% monocytes were significantly increased. Pathogen transmission may be affected by immunologic response to *A. americanum* infestation in cats, a finding that deserves further investigation.

**IM-7**

**INFESTATION BY AMBLYOMMA AMERICANUM ON CATS LEADS TO INCREASED LEUKOCYTE PHAGOCYTOSIS. M.K. Sherrill, A.E. DeClue, H.K. Outi, J.E. Thomas, M.V. Reichard, A.J. Birkenheuer, H.K. Outi, L.A. Cohn.¹University of Missouri College of Veterinary Medicine, Columbia, MO., ²Oklahoma State University College of Veterinary Medicine, Stillwater, OK., ³North Carolina State University College of Veterinary Medicine, Raleigh, NC.**

Tick feeding inhibits the host innate immune system in several animal species, which could in turn affect pathogen transmission. Previous ow (1) studies revealed that *Ixodes* salvia inhibits neutrophil adhesion, superoxide secretion, and phagocytosis of spirochetes. *Amblyomma americanum* serve as vector for the important and
often fatal feline pathogens *Cytauxzoon felis* and *Francisella tularensis*. The aim of this study was to characterize leukocyte number, phagocytosis and oxidative burst following infestation with *A. americanum* on healthy cats to determine if phagocytic functions were suppressed.

Ten healthy purpose-bred cats were experimentally infested with 25 pairs of laboratory raised *A. americanum* that were allowed to feed for 9 days. Leukocytes were quantified every third day by complete blood count for 3 weeks following infestation. Phagocytosis of opsonized *E. coli* and *E. coli*-induced oxidative burst were assayed using commercial kits on blood samples collected at baseline (prior to infestation), and at 5 (with ticks still attached), 12 (3 days after tick removal), and 19 days post infestation (dpi). The percentage of cells exhibiting phagocytosis, the number of bacteria phagocytized, and the percentage of granulocytes exhibiting oxidative burst at baseline was 79.6 ± 4.1%.

These results differ from previous reports of neutrophil dysfunction and immunosuppression in tick-infested animals of other species. Infestation by *A. americanum* led to increased leukocyte phagocytosis, which would be an appropriate innate immune response.

### Table 1. Treg cell levels in 7 dogs with generalized demodicosis

<table>
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<th>Case No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Average</th>
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</thead>
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<td>CD4+FoxP3+ Treg cells (n)</td>
<td>31.8</td>
<td>24.75</td>
<td>20.58</td>
<td>27.75</td>
<td>25.89</td>
<td>15.58</td>
<td>13.15</td>
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</tbody>
</table>

**ID-1 CHARACTERIZATION OF CANINE CORONAVIRUS ASSOCIATED WITH CANINE NEONATAL ENTERITIS AND MORTALITY IN THE UNITED STATES.** B. Lecru, E. Dubovi, G. Whittaker, G. Duhamel. Cornell University College of Veterinary Medicine, Ithaca, NY.

There are recent reports from the European Union of emergent canine coronavirus (CCoV) variants associated with severe clinical disease and mortality in dogs. The purpose of this retrospective study was to characterize CCoVs associated with 11 cases of fatal enteritis in canine neonates that were submitted to Cornell University between 2008 and 2013. 11 dogs met the inclusion criteria for CCoV associated viral enteritis on the basis of histopathologic changes and demonstration of viral antigen in intestinal tissues by immunohistochemistry. RT-PCR and sequencing were used to genotype the CCoV RNA isolated from formalin fixed paraffin embedded tissue. This study provides the first confirmation that emergent CCoV-IIa and CCoV-Ib variants related to those in Europe are associated with severe canine enteritis in the United States. CCoV antigen was restricted to the small intestine with the notable exception of two cases in which the large intestine was also affected. The majority of dogs in this study were living in high-density housing conditions, indicating that breeding facilities, kennels, and shelters are high-risk environments for CCoV infection. These findings will assist with rapid laboratory diagnosis of enteritis and enhance surveillance for emerging intestinal viruses of global significance.

### ID-2 COMPARATIVE EFFICACY OF FELINE LEUKEMIA VIRUS INACTIVATED WHOLE VIRUS VACCINE AND CANARY-POX VIRUS-VECTORED VACCINE BY MODERN MOLECULAR ASSAYS AND CONVENTIONAL PARAMETERS.** M. Patel1, K. Carritt 1, J. Lane 1, H. Jayappa 1, M. Stahl 2. 1Merck Animal Health, Elkhorn, NE., 2Merck Animal Health, Summit, NJ.

The purpose of this study was to compare the efficacy of two commercially available feline leukemia vaccines, Nobivac2 Feline 2-FeLV (inactivated whole virus vaccine) and PureVax2 Recombinant FeLV (live canarypox virus-vectored vaccine) following challenge with virulent feline leukemia virus.

Cats were vaccinated subcutaneously at 8 and 11 weeks of age with Nobivac2 Feline 2-FeLV vaccine (Group A, n = 11) or PureVax2 Recombinant FeLV vaccine (Group B, n = 10), three weeks apart per manufacturer’s label. Group C (n = 11) served as age-matched, unvaccinated controls. Three months after second vaccination, all cats were challenged with virulent FeLV-A 61E. Challenge outcome was monitored for 12 weeks post-challenge (PC) for the development of persistent viremia utilizing a commercial FeLV p27 ELISA. Circulating proviral DNA and plasma viral RNA loads were determined by quantitative PCR and real-time RT-PCR assay, respectively, from week 3 to 9 PC to determine whether FeLV vaccination would prevent nucleic acid persistence.
Persistent viremia was observed in 0 of 11 (0%) of Group A cats, 5 of 10 (50%) of Group B cats and 10 of 11 (91%) of Group C cats. Cats in Group A were significantly protected from persistent viremia compared to Group B cats ($P = 0.013$) and Group C cats ($P < 0.0001$). No significant difference was found between Group B cats and Group C cats ($P > 0.063$). Persistent viremia preventable fraction was 100% for Group A and 45% for Group B. At the end of 9 weeks post-challenge, proviral DNA and plasma viral RNA loads were also significantly lower in Group A cats, 6 of 10 (60%) Group B cats and 9 of 11 (82%) Group C cats. Proviral DNA and plasma viral RNA loads were significantly lower in Group A than Group C cats ($P < 0.01$). Group A cats had significantly lower proviral DNA loads than Group B cats from week 6 to 9 ($P < 0.02$). Detectable plasma viral RNA loads were also significantly lower in Group A cats than Group B cats from week 7 to 9 ($P < 0.01$). Proviral DNA loads as well as plasma viral RNA loads were strongly associated with the persistently viremic cats.

The results demonstrate that Nobivac® Feline 2-FeLV vaccinated cats were fully protected against persistent viremia and had significantly lower amounts of proviral DNA and plasma viral RNA loads compared to PureVax® Recombinant FeLV vaccinated cats and unvaccinated control cats.

**ID-3**

**EFFICACY OF AZITHROMYCIN AND COMPOUNDED ATOVQUOINE FOR TREATMENT OF BABESIA GIBSONI IN A LARGE-SCALE DOGFIGHTING CASE.** S.K. Kirk1, J.K. Levy2, P.C. Crawford3, C.M. Leutenegger1. 1The American Society for the Prevention of Cruelty to Animals, New York, NY, USA., 2Maddie’s Shelter Medicine Program, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL., 3IDEXX Laboratories, Inc, West Sacramento, CA.

Most cases of Babesia gibsoni in North America are diagnosed in pit bulls and fighting dogs. Transmission is believed to be primarily transplacental or via bite wounds. Ticks can transmit B. gibsoni, but the full extent of other modes of transmission is unknown. The purpose of this study was to chronicle the response to treatment for Babesia gibsoni in pit bull dogs seized as part of a large-scale dogfighting case.

All dogs were screened for blood-borne pathogens, including Babesia spp., by PCR at the time of intake to a temporary shelter. Positive samples (37) were confirmed by sequencing to identify Babesia species. Infected dogs were treated with atovaquone (13.4 mg/kg PO TID with a fatty meal) compounded into capsules and commercially available azithromycin (10 mg/kg PO SID) for 10 days. PCR testing was repeated at 1 and 2 months post-treatment to assess treatment efficacy.

Follow-up testing revealed that all but 1 dog were PCR-negative at 1 month and 2 months post-treatment; 4 dogs were lost to follow-up at 2 months. One dog that was PCR-negative at 1 month was PCR-positive at 2 months. Three of the PCR-positive dogs were treated during pregnancy. Their 22 puppies were PCR-negative at 1 month of age, as were the 20 puppies available for testing at 2 months.

Use of compounded atovaquone capsules was as efficacious, less expensive, and more practical than the commercially available liquid formulation for large-scale treatment of B. gibsoni. Treatment during pregnancy may prevent vertical transmission of infection.

**ID-4**

**EVALUATION OF THE ORAL AND CONJUNCTIVAL MICROBIOTA IN CATS WITH FELINE IMMUNODEFICIENCY VIRUS INFECTION AND UNINFECTED CONTROLS.** J. Weese1, J. Nichols1, A. Litster1. 1University of Guelph, Ontario, Canada., 2Purdue University, West Lafayette, IN.

Feline immunodeficiency virus (FIV) infection can be associated with various opportunistic infections; however, the effect on the commensal microbiota is unknown. The objective of this study was to describe and compare the oral and ocular microbiota of cats naturally infected with FIV and FIV-negative control cats.

Oral and conjunctival swabs were collected from 19 FIV-positive cats and 12 FIV-negative cats. The microbiota was assessed by next generation sequencing of the V4 region of the 16s rRNA gene and analysed using mothur. A total of 1,286,063 sequences from oral swabs and n = 657,873 from conjunctival swabs passed all quality control filters. The Proteobacteria phylum dominated in oral swabs from both groups, accounting for 75% of sequences. Firmicutes was the next most abundant phylum (15.5%), followed by Fusobacteria (1.8%), Bacteroidetes (1.7%), Spirochaetes (1.2%) and Actinobacteria (1.0%). Six other phyla accounted for <1% each. FIV-positive cats had greater relative abundances of Bacteroidetes ($P = 0.016$) and Fusobacteria ($P = 0.009$) and fewer Proteobacteria ($P = 0.036$). Numerous differences were observed at lower taxonomic levels, including in potential oral pathogens such as Porphyromonadaceae (higher prevalence in FIV-positive cats, $P = 0.01$) there was no difference in population diversity (inverse Simpson’s index $P = 0.55$). However, there were differences in microbial population structure (parsimony test $P = 0.01$). Twenty-eight out of 121 operational taxonomic units (OTUs) were identified as indicators of FIV-positive status. No OTUs were present at a relative abundance of 1% or more in all samples, but 3 (Pasteurella, uncultured Pasteurellaceae and Mammheinia) were present at that abundance in 23 (74%) samples.

The conjunctival microbiota was predominated by Firmicutes (61%), followed by Proteobacteria (28%) and Verrucomicrobia (2.4%). FIV-positive cats had significantly fewer Bacteroidetes, Fibrobacter, Spirochaetes, Tenericutes and Verrucomicrobia, and more Deinococcus-Thermus ($P < 0.03$ for each). Numerous differences were present at lower taxonomic levels, including a marked overabundance of Staphylococcus in FIV-positive cats (43% vs 13%, $P = 0.01$). Diversity was greatest in the FIV-negative group (inverse Simpsons 7.7 vs 3.0, $P = 0.03$) and there was a significant difference in population structure (parsimony test $P = 0.018$).

These data suggest that FIV infection is associated with alteration of the oral and conjunctival microbiota. The clinical relevance is unclear in this population, but given that FIV-infected cats are deemed to be “at-risk” for certain oral and respiratory diseases, these results suggest that FIV-associated microbiota alteration could be a clinical concern.

**ID-5**


Fighting dogs often receive minimal preventative health care, and the potential for spread of infectious diseases is high. The purpose of this study was to describe the prevalence of infectious diseases in dogs rescued from fighting operations in order to guide medical protocols for immediate and long-term care.

A total of 269 pit bull dogs were seized in a multi-state investigation. Testing performed at intake included PCV, fecal flotation, SNAP 4DX®, and PCR for blood-borne infections. Fleas were present on most dogs, but few ticks were observed.

Samples were PCR-positive for Babesia gibsoni (32%), Candidatus Mycoplasma haemocanis (33%), Mycoplasma hominis (30%); seropositive for Dirofilaria immitis (9%); and flotation positive for Ancyllostoma (23%). The presence of anemia (PCV<37%) was associated with B. gibsoni infection (56% of infected dogs) ($P < 0.05$), but not with hemotropic Mycoplasma or Ancyllostoma.

Fighting dogs often receive minimal preventative health care, and the potential for spread of infectious diseases is high. The purpose of this study was to describe the prevalence of infectious diseases in dogs rescued from fighting operations in order to guide medical protocols for immediate and long-term care.
pattern. Anemia was common, but many cases were not associated with documented infections or parasitism other than fleas. Empirical treatments for all dogs should include broad-spectrum intestinal and external parasiticides and monitoring for anemia. Dogfighting case responders should be prepared for mass screening and treatment of B. gibsoni and heartworm infections and should implement protocols to prevent transmission of infectious and zoonotic diseases in the shelter and following adoption. For-mer fighting dogs should not be used as blood donors.


In the two years since the initial discovery of Canine Circovirus, DogCV, there has been substantial attention drawn to its potential role in disease. Before the discovery of DogCV, the only species of the genus Circovirus known to infect mammals were the porcine circoviruses (PCV1 and PCV2). We have characterized the complete genome of dog circovirus (DogCV) from the liver of a dog with severe hemorrhagic gastroenteritis, vasculitis, and granulomatous lymphadenitis. By phylogenetic analysis, DogCV-LCD1 groups with porcine circoviruses, forming a distinct clade of mammalian circoviruses, while avian circoviruses cluster separately. In a combined retrospective and prospective analysis using a combination of PCR, in situ hybridization (ISH), immunohistochemistry (IHC), and transmission electron microscopy, we have detected DogCV in the tissues of 12 other dogs with vascular compromise and/or histiocytic inflammation. Each case was independent dating back to 2009, indicating that this is a newly identified, not necessarily a newly emergent virus. Sequence analyses of a subset of these virus isolates reveal that DogCV capsid genes from different dogs vary by up to 8%. The prevalence of DogCV is more widespread than disease association, with the virus detected by quantitative real-time PCR in fecal samples from 19/168 (11.3%) dogs with diarrhea and 14/204 (6.9%) of healthy dogs. Among DogCV-positive dogs with diarrhea, co-infection with other canine diarrheal pathogens was detected in 13/19 animals (68%). It has been established for PCV2 that the immune status of the host and the presence of co-pathogens also play an important role in pathogenesis of that virus. Our results indicate that in dogs, circovirus infection alone or as a component of a polymicrobial infection, might contribute to disease.

ID-7 LONGITUDINAL STUDY OF VECTOR-BORNE PATHOGEN SEROPREVALENCE IN MID-MISSOURI DOGS. L.A. Cohn1, J. Brafi2, B. Thatch3, M.J. Beall4, R. Chandrashekar2, A. Platt5. 1University of Missouri, College of Veterinary Medicine, Columbia, MO, 2IDEXX Laboratories Inc, Westbrook, ME.

Vector-borne infections are an important cause of morbidity and occasional mortality in dogs, but healthy seropositive dogs are commonly identified. There is little information regarding the changes over time in serologic status of dogs incidentally identified as seropositive for Ehrlichia, Anaplasma, or Lyme. This study used a convenience population of healthy dogs between the ages of 1 and 10 years belonging to students and staff at the University of Missouri to follow serologic status of dogs over time. At each evaluation, owners completed a detailed history and a physical examination was performed. In addition, samples were obtained for CBC, serum chemistry profile, urinalysis, and serology. Plasma was separated and analyzed using a multi-analyte SNAP research ELISA for antibodies to C6 (lyme), p30/p30.1 (E. canis/E. chaffeensis), VLPT (E. chaffeensis), p28 (E. ewingii), p16 (E. canis), enz1 (Anaplasma genus), p44 apl (A. platys), and p44 aph (A. phagocytophilum).

A total of 118 dogs were enrolled in the study; thus far, two years of evaluation and over 500 total testing events are completed. Of the original 118 dogs, 5 have died (2 with cancer, 1 with seizures, 1 after an acute neurologic episode, and 1 for aggression) while another 4 have dropped out due to relocation. Three seropositive dogs have received tetracycline antibiotics during the last two years (two for clinical signs potentially attributable to rickettsial disease, one with heartworms). No dog was seropositive for Lyme, but dogs were commonly positive for ehrlichiosis. For p30/p30.1, 23/118 dogs (19.5%) were seropositive (10 consistently, 5 seroconverted, 2 converted from seropositive to negative, and 6 were intermittently seropositive). For VLPT, 26/118 dogs (22.0%) were seropositive (7 consistently, 8 seroconverted, and 11 have been intermittently seropositive). For p28, 19/118 dogs (16.1%) were seropositive (12 consistently seropositive, 4 converted from seronegative to positive, 1 dogs converted from seropositive to negative, and 2 dogs have been intermittently seropositive). For p16, 3/118 dogs (2.5%) were seropositive (all dogs have been intermittently seropositive). Anaplasmosis was less common than ehrlichiosis; for the genus antibody enz1, 2/118 dogs (1.7%) were seropositive (both were intermittently seropositive). For p44 aph, 1/118 dogs (0.8%) were seropositive (converted from negative to positive). For p44 apl, 3/118 dogs (2.5%) were seropositive (converted from seronegative to negative, and 2 have been intermittently seropositive).

Serologic evidence of infection with E. chaffeensis and E. ewingii were common. Dogs without clinical evidence of disease due to ehrlichiosis may remain seropositive for at least 2 years, and intermittent positives are not uncommon. Anaplasmosis was very rare, and Lyme was not detected in these Missouri dogs.

ID-8 MINOCYCLINE PHARMACOKINETICS AND PHARMACODYNAMICS FOR TREATMENT OF MITHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS INFECTIONS IN DOGS. M.G. Maaland1, L. Guardabassi2, M.G. Papich3, 1Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark, 2College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

Minocycline, a tetracycline used in human medicine, has therapeutic potential as a second-line agent against infections caused by methicillin-resistant Staphylococcus pseudintermedius (MRSP) in dogs. MRSP often contain tet(K), a tetracycline resistance determinant conferring resistance to tetracyclines licensed for veterinary use, but not to minocycline. The aim of this study was to establish rational dosage recommendations for minocycline use in dogs.

Six healthy dogs from a research colony were administered 5 mg/kg (IV) and 10 mg/kg (PO) of minocycline hydrochloride in separate crossover experiments. Minocycline concentrations in plasma and intestinal fluid (ISF), as well as in vitro plasma protein binding, were analysed by high performance liquid chromatography (HPLC). Pharmacokinetics (PK) were analysed on plasma and ISF concentrations. Minocycline susceptibility in 168 S. pseudintermedius isolates was assessed by broth microdilution according to CLSI standards. With an AUC/MIC ratio of 23 as target for antibacterial efficacy, Monte Carlo simulation using the obtained PK and susceptibility data was performed to assess Target Attainment at different drug dosages.

Values are reported as the mean (standard deviation). After the IV dose, the half-life (T1/2), volume of distribution, and clearance was 6.02 (6.15) hr, 1.5 (0.86) L/kg, and 0.173 (0.15) L/kg/hr, respectively. After the oral dose, the T1/2 and peak concentration were 4.14 (0.5) hr, and 3.4 (1.1) µg/mL, respectively. The oral absorption was 50.3% (20.8). The penetration of free (unbound) minocycline to the tissue compartment was 50.1% (17.3) after the oral dose as assessed by a ratio of the AUC values. The mean plasma protein binding was 65.8% (fraction unbound 0.34). Diffusion into tissue was higher than expected from the protein binding and plasma concentration data. The S. pseudintermedius MIC distribution was bimodal, with MICs in the range of 0.031–0.25 µg/mL for susceptible isolates. A high probability of Target Attainment was seen at oral dosages of 5
and 10 mg/kg twice daily to treat dogs with infections caused by *S. pseudintermedius* with MICs of ≤0.25 and ≤0.5 µg/mL, respectively.

In conclusion, the data from this study supports an oral dosage of 5 mg/kg twice daily for treatment of infections caused by minocycline-susceptible MRSP in dogs.

**ID-9 MOLECULAR DETECTIONS OF A HIGHLY PATHOGENIC PIROPLASMA IN DOGS: RANGELIA VITALII**


The piroplasma *Rangelia vitalii* is either a new as an old kind of highly pathogenic hemoparasitosis for dogs. Old due to the fact that it was described between 1910 and 1914. New because of highly pathogenic hemoparasitosis for dogs. Old due to the age of 5 mg/kg twice daily for treatment of infections caused by *Rangelia vitalii* susceptibles MRSP in dogs.

The present study aims at compiling the clinical and hematological findings of 28 cases of naturally infected dogs with *Rangelia vitalii*, confirmed by molecular identification (PCR).

The suspected samples had DNA extracted and subjected to Real Time PCR for *Rangelia vitalii*.

Of the 28 samples, 19 were from the state of Rio Grande do Sul, 6 from São Paulo, 2 from Minas Gerais and 1 from Santa Catarina. There was no prevalence between gender and the patients were 4 months to 11 years old. Hematological and clinical data were provided by the sample senders, indicate apathy (100%), anorexia (100%), pale mucous membranes (85%), fever (77%), jaundice (56%), splenomegaly (59%), dehydration (54%) and bloody diarrhea (52%). The most frequent hematologic changes were thrombocytopenia (100%) and reduction in hematocrit (88%), hemoglobin (81%) and RBC (77%). The leukocyte count was variable among individuals, without a standard. Of the 28 dogs, at least 61% were from rural or peri-urban areas and 76% of them had had recent contact with ticks. Of 20 cases that were followed, 35% dogs died.

The results indicate that *Rangelia vitalii* circulates among the canine population in southern and southeastern Brazil, and the disease has a high lethality.

**ID-10 PERFORMANCE COMPARISON OF SNAP® 4DX® PLUS AND ACCUPLEX® 4 FOR THE DETECTION OF ANTIBODIES TO B. BURGDORFERI AND A. PHAGOCYTOPHILUM.**

R.E. Goldstein1, M.D. Eberts2, M.J. Beall3, B. Thatcher3, R. Chandrashekar2, A.R. Alleman2, 1Animal Medical Center, New York, NY., 2Lakeland Veterinary Hospital, Baxter, MN., 1IDEXX Laboratories Inc, Westbrook, ME., 3Florida Fish and Wildlife Conservation Commission, Gainesville, FL.

The purpose of this study was to compare the performance of two vector-borne disease screening tests using a broad population of well characterized canine samples. Four hundred and sixty-four canine serum samples were included; 99 control, purpose bred research dogs with no exposure to ticks and 365 field-infected dogs from a broad geography to target *Borrelia burgdorferi* (>Bb) and *Anaplasma phagocytophilum* (>ApH) endemic areas. All samples were tested on SNAP 4Dx Plus (IDEXX Laboratories, Inc.) and AccuPlex4 (Antech Diagnostics, Inc.). A subset of samples was tested on *Bb* Western blot and *ApH* IFA. Repeatability of SNAP 4Dx Plus and AccuPlex4 was assessed by repeated testing (twice) of 56 samples and comparing *Bb* and *Anaplasma* results.

Percent agreement between the two tests for *Bb* and *Anaplasma* was 86% and 81%, respectively. Five of the 99 control research dogs tested positive for *ApH* on AccuPlex4 and were negative on SNAP 4Dx Plus. Four of these samples were also tested by *ApH* IFA and were negative. Of the 56 samples that were tested twice on the AccuPlex4 test, 18/112 (16%) test results for *Bb* or *ApH* differed from the first test event while SNAP 4Dx Plus had only 5/112 (4%) results that differed.

Clinically-significant differences between the SNAP 4Dx Plus and AccuPlex4 tests were recognized in this direct comparison. The SNAP 4Dx Plus Test had significantly better sensitivity and specificity for antibodies to *Bb*, fewer false positive for antibodies to *Anaplasma* and better test-to-test repeatability.

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<th><em>B. burgdorferi</em> Western blot (n=135)</th>
<th><em>A. phagocytophilum</em> IFA (n=159)</th>
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<td><strong>SNAP 4Dx Plus</strong></td>
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<td>** Accuracy**</td>
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**ID-11 PRELIMINARY DATA ARISING FROM A NOVEL FLORESCENCE IN SITU HYBRIDIZATION ASSAY TO DETECT FELINE PAPILLOMAVIRUSES.**

L.E. Demos1, M.D. Bennett1, J.S. Munday2, 1Waterford, Michigan, 2School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia, 3Department of Pathobiology, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand.

This report presents the preliminary data arising from the development of a novel fluorescence in situ hybridization (FISH) assay targeting FePVs. Papillomaviruses (PVs) are ubiquitous; found worldwide, they display high host specificity and can be isolated from humans as well as various domestic and wildlife species. Research suggests that PV infections of cats are associated with cutaneous and oral papillomas, viral plaques, Bowenoid in situ carcinomas and sarcomas. Currently, three PV genomes of domestic feline hosts have been fully sequenced. However, with increased reports of FePV-positive symptomatic cats in recent years, there is a need to investigate what role, if any, FePVs play in feline dermatoses. The link between these feline papillomaviruses (FePVs) and clinical disease is difficult to confidently assert due to the lack of an appropriate detection method.

FISH probes were generated from PCR amplicons of Felis domesticus papillomavirus type 2 (FdPV2) DNA, fluorescently labeled, then applied to formalin-fixed paraffin-embedded feline skin biopsies. Probe detection was facilitated via anti-digoxigenin Fab fragments conjugated with alkaline phosphatase (AP) and visualized with an AP-substrate fluorescent chromagen. Binding was interpreted as positive when confined to the nuclei of epithelial keratinocytes. Preliminary test cases were selected from lesions with previous histopathological diagnoses of PV infection that were concomitantly PCR positive for FdPV2 (n = 3). All test cases produced positive FISH results. This suggests that FISH can be a rapid and useful method for the detection of FePVs in situ. This, in turn, may have positive implications in prognosticating patient outcomes and devising treatment strategies.

**ID-12 PREVALENCE OF BORRELIA SPP. IN HOST-SEEKING TICK POPULATIONS AND WHITE-TAILED DEER IN NORTH CENTRAL FLORIDA.**

C. Boyce1, K. Sayler1, R. Chandrashekar2, M. Beall1, B. Clemons3, M. Cunningham3, A. Alleman1, 1University of Florida, College of Veterinary Medicine, Gainesville, FL., 2IDEXX Laboratories, Inc., Westbrook, ME., 3Florida Fish and Wildlife Conservation Commission, Gainesville, FL.

Many species within the *Borrelia burgdorferi* sensu lato genus group are responsible for the most frequently reported vector-
borne disease in the United States, Lyme borreliosis. Increasing numbers of human and canine cases are reported annually, including in the southeastern US which is not typically thought of as a Lyme disease endemic area. According to the Florida Department of Health, 76 new human Lyme disease cases were confirmed between Jan.1, 2012 and Sept. 27, 2013. Of these, 31 cases were confirmed to be contracted in the state, primarily in North Central (NC) FL. Further, the seroprevalence of *B. burgdorferi* in dogs in FL is estimated to be only 0.72% (902 positives out of 124,407 tested). The travel history of this canine population is not known and many part-time residents of FL spend spring and summer months (tick seasons) in the North Eastern parts of the US (Lyme endemic areas). Thus, a portion of the seropositive animals could have been exposed elsewhere.

The lone star tick (LST), *Amblyomma americanum*, accounts for more human tick bites in FL than all other tick species combined. Tick bites from the deer tick (*Ixodes scapularis*), the vector for *Borrelia burgdorferi*, are rarely reported by Florida residents. This raises questions regarding the causative agent(s) of Lyme disease-like illness in parts of the US inhabited by the LST. In this study, 740 nymphal LSTs, 310 adult LSTs and 100 adult deer ticks were collected from state parks in three counties located in NC FL and evaluated for the presence of *Borrelia* spp. Aclin and *Bartonella* exposure in client-owned cats. Genus-specific primers were used in a nested PCR assay targeting a hypervariable region of the flagellin B gene (*FlB*) of *Borrelia* spp. Amplicons were sequenced and analyzed using BLAST and ClustalW.

*Borrelia* spp. were identified in 13/74 (17.6%) pools of nymphal LSTs and 10/310 (3.2%) adults. Sequence analysis of amplicons resulted in 97% to 99% identity to *B. lonestari*. No *Borrelia* spp. were identified in any of the *I. scapularis* ticks or deer blood. None of the deer were seropositive for antibodies to *B. burgdorferi*.

In conclusion, *B. lonestari* was the only *Borrelia* spp. identified in LSTs found in NC FL. Additionally, though current numbers are limited, *B. burgdorferi* was not identified in any of the *I. scapularis* ticks tested or WTD. Our findings indicate that in NC FL, and possibly other areas where the LST is the predominant tick vector, the potential for exposure to *B. lonestari* and possibly other areas where the LST is the predominant tick vector is limited, in LSTs found in NC FL. Additionally, though current numbers are limited, *B. burgdorferi* was not identified in any of the *I. scapularis* ticks tested or WTD. Our findings indicate that in NC FL, and possibly other areas where the LST is the predominant tick vector is limited.

**ID-13**

**RELATIONSHIP BETWEEN DEGENERATIVE JOINT DISEASE, PAIN AND BARTONELLA EXPOSURE IN DOMESTICATED CATS**. A. Tomas1, E.L. Pultorak2, M.E. Gruen1, E.B. Bretschwerdt1, B.D.X. Lascelles1. 1Comparative Pain Research Laboratory, 2Intracellular Pathogens Research Laboratory, North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Given the association between *Bartonella* exposure and arthritides in other species, we evaluated association between DJD, pain indices in cats. Whole blood was collected in EDTA blood samples stored at -80°C. DNA was extracted from individual adult ticks, pools of 10 nymphal ticks in-clinic assay (IDEXX Laboratories). Genomic DNA was extracted from individual adult ticks, pools of 10 nymphal ticks using Qiagen DNeasy Blood & Tissue Kit. DNA was also tested for antibodies to *B. burgdorferi* using SNAP4Dx® in-clinic assay (IDEXX Laboratories). Genomic DNA was extracted from individual adult ticks, pools of 10 nymphal ticks and whole blood using Qiagen DNeasy Blood & Tissue Kit. Genus-specific primers were used in a nested PCR assay targeting a hypervariable region of the flagellin B gene (*FlB*) of *Borrelia* spp. Amplicons were sequenced and analyzed using BLAST and ClustalW. *Borrelia* spp. were identified in 13/74 (17.6%) pools of nymphal LSTs and 10/310 (3.2%) adults. Sequence analysis of amplicons resulted in 97% to 99% identity to *B. lonestari*. No *Borrelia* spp. were identified in any of the *I. scapularis* ticks or deer blood. None of the deer were seropositive for antibodies to *B. burgdorferi*.

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**ID-14**

**REPEATED TESTING INCREASES DETECTION OF VECTOR-BORNE INFECTION IN DOGS WITH CLINICAL SIGNS OF IMMUNE-MEDIATED DISEASE**. L. Kidd1, B. Quarollo2, M. Lappin3, R. Maggi2, C. Osmond4, J. Hart5, S. Hill5, K. Richter5, E. Breitschwerdt2. 1Western University of Health Sciences, Pomona, CA, 2VetCollege of Veterinary Medicine, Raleigh, NC, 3Center for Companion Animal Studies, Fort Collins, CO, 4California Veterinary Specialists, Carlsbad, CA, 5VetCollege of Veterinary Medicine, Pomona, CA.

Clinical abnormalities associated with canine vector-borne diseases (CVBDs) and primary immune-mediated diseases (PIMDs) in dogs overlap. Failure to detect underlying infection in PIMDs contributes to treatment failure, particularly in association with immunosuppression. We previously reported the CVBD prevalence in 53 dogs with clinically significant signs presenting to specialty hospitals in southern California. Dogs were tested at presentation using PCR for *SFG Rickettsia, Bartonella, Babesia, Ehrlichia, Anaplasma and Mycoplasma*. Serum was tested for antibodies to *B. phagocytophilum, E. canis* and *B. burgdorferi* using ELISA (SNAP4Dx®), and *R. rickettsii*, *E. canis*, *B. henselae*, *B. vinsonii* spp. *berkhoffii*, and *B. canis* using IFA. Convalescent serum samples from 34 of the 53 dogs were tested for antibody to *R. rickettsii*. 10/53 (19.8%) dogs had evidence of exposure or infection to one or more of the organisms. The objective of this study was to determine if repeated testing is diagnostically valuable in the clinical setting. Convalescent serum and EDTA blood samples were PCR-tested at -80°F from 33/53 dogs (62%) available and tested using a comprehensive CVBD serology and PCR panel. Acute EDTA blood samples from these 33 dogs were also re-tested for *Babesia* species DNA using PCR.

For the 33 dogs included in this study, previous testing had revealed evidence of exposure or infection in 5 dogs (15%) overall. The additional testing of convalescent and acute samples provided evidence of CVBD infection in 9 dogs (27%) overall. PCR identified *B. gibsonii* in 1 and *Ehrlichia sp.* in 5 convalescent samples. In the initial study, the acute samples from 5 of these 5 *Ehrlichia* infected dogs and *B. gibsonii* infected dog tested PCR-. Three banked acute samples from the 33 dogs were PCR+ for *B. canis*. Samples from 2 of these 3 *B. canis* infected dogs were PCR- initially. None of the 3 *B. canis* infected dogs seroconverted to *Babesia* species, two were receiving immunosuppressive medication. Repeat testing of convalescent serum samples from 2 dogs with an initial borderline positive titer of 1:64 to *R. rickettsii* was negative. Thrombocytopenia was reported more frequently in dogs positive for infection with *CVBD* (6/9) compared to negative dogs (5/24) (χ²=6.19, p = 0.012).

Detection of *Ehrlichia* and *Babesia* species DNA increased when PCR was performed more than once and/or on more than one sample. That these organisms can circulate in low numbers and intermittently may explain this finding. Seroconversion did not occur in the *Babesia* infected dogs, a phenomenon that has been described previously in immunosuppressed patients. Clinicians should consider repeated testing for vector-borne agents in patients with suspected PIMD.
ID-15 DEVELOPMENT AND EVALUATION OF AN ENZYMELINKED IMMUNOSORBENT ASSAY FOR THE QUANTIFICATION OF ANTI-LAGENIDIUM SP ANTIBODIES IN DOGS. J.N. Hartfield, K.J. Waite, A.M. Grooters. Louisiana State University School of Veterinary Medicine, Baton Rouge, LA.

Lagenidium sp infection causes severe skin disease that is clinically and histologically similar to pythiosis. Currently, diagnosis requires culture and rRNA gene sequencing. The purpose of this study was to develop and evaluate an ELISA for quantitation of anti-Lagenidium sp antibodies in canine serum.

Antigen was prepared from soluble mycelial extract of a Lagenidium sp isolate (CBS 135280) previously cultured from canine tissue. Checkerboard titration was used to determine optimal antigen and antibody concentrations. Samples evaluated in the ELISA included sera from 34 dogs with lagenidiosis; 18 dogs with pythiosis; 26 dogs with non-oomycotic fungal infection, non-fungal skin disease, or lymphadenopathy; and 10 healthy dogs.

Results were expressed as percent positivity (PP) relative to a strong positive control serum included on each plate. Mean well-to-well, plate-to-plate, and day-to-day coefficients of variation were 4.5%, 3.8%, and 13.1%, respectively. Medians and ranges for PP for each of the groups were: lagenidiosis (70.6%, 21.0-108.9%), pythiosis (31.3%, 15.8-87.5%), non-oomycotic fungal infection, non-fungal skin disease, or lymphadenopathy (19.2%, 0.4%-70.0%), and healthy dogs (9.9%, 7.6%-24.7%). When using a cutoff value of 40% PP, sensitivity and specificity (with 95% CI) of the ELISA in clinically affected dogs was 79.4% (63.2%-89.7%) and 81.8% (68.0%-90.5%), respectively. When excluding dogs with serologic evidence of pythiosis, specificity was 92.3% (75.9%-97.9%).

Quantification of anti-Lagenidium sp antibodies has moderate sensitivity and specificity for the diagnosis of lagenidiosis in dogs, but must be interpreted in conjunction with results of anti-Pythium insidiosum serology because of serologic cross-reactivity in dogs with pythiosis.

While all of the cats became infected with the respective Bartonella spp., none of the cats developed azotemia and the urine specific gravity of all samples was greater than 1.045. However, after inoculation there was a statistically significant (p = 8.6 x 10^-4, p = 2.5 x 10^-3) increase in creatinine concentrations in both groups of cats over time when compared to the pre-inoculation values. Bartonella clarridgeiae DNA was amplified from the urine of one cat on one date; a threefold increase in hematuria (20-50 RBC/hpf) compared to pre-inoculation was detected concurrently.

The increasing creatinine concentrations over time in Bartonella spp. infected cats suggests that additional studies should be performed assessing the role Bartonella spp. infections may play in CKD in cats. The concurrent detection of B. clarridgeiae DNA in blood, B. clarridgeiae DNA in urine, and hematuria suggests further studies of Bartonella spp. infections in cats with hematuria should be performed.

ID-16 EVALUATION FOR ASSOCIATIONS OF BARTONELLA SPECIES WITH AZOTEMIA AND HEMATURIA IN CATS. S.B. Shropshire, M. Brower, J.R. Hawley, M.R. Lappin. Colorado State University College of Veterinary Medicine, Fort Collins, CO.

Chronic kidney disease (CKD) and hematuria are common clinical problems in cats which are often due to unknown causes. Bartonella henselae DNA has been amplified from renal tissues from experimentally inoculated cats with concurrent mild lymphocytic interstitial nephritis. It has also been shown that B. henselae antibodies are associated with hematuria in sick cats. Another study showed an increased frequency of diseases of the kidneys and urinary tract in sick cats positive for B. henselae antibodies. The purpose of this study was to determine if there are associations between Bartonella species and azotemia and/or hematuria in experimentally inoculated cats.

Young adult, SPF cats were divided into two groups of five cats and then inoculated IV with Bartonella clarridgeiae or Bartonella henselae infected blood. Whole blood, sera, and urine (free catch) were collected before inoculation and then once weekly for four weeks. Each whole blood and urine sample was assessed for DNA of B. henselae and B. clarridgeiae using a previously reported PCR assay. A limit of detection experiment was performed with both agents and showed the sensitivity of the assay using urine to be similar to PBS. Serum creatinine concentrations, urine specific gravity, and urine RBCs/hpf were determined weekly. A paired t-test was used to evaluate for changes in creatinine concentrations over time with P < 0.05 considered significant.

ID-17 EFFECTS OF SUBCUTAEOUS OR INTRANASAL VACCINE ADMINISTRATION ON CLINICAL SIGNS IN FHV-1 INFECTED CATS WITHOUT PREVIOUS VACCINATION. S.A. Wennogle, M.R. Lappin. Colorado State University, Fort Collins, CO.

In previous studies of SPF kittens, administration of intranasal (IN) vaccines containing modified live strains of feline herpesvirus 1 (FHV-1), feline calicivirus (FCV), and panleukopenia virus (FPV) induced significant protection against FHV-1 on challenge as soon as 4 days after 1 dose of vaccine. In addition, IN vaccines have been used successfully as immunotherapy in some cats with chronic viral infections. In contrast, kittens administered one SQ dose of a modified live FHV-1, FCV, and FPV vaccine and challenged with FHV-1 on Day 7 trended towards having more severe clinical signs of FHV-1 than unvaccinated controls. While a transient state of immunosuppression after SQ vaccination has been reported in the dog, similar information is not available for cats. The objective of this study was to determine the effects of one dose of IN or one dose of SQ modified live virus containing vaccine on the clinical signs of FHV-1 in cats with chronic infection.

Mixed sex, 7 month-old kittens (n = 12) with mild FHV-1 associated illness from a previously completed FHV-1 treatment study (primary infection by ocular inoculation of FHV-1) were randomly divided into 2 groups of 6 kittens (3 males, 3 females) and housed and handled separately to avoid cross-contamination between groups. The kittens had not been previously vaccinated. Total clinical scores, ocular scores, respiratory scores, and FHV-1 DNA shedding (pharyngeal swabs collected twice weekly) were determined before (Days 0 – 6) and after (Days 7 – 20) vaccination with either a FHV-1, FCV, and FPV containing SQ vaccine (Felocell 3; Pfizer Animal Health, New York, NY) or a FHV-1 and FCV containing IN vaccine (Felocell FVR C; Pfizer Animal Health). On the day of vaccination (Day 7), the IN vaccinated kittens were administered the vaccine and 1 ml of sterile 0.9% NaCl SQ and the SQ vaccinated kittens were administered the vaccine and 0.5 ml of sterile NaCl IN.

Ocular scores in kittens vaccinated SQ were significantly greater Days 7 – 20 when compared to Days 0 – 6 (Mann Whitney U test; p = 0.041); this finding was not apparent in the kittens vaccinated IN. Total clinical scores, total respiratory scores, and viral DNA shedding were not different between the groups within the time periods.

Although mild, the findings suggest that kittens infected with FHV-1 prior to vaccination may have clinical signs exacerbated transiently after administration of SQ modified live vaccines. The mechanism for this finding is not currently known. Use of IN modified live FHV-1 and FCV containing vaccines should be considered in kittens with known or suspected FHV-1 infections.
**B. K. Curtis 1, P. Foster 1, P. Smith 1, M. Monn 1, B. Stil-**

Borrelia burgdorferi (Bb) vaccines are commonly administered to prevent Bb infection and clinical Lyme disease in endemic areas. Numerous published studies have documented that assays based on Bb VlsE derived C6 peptide do not react with sera from vaccinated animals. However, because of variability in vaccine formulations, study designs and possible variability in interpretation of confirmatory assays, evaluation of samples derived from a controlled experimental vaccination study was warranted.

Twelve purebred specific pathogen-free beagles were utilized. Groups of three dogs were assigned to a vaccine group and administered one of four vaccines [Recombikine™ (Lyme) (Merial), LymeVax® (Fort Dodge Laboratories), Galaxy® Lyme (Schoring-Plough Animal Health) and Nobivac® (Lyme) (Merk)]. The first three vaccines were administered on weeks 0, 2, 33 and 39 to generate high titer serum. The Nobivac Lyme Vaccine was administered on weeks 0 and 3. Blood samples collected before and after vaccinations at all time points were processed and serum was tested using Bb IFA (IDEXX Reference Laboratories), Quart C6® ELISA (IDEXX Reference Laboratories) and SNAP 4D® Plus.

Sera from all dogs had positive IFA titers, ranging from 1:800 five weeks post vaccination to 1:6400 following additional vaccinations. The Lyme Quant C6 Test and the SNAP 4D® Plus test were negative for all samples from all vaccinated dogs at all time points including at peak IFA titers.

The results of this study document that the Bb. vaccines studied did not induce antibodies detectable in C6 based immunassays, even when dogs are hyper-vaccinated.

**ID-19 PERFORMANCE OF AN IN-CLINIC ELISA FOR THE DETECTION OF LEPTOSPIRA SPECIFIC ANTIBODY IN DOGS. K. Curtis1, P. Foster1, P. Smith2, M. Monn1, B. Stillman1, R. Chandrashekar1, S. Tsai2, R. Goldstein1. 1IDEXX Laboratories, Inc., Westbrook, ME., 2The Animal Medical Center, New York, NY.**

The SNAP® Lepto test is an enzyme-linked immunosorbsorbent assay (ELISA) for the detection of Leptospira specific antibodies in canine serum. The assay utilizes LipL32 as the target, an abundant and highly conserved protein found only in pathogenic Leptospira. The purpose of this study was to evaluate the performance of SNAP Lepto using samples characterized by the microscopic agglutination test (MAT). A total of 403 serum field samples that were originally submitted to IDEXX Reference Laboratories, Inc. for MAT testing were used to evaluate the sensitivity and specificity of SNAP Lepto. Of these, 201 were MAT negative and 202 were MAT positive with a peak MAT titer ≥1:800. The sensitivity and specificity of SNAP Lepto relative to MAT was 83.2% and 82.1%, respectively. SNAP Lepto detected samples with peak MAT titers to serovars Pomona, Icterohaemorrhagiae, Grippotyphosa, Canicola, Bratislava and Autumnalis.

In order to better understand the performance of SNAP Lepto with MAT negative samples, 150 MAT negative sample pools from healthy dogs in a non-endemic area (Alaska) were tested; the specificity of SNAP Lepto in this population was 96.0%. Additionally, 52 Lyme positive (Lyme Quant C6) canine serum samples were tested for cross-reactivity, of which 51 (98.1%) were negative on the SNAP Lepto test.

Results from this study demonstrated the value of SNAP Lepto for the detection of Leptospira specific antibodies with performance comparable to MAT. SNAP Lepto can be used to aid in the clinical diagnosis of leptospirosis in canine cases where Leptospira infection is considered a top differential.

**ID-20 STAPHYLOCOCCUS PSEUDINTERMEDIUS CELL WALL-ASSOCIATED PROTEINS IN DOGS: 374 ISOLATES (2010-2012). R.M. Gold1, N.D. Cohen1, S.D. Lawhon1. 1Department of Veterinary Pathobiology and Texas A&M University, College Station, TX., 2Department of Large Animal Clinical Sciences, Texas A&M University College of Veterinary Medicine, College Station, TX.**

Staphylococcus pseudintermedius is considered the predominant cause of superficial pyoderma in dogs. Cell wall-associated proteins (CWAPs) aid in the binding of bacteria to the surface of host cells promoting colonization. Eighteen CWAPs of the family sps have demonstrated a role in staphylococcal strain virulence; however, four CWAPs have exhibited variability in strains of S. pseudintermedius. The objectives of this study were to determine the prevalence of methicillin-resistant S. pseudintermedius (MRSP), methicillin-susceptible S. pseudintermedius (MSSP), and four CWAPs isolated from S. pseudintermedius in dogs, and to determine whether there is an association between these CWAPs and methicillin resistance.

Medical records of hospital population dogs culturing positive for S. pseudintermedius were reviewed retrospectively. Sixty-nine MRSP and 305 MSSP isolates cultured from 294 individual dogs between September 2010 and February 2012 were enrolled. Bacterial cultures were analyzed by polymerase chain reaction for the presence of mecA, SCCmec type I-VI, and sps genes F, O, P, and Q.

Of the 69 MRSP isolates, 64% (44/69) carried the mecA gene on a staphylococcal cassette chromosome SCCmec type V. Isolates that were methicillin-resistant were found to be significantly more likely to be associated with diseased dogs than isolates that were methicillin-susceptible (P < 0.0001). The proportion of MRSP isolates bearing the CWAP gene spsP (32%; 22/69) was significantly greater compared to the proportion of MSSP isolates bearing spsP (14%; 42/305; P = 0.0006). The proportion of MRSP isolates bearing the CWAP gene spsQ (35%; 24/69) was significantly greater than the proportion of MSSP isolates bearing spsQ (16%; 48/305; P = 0.0006). While there was no significant association between MRSP and the site that was cultured, there was a tendency for MRSP isolates to be more likely from a dog with pyoderma (odds ratio [OR] = 3.1; 95% CI, 0.8 to 11.8; P = 0.1051) or from a dog with an infected wound (OR = 3.0; 95% CI, 0.8 to 12.3; P = 0.1219) than from other sources.

In this study there was a significant association between the CWAPs sps genes P and Q and methicillin resistance, both as individual genes and in combination. Therapeutic targeting of these genes may prove to be a potential means of treatment for cases where antibiotics fail and also as a preventative measure by way of producing a vaccine.

**ID-21 EXPERIMENTAL INFECTION OF CATS WITH CRYPTOSPORIDIUM FELIS. A.V. Scorza1, P. Tyrell2, S. Wensnegle2, R. Chandrashekar3, M.R. Lappin1. 1Department of Clinical Sciences, Colorado State University, Fort Collins, CO., USA., 2IDEXX Laboratories Inc., Westbrook, ME.**

Cryptosporidium felis infection is common in cats but few data after experimental infection exist. The objectives of this study were to infect cats with a genetically characterized C. felis field isolate and follow fecal diagnostic test results and clinical findings over time.

Cats shown to be C. felis after sequencing of the SSU RNA gene were concentrated from the feces of a naturally infected cat. Domestic short-hair laboratory reared cats (8 months old) were shown to be negative for C. felis using a commercially available Cryptosporidium spp. fluorescent antibody assay (FA), Merilhuor Cryptosporidium/ Giardia Meridian Diagnostics) and fecal flotation (FF) after sugar centrifugation and then inoculated with approximately 5,000 C. felis oocysts diluted in water by stomach tube while sedated. Food was withheld for 24 hr and then the cats were housed separately, fed a commercial feline diet ad libitum, and observed daily for the presence of gastrointestinal signs (inappetence, diarrhea, vomiting). Fecal sam-
ple from all 6 cats on Day 0, Day 9, and one sample per cat collected between Days 18-21 were evaluated by FA, FF, and for C. felis DNA using a fluorogenic PCR assay that amplifies a fragment of the COWP gene. On Day 31, 2 cats negative for C. felis oocysts by FF and FA were administered methylprednisolone acetate at 5 mg/kg IM and all assays repeated on Days 32, 33, and 35.

None of the cats were positive for C. felis oocysts by FF or FA in samples collected Days 0, 9, or 18-21. However, C. felis DNA was amplified by PCR assay from one sample collected prior to inoculation and from 11 of the other 12 samples collected during these times after inoculation. The cats administered methylprednisolone acetate had detectable oocysts only on Day 33. In contrast, both cats were positive for C. felis DNA in all samples collected on Days 31, 32, 33, and 35. Additionally, varying numbers of Isospora felis oocysts were shed by each of the 6 cats over the course of the study. However, none of the cats showed clinical signs of gastrointestinal disease before or after glucose treatment.

Cats infected with this dose and strain of C. felis failed to develop clinical signs of gastrointestinal disease even when co-infected with I. felis and administered glucocorticoids suggesting that the organism is not pathogenic for otherwise healthy young adult cats. The extent of the infection and FA tests used are inadequate for screening cats for subclinical C. felis infection. While the PCR assay is more sensitive than FF and FA, positive test results did not correlate with the presence of clinical disease. Additional data are needed to determine the predictive value of these tests in cats with naturally occurring C. felis infections.

ID-22 EXPERIMENTAL INFECTION OF CATS WITH ISOSPORA FELIS, A.V. Scorza 1, P. Tyrrell 2, S. Wennogle 1, R. Chandrashekar 1, 2, M.R. Lappin 1. 1Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA. 2IDEXX Laboratories, Westbrook, ME.

Isospora felis is commonly believed to induce diarrhea in kittens but few experimental data are available from young adult cats. The objective of this study was to describe the results of fecal diagnostic tests and clinical presentation of cats experimentally infected with I. felis.

Isospora felis oocysts were identified morphologically and then concentrated from the feces of a naturally infected kitten with diarrhea following a published protocol. No other parasites were detected after sugar centrifugal flotation and assessment by a fluorescent antibody assay (FA; Merifluor Cryptosporidium/Giardia, Meridian Diagnostics) for Giardia spp. and Cryptosporidium spp. Six kittens, 4 short-hair cats (8 months old) and 2 Siamese cats (6 months, 2 short-hair cats (8 months old) were purchased from a commercial breeder, housed individually, and shown to be negative for fecal parasites three times. A total of 5,000 I. felis sporulated oocysts were then administered to each cat by stomach tube or by drop feeding. Food was withheld for 24 hrs and then the cats were fed a commercial feline diet ad libitum and observed daily for the presence of gastrointestinal signs. Fecal samples were evaluated by fecal flotation and FA up to three times per week post inoculation (PI) to Day 27. An oocyst score was assigned after fecal flotation: Score 0 = no oocysts (per slide); Score 1 = 1 to 250 oocysts; Score 2 = 250 to 500 oocysts; Score 3 = greater than 500. A total of 36 samples collected prior to inoculation and from Days 8, 10, 13, 15, and 20 PI were assayed using a recently optimized ITS1 PCR assay.

All cats were negative for I. felis by all assays prior to inoculation. Oocysts were seen in 4 of 6 cats on Day 8 PI (Score 1), all 6 cats shed oocysts on day 10 PI (Score 3), and 4 of 6 cats were shedding oocysts on Day 14 PI (Score 1). On all other time points assessed (Days 17, 19, and 20) all cats were negative after fecal flotation. After I. felis inoculation, 24 of 30 fecal samples tested were positive for I. felis DNA in feces. Oocysts were not detected in 3 of the selected samples that were positive for I. felis DNA (Days 15 or 20). None of cats showed clinical signs.

Young adult cats orally inoculated with I. felis sporulated oocysts in this model developed self-limited, subclinical infections suggesting this I. felis isolate was host adapted and unlikely to associated with clinical disease. Fecal fluctuation is a sensitive and convenient assay for detection of I. felis but may occasionally give false negative results when compared to the ITS1 PCR assay.

ID-23 FIELD COMPARISON OF SERESTO® (10% IMIDACLOPRID/4.5% FLUMETHRIN) COLLAR AND A PLACEBO COLLAR PLACED ON CATS, H.K. Fink 1, S. Wennogle 1, W.J. Davis 1, C. Von Simonson 1, M.R. Lappin 2. 1Department of Clinical Sciences, Colorado State University, Fort Collins, CO, 2Bayer Animal Health, Shawnee Mission, KS.

In addition to controlling flea and tick infestations for eight months, the Seresto® collar (Bayer Animal Health) has been shown to be safe and effective in preventing transmission of Bartonella henselae and Cytauxzoon felis among cats. While collars avoid some of the compliance issues associated with topical products, some cats object to wearing collars. The purpose of this study was to evaluate tolerance of client-owned cats for the Seresto® collar or a placebo collar. A total of 96 cats owned by veterinary students or employees of the Veterinary Teaching Hospital were enrolled in the study. All cats were greater than 10 weeks of age and considered in good health; cats that were systemically ill on Day 0, from the hairless breeds, or declined in all four limbs were excluded. Cats were randomized by household to wear a placebo collar for 14 days followed by a Seresto® collar for 14 days or a Seresto® collar for 28 days. All cats in a household received the same collar treatment. Examinations by a veterinarian were performed on Days 0, 14, and 28. Owners recorded daily systemic and local health observations.

There were no systemic clinical signs of disease attributed to the collars. One cat in the Seresto® group had the collar entangled over the mandible on Day 1. None of the placebo cats completed the 28 day study. Licking at the collars and a few local lesions that were apparently associated with the collars were noted in the first 14 days of the study (Table). None of the placebo cats had reports of local lesions after crossing to the Seresto® collar and only one Seresto® collar cat had a reported side effect after Day 14 (licking). One cat required removal of the collar for treatment (Seresto®) for local pyoderma. Housing status, single or multiple cat household, and whether a collar had been worn previously were not associated with side-effects.

Adverse events detected for cats wearing Seresto® collars were similar to that for cats wearing placebo collars and to cats wearing identification collars reported in a previous study. While wearing Seresto® collars were more likely to lick the collar area in the first 14 days of placement, the data suggest that cats originally intolerant of collars will become more receptive over time.

<table>
<thead>
<tr>
<th>Group</th>
<th>Licking</th>
<th>Crusting (CR)</th>
<th>Alopecia (A)</th>
<th>Itch (I)</th>
<th>Sleep (S)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 48)</td>
<td>4.8 (5.3%)</td>
<td>12.2 (12.2%)</td>
<td>24.2 (24.2%)</td>
<td>12.1 (12.1%)</td>
<td>2.4 (2.4%)</td>
<td>1.012 (1.012%)</td>
</tr>
<tr>
<td>Seresto (n = 47)</td>
<td>1.7 (1.5%)</td>
<td>4.2 (4.2%)</td>
<td>10.4 (10.4%)</td>
<td>21.7 (21.7%)</td>
<td>2.5 (2.5%)</td>
<td>0.051 (0.051%)</td>
</tr>
</tbody>
</table>


Dipylidium caninum is vectored by Ctenocephalides felis and so is very common in cats and dogs in areas of the world endemic for this flea. Recently, PCR assays that amplify specific genes of D. caninum have been optimized for use is diagnostic assays and prevalence studies. The purpose of this study was to report the prevalence of D. caninum DNA and DNA of other C. felis associated infectious disease agents in select groups of C. felis collected off cats in the United States.
DNA extracted from *C. felis* from cats in previously published studies in the United States that reported the prevalence rates for *Bartonella* spp., hemoplasmas, and *Rickettsia felis* had been maintained at ~80C. The *C. felis* were collected from cats in Alabama and Florida and were pooled in groups of a maximum of 5 fleas before DNA extraction. Aliquots of total DNA were shipped on ice to the ClinVet molecular biology laboratory for evaluation in a proprietary conventional PCR assay that uses primers specific to the *D. caninum* rDNA region. The targeted region is present in multiple copies per genome and is present in all stages of *D. caninum*. The detection sensitivity of the assay is 21 fg *D. caninum* genomic DNA per PCR; up to 400 ng of isolate DNA served as template for PCR amplification of the target region. PCR products were analyzed using agarose gel electrophoresis and positive amplicons were evaluated by genetic sequencing.

Overall, 9 of the 55 flea sets were positive for *D. caninum* DNA (16%), all positive samples were from Alabama, and 3 cats were used exclusively indoors. Adequate DNA for sequencing from 7 of 9 flea groups was evaluated with multiple pairwise sequence alignment and distance tree analysis and showed 2 distinct groups that clustered together. One group was greater than 99% identical to the GenBank *D. caninum* sequence AF203120 whereas the owners of the other group was approximately 93% identical to this sequence but had unique deletions and substitutions. Of the 9 flea group DNA samples, 9 groups, 6 groups, and 5 groups were concurrently positive for *Bartonella* spp., hemoplasma, and *R. felis* DNA.

*Diopyldium caninum* DNA is common in *C. felis* collected from cats in Alabama and 2 distinct genotypes exist. Coinfections with other agents capable of causing disease in cats or people exist commonly. The results support recommendations that flea control be maintained on cats continuously in endemic areas.

### ID-25

**ANAPLASMA PHAGOCYTOPHILUM AND BORRELLA BURGDORFERI ANTIBODIES IN NATURALLY EXPOSED CATS IN MAINE.**

K. Hoyt, R. Chandrashekar, E. Brejtschwerdt, M.R. Lappin. 1Cats on Call, Portland, ME, 2IDEXX Laboratories, Inc., Westbrook, ME, 3North Carolina State University, Raleigh, NC, 4Colorado State University, Fort Collins, CO.

Antibodies against *A. phagocytophilum* (Aph) and *B. burgdorferi* (Bb) can be detected in serum of cats using a commercially available test originally developed for dogs (SNAP® 4DX®, IDEXX). Client-owned cats in areas endemic for *Ixodes scapularis* can be Aph or Bb seropositive, but little information is available concerning clinical manifestations of Lyme disease or anaplasmosis in cats. The objective of this study was to evaluate Aph and Bb antibody prevalence rates in cats with and without clinical signs associated with these infections.

Between October 2009 to August 2013, owners of a healthy cats and cats with clinical findings consistent with Aph or Bb infection (fever, lameness, lethargy, inappetence, presence of I. scapularis) in Portland, ME were offered Aph and Bb serological testing (SNAP® 4DX®) on a research basis. Age, housing status (exclusively indoors or outdoors at least part of the time), and weather the owners had purchased a tick control product in the last 6 months was recorded and results between groups and findings were compared.

Aph and Bb antibodies were found in healthy and sick cats (Table). Cats with clinical signs consistent with Aph or Bb were more likely than healthy cats to have Aph antibodies (gray cells are statistically different at p < 0.05). The majority of Aph and Bb seropositive healthy (81.3%) or sick cats (80%) were allowed outdoors. When the results from both groups were combined, Aph or Bb antibody prevalence rates were not significantly different in cats with owners that did (37%) or did not (23.3%) purchase tick control products.

The finding that Aph antibodies were over-represented in clinically ill cats compared to healthy cats suggests that Aph can be a cause of these clinical manifestations in the region. PCR testing on blood can be used to document active Aph infections. Also, the importance of using tick preventives should be emphasized to cat and dog owners.

### ID-26

**FHV-1 OR MYCOPLASMA SPP. PCR ASSAY RESULTS FROM CONJUNCTIVAL CELLS FAIL TO PREDICT RESPONSE TO DIFFERENT TREATMENTS.**

M.R. Lappin1, W. Rekers1, D. Zirofsky2, C. Powell1, J. Veir1, M. Brewer, J.R. Hawley1, 2Colorado State University, Fort Collins, CO., 3Cat Adoption Team, Sherwood OR.

Feline herpesvirus 1 (FHV-1) and *Mycoplasma* spp. are two of the most common causes of conjunctivitis in cats. Modified live vaccines containing FHV-1 can induce positive test results in FHV-1 PCR assays and both organisms can be carried by normal cats. Thus, positive PCR assay results for FHV-1 and *Mycoplasma* spp. may not always correlate to the presence of disease. The objectives of this study were to determine whether quantitative PCR assay results for FHV-1, conventional PCR assay results for FHV-1, or conventional PCR assay results for *Mycoplasma* spp. can be used to predict treatment responses.

Cats with conjunctivitis, with or without rhinitis, that were housed in two animal shelters were used. Conjunctival cells were collected by rubbing a sterile cotton swab gently in the conjunctival fornix of an affected eye after application of topical 1% proparacaine. The cats were then randomized to be administered 0.5% cidofovir in methylcellulose, OU, q8-12 hours or tetracycline ointment (Terramycin), 1/2 inch of ointment OU, q8-12 hr for 7 days. Samples were assayed for total DNA, GAPDH, and FHV-1 DNA by quantitative PCR as well as for FHV-1 DNA by conventional PCR, *Mycoplasma* spp. DNA by conventional PCR, and feline calcivirus (FCV) RNA by conventional reverse transcriptase PCR. Cats for which the total ocular score, based on the degree of conjunctivitis, degree and type of ocular discharge, and presence or absence of blepharospasm, decreased at least 2 points from Day 0 by Day 7 were considered positive responses. Associations amongst PCR assay results and response to treatments were evaluated.

DNA of FHV-1 was amplified alone (13 cats) or in combination with DNA of a *Mycoplasma* spp. (9 cats) for a total prevalence of 36.7%. DNA of a *Mycoplasma* spp. was amplified alone (17 cats) or in combination with DNA of FHV-1 (8 cats) for a total prevalence of 41.7%. FCV RNA was amplified from 2 samples, both were concurrently positive for *Mycoplasma* spp. DNA. Treatment responses were not associated with the PCR assay result for either organism and the magnitude of the FHV-1/GAPDH ratio did not associate with response to cidofovir (Table).

Use of PCR assays for *Mycoplasma* spp. or FHV-1 to attempt to predict treatment responses in these shelter cats was of no clinical benefit.

### ID-27

**ISOLATION OF MICROSPORUM CANIS FROM THE HAIR COATS OF SHELTER CATS IN SOUTHEAST OF BRAZIL.**

C. Nitta1, C.E. Larsson1, C.P. Taborda2, A.G.T. Daniel1, 1School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil., 2Patogetic Fungi Laboratory of Biomedical Sciences Institute, University of São Paulo.

All over the world asymptomatic cats are considered the main reservoirs and sources of infection of dermatophytes, especially *Microsporum canis*. In humans, 15% of tinea cases have zoonotic
origin, and often envelop with contact with cats with no skin lesions. At the Dermatology Service of HOVET/USP, fungal dermatitis represent 30% of all dermatologic diseases, mainly diagnosed on Persian cats (94%). In order to quantify the potential risk of human infection after contact with apparently healthy Persian cats, from commercial catteries, 61 hair coat samples were collected (18 males and 43 females) from Persian cats using Mariat Adam Campos technique. The animals came from three different catteries, had a mean age of 38 months, and none of them had skin lesions, all were negative at Wood’s Lamp examination. All of the 18 human contactants were inquired about the presence of dermatophytic typical skin lesions recently or at the time of sample collection. The obtained samples were cultured on Micosel Agar (Difco) and incubated at 25°C for 21 days. Fungal growth was present on the samples in 51 (83.6%) cases, and M. canis was the only agent isolated. Among humans contactants, respectively 8 (44.4%) and 3 (16.7%) had shown in the past or have at the moment typical tinea corporis skin lesions. The obtained results characterize the potential risk of human infection with Microsporum species to owners that had acquire apparently healthy cats from commercial catteries in São Paulo, Brazil.

**ID-28**

**CANINE INFECTION WITH DIROFILARIA IMMITIS, BORELIA BURGDORFERI, ANAPLASMA SPP., AND EHRlichia SPP, IN THE UNITED STATES, 2010-2012. S.E. Little1, M.J. Beall2, D.D. Bowman3, R. Chandrashekar4. 1Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK; 2Department of Clinical Sciences, Colorado State University, Ft. Collins, CO, USA., 3Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY.

To provide an updated, expanded assessment of geographic trends in canine infection with Dirofilaria immitis, Borelaria burgdorferi, Ehrlichia spp., and Anaplasma spp., we evaluated more than 10 million canine test results generated by veterinarians from throughout the United States from 2010 – 2012. As in an earlier summary report, the percent positive test results varied by agent and region, with antigen of D. immitis and antibody to Ehrlichia spp. most commonly identified in the Southeast and antibody to both B. burgdorferi and Anaplasma spp. most commonly identified in the Northeast and upper Midwest. Percent positive test results for D. immitis antigen were lower in every region considered, including in the Southeast, than previously reported (P < 0.0001). Percent positive test results for antibodies to B. burgdorferi and Ehrlichia spp. were higher nationally than previously reported, and, for antibodies to Anaplasma spp., were higher in the Northeast but lower in the Midwest West than in the initial report (P < 0.0001). Percent positive test results for each tick-borne disease agent corresponded to annual reports of human disease caused by each organism considered. Within endemic areas, evidence of geographic shift in percent positive test results for all three tick-borne agents was also identified. Continued national monitoring of canine test results for vector-borne zoonotic agents is an important tool for accurately mapping the geographic distribution of these agents, and greatly aids our understanding of the veterinary and public health threats posed by these organisms.

**ID-29**

**CANINE SERUM ANTIBODIES AGAINST EHRLICHIA EwINGII PROTEIN OMPI-15, S. Moroff1, J. Sokolchik1, T. Woodring1, C. Woodruff1, M. Lampert1, 1Dynamet Diagnostics, Lake Success, NY, 2Department of Clinical Sciences, Colorado State University, Ft. Collins, Colorado.

Ehrlichia ewingii (EC) is a cause of granulocytotropic ehrlichiosis in dogs in areas endemic for Amblyomma americanum. Antibodies against EC have variable cross reactions with antigens from other Ehrlichia spp. The purpose of this study was to evaluate 4 peptides from E. ewingii for potential use in a new automated fluorescent system (Accuplex®; Antech Diagnostics).

Client-owned dogs (n = 20) in Oklahoma were screened for antibodies against E. canis, Borellia burgdorferi (5 antigens), Anaplasma phagocytophilum, and Rickettsia rickettsii as well as for DNA of E. ewingii, E. canis, E. chaffeensis, A. phagocytophilum, A. platys, R. rickettsii, Neorickettsia risticii, Babesia canis, B. gibsoni, Babesia sp. (Coco), Babesia conradae, Mycoplasma haemocanis, ‘Candidatus M. haematoparvum’, Bartonella vinsonii berkholffii, B. henselae, and B. clarridgeiae by PCR assay (FastPanel™, Antech Diagnostics). One dog was identified that was positive for E. ewingii DNA (sequence confirmed) but negative in all other tests. Heparinized blood from this dog was used to inoculate 2 young adult research beagles that were negative in all tests above. The dogs were monitored daily for clinical abnormalities and both serum and blood were collected on Days 0, 11, 27, 32, 34, 41, 48, 62, 69, 83, 125, 138, and 172. The E. ewingii outer membrane proteins OMPI-13, OMPI-14, OMPI-15, and OMPI-16 were synthesized. Sera from the dogs described above as well as another client-owned dog shown to be PCR positive for E. ewingii but no other known pathogens were used in conjunction with serum from SPF beagles in checkboard titrations to determine the optimal peptide and conditions in a well based ELISA.

The 15 peptide OMPI-15 demonstrated optimal reaction with sera of all E. ewingii infected dogs and was chosen for use in the optimized Accuplex™ E. ewingii antibody assay. Both experimentally inoculated dogs were normal throughout the study, became positive for E. ewingii antibodies on Day 34 after inoculation, and remained antibody positive throughout the study, even after the administration of doxycycline at 10 mg/kg, PO, daily on Days 63 – 97. The dogs were PCR positive for E. ewingii, but not other agents, by Day 18 after inoculation. In other experiments, sera from SPF dogs experimentally inoculated with E. canis or A. phagocytophilum did not react in the Accuplex™ E. ewingii antibody assay.

The Accuplex™ E. ewingii antibody assay is specific and in this inoculation model was positive within 16 days of the first PCR assay positive result in both dogs studied longitudinally.

**ID-30**

**PREVALENCE OF ZOONOTIC PARASITES IN SHELTER DOGS IN VERACRUZ, MEXICO. M.Y. Nakamoto1, A.V. Scorzal2, M. Aguilar Domingez2, D. Romero Salas2, M.R. Laporta3, 1Department of Clinical Sciences, Colorado State University; and, 2Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, Veracruz, Mexico.

The objectives of this study were to determine the prevalence of enteric parasites in shelter dogs in Veracruz, Mexico and to genetically characterize the Giardia duodenalis and Cryptosporidium spp. isolates. Fecal samples were submitted to the University of Veracruz, Mexico and parasites were microscopically identified after fecal flotation (FF) by zinc sulfate centrifugation. The samples were then shipped on ice to Colorado State University for Giardia (G) and Cryptosporidium (C) immunofluorescence assay (FA) performed following the manufacturer’s guidelines (Meridian Diagnostics) and molecular analysis. Total DNA was extracted from FA positive samples following a published protocol. PCR amplification of the SSU RNA and the heat shock protein 70 (hsp70) genes of Cryptosporidium spp. were performed on Cryptosporidium FA positive samples while PCR amplification of the triose phosphate isomerase (TPI), glutamate dehydrogenase (GDH), and beta-giardin (BG) genes were performed on Giardia FA positive samples.

Of the 222 dogs examined from the La Roca shelter in Veracruz, Mexico and to over 60% were tested for multiple parasites: Ancylostoma canum (AC); Strongyloides stercoralis (ST); Uncinaria stenocephala (US); and Trichuris vulpis (TV) were common (Table 1). Five Cryptosporidium spp. and two Cryptosporidium canis isolates were amplified by the 18S rRNA and by the HSP-70 assays respectively. Of the 30 Giardia spp. isolates, five, and one samples were amplified by the GDH, TPI, and BG genes, respectively. Fourteen of the 19 (73.7%) PCR positive isolates typed as...
The prevalence of enteric parasites of stray dogs in the La Roca shelter in Veracruz is high. The majority of Guardia assemblages were dog host adapted, but two were the human adapted assemblage A, suggesting the dogs were exposed to human feces.

**ID-31**

PREVALENCE OF SELECTED VECTOR BORNE DISEASES IN PET AND HUNTING DOGS IN FINLAND. C. Pérez Vera1, S. Junnikkala2, S. Kaptanen1, T. Spillmann1, O. Vapaatalo1, 1Department of Virology, Haartman Institute, Helsinki, Finland., 2Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland., 3Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland., 3Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland.

For multifactorial reasons the epidemiology of vector borne diseases in Europe is gradually changing. Due to climate changes of the last decades, ticks have progressively spread into higher latitudes in northern Europe. In Finland, the estimated numbers of cases of Borrelia burgdorferi infections in humans is over 5000 (population 5.5 million) per year. However, in Aland and in the southern archipelago of Finland the annual incidence exceeds 200 per 100,000 people. To date there is limited information regarding the prevalence of arthropod borne diseases in dogs and cats, and thus the majority of available data comes from studies in human medicine.

The objective of this study was to determine the serological and molecular prevalence of Anaplasma phagocytophilum, Ehrlichia canis and Bartonella infections in pet dogs as well as hunting dogs in Finland.

EDTA-blood and serum samples were collected from pet dogs (n = 219) evaluated in private practices around Finland and hunting dogs (n = 50) between September and November of 2011 and 2012. Additionally, 121 blood bank samples collected in the fall of 2011 and stored at -30°C were included in the study. Serum samples were tested for the presence of Ehrlichia canis, Anaplasma and Borrelia burgdorferi antibodies as well as Dicrofilaria immitis antibodies using a qualitative dot-ELISA (SNAP®-2.3). The EDTA-blood samples were also sequenced by real time Anaplasma PCR and for the intergenic transcribed spacer of the genus Bartonella. Positive amplions were sequenced and compared to sequences of other organisms. In total, 18/340 (5.3%) and 10/340 (2.94%) pet dogs had positive Anaplasma and Borrelia burgdorferi antibodies, respectively. One dog was E.canis seropositive. In Aland, a region in Finland where human Lyme disease is endemic, 45% and 20% dogs were Anaplasma and Borrelia seropositive, respectively. In the hunting dogs group, 2/30 (4%) were Anaplasma-seropositive and one dog was seroreactive to Borrelia burgdorferi antigens. Two dogs that were PCR positive for A. phagocytophilum (1 pet dog and 1 hunting dog) were seronegative in the SNAP®-4DX, suggesting an acute infection. Bartonella DNA was not detected by PCR from any of the dog’s samples.

**ID-32**

A DESCRIPTIVE ANALYSIS OF CLINICAL MANIFESTATIONS IN DOGS WITH TICK-BORNE PATHOGEN CO-INFECTIONS AND CO-EXPOSURES. B.A. Qurollo1, R. Chandrashekar2, B.C. Hegarty1, M.J. Beall3, B. Stillman3, J. Liu2, B. Thatcher2, A. Comyn1, E.B. Breitschwerdt1, 1Intracellular Pathogens Research Laboratory, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina., 2IDEXX Laboratories, Inc., Westbrook, Maine.

Canine tick-borne pathogens are responsible for a variety of clinical disease manifestations, with variations in type and severity among dogs co-infected with more than one organism. The purpose of this study was to identify dogs with vector-borne pathogen co-exposures (serology) and co-infections (PCR) and to characterize clinical abnormalities in co-infected dogs.

Archived serum from a population of dogs being tested for tick-borne diseases (IFA for Ehrlichia canis, Rickettsia rickettsii, Babesia canis, Bartonella henselae, Bartonella vinsonii subsp. berkhoffii, and SNAP®-4DX for E. canis, Anaplasma spp, Borrelia burgdorferi, D. immitis) were included for retrospective screening using SNAP®-M-A, an experimental, diagnostic ELISA assay that uses synthetic, species-specific peptides to differentiate between exposure to E.canis, E.chaffeensis, E.ewingii, Anaplasma phagocytophilum, A.platys and Borrelia burgdorferi. A total of 85 dogs with evidence of co-exposure had accessible clinical data. Blood samples were available for 57/85 (67%) cases for retrospective PCR testing to confirm co-infections. By IFA and SNAP®-4DX testing, 36/85 (42%) dogs were co-exposed to more than one tick-borne pathogen, whereas retrospective testing using SNAP®-M-A identified 66/85 (78%) co-exposures. SNAP®-M-A identified exposures to pathogens in 74/85 (87%) dogs that had not previously been detected by the initial IFA and SNAP®-4DX assays. When original and retrospective serology results were combined, dogs were co-exposed to 2 to 5 pathogens. Pathogen DNA was PCR amplified from 14/57 (25%) whole blood samples. Two different pathogens were amplified from only 2/14 (14%) blood samples, whereas PCR identified a previously undetected infection in 12/14 (86%) dogs, not detected in the initial work-up. E.ewingii, the most prevalent infection, was found in 8/14 (57%) dogs. One dog was co-infected with E.ew ingii and E.canis and one dog with E.ewingii and A wolbachia sp. Among the 14 PCR+ dogs, clinical manifestations included IMHA and/or ITP in 3; polyarthritis in 2; vasculitis in 2; trigeminal neuritis in 2; dermatitis in 1, cutaneous neoplasia in 1; and 3 dogs were healthy, blood donors. The most prevalent hematological changes observed were abnormal lymphocytes 10/14 (71%), hyperglobulinemia 8/14 (57%), and hypoalbuminemia 7/14 (50%). These results underscore the importance of a multi-modal diagnostic approach to detect tick-borne pathogens and the need for clinicians to understand test limitations when attempting to confirm tick borne disease infections and co-infections.

**ID-33**

PERSISTENT EHRLICHIA EWINGII INFECTION IN DOGS. L.A. Starkey1, A.W. Barrett1, M.J. Beall1, R. Chandrashekar2, B. Thatcher2, P. Tyrell1, S.E. Little1, 1Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK and, 2IDEXX Laboratories, Westbrook, ME.

To evaluate the persistence of natural infection with Ehrlichia ewingii, four beagles exposed to ticks via weekly walks through tick habitat in north central Oklahoma and positive for infection with E. ewingii by sequence-confirmed PCR and peptide-specific serology continued to be evaluated for a total duration of 733 days post initial infection (dpi). Whole blood was collected once weekly for nested and/or real-time PCR. Serum was collected once monthly for detection of antibodies to E. canis (p16), E. chaffeensis (IFA and VLP), and E. ewingii (p28). All dogs (4/4) became infected with Ehrlichia spp. as evidenced by seroconversion on IFA to E. chaffeensis (4/4; maximum inverse
**ID-34**

**CHARACTERIZATION OF PARVOVIRUSES FROM CATS WITH PANLEUKOPENIA IN NORTHERN COLORADO.**

Feline panleukopenia (FPV) is caused by parvoviral infections in immunologically naïve cats. It has been shown that variants of the canine parvovirus type 2 can infect and replicate in the feline as well as the traditional canine host. However, clinical disease associated with infection from canine parvovirus in felines has not been well described in the literature. Detecting canine parvovirus type 2 in cats with clinical disease would have important implications for housing and infection control. The objective of this study was to genetically characterize parvoviruses from shelter cats with panleukopenia in Northern Colorado.

Whole blood samples from 20 cats with suspected panleukopenia from which a parvovirus had been amplified by a nonspecific endpoint parvovirus PCR were evaluated. A previously published quantitative polymerase chain reaction (qPCR) using minor groove binding probe targeting the capsid VP2 protein (DeCaro 2008) was used to differentiate canine and feline parvoviruses.

Using the differentiating qPCR, a detectable signal was generated from 13 samples. Using a second qPCR targeting an alternate region of the genome, viral copies in the positive samples ranged from 138 copies/μL to 211,333 copies/μL. Those samples in which virus was not detected by the differentiating qPCR all had <10 copies/μL using the alternate qPCR. This suggests that the differentiating assay is less sensitive than the endpoint PCR and alternate qPCR assays. DNA from all 13 samples from which a parvovirus was amplified using the differentiating qPCR assay were consistent with a feline panleukopenia virus. Sequencing of the differentiating qPCR product was consistent with feline parvovirus in all but two samples. However, the feline sequences could not be differentiated from parvoviruses of other species (fox, mink) in 11 of the 13 samples.

Canine parvovirus type 2 was not amplified based on analysis of the VP2 gene and so it was not likely to be a clinically important cause of the panleukopenia syndrome in this group of cats. Analysis of other genes should be evaluated to determine if other species of parvovirus can be the cause of feline panleukopenia.

**ID-35**

**MICROBIOLOGICAL EVALUATION OF FLEXICULT™VET AS A POINT-OF-CARE CANINE URINE CULTURE METHOD.**
A. Uhl, F.A. Hartmann, K.R. Viviano. Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI.

The gold standard for documenting bacteriuria is a quantitative urine culture. The purpose of this study was to evaluate the Flexicult™Vet urine culture kit (Atlantic Diagnostics) as an in-clinic screening test for bacteriuria in dogs. In this prospective study, client-owned dogs were enrolled if bacteriuria was suspected and urine was collected for a routine quantitative bacterial culture and antimicrobial susceptibility testing. For the purposes of this study urine culture results were compared between a quantitative culture in which the urine was refrigerated overnight to mimic sample shipment prior to plating at a reference laboratory and the immediate Flexicult™Vet culture.

Of the four canine urine samples were evaluated from 59 dogs with 36 bacterial isolates identified by the quantitative culture. The Flexicult™Vet bacterial isolates were consistent with the quantitative cultures in 26/36 positive cultures and yielded no growth in 36/36 of the negative cultures. The Flexicult™Vet correctly identified the bacterial isolate to the level of the genus/species in 16/26 of the positive cultures. The antimicrobial susceptibility data from the 16 cultures with a single bacterial isolate were consistent between the quantitative and Flexicult™Vet cultures in 8/16 cases with the majority of the inconsistencies with beta-lactams.

The Flexicult™Vet offers an in-house culture technique for bacteriuria in dogs with a sensitivity of 72% and specificity of 100%. Significant limitations occurred in patients with low levels of bacteriuria, mixed bacterial infections, or those receiving antimicrobial therapy at the time of urine culture and the susceptibility data for the beta-lactams.

**ID-36**

**DEVELOPMENT OF REAL-TIME PCR ASSAYS FOR CRYPTOSPORIDIUM CANIS AND CRYPTOSPORIDIUM FELIS.**
P. Tyrrell1, C.M. Leutenegger2, M. Lappin3, R. Chandrashkar4, IDEXX Laboratories, Inc., Westbrook, ME., IDEXX Reference Laboratories, West Sacramento, CA., Colorado State University, Fort Collins, CO.

The protozoan parasite Cryptosporidium can be the cause of diarrhea in humans and companion animals. The presence of Cryptosporidium oocysts may be determined through evaluation of fecal smears. Observation of Cryptosporidium oocysts will provide identity only at the genus level. To determine the species of Cryptosporidium present in canine and feline fecal samples, specific real-time PCR assays were developed. The targets for the assays were the Cryptosporidium canis Hsp90 and Cryptosporidium felis COWP genes. Analytical sensitivity, based on 10-fold dilution series of plasmid controls, was determined to be 10 copies for each assay. Specificity was confirmed through the testing of C. canis and C. felis positive samples. The C. canis positive samples were negative with the C. felis PCR assay and the C. felis positive samples were negative with the C. canis PCR assay. A set of Cryptosporidium spp. positive samples (as determined by a Cryptosporidium spp. real-time PCR assay, IDEXX Reference Laboratories, Inc.) consisting of 50 canine and 50 feline fecal DNA samples were selected to determine the performance of the assays. The C. canis real-time PCR assay detected C. canis DNA in 13 of the 50 canine samples. A total of 47 feline samples tested positive for the presence of C. felis DNA with the C. felis real-time PCR assay. The C. canis real-time PCR negative samples were tested for the presence of Cryptosporidium parvum with a conventional PCR assay targeting the COWP gene. One sample of this group was positive for the presence of C. parvum DNA. The remaining eighteen samples may represent other Cryptosporidium species or the C. canis variants not detected by the current assay. These results represent the successful development of the first real-time PCR assays for the detection of C. canis and C. felis.
NU-2

COMPARISON BETWEEN URINE PROTEIN:CREATININE RATIOS OF SAMPLES OBTAINED AT HOME AND IN A HOSPITAL SETTING: A PILOT STUDY. M. Duffy, A. Specht, R. Hill. University of Florida College of Veterinary Medicine, Gainesville, FL.

The urine protein:creatinine ratio (UPC) is a standard test for quantifying urine protein and UPC values form the basis for recommendations about monitoring and treatment of glomerular proteinuria. The objective of this pilot study was to compare UPC values of paired urine samples collected from individual dogs at home and in a hospital setting on the same day. Based on anecdotal experience from a few individual patients, we hypothesized that UPC values measured in samples obtained at home would be lower than those obtained in hospital. Clients with canine patients seeing the small animal internal medicine service were asked to collect urine samples at home the morning of the appointment. A second sample was collected while the dog was at the hospital (within 12 hours of the first sample). Inclusion in the study required paired urine samples of adequate volume and clarity from patients not receiving steroid or angiotensin converting enzyme inhibitor (ACEi) medications, ≥ trace dipstick protein from either sample, and inactive urine sediment in both samples. Urine samples were refrigerated as soon as possible after collection, dipstick and sediment evaluation was completed within 12 hour s, and a portion of each sample was frozen at -80°C within 12 hours. UPCs were performed within 2 months of storage.

Forty canine patients presented with urine samples collected at home. Twenty three were excluded (2 had grossly visible debris, 6 did not have hospital samples collected, 2 had inadequate volumes, 7 had no dipstick protein, 4 had active sediment, 1 was receiving prednisone, and 1 was receiving an ACEi). Of 17 eligible sample pairs (see Figure 1), 10/17 (58.8%) had higher UPC values from hospital samples than home samples, 3/17 (17.6%) had the same value, and 4/17 (23.5%) had lower values in hospital. Urine protein:creatinine ratio values were significantly higher in hospital samples compared to home samples (Wilcoxon signed rank test; p = 0.005). The median difference in UPC was 0.07 (range = -0.04 to 3.7).

It is not clear from this study whether higher median UPC values in hospital compared to home samples is clinically significant. However based on these observations, further study involving a larger number of patients may be warranted to confirm this finding and attempt to determine the potential cause(s) of the observed difference.

NU-3

IRON PARAMETERS OF CATS WITH CHRONIC KIDNEY DISEASE. J. Gest1, C. Langston1, A. Eatoff2. 1The Animal Medical Center, NY, NY, 2 Blue Pearl Veterinary Partners, NY, NY.

Iron deficiency is a proposed mechanism for the non-regenerative anemia that occurs in feline chronic kidney disease (CKD). However, little research investigating the iron status of these patients has been performed. The objective of this study was to assess iron parameters in healthy cats (Group 0, n = 39) compared to cats with CKD (Group 1, n = 40) and cats with non-renal illness (Group 2, n = 34). Complete blood counts, serum chemistries, serum iron, total iron-binding capacity (TIBC), and ferritin were measured and percent transferrin saturation (TSAT) was calculated. Exclusion criteria included prior iron administration, blood transfusion, or erythropoietin stimulating agent administration. Cats with concurrent CKD and non-renal related illness were excluded. Data were analyzed using non-parametric statistical testing.

No statistically significant differences were detected among groups for iron (p = 0.5001), ferritin (p = 0.4678), or TIBC (p = 0.1940). TIBC was significantly lower in Group 1 (median 262 ug/dL; IQR 233, 302; range 165, 488) compared to Group 0 (median 316 ug/dL; IQR 272, 345, range 296, 464); (p = 0.0030). When comparing anemic (hemoglobin < 9.5 g/dL) versus non-anemic cats with CKD, TSAT was significantly lower (p = 0.0334) in anemic cats (median 20.2%; IQR 17.8, 34.5; range 17.6, 35.9) compared to non-anemic cats (median 29.0%; IQR 25.5, 44.1; range 11.5, 94.4). No statistically significant differences were found for ferritin (p = 0.9445), iron (p = 0.2103) or TIBC (p = 0.9700). These results suggest that if an iron deficient state exists in anemic cats with CKD, it is more likely functional (e.g. secondary to decreased erythropoiesis or chronic inflammation) rather than absolute.
ACUTE PHASE PROTEINS AND IRON METABOLISM IN FELINE CHRONIC KIDNEY DISEASE. R. Javad, C. Grimes, L. Bau, G. Beauchamp, M. Dunn. Faculty of Veterinary Medicine of the University of Montreal, Saint-Hyacinthe, QC, Canada.

Anemia is a common finding in chronic kidney disease (CKD) and its etiology is multifactorial. In people, it has been shown that CKD is a chronic pro-inflammatory state contributing to iron sequestration and consequently anemia. However, the role of inflammation in feline CKD remains unknown.

In this prospective, observational, control study, we aimed to assess the role of inflammation, erythropoietin (EPO) deficiency, iron metabolism and hepcidin in cats with naturally occurring CKD, compared with a control population of healthy cats.

Twelve healthy cats (Group C) and 21 CKD cats (Group CKD) were recruited, according to IRIS classification. A complete physical examination along with hematology, biochemistry, serum amyloid A (SAA), haptoglobin (HAP), EPO, hepcidin, iron, TIBC and ferritin were performed using routine laboratory analyses and ELISA-assays.

Median serum SAA and HAP were significantly higher in the CKD group (p = 0.02 and p < 0.001, respectively) and median serum iron was significantly lower (p = 0.03) in the CKD group, compared to group C. In the CKD group, there was a significant association between serum creatinine and SAA (p = 0.008), HAP (p = 0.02), ferritin (p = 0.03) and TIBC (p = 0.001). SAA was also significantly associated with HAP (p < 0.05), ferritin (p < 0.05) and TIBC (p = 0.01). A significant inverse correlation was found between EPO and HAP (p = 0.01). Multiple linear regressions demonstrated that serum creatinine was positively associated with SAA and negatively associated with TIBC (R² = 0.67, p < 0.001).

These preliminary results reveal that feline CKD is a pro-inflammatory state, having significant impact on iron metabolism. Hepcidin analysis is underway and may help better characterize these interactions.
cats were significantly lower than control cats (P < 0.05). Additionally, F2-IsoP concentrations for stage II, III, and IV cats were significantly lower than stage I cats (P < 0.05, P < 0.05, P < 0.01, respectively).

The preliminary results of this study suggest that F2-IsoP concentrations do not increase with advancing IRIS stage in cats with CKD. Interestingly, there is a trend for decreasing F2-IsoP with advancing IRIS stage in this species. A larger population is being recruited to see if these observations are supported. Further studies will be needed to elucidate the differences in renal oxidative stress in cats compared to humans.

NU-8


In human medicine validated psychometric tools have shown that QoL is decreased in patients with chronic kidney disease (CKD). The aim of this study was to develop a quantitative, individualised and validated tool for assessment of owner-perceived QoL in cats (CatQoL) and to use this tool to compare the QoL between cats with CKD and healthy older cats.

Discussions and pilot surveys were conducted with owners of young (YH) (<9 years) and older healthy (OH) cats (≥9 years), and cats diagnosed with CKD, veterinarians and technicians. As a result, 18 questions/items were formulated. Each question could be scored according to severity of the item (range: never-the time, very abnormal-normal or terrible-fantastic) and the perceived importance (range: very important-not important at all) (b). The item-weighted-impact-score (IWIS) was calculated for each item by multiplying (a) and (b) and the average-weighted-impact-score (AWIS) by averaging all IWISs (both expressed as mean±SD). One overall question asked the owner to assign their cat's QoL a score between 1 ('cannot get worse') and 10 ('cannot get better'). Principal components analysis (PCA), assessing the tool's uni-dimensionality, factor loadings and extraction communalities, was used to validate the CatQoL and internal consistency reliability was assessed by calculating Cronbach's α. Spearman's rho was used to measure the correlation between AWIS and the overview question. A comparison of AWIS between YH, OH and CKD cats was performed using a Kruskal-Wallis test. All OH and CKD cats had a blood sample available ≤3 months prior to completing the CatQoL. CKD was diagnosed based on plasma creatinine concentration ≥2.0 mg/dl either on two consecutive visits or in conjunction with USG ≤1.035.

The survey was completed by 184 owners (105 YH; 23 OH; 56 CKD cats, of which 42 were IRIS stage 2 and 14 stage 3). The CatQoL showed a good internal consistency reliability (Cronbach’s α=0.77) and PCA revealed adequate uni-dimensionality with significant loadings (0.13-0.74) and communalities (all ≥0.43). Combined with the owners' perceived importance of the items, inclusion of all but one item ('sleeping') could be justified. 'Low activity levels' had the most negative impact on QoL (IWIS: -1.60 ± 1.4), followed by 'going to the vet' (IWIS: -1.58 ± 1.7), 'Liking food' was the most positive item (IWIS +5.41 ± 3.8), followed by 'interaction' (IWIS +4.46 ± 3.7). Moderate correlation was found between the overview question and AWIS (r=0.52, P < 0.01). YH cats had a significantly better AWIS than OH and CKD cats (1.38 ± 0.79 vs 0.75 ± 0.79 vs 0.30 ± 1.20, P < 0.001). The scores of old healthy cats and CKD cats did not differ significantly.

These results suggest that the CatQoL is a valid tool in assessing owner-perceived QoL in cats. The CatQoL may prove useful to evaluate disease management in addition to biological parameters. Cats with CKD did not have a significantly lower QoL score than old healthy cats. Inclusion of a larger number of cats, including those with a later stage of CKD, might alter this.

NU-9

ASSOCIATION BETWEEN UROLITHS AND RENAL DISEASE IN CATS. A. Cleroux-Gaboury, K. Alexander, G. Bouchamp, M. Dunn. Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Canada.

Chronic kidney disease (CKD) is common in feline patients and many also have nephrolithiasis. In human medicine, an association has been shown between uroliths and CKD and several risk factors, notably nephrolithiasis, have been identified in the development and progression of this disease.

The objective of this retrospective study was to determine the association between uroliths and CKD in cats.

Fifty-nine cats with an ultrasonographic diagnosis of uroliths for which serum creatinine and urine specific gravity (USG) were available at time of diagnosis were included in the study group. The control group was constituted of 65 randomly selected cats who underwent abdominal ultrasound without evidence of urolithiasis and who had serum creatinine and USG obtained at the time of the ultrasound. The groups did not differ in age, weight, sex nor breed.. The IRIS classification was used to determine the prevalence of CKD in both groups and the individuals were considered to have CKD when their serum creatinine was 140μmol/L or higher with a USG less than 1.035 (IRIS stage 2 and ≥3).

The prevalence of CKD was significantly higher in the study group (p = 0.003). The prevalence of CKD was significantly decreased when uroliths were located in the bladder (p = 0.02). However there was no significant association between uroliths and CKD.

The results confirmed our hypothesis. There is a statistically significant association between uroliths and CKD in cats.
NU-11
ENDOSCOPE-ASSISTED SURGICAL NEPHROLITHOTOMY FOR NEPHROLITHIASIS IN 11 DOGS. Y. Kuwahara, A. Ishino, N. Kuwahara. Kuwahara Animal Hospital, Nagoya, Japan.

Nephroliths are preferable to be removed in order to prevent or to resolve pyelonephritis and ureteral obstruction. Nephrotomy, which is the standard-of-care for removal of nephroliths in veterinary medicine, cause not a little decrease of renal function.

We use endoscope to minimize renal damage when removing nephroliths surgically in dogs. The purpose of this study is to demonstrate the procedure, efficacy and complications of our method for nephrolithotomy.

Our endoscope-assisted surgical nephrolithotomy (ESN) was performed as the following procedure. Bilateral nephrolithiasis had twice ESN with more than 1 month interval. After abdominal midline incision, a 16 gauge needle was punctured from surface of kidney toward nephrolith using ultrasonography guidance. A guide wire was inserted through the needle, and the needle was then removed. The puncture hole was dilated up to 21Fr by metallic dilator-tubes inserted over the guide wire. Nephroliths were observed by a rigid cystoscope through the dilator-tube, and were removed by forceps inserted directly through the puncture hole or inserted through cystoscope working channel.

The nephrolith, which had larger diameter than 7 mm, were removed after lithotripties with a pneumatic lithotriptor. After washing renal pelvis with saline using catheter inserted from both of puncture hole and ureteral orifice, observation of pelvis by cystoscope were performed to determine if remanence of nephroliths. The puncture hole was closed with absorbable suture. Only one dog which had azotemia preoperatively received also insertion of bilateral ureteral stents.

Eleven dogs (17 kidneys) had ESN. Median weight was 5.2 kg (2.5-16.1). Stone composition was calcium oxalate (6), mixed struvite-calcium phosphate (3) and calcium oxalate-calcium phosphate (2). Median stone size was 4 mm (1-21). Median procedure time was 132.5 minutes (90-240). In all cases, removals of all stones (1-5 each) were accomplished. There was no case with increase of plasma creatinine concentration after surgery. One case died postoperatively to the 7th day with acute pancreatitis. It was considered as a surgery-related complication. Another case died with acute gastroenteritis at 3rd month after surgery. Other 9 cases have median follow-up time of 42 months (9-67) and are still alive without recurrence of the nephrolithiasis.

ESN might be safe if careful of handling pancreas and be the procedure which could remove nephroliths reliably.

NU-12
CHARACTERIZATION OF PROTEINURIA IN DOGUE DE BORDEAUX DOGS USING ELECTROPHORESIS AND URINARY BIOMARKERS. R. Lavouè, P. Smets, C. Trumel, J.P. Braun, S. Daminet, D. Feeters. National Veterinary School of Toulouse, France., 1Faculty of Veterinary Medicine of Ghent, Belgium., 2Faculty of Veterinary Medicine of Liege, Belgium.

Dogue de Bordeaux dogs (DDBs) are predisposed to a juvenile glomerulonephropathy. An observational study revealed proteinuria in 30% of clinically healthy adult DDBs. Our objectives were to retrospectively characterize proteinuria in 47 non-proteinuric, 20 borderline-proteinuric and 33 proteinuric healthy DDBs using urinary albumin (uAlb), N-acetyl-b-glucosaminidase (uNAG), and C226, a newly developed anti-proteinuria diagnostic marker. SDS-AGE was used to classify proteinuria as physiological, non-proteinuric, borderline-proteinuric, and proteinuric.

SDS-AGE is useful to screen dogs at risk.

NU-13
TREATMENT OF CONGENITAL URETEROVESICULAR JUNCTION STENOSIS BY ENDOSCOPIC LASER-ABLA-TION IN DOGS: 10 CASES (2010-2013). E. Meier, A.C. Berent, C. Weisse, M. Dunot. Animal Medical Center, New York, City, NY, USA., 2University of Montreal, Faculty of Veterinary Medicine, St-Hyacinthe, QC, Canada.

Congenital ureterovesicular junction (UVJ) stenosis has rarely been reported in animals. The objective of this study was to report this condition, along with treatment, using cystoscopic-guided laser ablation (CLA), in dogs.

Cases were reviewed that were diagnosed with UVJ stenosis from 2010-2013 in the authors’ practice. A diagnosis was based on cystourethroscopy and ureteropellography and treatment was performed using CLA (diode or Ho:YAG).

Ten cases were identified (4 males; 6 females) with the most common breed being Labrador retrievers (n = 4). The median age was 12 months (3.5-96). Ureterovesicular junction stenosis was confirmed bilaterally in 4 and unilaterally in 6 cases (14 ureteral openings). Intramural ectopic ureteral openings were concurrently identified at the site of stenosis in 7 cases (10 ureteral openings). CLA was able to enlarge the stenotic opening in all cases, while concurrently fixing the ectopic ureters, if present. No complications were seen. Median anesthesia time was 90 minutes (range: 60-150). Median length of hospitalization was 22 hours (range: 10-48).

At the time of writing, 8 animals were alive and 2 dogs were lost to follow-up with a median follow-up time of 6 months (range, 1-33.5) and a mean of 15.7 months. Eighty-seven percent of the responding owners felt the procedure improved their pets quality of life.

Congenital UVJ stenosis should be considered in patients with distal ureteral obstruction, especially in young dogs. The use of a minimally invasive endoscopic laser-ablation procedure can be safe and effective in opening the orifice in dogs.

NU-14

Cerenia® is commonly used for acute vomiting. A recent pharmacokinetic and toxicity study in cats indicated that longer-term usage appears safe. The aim of this study was to assess the efficacy of Cerenia® for management of chronic vomiting and inappetence associated with feline chronic kidney disease (CKD).

Forty-one cats with stable IRIS Stage II or III CKD, no known concurrent illness and a complaint of chronic vomiting and inappetence attributed to CKD were enrolled in a placebo-controlled, blinded clinical study. A CBC, serum biochemistry, urinalysis, urine culture, T4 and blood pressure were required for entry. Cerenia® was administered at a dose of 1.1 mg/kg, range 0.6-2.9 mg/kg daily for 2 weeks. Owners kept daily logs of vomiting incidence, appetite and activity scores. Physical exam, weight, body condition score, and serum biochemistry were performed before and after the trial period. Mann Whitney statistics were used to compare treatment groups.

Thirty-three cats successfully completed the trial: 21 cats received drug (9 Stage II cats, 12 Stage III cats) and 12 cats
NU-15
RENAL PELVIC AND URETERAL ULTRASONOGRAPHIC CHARACTERISTICS OF NORMAL CATS, AND CATS WITH STABLE CHRONIC KIDNEY DISEASE, PYELONEPHRITIS AND URETERAL OBSTRUCTION.

Renal ultrasound is commonly used to assess cats with azotemia. The purpose of this study was to determine ultrasonographic characteristics of cats with stable chronic kidney disease (CKD) and determine if these were significantly different enough from cats with pyelonephritis and ureteral obstruction (UO) to aid in clinical diagnosis of the latter.

Fifty-four cats with clinically stable CKD and a negative urine culture were prospectively enrolled as well as normal control cats (10), cats with a clinical diagnosis of pyelonephritis (9) and cats with UO confirmed by surgical resolution (11). Renal ultrasound was performed and routine still images and cine loops were obtained for analysis. Analysis included degree of pelvic dilation and presence and degree of ureteral dilation. Measurements were compared between groups using nonparametric one way ANOVA with Dunn's post hoc analysis.

65% of stable CKD cats had measurable pelvic dilation compared to 78% of pyelonephritis cats and 100% of UO cats. There was no statistically significant difference between pelvic diameter in CKD cats vs. pyelonephritis cats. UO cats had significantly greater pelvic dilation than CKD cats (p < 0.05). Proximal ureter diameter was significantly greater in pyelonephritis and UO cats than CKD cats (p < 0.05).

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<thead>
<tr>
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<th>Sagittal Pelvic Diameter (mm)</th>
<th>Transverse Pelvic Diameter (mm)</th>
<th>Proximal Ureter Diameter (mm)</th>
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<tr>
<td>Normal</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>CKD</td>
<td>0 – 14.5</td>
<td>0 – 12.5</td>
<td>0 – 8.1</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>2.5</td>
<td>0 – 8.3</td>
<td>2.7</td>
</tr>
<tr>
<td>UO</td>
<td>5.2</td>
<td>0 – 26.2</td>
<td>5.6</td>
</tr>
</tbody>
</table>

A significant number of cats with clinically stable CKD had renal pelvic changes that are similar to cats with pyelonephritis yet suffer no apparent clinical consequence. This data suggests CKD cats should have a baseline ultrasonography performed so that changes documented during a uremic crisis can be better interpreted.

NU-16
COMPARING INVASIVE VS. NON-INVASIVE BLOOD PRESSURE: WHAT IS THE BEST ANATOMICAL LOCATION FOR COMPARISON OF INDIRECT VS. DIRECT BLOOD PRESSURE MEASUREMENTS?
S.Ramos, M. Dominigue, A. Shelby, M. Acierno, A. da Cunha. Louisiana State University School of Veterinary Medicine, Baton Rouge, Louisiana.

In companion animal medicine, noninvasive blood pressure methods are relied upon to monitor patients under anesthesia; however, blood pressure is likely to be dynamic through out the body and may be dependent upon the anatomical location measured. The objective of this study was to investigate the agreement between indirect blood pressure measured at the brachial area and direct blood pressure values from four different arteries in order to identify the best anatomical location for comparison of indirect vs. direct blood pressure measurements.

Twenty dogs from the LSU research colony were used in this study. 20G catheters were placed in four peripheral arteries: dorsal pedal artery, median sacral artery, intermediate auricular artery and superficial palmar artery. Direct blood pressure at the four sites was measured using electronic transducers and multifunction monitors. An indirect blood pressure cuff was placed in the brachial area and four sets of direct and indirect blood pressure measurements were simultaneously collected every two minutes. Linear mixed model with Tukey adjustment indicated that overall, indirect blood pressure as measured at the brachial area tends to have the best agreement with direct blood pressure measured at the median sacral artery. In addition, direct blood pressure measured at the intermediate auricular and superficial palmar were significantly lower than measurements taken from dorsal pedal and median sacral arteries.

The best anatomical location for comparison between indirect blood pressure measured at the brachial area and direct blood pressure is the median sacral artery.

NU-17
PLASMA ANALYTES IN HEALTHY CATS: WITHIN-DAY VARIATIONS AND EFFECTS OF A STANDARD MEAL.
B.S. Reynolds, C. Brosse, E. Jeunesse, H.P. Lefebvre. Clinical Research Unit and 2UMR 1331 Toxalim, INRA, University of Toulouse, INP, National Veterinary School, Toulouse, France.

Limited information is available on pre-analytical variations of plasma analytes in cats. The objectives of this study were to assess the effects of the time of sampling and a standard meal on plasma analytes in healthy cats.

Eight healthy adult fasted cats underwent blood sampling every 2 h from 8 am to 8 pm twice at 12-day interval. On the days of sampling, 4 cats were kept fasted (F) and the others were fed just after the first sample, in a cross over design. Plasma glucose, urea, creatinine, sodium, potassium, chloride, CO₂, calcium, phosphate, proteins, albumin, cholesterol and triglycerides, ALT and ALP were assayed. Effects of time of sampling and meal on plasma biochemistry results were tested using a general linear model.

Within-day variations were negligible in F cats except for urea and creatinine with mean plasma concentrations higher (15% and 18%, respectively) in the afternoon than in the morning. Mean phosphate (+22%) and creatinine (-23%) concentrations varied noticeably 8 and 10 h after the meal, respectively. Post-prandial increases of indisputable clinical relevance were observed for CO₂ and urea (+34% and +38% after 2 and 8 h, respectively).

In conclusion, meal and time of sampling can affect clinical interpretation of some plasma analytes in cats.

NU-18
SIZE OF THE URINARY BLADDER IN HEALTHY CATS: COMPARISON OF ULTRASONOGRAPHY AND MANUAL PALPATION.
B.S. Reynolds, L. Gregoire, O. Dossin, M. Aumann, D. Concordet, A. Nicolle, H.P. Lefebvre. Clinical Research Unit, 1Department of Clinical Sciences, and. UMR 1331 Toxalim, INRA, University of Toulouse, INP, National Veterinary School, Toulouse, and, Mirepoix France.

Estimation of the bladder size by manual palpation or ultrasonography is frequently used in clinical settings. The objective of this study was to compare these two techniques and the effect of the investigator on bladder size measurements.

Two groups of six healthy adult cats were used. Four well-trained investigators measured the bladder size, 3 with manual palpation (MP), 1 with ultrasonography (US). The measurements were performed randomly and blindly on two different days. Each MP investigator examined 6 cats at 3 different times on a given day. After each palpation, the MP investigator estimated
the transversal bladder diameter using a visual analogue scale. The diameter was also estimated by US measurement (Vivid i, GE healthcare; 10 MHz probe). Within- and between-investigateur variability and difference between manual and ultrasonographic measurements were determined.

The smallest within-investigator variability was observed for US (Table 1).

**Table 1. Mean bladder size and within-investigator CV**

<table>
<thead>
<tr>
<th></th>
<th>MP1</th>
<th>MP2</th>
<th>MP3</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (cm)</td>
<td>4.3</td>
<td>4.7</td>
<td>2.8</td>
<td>4.2</td>
</tr>
<tr>
<td>CV (%)</td>
<td>10.0</td>
<td>9.9</td>
<td>15.4</td>
<td>4.4</td>
</tr>
</tbody>
</table>

The measured size was significantly different between US and MP2 (P = 0.02) or MP3 (P < 0.001) and between MP investigators (P < 0.001).

In conclusion, bladder diameter estimation is repeatable, but depends on the investigator.

**NU-19**

**ASYMPTOMATIC BACTERIURIA IN OLDER CATS: ASSOCIATION WITH SURVIVAL.** J. White, N. Cave, D. Thomas, A. Grinberg, C. Heuer.

Asymptomatic bacteriuria (ASB) was identified in 10 to 13% of healthy cats aged 7 years or older. A prospective, observational study was performed to evaluate the association between ASB and survival among the same cohort of cats.

Cats from the Feline Nutrition Unit at Massey University were tested on five occasions over three years. Urine samples were obtained by cystocentesis for quantitative urine culture and blood samples were obtained for creatinine determination. Cats were grouped housed and weighed weekly. Cats with persistent weight loss of 10% or greater were evaluated by a veterinarian. Episodes of ASB were not treated but medical care otherwise followed standard veterinary practice. Veterinarians involved in evaluation and euthanasia of cats were unaware of the study's results.

Survival of cats was calculated from the date of the first urine culture to the date of euthanasia for medical reasons. Cats were censored if alive 6 months after the final urine collection. The association between urine culture results and survival was evaluated while accounting for the potential confounding effects of age and sex and creatinine concentration using a Cox's proportional hazard model with a counting process to allow for the repeated measures on each cat.

A total of 256 urine samples were obtained from 67 cats over 3 years. There were 28 positive cultures from 11 cats. Five of eleven urine culture positive cats and 30 of 56 urine culture negative cats were euthanised for medical reasons during the study. Only age was significantly associated with survival. Each increase in year of age was associated with a 1.7 fold increase in the hazard of dying (95% CI 1.4 – 2.1, p < 0.001). ASB was not significantly associated with survival.

Considering the observed mortality rate of 54% over 3.5 years in the culture negative cats and with 11 of 67 cats having at least one positive urine culture, the study had adequate power (80%) to detect a 1.5 fold difference in mortality between culture positive and culture negative cats. This study does not support the routine treatment of ASB. However, studies involving larger numbers of cats would allow detection of smaller differences in survival between culture positive and culture negative cats.

**NU-20**

**EFFECT OF ESTRIOL ON URODYNAMIC FINDINGS 24 HOURS AFTER DOSES IN FEMALE SPAYED RESEARCH BEAGLES.** L.E. Ferguson, L. Martin, M.R. Lappin.

Estrol is a short acting estrogen used for the management of urinary incontinence that is available as a commercial product in many countries (Incurin®, Merck Animal Health). While safe when administered daily, some dogs maintain continence on Incurin® administered every other day. The objective of this study was to describe select urodynamic findings in dogs 24 hours after administration of Incurin®.

Research beagles (n = 5) that had been used in a previous vaccine study had an ovariohysterectomy performed 10 months before this study. The maximum urethral closure pressure (MCUP) and functional profile length (FPL) were determined 3 times over 2 weeks prior to starting Incurin® administration. Incurin® was dosed at 2 mg, PO, daily in the morning for 14 days. On the morning of the 15th day, each dog received xylazine 1 mg/kg and atropine 0.02 mg/kg subcutaneously. A triple lumen urinary catheter was placed with the guidance of a speculum and a urethral pressure profile (UPP) was obtained using standard operating procedures at the Veterinary Teaching Hospital. The percentage increase in MUCP and FPL were determined by comparing the measurements obtained 24 hours after the 15th dose of Incurin® compared to the last baseline measurements before Incurin® therapy was begun.

Twenty-four hours after the 15th daily dose of Incurin®, the MUCP and FPL were numerically increased compared to baseline in 4 of 5 dogs. The MUCP increases ranged from 0 to 222.2% (Mean = 83.8%; Median = 17.6%). The FPL increases ranged from 0 to 40% (Mean = 13.6%; Median = 12.7%). A urinalysis collected at the time of data collection was normal for all dogs.

The results support the clinical observations that Incurin® induces urodynamic changes that would promote continence for greater than 24 hours in many dogs.

**NU-21**

**EVOLUTION OF ANTIMICROBIAL RESISTANCE PATTERNS AND CLINICAL DATA IN CANINE URINARY TRACT INFECTIONS AT A VETERINARY TEACHING HOSPITAL.** D.A. McGovern, F. Gashen, M. Acerno, A. Roy.*

This retrospective study was designed to investigate clinical phenotype of canine urinary tract infections (UTI) and resistance patterns of causative bacteria in a veterinary teaching hospital over a six-year period.

Signalment and clinical data were collected from the medical records of dogs with a positive urine culture during the years 2006, 2009, and 2012. Sensitivity patterns of isolated bacteria (disk diffusion) were plotted. The Somers’ D test was used to detect associations between time and resistance patterns with significance set at p < 0.05, chi-square statistics were used as appropriate for other data.

Positive urinary cultures were retrieved (n = 385). Moderate statistically significant increases in resistance over time were detected for *E. coli* (amikacin, tobramycin), *Proteus* spp. (cefovecin, cepodoxime), *Enterobacter* spp. (ampicillin, marbofloxacin), and *Enterococcus* spp. (timentin, tobramycin). Decreased resistance was observed for *E. coli* (cephalothin, ampicillin), *Enterobacter* spp. (ampicillin, marbofloxacin, tobramycin), and *Enterococcus* spp. (enrofloxacin).

Clinical data from 218 cases was reviewed. The three most common organisms cultured were *E. coli* (45%), *Staphylococcus* spp. (12%), *Enterococcus* spp. (11%), with an increase in the proportion of *Staphylococcus* spp. infections over time. Signs of lower urinary tract infection were present in 28% of cases. UTI were acquired during hospitalization in 31 dogs (14%), 17 had an indwelling urinary catheter. Predisposing factors were...
identified in 89% of dogs. Urinalysis revealed pyuria in 69% of cases, and bacteruria in 73%.

This study showed only mild changes in the antimicrobial resistance pattern of bacteria causing UTI in this cohort consisting of first, second and third opinion cases.

NU-22 ELECTROPHORETIC PATTERN OF URINARY PROTEINS IN YOUNG DOGS WITH CHRONIC KIDNEY DISEASE, AND IN DOGS WITH RENAL GLUCOSURIA. D.S. Carageasoc, C.R. Martorell1, F.C. Chacar1, M.M. Kogikas, L. Andrade2. 1School of Veterinary Medicine and Animal Science, University of Sao Paulo, 2School of Medicine – University of Sao Paulo, Brazil.

Proteins filtered normally in the glomerulus are almost completely removed from tubular fluid by megalin and cubilin receptor-mediated endocytosis process. Therefore, in tubular disease some low molecular weight proteins (LMWP; < 60 kDa) fail to be reabsorbed and can be found in urine. Otherwise, in glomerular disease, high molecular weight proteins (HMWP; > 60 kDa) can be detected due to loss of macromolecules through the glomerular barrier. Urine protein electrophoresis by sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) determines the molecular weights. The goal of this study was to evaluate the electrophoretic pattern of urinary proteins in young dogs with chronic kidney disease (CKD), and in dogs with renal glucosuria. Group A was composed of five young dogs with CKD (24 to 36 month-old and various breeds), and Group B of five dogs with renal glucosuria (72 to 120 month-old and various breeds). Ten clinically normal dogs were enrolled as control group. Urinary protein-to-creatinine (UPC) ratios were confirmed according to the guidelines. Electrophoresis was performed in the last urine sample that UPC ratio was confirmed. The exclusion criteria were any other condition that could cause secondary renal proteinuria. In the Group A, mean of UPC (± SEM) was 2.1 ± 1.2 (min= 0.4 and max= 6.7), and 3 out of 5 dogs had UPC > 0.5 (control = 0.12 ± 0.03, min= 0.03 and max= 0.40). All dogs of Group A had higher percentages of LMWP (63.7 ± 2.5%); min=54.5% and max=69.4%; t test p < 0.05) compared to control (40.6 ± 2.7%, min= 31.1% and max= 54.0%) and 3 to 5 bands of LMWP (control dogs had 2 to 5 bands). In Group B all dogs had UPC > 0.5 (2.2 ± 2.3; min= 0.6 and max= 4.1; p < 0.05), as well as higher percentages of LMWP (53.7 ± 6.1%); min= 30.0% and max= 64.2%; p < 0.05) and 2 to 4 bands. Proteins bands of 18 to 25 kDa were found in higher percentages in Group A (26.9 ± 9.1%; p < 0.05) compared to control (9.9 ± 2.5%); min= 0 and max= 20.4); as well as in Group B (21.7 ± 4.2%; p < 0.05) versus control. The LMWP of 18 to 21 kDa observed in groups A and B may suggest that those proteins could be related to retinol binding protein (RBP) which is a marker of tubular abnormality that resulted from the impairment of LMWP reabsorption. HMWP were found as well, however the percentages of HMWP in Groups A and B were lower comparing to control (p < 0.05). The limitation of this study was to have not exactly identified those proteins, and further investigations are needed e.g. using western blotting or proteomic analysis. In conclusion, the evaluation of urinary proteins by molecular weights showed that tubular proteinuria predominated, and it might add more information about the origin of that proteinuria, and not only based on UPC, as the quality of proteinuria could be associated to the segment of the nephron that is damaged.

NU-23 ULTRASONOGRAPHIC MEASUREMENT OF RENAL COR-TICAL THICKNESS INDEX APPLYING TO ACUTE AND CHRONIC KIDNEY DISEASES IN CATS. M. Choi1, N. Lee1, S. Key2, H. Kim1, M. Choi1, Haemaru Referral Animal Hospital, Seong-Nam, Korea, 2Seoul National University, Seoul, Korea.

Ultrasonography is an effective modality for the detection of kidney disorders providing information of qualitative and quantitative data. The renal length is commonly used quantitively but varies depending on sex, age, weight and needs another objective evaluation method. In human, as the change in renal cortical thickness (RCT) is important because it is useful for differentiating between acute (AKI) and chronic (CKD) renal disease as well as more closely related to eGFR (estimated glomerular filtration rate) than renal length. The aim of this study was to determine whether RCT was effective for applying feline kidney disease as human medicine.

Nineteen clinically healthy cats, 19 AKI and 39 CKD patients (IRIS 1: 7, IRIS 2: 20, IRIS 3: 6, IRIS 4: 6) were measured RCT (cm) and each results adjusted using body surface area (RCT index). It was measured in the sagittal or dorsal plane, perpendicular to the capsule as shortest distance from the medulla. Mean RCT index of normal and AKI patients was 1.54 ± 0.22 and 1.64 ± 0.30. In CKD patients, IRIS 3 (2.30 ± 0.61) and 4 (3.12 ± 0.81) was higher than normal (p < 0.05). Between AKI and CKD (IRIS 3 and 4) patients, RCT index of CKD cats was significantly higher than AKI (p < 0.05). Those results suggested that CKD patients showed higher RCT index and it could distinguish from AKI patients. Although the population of the study was small and need more clinical data (histopathologic examination, GRF), RCT index may be a useful method for evaluating kidney objectively.


Chronic kidney disease is an irreversible, progressive loss of renal function, eventually leading to azotemia and uremia. The prevalence of kidney disease increases as cats get older, and approaches an incidence of 9% in cats over 10 years of age. Nutritional management of chronic kidney disease in cats has been the established treatment for several decades. Therefore, palatability and acceptability are important attributes of a renal therapeutic food. The objective of this study was to validate the palatability and food transition success of a renal therapeutic food and to substantiate the use of the product for management of renal disease at all IRIS stages.

This was a blinded, prospective 12 month clinical study of 128 client-owned adult cats with varying stages of renal disease. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee, Hill’s Pet Nutrition, Inc., Topeka, KS, USA. All cats were maintained in their owner’s home during the length of the study. The eligibility of each animal was assessed by medical, drug, and dietary histories, physical examination, and laboratory analysis of blood and urine. Cats were assigned to an IRIS stage based on elevated blood creatinine and the presence of decreased kidney size, or dilute urine, or elevated UPC. All cats were transitioned over a seven day period from a grocery brand food to the test food with controlled levels of protein and phosphorus. Dermatological and physical examination, body weight and body condition assessments were performed by veterinarians at 1, 3, 6, 9, and 12 months. Concurrently, pet owners recorded changes in overall health, quality of life, energy level, youthfulness and vitality, desire for attention, socializing with other animals, and playfulness. Pet owners also evaluated food liking, food consumption, eating enthusiasm, skin and coat, and stool quality.
Sixty-seven cats completed the study according to protocol. Most (95%) cats successfully transitioned onto the test food. A majority of pet owners reported that their cats liked (90%) the renal therapeutic food, ate enthusiastically (69%), and consumed most or all of the food offered (55%). Body weights increased slightly (p = 0.01), while a modest decrease in BCS was reported (p = 0.002). Veterinarians observed improvements in overall skin and coat (p = 0.01), luster (p < 0.0001), texture (p = 0.0004), and reduced shedding (p = 0.0002). Pet owners noticed significant improvements in overall health (p ≤ 0.008), quality of life (p ≤ 0.005), energy level (p < 0.0001), youthfulness/vitality (p < 0.0001), desire for attention (p < 0.0001), socializing (p ≤ 0.004), and playfulness (p < 0.0004) during the first six months. Blood creatinine and urea nitrogen levels remained relatively unchanged over time, with a slight decrease in blood creatinine for cats with advanced stages of renal disease.

NU-28
A FOOD WITH CONTROLLED PROTEIN AND PHOSPHORUS HAS BENEFICIAL EFFECTS FOR DOGS WITH VARYING STAGES OF RENAL DISEASE. D.A. Fritsch, D.E. Jewell. Hill’s Pet Nutrition, Inc. Science and Technology Center Topeka, KS.

Chronic kidney disease (CKD) is an irreversible, progressive loss of renal function, eventually leading to azotemia and uremia. The prevalence of kidney disease increases as dogs get older, and approaches an incidence of 3% in dogs over 10 years of age. Nutritional management of chronic kidney disease in dogs has been the established treatment for several decades. Signs typically associated with CKD include anorexia, vomiting, and weight loss. Therefore, palatability and acceptability are important attributes of a renal therapeutic food. The objective of this study was to validate the palatability and food transition success of a renal therapeutic food and to substantiate the use of the product for management of renal disease at all IRIS stages.

This was a blinded, prospective 12 month clinical study of 73 client-owned adult dogs with varying stages of renal disease. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee, Hill’s Pet Nutrition, Inc., Topeka, KS, USA. All dogs were maintained in their owner’s home during the length of the study. The eligibility of each animal was assessed by medical, drug, and dietary histories, physical examination, and laboratory analysis of blood and urine. Dogs were assigned to an IRIS stage based on elevated blood creatinine and either the presence of decreased kidney size, or dilute urine or, as defined by UPC. All dogs were transitioned over a seven day period from a grocery brand food to the test food with controlled levels of protein and phosphorus. Dermatological and physical examination, body weight and body condition assessments were performed by veterinarians at 1, 3, 6, 9, and 12 months. Concurrently, pet owners recorded changes in overall health, quality of life, energy level, youthfulness and vitality, desire for attention, socializing with other animals, and playfulness. Pet owners also evaluated food liking, food consumption, eating enthusiasm, skin and coat, and stool quality.

Thirty-three dogs completed the study according to protocol. Most (99%) dogs successfully transitioned onto the test food. A majority of pet owners reported that their dogs liked (92%) the renal therapeutic food, ate enthusiastically (75%), and consumed most or all of the food offered (84%). Body weights increased slightly (p = 0.003) while no significant change in BCS was reported. Veterinarians observed improvements in coat luster (p = 0.005), coat texture (p = 0.001), and reduced shedding (p = 0.003). Pet owners of dogs with renal dysfunction noticed significant improvements in overall health (p ≤ 0.004), quality of life (p ≤ 0.03), energy level (p < 0.0001), youthfulness and vitality (p ≤ 0.007), desire for attention (p < 0.0001) after one month of feeding. Likewise, blood creatinine (p ≤ 0.004) and urea nitrogen (p ≤ 0.0005) were significantly lower in dogs with renal dysfunction after one month of feeding.

NU-27
FEEDING REDUCED PROTEIN AND PHOSPHORUS FOOD TO DOGS WITH NATURALLY OCCURRING RENAL DISEASE REDUCES SERUM CONCENTRATIONS OF CREATININE AND SYMMETRIC DIMETHYLARGININE (SDMA). D.E. Jewell1, D. Fritsch1, M. Yerramilli2, E. Obara3, M. Yerramilli2, J.A. Hall4. 1Hill’s Pet Nutrition, Inc., Science and Technology Center, Topeka, KS; 2IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine; 3Department of Biomedical Sciences, Oregon State University, Corvallis, OR.

Sixty-two dogs with variable renal function, defined by serum blood urea nitrogen (BUN) and creatinine concentrations, urine protein to creatinine ratio, and urine specific gravity (USG) sub-
sequently had serum concentrations of SDMA evaluated initially and up to 12 months later while being fed a renal protective food (Prescription Diet k/d®). The study protocol was reviewed and approved by the Instrumental Animal Care and Use Committee, Hill’s Pet Nutrition, Inc., Topeka, KS. All dogs were maintained in their owner’s home throughout the length of the study. There was a significant correlation between serum SDMA and serum creatinine (r = 0.78, p < 0.0001) and BUN (r = 0.69, p < 0.0001) concentrations, as well as between serum SDMA concentration and USG (r = -0.28, p < 0.0002). Serum creatinine concentration was significantly correlated to BUN concentration (r = 0.68, p < 0.0001), and USG (r = -0.19, p < 0.01). There was a significant correlation between the change in serum SDMA and change in serum creatinine (r = 0.88, p < 0.0001) as well as the change in SDMA and change in BUN (r = 0.92, p < 0.0001). The change in serum creatinine was also significantly correlated to change in BUN (r = 0.90, p < 0.0001). There was a linear time-on-food related decline (p < 0.05) in serum SDMA concentration. Normal dogs had a small reduction in serum SDMA concentration (15% decline) in the first month, whereas dogs with severe loss of renal function had a 36% reduction in SDMA concentration during the first month. This study shows that serum SDMA concentration is a sensitive indicator of change in renal function, and decreases in dogs with renal disease that are fed a food with reduced protein and phosphorus content.

NU-29
SERUM ALDOSTERONE: INTRA AND INTER-ASSAYS, AND EVALUATION IN DOGS WITH CHRONIC KIDNEY DISEASE. M.M. Kogika 1, F.C. Chacar 1, M.F. Waki 1, C.R. Martorelli 1, D.S. Caragelasco 1, L.R.F. Rochelle 1. 1University College of Veterinary Medicine, Seoul, South Korea. 2KonKuk University, Seoul, South Korea. 3School of Veterinary Medicine and Animal Science, University of Sao Paulo. 4School of Medicine, University of Sao Paulo, Brazil.

Renin-angiotensin-aldosterone system (RAAS) may play a pivotal role in the progression of chronic kidney disease (CKD), since its activation could cause glomerular hypertension, hypertrophy and sclerosis. Aldosterone has been recognized as an important mediator of renal injury, according to the studies in rats and humans, and it induces renal vascular remodeling, generation of reactive oxygen species, fibrosis and proteinuria. The aim of this study was to perform intra and inter-assays for serum aldosterone (SA) as well as to measure sequentially SA in dogs with CKD. Pooled serum sample was separated in 20 aliquots and SA was determined by analyzing 5 aliquots per day, and it was repeated for 4 consecutive days. SA was measured by radioimmunoassay (TKALiCoat-a-Count; Siemens). Thirteen CKD dogs (various ages and breeds, and mongrels) were followed-up, clinical data recorded every 30 to 40 days (visit 1 to 4), and they were classified in stage 1 (n = 5), stage 2 (n = 4) and stage 3 (n = 4), and twelve clinically normal dogs (normotensive) were evaluated as control, and for all these animals SA was measured in duplicate. The mean (± SEM) percentages of coefficient of variation (CV) of the duplicate measurements were 9.5 ± 1.0% and 9.5 ± 1.5% for CKD dogs and control dogs, respectively. Intra-assay percentages of CV were: 10% on day 1, 5% on day 2, 31% on day 3, and 21% on day 4; good repeatability was observed on days 1 and 2, and on days 3 and 4 it was satisfactory. Inter-assay CV was 23% and it was satisfactory. CKD dogs of stages 1, 2 and 3 were normotensive, except one of stage 2 which was hypertensive and under amlopidine and ACE-inhibitor treatment. SA concentrations mean (± SEM) of each CKD stage (1, 2 and 3) were 74.4 ± 12.7 pg/mL (min= 1.4, max= 224.9, p=0.3880), 95.4 ± 24.8 pg/mL (min = 9.3, max= 379.5, p=0.9282) and 85.1 ± 24.2 pg/mL (min= 0.6, max= 212.7, p= 0.5696), respectively, and there was no significant different compared to the control group (98.9 ± 29.5 pg/mL; min = 7.3 and max= 318.0). One dog, normotensive however, in stage 2 presented the highest values of SA in visit 1 and 2 (279.3 and 379.9 pg/mL, respectively). The lowest SA levels (9.3 to 38.4 pg/mL) were detected in that dog of stage 2 that was under amlopidine and ACE-i. Serum levels of Na+ and K+ were normal in all dogs in all stages, and no correlations were observed between SA and Na+, K+, or systolic blood pressure. Herein, we conclude that SA values can be relatively unstable and measuring only one sample and punctually could misinterpret the data. Therefore, the serial SA measurements could give additional information for the better understanding of the role of RAAS during the course of CKD.

NU-30
ADMINISTRATION OF AUTOLOGOUS BONE MARROW DERIVED MESENCHYMAL CELLS CONTRIBUTES TO THE REGENERATION OF RENAL TUBULAR EPITHELIAL CELLS AND THE PREVENTION OF RENAL FIBROSIS AFTER ACUTE KIDNEY INJURY IN DOGS. C.Y. Lim, S.J. Moon, J.I. Han, S.G. Kim, M.H. Kang, H.M. Park. KonKuk University College of Veterinary Medicine, Seoul, South Korea. Acute kidney injury (AKI) is an abrupt loss of renal function that results in the retention of nitrogen wastes in the bloodstream. This study was conducted to evaluate the usefulness of mesenchymal stem cell (MSC) therapy for the treatment of AKI in dogs. A total of 6 healthy dogs were included in the study. All dogs were randomly divided into 2 groups; saline and MSC group. Cisplatin was used to induce AKI in dogs. Each group was administered intravenous saline or autologous bone marrow-derived MSC (BM-MSC) via cephalic vein immediately after cisplatin injection. During the experiment, CBC, serum biochemis-
try profiles, urinalysis, glomerular filtration rate and BM-MSC tracking by magnetic resonance imaging were performed. At day 4, all dogs were euthanized and histopathology, immunohistochemistry [for vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen (PCNA), and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)] and reverse-transcriptase polymerase chain reaction (RT-PCR) (for VEGF, TNF-α, TGF-β, SDF-1 and HGF gene) were performed. To assess the differences between groups, repeated measures analysis of variance (RM-ANOVA) or student’s t-test was used. Differences for all analysis were considered significant at *p* < 0.05.

Laboratory examination revealed no improvement in the renal function in the MSC group. BM-MSC was not detected at day 4 upon magnetic resonance imaging. Histopathology showed significant decrease of the inflammatory cell infiltration or fibrotic changes in the MSC group. Immunohistochemistry revealed significantly higher number of PCNA-positive cells (49.97 ± 3.80 cells per HPF) than that of the saline group (32.83 ± 2.26 cells per HPF, *p* < 0.05). The expression level of TNF-α or TGF-β was lower in MSC group compared with saline group.

This study demonstrated that MSC treatment in cisplatin-induced canine AKI accelerates renal regeneration and prevent renal injury through reduced inflammatory cell infiltration, less fibrosis, higher level of expression of PCNA, and lower expression of TNF-α and TGF-β with no negative effect on impaired renal function.

**NU-31 EFFECT OF GRANULOCYTE COLONY STIMULATING FACTOR THERAPY ON RENAL REGENERATION AFTER ACUTE KIDNEY INJURY IN DOGS.** C.Y. Lim, S.J. Moon, J.I. Han, S.G. Kim, H.M. Park. KonKuk University College of Veterinary Medicine, Seoul, South Korea.

Effects of granulocyte colony stimulating factor (G-CSF) on canine acute kidney injury are unknown. The study was conducted to evaluate the therapeutic efficacy of G-CSF on acute kidney injury in dogs.

A total of 6 healthy dogs were included in the study. All dogs were randomly divided into 2 groups (control and G-CSF). Following kidney injury caused by intravenous cisplatin injection (day 0), each group was immediately administered subcutaneous saline or G-CSF for 5 consecutive days (from day 0 to day 4). A complete blood count, serum biochemistry profile, and urinalysis were performed for each dog every day during the experiment. After 5 days, all dogs were euthanized. Histopathologic examination, immunohistochemistry for vascular endothelial growth factor (VEGF), proliferating cell nuclear antigen (PCNA), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), and reverse transcriptase-polymerase chain reaction (RT-PCR) assay for VEGF, tumor necrosis factor alpha (TNF-α), tumor growth factor beta (TGF-β), stromal cell-derived factor-1 (SDF-1) and hepatocyte growth factor (HGF) were performed. To assess the differences between groups, repeated measure analysis of variance (RM-ANOVA) or student’s t-test was used. Differences for all analysis were considered significant at *p* < 0.05.

In the G-CSF group, histopathology and immunohistochemistry showed less inflammatory cell infiltration and fibrotic area in the kidney, an increase of expression of VEGF, PCNA and TUNEL, suggesting less fibrotic change and increased proliferation of renal tubular epithelial cells. The RT-PCR showed decreased expression of TNF-α and TGF-β in the G-CSF group, suggesting that G-CSF decreases inflammatory response after acute kidney injury in dogs. CBC, serum biochemistry profiles and urinalysis did not show any improvement of renal function. The study demonstrated that the short-term administration of G-CSF accelerates renal regeneration after acute kidney injury in dogs.

**NU-32 A TWO YEAR-LONG PROSPECTIVE, RANDOMIZED, DOUBLE-MASKED STUDY OF NUTRITION ON THE RECURRENTINESS OF MAGNESIUM AMMONIUM PHOSPHATE UROLITHIASIS IN STONE FORMING CATS.** L. Dziełych1, J. Kruger1, J. MacLeay2, J. Merrill3, I. Paetkau-Robinson1, J. Brejda1, S. Steck4, S. Davidson1, C. Osborne1. 1University of Minnesota, St. Paul, MN., 2Michigan State University, E. Lansing, MI., 3Hills Pet Nutrition, Inc., Topeka, KS., 4Alpha Statistical Consulting, Inc. Lincoln, NE.

Struvite uroliths account for approximately 50% of feline stone submissions to the Minnesota Urolith Center. To date, no study has followed affected cats long term to determine the rate of recurrence, confounding factors and the effect of dietary intervention.

This study was a 2- year-long, randomized, double-masked and controlled study examining 2 foods for management of struvite urolithiasis. Compared to the Control (Ctl) food the Test food contained greater amounts of anti-oxidants and n3-fatty acids, and less Ca and Mg with a target urine pH of 6.2-6.4. The Ctl food was designed to be similar to popular grocery brands and had a target urine pH of 6.6-6.8. Foods were otherwise similar and met minimum AAFCO requirements for adult cats. Pro-tocols were ACUC approved. Cats with a recent history of confirmed struvite uroliths were recruited. Owners were given the choice of food form (wet, dry or combined) and then cats were randomized onto Test or Ctl foods. Cats were evaluated at 4 month intervals by physical examination, abdominal radiography and urinalysis and serum chemistries. 24-hour urine collections for chemistry and relative super saturation (RSS) calculation were conducted at 4 and 24 months or at detection of stone recurrence. Data was analyzed with repeated measures ANOVA and a generalized linear mixed model.

Thirty-seven cats completed the study (18 Ctl and 19 Test). Four Ctl food fed cats and 1 Test food fed cat had recurrence of a struvite urolith during the 2 year study. Urine specific gravity was similar in cats fed dry alone versus combined dry and wet formulations. The relative risk of forming a stone when fed the Ctl food was 4.2 times higher. At the study endpoint Struvite RSS was 2.97 times higher in Ctl food fed cats (*p* = 0.004). Urine pH was significantly lower in Test food fed cats (mean 6.46 (SE 0.05) compared to Ctl food fed cats 6.91 (SE 0.06, *p* < 0.001). Over time, the probability of struvite crystaluria in the Ctl group continually increased to over 50%. In contrast, this probability declined in the Test group to less than 10% and the change over time and between groups was significant (*p* = 0.0456).

In conclusion, the nutrition of the Test food was associated with a significantly lower struvite RSS and a substantially lower rate of struvite urolith recurrence compared to the Ctl food. Long term acceptance and compliance was high. Interestingly, the probability of struvite crystaluria progressively increased in the Ctl group, but decreased in the Test group over the 24 months of the study.
cise and social interaction in spacious rooms with natural light. Cats were fed 2 therapeutic foods that are available as wet and dry forms. They received one form (wet or dry) at a time for 1 month each. The therapeutic food is designed to minimize risk for urinary tract disorders and the other is intended for obesity and diabetes mellitus. Foods were fed to maintain body weight. A 24-hr urine collection was performed on day 28. Equil 2 software was used to calculate RSS CaOx and struvite from urine concentrations of NH₄⁺, Ca²⁺, Cl⁻, Citrate, creatinine, Mg²⁺, oxalate, PO₄³⁻, K⁺, Na⁺, SO₄²⁻ and urine pH. SAS® version 9.2 was used for the analysis and significance was p ≤ 0.05.

For all foods and forms the mean RSS Struvite and RBCaOx values were within ranges considered acceptable for management of urolithiasis in cats. However, subtle statistical differences were found. For the urinary food, RSS Struvite and CORI were not different between the wet and dry forms. The RSS CaOx was lower for the wet food (2.41 ± 0.55) compared to the dry (4.20 ± 0.60). For the obesity/diabetes mellitus food, RSS struvite and RSS CaOx was not different between wet and dry forms. However, CORI was significantly lower for the wet food (10.83 ± 13.09) than the dry food (75.01 ± 13.09). When the dry foods were compared, there was no difference in RSS Struvite and RSS CaOx but the urinary food had a significantly lower CORI than the obesity/weight loss food. When the wet forms were compared, the urinary food had a significantly lower RSS Struvite value but RBCaOx and CORI values were not significantly different.

Results of this study show that for the foods tested only minor differences in wet and dry forms existed. For overweight cats with a history urolithiasis, the weight loss/diabetes mellitus food may be used during weight loss and then the cat transitioned to the urinary food once a healthy weight is achieved.

NU-34 EFFECT OF DIET ACCLIMATIZATION AND LENGTH OF COLLECTION ON RELATIVE SUPER SATURATION FOR CALCIUM OXALATE AND STRUVITE AND THE CALCIUM OXALATE RISK INDEX IN CATS. H.M. Schiefelbein¹, J.M. MacLeay¹, J.V. Raymond-Lober¹, S.J. Davidson¹. ‘Hill’s Pet Nutrition, Inc., Topeka, KS,’ ‘Aerotek Inc., Raleigh, NC.’

Routine urine evaluation in cats with a history of urolithiasis can provide valuable information on the success of an intervention strategy. However, the gold standard is to test a urine sample collected over 24-48 hours after a cat has been consuming a food for weeks to months. It is generally assumed that a 24-hour urine sample provides the best overall picture of urinary health, but it does not represent a “real world” urine collection strategy. Therefore, the objective of this study was to examine a five-diet, randomized, double-masked and controlled clinical trials (dietary prevention of idiopathic cystitis (IC), struvite urolithiasis or calcium oxalate (CaOx) urolithiasis) that used the same foods where dietary recommendations were made at each visit. Study duration was either 1-year (IC) or 2 years (urolith prevention). Compared to the Control food (Ctl), the Test food contained greater amounts of anti-oxidants and n-3 fatty acids, and less Ca and Mg with a target urine pH of 6.2-6.4. The Ctl food was designed to be similar to popular grocery brands and had a target urine pH of 6.6-6.8. Foods were otherwise similar and met minimum AAFCO requirements. Cats enrolled in the IC study were fed either wet or dry foods. Cats enrolled in the urolith studies were fed either dry or wet plus dry foods based on owner preference. Wet and dry foods for each treatment were of similar nutritional profile. Caloric density (dry matter basis) of foods were Test dry=4,100 kcal/kg, Ctl dry=4,000 kcal/kg, Test wet=4,260 kcal/kg and Ctl wet=4,060 kcal/kg. Cats enrolled in the IC study had body weight and body condition score evaluated at baseline, 1, 2, 3, 6, 9 and 12 months. Cats enrolled in the urolithiasis studies were evaluated at baseline, 1, 4, 8, 12, 16, 20 and 24 months. Analyses included 25 FIC study cats, 35 Struvite study cats and 32 CaOx study cats. Most cats entered the study with a body condition score in excess of 3.5 and upon study completion, the body condition score was not different between treatments and did not change significantly over time. There was a significant but modest increase in weight in both the Ctrl and Test cats in the 2-year studies, but not in cats enrolled in the 1-year study. In the CaOx study; mean baseline and final body weights were 5.58 kg and 5.80 kg for the Ctrl group, and 5.88 kg and 6.37 kg for the Test group. In the Struvite study; mean baseline and final body weights were 5.64 kg and 5.87 kg for the Ctrl group, and 6.23 kg and 6.46 kg for the Test group (p < 0.05). There was no difference between treatments. Considering cats which completed the full length of study, compared to baseline, 43% of Test and 38% of Ctrl cats had lost weight at 12 months and 46% of Test and 33% of Ctrl had lost weight after 24 months. Only 3 cats had a weight gain in excess of 1.25 kg (2 Test, 1 Ctrl). Our results indicate that significant weight gain did not occur in cats fed either the Test or Ctrl foods. In fact, over a third of cats successfully lost weight. This may have resulted from the frequent interactions between owners and veterinarians and strong recommendations of how much to feed over the course of the studies.

There is a need to develop a test that can predict the risk that an animal may reform a calcium oxalate (CaOx) stone to provide feedback to pet owners that a given intervention is lowering risk. Relative super saturation (RSS) for CaOx is regarded as the best method to evaluate urine. However, the test is not commercially available to the veterinary community, is labor intensive and costly. Further, RSS uses a limited number of urine constituents instead of whole urine and therefore cannot fully evaluate the complex system where many promoters and inhibitors interact.

The human Bonn Risk Index uses whole urine and better reflects the risk of stone formation. The Calcium Oxalate Risk Index (CORI) is similar in principle to the human Bonn Risk Index. Briefly, whole urine is titrated with a sodium oxalate solution and monitored at 585 nm until precipitation occurs. The CORI value is determined by dividing the urine [Ca$^{2+}$] by the resultant amount of oxalate added at the point of precipitation. We tested the method for range, system suitability, repeatability, reproducibility, robustness, limits of Detection and Quantitation.

A panel of 12 healthy adult cats was used in this ACUC approved study. Cats were individually housed with group exercise and social interaction in spacious rooms with natural light. The cats were fed the same food for 28 days and urine was collected into pans with plastic beads which drained into a container containing thymol sitting in a 38°C water bath. Samples were tested in triplicate on days 1, 2, 5 and 15. Cats were then fed a food of differing nutrient composition for 28 days, urine was collected and testing repeated. Overall the range of CORI values was 3.2 – 174.8/L. The intraday variability on each day of testing was 5.50% (n = 12), 9.13% (n = 12), 12.71% (n = 12), and 11.37% (n = 5), respectively. The variation of the method across all days was 9.78% (n = 41). However, on days 5 and 15, the CORI result was observed to be 17.48% (n = 12) and 27.42% (n = 5) different on average from the same sample titrated on the day of collection. As much as 36.9% difference (n = 12) was observed by allowing the samples to cool to room temperature. There was some recovery upon reheating the samples but the results were still different by 12.8% (n = 11) on average. Centrifugation was found to be preferable over filtration for preparation of samples. The experimental system was sensitive and able to detect the addition of as little as 10.4 μmol of oxalate to a 2 mL urine sample. Results support that, kept at body temperature, the urine can be held for up to 48 h with reproducible results.

The CORI method is a practical, cost effective, reproducible and reliable test compared to RSS CaOx. It can provide veterinarians and pet owners with rapid feedback concerning the success of an intervention and, most importantly, takes into account the entire milieu of urinary constituents that affect the propensity of an individual animal to form a calcium oxalate stone.

NU-37  RISK FACTORS FOR CALCIUM CARBONATE UROLITHS IN GOATS: 368 CASES (1984 - 2012), E. Nwaokorie1, C. Osborne2, J. Lukich1, C. Wolf2, F. Thomas3. 1Minnesota Urolith Center, Veterinary Clinical Sciences Department, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108; 2Department of Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108; 3Department of Veterinary Biosciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN.

To determine the predominant mineral composition of naturally occurring uroliths from goats; to determine whether age, breed, sex, reproductive status, geographic location, season of the year, and anatomic location are risk factors associated with urolith formation in goats; and to determine whether the rate of urolith submissions varied over time. 384 goats with uroliths of which 368 had calcium carbonate uroliths, and 16,866 control goats. Information about breed, age, sex, reproductive status, season of submissions, geographic location and anatomic location in goats were used to identify risk factors. Changes in the yearly urolith submission frequencies were also evaluated. Breeds of African descent (Pygmy, Boer, Anglo-Nubian, and Nigerian Dwarf) had a significantly higher risk for calcium carbonate uroliths (65%; n = 239) than the combination of all other breeds evaluated in this study (35%; n = 129). Neutered male goats had a significantly increased risk of developing calcium carbonate uroliths compared with control goats. A significant association was found between increasing age, geographical location, season, anatomic location and detection of calcium carbonate uroliths. Calcium carbonate urolith submission rates significantly increased during the study period from 5% in 1984, to 43% in 2012. The prototype goat with calcium carbonate uroliths was a neutered male, 2 to 3 years-old and of African descent. While these observations indicate risk factors for calcium carbonate urolithiasis in goats they do not represent cause-and-effect associations.


The study of proteinuria in chronic kidney disease (CKD) is important because its occurrence may be a consequence of the disease and also can be a perpetuating factor of injury, exacerbating renal disease. Pathological renal proteinuria may have three origins, i) glomerular, due to lesions that alter the permeability and selectivity properties of glomerular wall, ii) tubular, which occurs as a result of injury that allows reabsorption of proteins that normally pass through the glomerulus, or iii) interstitial, when occur inflammatory lesions or pathological processes that cause exudation of proteins in the urinary space. Glomerular proteinuria is characterized by loss of high molecular weight proteins (HMW > 60 kDa) and tubulo-interstitial proteinuria is characterized by loss of proteins of low molecular weight (LMW <60 kDa). The aim of this study was to evaluate the molecular weight of urinary proteins in dogs with naturally acquired CKD by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), determining the proportion of albumin (≈ 69 kDa), HMW and LMW proteins, to allow the identification of the damaged nephrons segments in different stages of the disease. The protein-to-creatinine ratio (UPC - pira-galol) was used as a quantitavite method of proteinuria. Study animals were divided in four groups according to CKD staging proposed by the International Renal Interest Society (IRIS). Group I was composed of 8 dogs, group II included 6 dogs and groups III and IV were made by 9 and 5 animals, respectively. The control group was composed of 5 clinically healthy dogs that did not present any changes in laboratory tests. All groups were composed by elderly dogs (more than 7 years-old). Animals diagnosed with any concomitant disease were excluded, as were cases of acute kidney injury and causes of pre-or post-renal azotemia. The statistical analysis was made with Dunn's test. In group I, only 2 of 8 dogs presented UPC > 0.5 (0.39 ± 0.29), by electrophoresis it was found 57.1 ± 14.2% of urinary proteins were HMW (LMW= 42.9 ± 14.2%) and albumin corresponded to 30.9 ± 18.1% of total proteins. In group II 4 of 6 dogs presented UPC > 0.5 (1.07 ± 0.87), it was found 59.8 ± 23.6% of HMW protein (LMW = 40.2 ± 23.6%) and albumin corresponded to 23.9 ± 21.2% of urinary proteins. In group III all animals had UPC > 0.5 (3.25 ± 2.65), HMW proteins corresponded to 65.7 ± 21.2% (LMW = 34.2 ± 21.1%), and albumin represented 29.7 ± 18.6% of all proteins. In group IV, also all dogs presented UPC > 0.5 (3.56 ± 3.40), the proportion were 57.0 ± 24.6% of HMW proteins (LMW = 43.0 ± 24.6%) and 28.7 ± 16.9% of albumin. In the control group all animals had UPC < 0.5 (0.18 ± 0.10), and the proportion found was 54.2 ± 14.5% of HMW proteins (LMW = 45.8 ± 14.5%) and 20.8 ± 10.4% of albumin. The analysis of urinary protein electrophoresis demonstrated a higher propor-
tional loss of albumin and a predominant loss of HMW proteins compared to LMW proteins in all groups of CKD. Electrophoresis also demonstrated a greater loss of LMW proteins in group IV (p < 0.05). These results suggest a predominance of glomerular injury in all stages of canine CKD, with a tubulo-interstitial injury more intense in the terminal stage of the disease. The results of this study allow to classify the electrophoresis of urinary proteins as an efficient technique for qualitative assessment of proteinuria, and suggest an important role in analyzing the extension and location of renal injury.

The paddle was effective for the identification of *E.coli*, but inconclusive for other bacteria isolated. A capacity analysis of isolation of pathogenic bacteria in urine samples from dogs, revealed that the paddle had a specificity of 97.3% and accuracy of 98.6% over the ability to identify bacteria. It was found that paddle is suitable for isolating bacteria from the urine of dogs and the identification of *E. coli*.

**NU-39**

**PREDICTING ASYMPOTOMATIC BACTERIURIJA ESCHERICHIA COLI IN CLINICAL CANINE UROPATHOGENS.**

K. Thungrat, D.M. Boothe. Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL.

*Escherichia coli* (*E. coli*) is commonly associated with urinary tract infections (UTI). UTIs express varying severities of clinical signs from absent (asymptomatic bacteriuria; ABU) to severe. Severity is based on the expression of virulence factors (VF) which allow microorganisms survival. Adhesin is considered as a critical VF which allows *E. coli* to colonize the urinary tract. In humans, ABU is an indication of non-antimicrobial therapy since treatment may increase antimicrobial resistance, and lead to infection with more pathogenic organisms. The purpose of this study was to characterize *E. coli* from canine UTIs, and predict ABU based on the expression of VF genes.

*E. coli* (*n* = 68) cultured from canine UTIs were classified as to severity: absent (ABU; *n* = 15), and non-ABU (*n* = 53). The levels of RNA expression of adhesins (*papG*, *papC*, *fimH*, and *focA*), toxins (*hlyD*, and *cnf1*), and siderophores (*ireA*) were determined by RT-PCR and then each gene was standardized to the housekeeping *gapA* gene and normalized to that of wild-type *E. coli*. Levels of VF gene expression were computed to principal component (PC) and the first 4 PCs were selected to further analysis of the linear discrimination function that were able to classify severity of UTI into two groups: ABU and non-ABU. This model estimated the severity of UTI with a 9.3% error rate and 0% false negative rate. These results suggest further characterization of the VF profiles may assist the pre-treatment identification of ABU that will support de-escalation of antimicrobial use and thus antimicrobial resistance.

**NU-40**

**THE USE OF PLASTIC “PADDLE” FOR ESCHERICHIA COLI ISOLATION IN URINE OF DOGS.**


*Escherichia coli* is the most commonly isolated bacteria in urine cultures of dogs. Urinary bacterial isolation, is traditionally done by conventional culture medium. This study evaluated the performance of the plastic paddle using the conventional culture as Gold Standard.

Asseptically urine samples from 5 healthy dogs and 38 dogs with clinical signs of cystitis of both sexes and various breeds were collected by cystocentesis. For paddle was used commercial kit containing the means Cled Agar, Citrate Agar and Chrome Gold Standard.

The paddle was effective for the identification of *E.coli*, but inconclusive for other bacteria isolated. A capacity analysis of isolation of pathogenic bacteria in urine samples from dogs, revealed that the paddle had a specificity of 97.3% and accuracy of 98.6% over the ability to identify bacteria. It was found that paddle is suitable for isolating bacteria from the urine of dogs and the identification of *E. coli*.

**NU-41**

**SYMMETRIC DIMETHYLARGININE (SDMA) INCREASES EARLIER THAN SERUM CREATININE IN CATS WITH CHRONIC KIDNEY DISEASE (CKD).**


SDMA has been shown to be an accurate and precise biomarker for calculating estimated glomerular filtration rate (GFR) in humans, as well as a more sensitive biomarker than serum creatinine for assessing renal dysfunction. SDMA is a byproduct of protein methylation. Subsequent protein degradation of methylated proteins yields individual methylated arginine amino acids. SDMA is excreted primarily by renal excretion. Because SDMA is eliminated by the kidneys, plasma concentrations are affected by changes in GFR.

CKD cats (*n* = 21) included are those persistently azotemic for ≥ 3 months (*n* = 15); nonazotemic cats with GFR >30% reduced from median GFR. SDMA levels were considered to have CKD, whereas cats above this threshold were selected from the same colony. SDMA concentrations were determined retrospectively from historical data or banked serum samples in azotemic cats, or at the time GFR (iohexol clearance) was measured in nonazotemic cats. All cats were maintained with high quality care, including optimal nutrition and veterinary health care. All cats were provided with regular opportunities for socialization and experienced behavioral enrichment through daily interaction and play time with caretakers and with daily opportunities to exercise, and play with toys.

SDMA increased above the normal reference interval upper limit of 14 μg/dL before serum creatinine increased above the normal reference interval upper limit of 2.1 mg/dL in 17/21 cats (mean 14.6 months; range 1.5 to 48 months). In 4/21 cats, banked serum samples were not available to measure SDMA concentration prior to the time cats developed azotemia. The lower 2.5 percentile for GFR in healthy cats (1.36 mL/min/kg; 30% reduction from normal) was used to establish the lower limit of normal. All cats with GFR below the lower 2.5 percentile were considered to have CKD, whereas cats above this threshold were considered to have normal renal function. Based on this gold standard, sensitivity and specificity were determined to be 100% and 91%, respectively, for SDMA, whereas creatinine had a sensitivity of 17% and specificity of 100%. For SDMA, the upper limit of 14 μg/dL corresponded to approximately 24% reduction in GFR. Using serum SDMA as a biomarker for CKD allows earlier detection of CKD in cats compared with serum creatinine.

**NU-42**

**SYMMETRIC DIMETHYLARGININE (SDMA) INCREASES EARLIER THAN SERUM CREATININE IN DOGS WITH CHRONIC KIDNEY DISEASE (CKD).**


SDMA is derived from intracellular methylation of L-arginine by protein-arginine methyltransferases (PRMT) and released into the circulation after proteolysis. SDMA is eliminated primarily...
by renal clearance. Therefore, concentrations of SDMA are affected by changes in glomerular filtration rate (GFR). SDMA represents a potential biomarker for diagnosing and monitoring chronic kidney disease (CKD). In humans, SDMA has been shown to be an accurate and precise biomarker for estimating GFR, with better sensitivity compared with serum creatinine for assessing renal dysfunction.

In a retrospective study, 8 dogs with CKD (diagnosis based on >30% reduction in GFR, measured by iohexol clearance) and with normal serum creatinine and SDMA at the time of diagnosis, were followed for 3 years. The aim of the study was to determine the point at which these dogs developed increased serum SDMA and creatinine concentrations, in order to determine if SDMA increases earlier than creatinine. All dogs were maintained with high quality care, including optimal nutrition and veterinary health care. All dogs were provided with regular opportunities for socialization and experienced behavioral enrichment through daily interaction and play time with caretakers and with daily opportunities to run and exercise, and play with toys.

SDMA concentrations were measured using a fully validated Liquid Chromatography-Mass Spectrometry (LC-MS) assay. Creatinine concentrations were measured using a clinical chemis-

try assay. Twenty of the 8 dogs currently have normal SDMA and creatinine concentrations. In all other dogs, SDMA increased above its normal reference interval upper limit of 14 mg/dL before creatinine increased above its normal reference interval upper limit of 1.8 mg/dL. This occurred by an average of 17 months (range 11 to 26 months). Our results suggest that serum SDMA is indeed an earlier biomarker than serum creati-
nine for diagnosing and monitoring CKD in dogs.

NU-43 ANALYSIS OF SERUM SYMMETRIC DIMETHYLARGININE CONCENTRATION IN PATIENT SAMPLES IDENTIFIED THROUGH RETROSPECTIVE TRENDING OF SERUM CREATININE. J. Braff, J. Aguair, M. Yerramilli, M. Yerramilli, E. Obare, M.J. Beall. IDEXX Laboratories, Inc., Westbrook ME.

Concentrations of serum symmetric dimethylarginine (SDMA) are known to increase in canine and feline patients with reduced renal function, and recent studies have indicated that this marker may have outstanding value in the early detection of chronic kidney disease. This study evaluates concentrations of serum SDMA among canine and feline patients whose serum creatinine concentrations have increased over time yet remain within normal limits and compares these results to SDMA concentrations in patients exhibiting stable serum creatinine.

A database was compiled comprising all current canine and feline samples submitted to three reference laboratories for serum creatinine testing from patients with four or more historical cre-

atinine test results available. Individual serum creatinine limits were calculated for each patient based on that patient's cumula-
tive rolling average. Thirty-nine canine patients and 48 feline patients were selected whose serum creatinine had never exceeded the individual limit yet remained within the reference normal range (Group 1). For comparison, 38 canine patients and 33 feline patients were selected whose creatinine had exceeded the individual limit yet remained within the reference normal range (Group 2). The current sample from each patient identified had SDMA analyzed by liquid chromatography-mass spectrometry.

Among canine patients in Group 1, 17.9% of patients had SDMA concentrations exceeding 14 μg/dL, which is the working upper limit for both canine and feline SDMA. In canine Group 2, 63.2% had serum SDMA concentrations higher than 14 μg/

DL. This represents a risk ratio of 3.52 (95% CI: 1.72 to 7.18). Among feline patients in Group 1, 8.3% of patients identified had SDMA exceeding 14 μg/dL compared with 42.4% in feline Group 2. For feline patients, this represents a risk ratio of 5.09 (95% CI: 1.84 to 14.10).

Results from this study demonstrate that SDMA is more fre-
quent over the reference limit among canine and feline patients whose serum creatinine is increasing over time yet remains within normal reference limits than in patients exhibiting stable creati-
nine concentrations. Prospectively monitoring for additional sam-
ple from patients included in this analysis will help to determine the predictive value of this renal biomarker.

NM-1 GENOME WIDE ANALYSIS LEADS TO A NOVEL METHOD TO INCREASE HYDRATION AND REDUCE URINE SPECIFIC GRAVITY IN THE CAT. J.A. Brockman, M. Huentelman, C.B. Kingsley, D.E. Jewell, T. Nettleton, Inc. Science and Technology Center Topeka, KS, "Neuroge-
nomics Division, Translational Genomics Research Institute, Phoenix AZ, Diabetes, Cardiovascular and Metabolic Diseases Division, Translational Genomics Research Institute, Phoenix AZ.

A genome wide association study (GWAS) using 533 cats identified an association between urine specific gravity and a locus containing the prostaglandin E synthase 3. We hypothe-
sized that urine specific gravity would be responsive to changes in dietary arachidonic acid (ARA). Two experiments; one with 81 older cats and one with 34 normal adult cats were used to evaluate the effect of change in dietary fatty acid on subsequent urine specific gravity. In the normal adult cats circulating fatty acid were also evaluated and showed a 42% increase in dietary intake and subsequent relative super saturation of urine. In both experiments the control foods had a dietary ARA to EPA ratio below one while the treatment foods had an ARA to EPA ratio over three. The study protocols were reviewed and approved by the Institutional Animal Care and Use Committee, Hill’s Pet Nutrition, Inc., Topeka, KS, USA. All cats were housed individ-
ually and allowed exercise in indoor runs. Cats had access to natural light that varied with seasonal changes. All cats were provided with regular opportunities to exercise, with access to toys. There was a reduction in urine specific gravity in the cats fed the high ARA to EPA ratio (p < 0.05). In the older cats there was also an increase in hydration (estimated by the calculat-
ing osmolality using concentration of circulating sodium, glucose, and urea) while there was no change in the osmolality of the normal adult cats. There was a reduction (p < 0.05) in the relative super saturation of oxalate in urine as circulating ARA to EPA ratio in the blood increased. These studies demonstrate that GWAS can identify targets for nutritional intervention, fur-
thermore, dietary changes in essential fatty acids of the cat was a successful method for reducing urine specific gravity and oxalate stone risk in the cat.

NM-2 INTERMITTENT CALORIC RESTRICTION IS MORE EFFECTIVE THAN CHRONIC CALORIC RESTRICTION IN PROMOTING WEIGHT LOSS IN OVERWEIGHT CATS. Y. Pan, N. Purina Research, Checkerboard Square, St. Louis, MO.

Obesity is a major health problem in the cats between 5 and 10 years of age in developed countries. Chronic calorie restriction (CCR) has been employed to treat obesity in cats. Cats reduce their resting energy expenditure in response to CCR, which can slow down weight loss and predispose the cats to rebound after weight loss. The objective of this study is to compare the effects of CCR and intermittent caloric restriction (ICR) on weight loss, body fat loss, and lean body mass loss in overweight cats.

After the baseline maintenance energy requirement (MER) and body composition for each cat were determined, twenty eight over-
weight cats were randomized into two groups based on baseline MERs, body weight, and% body fat. The cats in the CCR group were fed 75% of their MERs for 6 months, while the cats in the ICR were fed 75% of their MERs during the first 2 weeks and 100% of their MERs during the 2nd 2 weeks of the months for 12 months. Daily food intake, weekly body weight, and monthly quantitative magnetic resonance for body composition were per-
fomed during the trial. When the results were compared based on equal caloric deficiency, the ICR was more effective than the CCR

1085
in promoting loss of body fat and % body fat. In addition, more cats reached their ideal body condition scores in the ICR group (82%) than in the CCR group (36%) at the end of the study.

NM-3
METABOLICOS PROFILES OF AGED, MEMORY-Impaired DOGS. Z. Ramadan, B. Zanghi, Nestlé Purina Research, St. Louis, MO.

Visuospatial memory declines with age and may be exacerbated in dogs with Cognitive Dysfunction Syndrome (CDS) because of impaired spatial orientation, housetraining, and recognizing of human family members. The present study sought to characterize serum metabolite profiles in aged dogs with varying degrees of visuospatial memory performance and identify biomarkers associated with memory performance using the variable Delay-Non-Matching-to-Position (vDNMP) task. A non-targeted metabolomics approach based on high-resolution, Flow-Injection Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (1F-FTICR-MS) was used to generate comprehensive metabolomic profiles containing 4990 accurate mass measurements from the serum of 60 Beagles (ages: 7-16 yrs) divided into 3 groups based on a combined vDNMP score (HMP=88-93% accuracy [N=12]; MMP=79-86% accuracy [N=10]; LMP=61-78% accuracy [N=24]) using 2 objects with 20-sec and 90-sec delay. Statistical analysis revealed several metabolic differences between the groups; largely with LMP differing from MMP and HMP. LMP dogs had significantly (p < 0.05) altered lipid metabolism, as evidenced by elevated levels of sterol metabolites, cholesterol, hydroxylated triacylglycerols, alkylacylglycerols, and α-Tocopherol (vitamin E). The HMP dogs had elevated (p < 0.05) indole-3-propanoic acid (IPA) and leukotriene C5 compared to MMP and LMP. This data indicates that serum biomarkers exist that facilitate categorization of memory performance in aged, kennel-housed Beagle dogs, yet further work is required to determine if similar metabolic differences exist in home-living dogs diagnosed with CDS regardless of breed. Ultimately, it may be possible to use serum biomarkers, particularly IPA and/or sterol metabolites, for identifying dogs with elevated risk of memory impairment, or hopefully early onset of CDS.

NM-4
SUCCESSFUL WEIGHT REDUCTION IN SEVERELY OBSESE CATS. A. Witzel1, I. Paetau-Robinson2, C. Kirk1. University of Tennessee, Knoxville, TN, 2Hill’s Pet Nutrition Inc., Topeka, KS.

With an increase in the population of overweight and obese cats it is critical to provide cat owners with foods that are efficacious for weight loss. The current study was designed to show the factors that are important for successful weight loss and achieving an ideal body condition (IBC), of a body condition score of 3 on a 5-point scale.

Thirty-two client-owned, overweight or obese cats were enrolled in this weight loss case series. The cats ranged in initial body fat from 29.5% to 61.3% determined by DEXA. After the initial health assessment and collection of the signed informed owner consent all cats were fed a therapeutic weight loss food. The food consisted of wet, dry or a combination of wet and dry. The food was based on the resting energy requirement (RER) and was adjusted in calories to meet the caloric intake for successful weight loss was less than the resting energy requirement for the majority of the dogs (range 0.5 x to 1.2 x RER). Significant improvements in serum glucose, cholesterol, hemoglobin, and hematocrit were observed. The owners reported significant improvements in overall health, quality of life, activity level, and playfulness.

The present study demonstrates that this therapeutic weight-loss food is safe and efficacious in severely obese dogs when an

NM-5
SUCCESSFUL WEIGHT REDUCTION IN SEVERELY OBSESE DOGS. A. Witzel1, I. Paetau-Robinson2, C. Kirk1. University of Tennessee, Knoxville, TN, 2Hill’s Pet Nutrition Inc., Topeka, KS.

The population of obese dogs in the US has increased and many pet owners are unsuccessful in reducing their pet’s body weight. A protocol of weight loss foods have a reputation of being ineffective. This study was done to show the critical components of successful weight loss and achieving an ideal body condition (IBC) with a body condition score of 3 on a 5-point scale.

Twenty seven client-owned, obese dogs were enrolled in this weight loss case series. The dogs ranged in initial percent body fat from 34% to 64%, determined by DEXA. After the first health assessment and collection of the signed informed owner consent all dogs were fed a commercial, therapeutic, weight loss food, wet, dry or a combination of wet and dry. The food was low fat, low in fiber, lysine, and carnotine compared to standard maintenance foods. The initial food dose of one time the resting energy requirement (RER; RER=0.75 x [IBW/0.75]) was based on the ideal body weight (IBW). The IBW was calculated by setting the DEXA-determined lean body mass plus bone mineral content at 80% of the total ideal weight and assuming ideal body fat content to be 20% of the total weight. Body weights were assessed monthly for the first year and every other month during the second year. Whole blood samples were collected every 8 weeks to analyze blood chemistry and complete blood counts. Owners completed a pet health & behavior questionnaire at weeks 0, 8, 16, and 24. The dogs remained on the study until they reached their IBC or for 104 weeks, whatever came first. The study protocol and procedures were approved by the Institutional Animal Care and Use Committees at the University of Tennessee and Hill’s Pet Nutrition, Inc.

Twenty dogs completed the study; of those, 65% (13/20) and 95% (19/20) had achieved an IBC by the end of the first and second year, respectively. Of the 27 dogs that started the study, four dogs were dismissed for compliance issues and three were dismissed for other medical conditions. Owner compliance was 83% (20/24). The average weekly rate of weight loss was 1.5% during the first two weeks and then fell to 1% at week 8; beyond 12 weeks the average weekly weight loss rate was < 1%. The caloric intake for successful weight loss was less than the resting energy requirement for the majority of the dogs (range 0.5 x to 1.2 x RER). Significant improvements in serum glucose, cholesterol, hemoglobin, and hematocrit were observed. The owners reported significant improvements in overall health, quality of life, activity level, and playfulness.
accurate estimate of ideal body weight is available to calculate required food doses. Close monitoring by a veterinarian is important for owner compliance and successful weight loss.

NM-6 PM-SUPPLEMENTATION WITH MELATONIN, ZINC, AND HAEMATOMOCoccus PLUVIALIS SELECTIVELY IMPROVES ATTENTION AND MOTOR LEARNING IN AGED, MEMORY-IMPAIRED DOGS. B. Zanghi1, J. Araujo2, M. Hesta2. 1Labora-

D. Jewell, A. Floerchin. 3Department of Clinical Chemistry, Laboratory of Metabolic Disorders, University Hospital Ghent, Belgium. 3Affinity-Petcare, Barcelona, Spain.

NM-7 DIETS CONTAINING BIOACTIVE FOOD FACTORS AME-


Aging in felines has detrimental health consequences with pathophysiological significance and impact on the feline/pet parent relationship. Of clinical importance are inflammation, reduced renal health and sarcopenic obesity. Additionally, decreased skin and coat quality might indicate dermatological pathology and can discourage physical and emotional interaction between cats and their owners. In this study, cats were fed a control food (CD), previously shown to improve indices of age-related pathology, or one of two diets (ED1 & ED2; n = 27 each diet) that contained test ingredients containing bioactive food factors (broccoli, tomato, oat, pea, beet, fish oil). The protocol was approved by IACUC. Cats were housed individually and allowed exercise in indoor runs. Cats had access to natural light that varied with seasonal changes. All cats were provided with regular opportunities to exercise, with access to toys. Health was monitored via clinical and physical indices. Results from ED1 & ED2 were the same and were significant with P < 0.05, unless noted. Levels of the inflammatory marker prostaglandin E2 decreased in cats fed CD and ED2. Renal health improved to various degrees, as indicated by improved glomerular filtration rate (GFR) in cats fed ED1 or ED2 and reduced levels of symmetrical dimethylarginine in cats fed CD or ED2, but not ED1 (P = 0.07). Possible implications for sarcopenic obesity were indicated by reduced fat mass in cats fed ED1 & ED2 and retained (ED1) or increased (ED2) lean mass. Overall dermatological quality was improved on all diets, as indicated by increased quality of skin and coat as well as by decreased shedding. Thus, diets containing plant secondary metabolites, fiber and omega-3 oils improved indices of age-related inflammation and renal health, reduced fat mass while preserving lean mass. Further these diets might promote better interactions between dermatologically challenged pets and their pet parents by improving skin and coat quality and reducing shedding.

NM-8 FERMENTABLE FIBERS MODULATE AMINO-ACID METABOLISM THROUGH INTESTINAL FERMENTATION IN HEALTHY DOGS FED A LOW-PROTEIN DIET. G. Quist-Rybachuk1, I. Jeusette1, B. Wuyts3, M. Hesta2, Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Belgium. 3Affinity-Petcare, Barcelona, Spain.

A recent study (Verbrugghe et al, 2010) has demonstrated that propionate production through soluble fiber fermentation induces an amino-acid sparing effect in healthy cats, due to a shift in substrates for gluconeogenesis. The aim of the present trial was to assess the existence of an amino-acid sparing effect in healthy dogs fed a low-protein diet containing a mix of fermentable fibers, sugar beet pulp and guar gum, as compared to cellulose, an insoluble non-fermentable fiber source.

Eight healthy adult Beagle dogs (age 2-11 years) were randomly assigned to one of two groups and received successively, in a cross over design, two low-protein (4 g /100 kcal ME) extruded dry diets similar in nutrient composition but containing either sugar beet pulp and guar gum mix or cellulose (total dietary fiber: 2 g/100 kcal ME). Dogs were fed each diet for 4 weeks in the amounts needed to maintain stable body weight. At the end of each 4 week-period, a meal response test was performed after an overnight fast. Plasma acylcarnitine and amino-acid (glycine, alanine, valine, leucine, methionine, phenylalanine, tyrosine) profiles were measured pre- and up to 6 hours postprandially. A-carnitine profile was used as a marker for fatty acids oxidation in the mitochondria, while amino-acids were measured to evaluate if they were spared in favor of other substrates of the TCA cycle. The areas under the curve (AUC) were calculated for each parameter and statistically analyzed (mixed model with diet sequence and period effects, SPSS). Values were considered as significantly different for P value < 0.05.

Compared to cellulose, fermentable fibers clearly affected the acylcarnitine profile, significantly increasing acetyl-, propionyl- and butyryl-carnitine's AUC, as well as the amino-acid profile, significantly increasing alanine, leucine, phenylalanine and tyro-
sine's AUC.

In conclusion, these results suggest that guar gum and sugar beet pulp fermentation in dog's large intestine could diminish postprandial use of amino-acids favoring instead the use of short-chain fatty acids as substrate for the TCA cycle. This amino-acid sparing effect of fermentable fibers is especially beneficial when feeding a low-protein diet.


Obesity is an increasingly common health concern that is frequently linked to other serious diseases in dogs. The purpose of
this study was to determine the weight loss and weight maintenance benefits of a reduced calorie, high fiber test food with added coconut oil, L-carnitine, lipoic acid, lysine, and leucine designed to support fat metabolizing pathways in overweight or obese dogs. The protocol was approved by an IACUC and participating dogs were group housed for social interaction in spacious rooms with natural light. The treatment group was composed of 20 dogs with a body condition score of 4 or 5/5 and >30% body fat as determined by DEXA. This group was compared to the colony average (n = 341) and a colony derived subset of 20 adiposity matched controls. The treatment group was fed to achieve 1-2% body weight loss per week for 4 months, or until they achieved 15-25% body fat, followed by 4 months feeding to maintain body weight (BW) whereas the colony was fed a variety of foods for maintenance throughout. DEXA scan data was available for all dogs (n = 361). In addition, Test food fed dogs were weighed weekly and DEXA scanned monthly. Ideal body weights (IBW) were calculated from baseline DEXA as 1.25*lean body mass. Energy intakes were also recorded throughout and caloric intake/kg IBW was compared between groups. All study foods met minimum AAFCO requirements for adults.

All 20 dogs consuming the Test food completed the study with maintenance of body weight throughout. Furthermore, the dogs averaged a total weight loss rate of approximately 1.44% BW per week during the weight loss phase. After 4 months of maintenance feeding, DEXA revealed that the Test group had a mean percent body fat of 23.0 ± 1.6%. Compared to the beginning of the weight maintenance phase, mean lean body mass increased by about 2.6% (248 ± 123 g, p ≤ 0.04), mean body fat mass decreased by about 13% (374 ± 199 g, p < 0.03), and mean body weight decreased by about 2% but this was not significant. The Test food fed group consumed significantly fewer calories than the other groups during months 1–5 (p ≤ 0.01) and significantly more calories during months 7 (p ≤ 0.01) and 8 (p < 0.01). Interestingly, despite losing fat mass, the Test food fed dogs consumed 16% more calories/kg IBW compared to the colony and adiposity matched control groups in month 7 and 24% and 33.7% more calories/kg IBW in month 8, respectively. Results support that the food in this study led to successful weight loss and maintenance. Body condition improved through loss of fat and gain of lean muscle during the weight maintenance phase. Furthermore, metabolic rate appeared to increase over time as evidenced by preservation of body weight despite increased caloric intake compared to a large control group.

**NM-10**


Obesity is an increasingly common health concern that is frequently linked to other serious diseases in cats. The purpose of this study was to determine the weight loss and weight maintenance benefits of a reduced calorie, high fiber test food with added coconut oil, L-carnitine, lysine, and leucine designed to support fat metabolizing pathways in overweight or obese cats. The protocol was approved by an IACUC and participating cats were group housed for social interaction in spacious rooms with natural light. The treatment group was composed of 20 cats with a body condition score of 4 or 5/5 and >30% body fat as determined by DEXA. This group was compared to the colony average (n = 418) and a colony derived subset of 20 adiposity matched controls. The treatment group was fed to achieve about 1% body weight (BW) loss per week for 4 months, or until they achieved 15-25% body fat, followed by 4 months of feeding to maintain body weight. The colony was fed a variety of foods for maintenance of body weight throughout the study. Baseline DEXA scan data was available for all cats (n = 438). Test food fed cats were weighed weekly and DEXA scanned monthly. Ideal body weights (IBW) were calculated from baseline DEXA as 1.25*lean body mass. Energy intakes were recorded throughout and caloric intake/kg IBW was compared between groups. All study foods met minimum AAFCO requirements for adult cats.

Twenty Test food fed cats completed the weight loss phase and 19/20 completed the weight maintenance phase without complications. Cats lost approximately 1.25% BW per week during the weight loss phase. After 4 months of maintenance feeding, DEXA revealed that the Test group had a mean percent body fat of 20.7 ± 1.7%. Compared to the beginning of the weight maintenance phase, mean lean body mass increased by 4.4% (152 ± 42 g, p < 0.01), mean body fat mass decreased by 21.0% (276 ± 52 g, p ≤ 0.01), and mean body weight decreased by 2.5% (123 ± 57 g, p = 0.04). The treatment group consumed significantly fewer calories than the colony during months 1–6 (p < 0.01) and the adiposity matched control group during months 2–4 (p < 0.01). Interestingly, despite losing fat mass, Test food fed cats consumed 24% more calories/kg IBW on average than the colony during month 8 (p < 0.01) and 34.5% and 55% more than the adiposity matched controls in months 7 and 8 (p < 0.01), respectively.

Results support that the food in this study led to successful weight loss and maintenance. Body condition improved through loss of fat and gain of lean muscle during the weight maintenance phase. Furthermore, metabolic rate appeared to increase over time as evidenced by preservation of body weight despite increased caloric intake compared to a large control group.

**NM-11**


Increased inflammation and oxidative stress are key features of aging that may contribute to adverse effects on general health including renal, cardiovascular, neurological and dermatological problems. The effect of diets supplemented with ingredients having anti-inflammatory effects including carrots, spinach, tomato pomace, carmine, and α-lipoic acid was tested in older dogs. Animals (n = 81) were maintained on one of the three diets (controls, test1, or test2 diets with added ingredients for six meals per ten meal) for six months. Urine, blood and DEXA analysis were conducted prior to administering the diets and at 3 and 6 months. The experimental protocol was reviewed and approved by IACUC. Dogs were housed in pairs in indoor runs or in spacious rooms with natural light that varied with seasonal changes. All dogs were exercised daily, and were provided with regular opportunities for socialization and environmental enrichment. Dogs experienced behavioral enrichment through interactions with each other, by daily interaction and play time with caretakers. Body lean was maintained on all foods. When compared to the control diet there was an increased level of peroxiredoxins, antioxidant enzymes, with test2 but not test1 diet. Concentrations of serum DHA, an α3 fatty acid, was increased with both test diets at 6 months when compared to control. A reduction in prostaglandin E2 was observed in all three groups. While there was a significant reduction in 8-OH-DG, a marker of DNA damage, in both test diets, a reduction in serum SDMA, a marker of progressive kidney dysfunction, was only observed with test1 but not test2 diet. Harmful microbial byproducts including p-cresol sulfate and catechol sulfate were also decreased in both experimental groups. Markers of muscle and cartilage degradation including 3-methyl histidine and 4-hydroxyproline respectively, were lower in the experimental groups. A significant reduction in dry matter waste in the stool was observed with both test diets when compared to control. These data indicate that diets supplemented with potential antioxidant and anti-inflammatory ingredients may have beneficial health effects in older animals.
NM-12
A NON-INVASIVE METHOD TO ESTIMATE PERCENT BODY FAT IS AS GOOD AS DEXA TO CALCULATE AN IDEAL BODY WEIGHT IN OBESE DOGS.

The population of obese dogs in the US has increased and many pet owners are unsuccessful in reducing their pet’s body weight. A critical component of a successful weight loss regimen is a good estimate of the ideal body weight (IBW) as the starting point to calculate a food dose. A recently developed method called the Body Fat Index (BFI) Risk Chart differentiates between levels of obesity and establishes a link between the BFI and an IBW. Dual-Energy X-ray Absorptiometry (DEXA) provides the most accurate way of measuring percent body fat. The current study compares IBW estimations and calorific resting energy requirements (RER) based on the BFI chart and on DEXA results for a group of obese dogs. The protocol and procedures were approved by the institutional animal care and use committee.

Seventeen obese dogs (6 male, 11 female; mean age of 6 y) were screened for a weight loss study. All dogs were group housed to allow for socialization and had access to outdoor runs. Three animal care technicians independently determined the BFI for each dog. The BFI chart included images and descriptions that were used to determine the dog’s percent body fat. The average BFI, together with the current body weight was used to establish an IBW using the chart provided with the BFI Risk Chart. The dogs underwent a DEXA scan to obtain exact body composition information. Assuming the lean body mass and the mineral content together make up about 80% of a normal-weight dog, the value for lean body mass plus bone mineral content obtained from the DEXA scan was multiplied by 1.25 to obtain an ideal weight. The RER was calculated as 70% (ideal body weight in kg). The mean percent body fat determined by DEXA and BFI chart were 37.1% and 38.8%, respectively. The Pearson Correlation coefficient for IBW based on the BFI chart (IBW_BFI) and the IBW based on DEXA results (IBW_DEXA) was 0.91. In 65% of the dogs the IBW_BFI was within 5% of the IBW_DEXA; in 76% it was within 10% of the IBW_DEXA and in 100% of the dogs it was within 15%. For eight dogs the IBW_BFI was lower than the IBW_DEXA; in one dog it exceeded the IBW_DEXA by more than 10%. The caloric difference for calculated RERs based on BFI and DEXA ranged from a 41-kcal shortfall to a 27-kcal excess; or, in other terms, a 14-g shortfall and 9-g excess for a diet having a metabolizable energy content of 3000 kcal/kg.

This experiment demonstrates the usefulness of a new method, the BFI Risk Chart, to estimate percent body fat in overweight dogs when a DEXA instrument is not available, and to establish an ideal weight. This allows the estimation of a food dose that will lead to successful weight loss.

NM-13
ANALYSIS OF LEPTIN, ADIPONECTIN AND SEROTONIN LEVELS IN OBESE DOGS.

Serotonin (5-hydroxytryptamine, 5HT) is associated with numerous behavioral and psychological symptoms and is a biochemical marker of mood. 5HT is involved in the hypothalamic regulation of energy consumption and serotonin levels in the central nervous system are influenced by energy conditions. Several human studies have evaluated peripheral circulating serotonin and obesity. The microbiota of obese patients may be comprised of organisms that are more expeditious at extracting energy from food compared with the microbiota of lean patients. Gut microbiota also can regulate the brain-gut axis. The hypothalamus and brain stem are sites of central regulation of appetite. Systemic endotoxemia which is related to gut microbiota can alter neuronal function including the function of vagal afferent neurons. This study evaluated differences, and co-relations among peripheral concentrations of leptin, adiponectin, and serotonin, and the lipid profiles of lean and experimentally induced obese dogs by ad libitum feeding. We also examined differences in the gut microbiota composition of lean and experimentally induced obese Beagle dogs similar ages (3-5 years old). In the obese group, Beagle dogs (n = 7) were fed commercial dog food ad libitum (Adult small dog8, Royal Canin Ltd, France; and Cesar® Mars Ltd, Australia). In the lean group, beagle dogs (n = 7) were fed a restricted amount of the same diet to maintain optimal body condition during a 6-month period. Serum leptin and adiponectin levels, and plasma 5HT and cerebrospinal fluid (CSF)-5HT levels were measured using a commercially available enzyme-linked immunoabsorbent assay (ELISA) kit. Targeted pyrosequencing of the 16S rRNA gene was performed by Macrogen Inc. (Seoul, Korea) on a Genome Sequencer FLX plus system (454 Life Sciences, CT, U.S.A.). Using the BLASTN algorithm, all sequence reads were compared to the Silva rRNA database. Sequence reads in which sequence similarity was described by an E-value below 0.01 were regarded as partial 16S rRNA sequences. Leptin, cholesterol, and cortisol concentrations were higher in obese group than lean group (P < 0.05). Adiponectin, CSF 5HT, and total T4(tT4) concentrations were higher in the lean group than in the obese group (P < 0.05). In the obese group, related to the body condition score (BCS), CSF-5HT levels were negatively correlated (r = -0.933, P < 0.05). However, CSF-5HT was positively correlated with cholesterol in obese animals (r = 0.827, p < 0.01) and cholesterol (r = 0.419, p < 0.01) were positively correlated with BCS, and adiponectin (r = 0.446, p < 0.01) and 5HT (r = -0.490, p < 0.01) were negatively correlated with BCS. Leptin was negatively correlated with adiponectin (r = -0.294, p < 0.01) and 5HT (r = -0.343, p < 0.01), but leptin was positively correlated with cholesterol (r = 0.516, p < 0.01) and TG (r = 0.563, p < 0.01). 5HT was negatively correlated with leptin (r = -0.343, p < 0.01), TG (r = -0.268, p < 0.05) and cholesterol (r = -0.357, p < 0.05). Peripheral 5HT concentrations in the lean group are significantly lower than lean group in this study, and this is similar with human study results. Enterochromaffin (EC) cells in the intestinal epithelium release 5HT according to mechanical stimulation, to promote transit. Experimentally diet-induced obesity model showed decreased 5HT levels with decreased the number of EC cells. The inflammation associated with changes in the gut microbiota is considered as the reason of decrease 5HT availability in obese status 5HT is an important appetite control neurotransmitter, but, there are limited studies for 5HT levels related obesity in dogs. To our knowledge, this is the first study of evaluating peripheral 5HT levels in obese dogs. From this research, we can assume that 5HT may be correlated with canine obesity. Further studies will be needed to find out the role of low serum 5HT levels in canine obesity.
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R. Applegate, J. Weigel, F. Magnetta, Sved3, S. Lind

NM-15 URINE METABOLITE PROFILES IN NORMAL WEIGHT AND OVERWEIGHT DOGS. J. Söder1, R. Hagman”, J. Dick-vad1, S. Lindäs2, P. Agback3, K. Malmö1, S. Wernersson1
1Department of Anatomy, Physiology and Biochemistry, 2Department of Clinical Sciences, 3Department of Animal Nutrition and Management, 4Department of Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Obesity in dogs is increasing and lifestyle-related diseases affect pets as well as pet owners. Urine metabolite profiles have earlier shown to be specific for both dog phenotypes and disease status. The objective of this study was to compare urine metabolite profiles in normal weight and overweight Labrador retrievers in fasting and postprandial samples. A total of 28 healthy intact male dogs aged 1-9 years with body condition score 4-8 (BCS, scale 1-9) were included in the study. Of these dogs 16 were classified as overweight (BCS 6-8) and 12 were classified as normal weight (BCS 4-5). An overnight fasting period of 14-17 hours followed by collection of free catch morning urine. Thereafter each dog was fed a high energy diet containing half its daily maintenance requirements, based on body weight (BCS 4-5) and calculated ideal body weight (BCS 6-8). Three hours after the meal, a second urine sample was collected. Proton nuclear magnetic resonance spectroscopy combined with multivariate analysis was used for urine evaluation at both time points.

Principal component analysis of metabolite profiles showed less variation among postprandial samples than among fasting samples and metabolite profiles changed with age irrespective of sampling time point. Moreover, a difference was seen between normal weight and overweight dogs in postprandial samples. A total of 28 healthy intact male dogs aged 1-9 years with body condition score 4-8 (BCS, scale 1-9) were included in the study. Of these dogs 16 were classified as overweight (BCS 6-8) and 12 were classified as normal weight (BCS 4-5). An overnight fasting period of 14-17 hours followed by collection of free catch morning urine. Thereafter each dog was fed a high energy diet containing half its daily maintenance requirements, based on body weight (BCS 4-5) and calculated ideal body weight (BCS 6-8). Three hours after the meal, a second urine sample was collected. Proton nuclear magnetic resonance spectroscopy combined with multivariate analysis was used for urine evaluation at both time points.

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Metronidazole and doxycycline can cause olfactory dysfunction in humans. Olfactory dysfunction in working dogs can potentially result in loss of working ability or in catastrophic loss of life or infrastructure. The objective of this study was to determine if oral metronidazole or doxycycline causes olfactory dysfunction in explosives detection (ED) dogs.

Metronidazole (25 mg/kg PO every 12 hours) was administered for 10 days to 18 trained ED dogs. Odor detection threshold was measured on days 0 (prior to drug administration), 5 and 10, and using a standard scent wheel configuration and three explosive scents. Dogs were tested using odor weights ranging from 1 to 500 milligrams, with the lowest repeatable measure recorded as the detection threshold. After completion of metronidazole and a ten day washout period, doxycycline (5 mg/kg PO) was administered for 10 days, and the same testing protocol was repeated. Degradative changes in the threshold level detected by a dog between days 0, 5, and 10 of drug treatment were assessed by the McNemar test of paired proportions using an exact binomial probability distribution. Significance was set at p < 0.05.

During metronidazole administration, degradation in detection threshold was noted for 2 of 3 odors, and 9/18 dogs (p = 0.004) experienced degraded performance in response to one or more odor. There was no significant degradation in ability to detect any odor during doxycycline administration.

The degradation in ability to detect explosive odors during metronidazole administration presents a potential risk for the use of the drug in ED dogs. Doxycycline, at the tested dose, is likely safe for use in ED dogs.

**OT-3**

**COMPARISON OF SERUM CORTISOL IN CATS EXAMINED IN A CLINIC VERSUS A HOME SETTING.** B. Niblett, J. Ketzis, E. Grigg. Ross University School of Veterinary Medicine, Basserette, St. Kitts, West Indies.

Serum cortisol levels were used as a measure of stress experienced by cats examined using equivalent low stress handling techniques in two different environments; their home and an idealized veterinary clinic setting.

Healthy cats (n = 18) were examined in a randomized cross-over study design: half were examined in a clinic setting first and the other half examined in the home first. One week later, at the same time of day, each cat was examined in the alternate environment. A standardized process including timeline, personnel and procedures was followed: 30-60 minutes of confinement/travel; 5 minute room acclimatization; and a 20 minute examination. The examination included a physical exam, measurement of weight, blood pressure, and rectal temperature and concluded with venipuncture. Minimal restraint and toweling techniques were utilized along with food and facial pheromone. Seeing or hearing other cats or dogs and restraint by 'scruffing' were not allowed.

Serum cortisol was not significantly different between the two examination environments. However, cortisol was lower for all cats on their second visit regardless of exam environment (paired t-test p < 0.05). Analysis of variance of temperature, pulse, respiration, blood pressure, blood glucose and lymphocyte count did not identify any significant differences.

In conclusion, the exam environment was not a significant factor in serum cortisol levels. Continuing research focuses on potential influence of low-stress handling techniques on cats' behavior in subsequent veterinary visits.

**OT-4**

**PERIPHERAL AND CENTRAL VENOUS BLOOD GLUCOSE CONCENTRATIONS IN ACUTE ARTERIAL THROMBOEMBOLISM IN DOGS AND CATS.** S. Klaiman1, E. Koval1, T. Bdaloh-Abram1, G. Segel1, D. Aroch1. 1Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel.

Acute limb paresis caused by arterial thromboembolism (ATE) occurs commonly in cats, and is less frequent in dogs. ATE is typically diagnosed by physical examination, although occasionally additional tests (i.e., advanced imaging) are needed. We hypothesized that local, affected limb venous glucose concentration is decreased in ATE, while its systemic concentration is within or above reference interval. The study included 3 groups for each species: ATE cases, animals with limb paralysis of orthopedic or neurologic origin (non-ambulatory controls), and ambulatory animals with different diseases (ambulatory controls). Systemic and peripheral, affected limb blood glucose concentrations were measured and their absolute and relative differences (%ΔGlu and ΔGlu, respectively) were compared among groups. There were no complications or pain associated with the procedure. Peripheral blood glucose concentrations were significantly (P < 0.006) decreased only in the ATE groups, in both cats and dogs. ΔGlu and %ΔGlu were significantly higher in the ATE groups in both cats and dogs compared to both of their respective control groups (P < 0.0001 and P < 0.001, respectively), with no differences between the control groups. Receiver operator characteristics analysis of ΔGlu and %ΔGlu as predictors of ATE in cats had area under the curve of 0.96 and 1.00, respectively, and 0.99 and 1.00, in dogs, respectively. ΔGlu and %ΔGlu are extremely accurate, readily-available, simple diagnostic markers of acute ATE in cats and dogs. ΔGlu cutoffs of 30 mg/dL and 16 mg/dL, in cats and dogs, respectively, corresponded to sensitivity and specificity of 100% and 90% in cats, respectively, and 100% in dogs.

**OT-5**

**CLINICAL TRIAL APPLICATION OF A CLINICAL PHENOMENON: DETERIORATION FOLLOWING WITHDRAWAL OF ACTIVE MEDICATION FOR THE TREATMENT OF CHRONIC PAIN IN CATS WITH DEGENERATIVE JOINT DISEASE.** M. Grisen1, W. Simpson2, A. Thomas1, E. Griffith1, B.D.X. Linselles1. 1North Carolina State University College of Veterinary Medicine, Raleigh, NC., 2Morrisonville Cat Clinic, Morrisonville, NC., 3Department of Statistics, North Carolina State University, Raleigh, North Carolina.

Detection of clinically relevant pain relief in cats with degenerative joint disease (DJD) has been complicated by a profound placebo-by-proxy effect. Worsening clinical signs following withdrawal of medication is often discussed as a clinical phenomenon, but to date, has not been included in clinical trial design.

Deterioration of clinical signs following withdrawal of active medication was assessed in a double-masked, placebo-controlled study. Cats with DJD-associated pain and mobility impairment received active treatment (meloxicam, 0.035 mg/kg) or placebo for 21 days followed by a masked washout period of 21 days (placebo administered). Improvement during treatment as well as a return of clinical signs (deterioration) during the washout were evaluated using two owner-completed clinical metrology instruments (CMI: Feline Musculoskeletal Pain Index, FMPI; Client Specific Outcome Measures, CSOM) and objective accelerometry data.

Fifty-eight cats were assessed. After the first treatment period, both meloxicam and placebo treated cats showed significant improvement (p < 0.0001) on both CMI, but no significant difference between the groups. Following washout, cats that had received meloxicam had a greater return of clinical signs than the placebo treated cats (CSOM: p = 0.048; FMPI: p = 0.021). Cats receiving meloxicam had higher activity (p < 0.0001) than the placebo group during the treatment period and showed a significant decrease in activity during washout (p = 0.019) such that there was no difference in activity between groups during the washout (p = 0.938).
The placebo-by-proxy effect complicates detection of efficacy over placebo in analgesic treatment trials. However, these data suggest a novel trial design method for mitigating this effect - the masked washout period.

**OT-6**  
**MODE OF ACTIVATION SIGNIFICANTLY IMPACTS THROMBOELASTOGRAPHIC RESULTS AND ASSAY VARIABILITY.** M. Shelton, E. Griffith, J. Spencer, R. Hanel. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

The objective of this prospective study was to evaluate the impact of mode of activation on thromboelastography (TEG). Forty-eight client-owned dogs deemed healthy by physical examination, complete blood count, serum chemistry and coagulation panel were included. TEG was performed by a single operator on citrated whole blood using four activators: kaolin, tissue factor (TF), diluted 1:3600, TF diluted 1:5000, and a TF/kaolin mixture.

Inter assay variability was assessed using all four activators in a subset of eight dogs analyzed on four separate occasions. Intra assay variability was evaluated by repetitive evaluation of a single activator on four separate channels analyzed on two separate occasions. For all assays, five TEG variables were analyzed: reaction time (R), clotting time (K), alpha angle (α), maximum amplitude (MA), and global clot strength (G).

Overall differences in TEG variables were analyzed with repeated measures ANOVA followed by the Tukey-Kramer adjustment for multiple comparisons. Significant differences between modes of activation were found for all variables (p < 0.05). Interassay and intraassay evaluations showed that samples activated by kaolin and TF at a 1:3600 dilution had the lowest degree of variability, and the lowest coefficients of variation (<10%) were detected with α and MA. The results of this study suggest that the use of strong activators and more reliance on MA and α in interpretation may improve the utility of TEG in clinical practice.

**OT-7**  
**EFFICACY AND SAFETY OF A SUBCUTANEOUS HIGH DOSE PROPRIOETARY FORMULATION OF BUPRENORPHINE FOR 72-HOUR CONTROL OF POST-OPERATIVE PAIN ASSOCIATED WITH SOFT TISSUE SURGERY IN CATS.** J. Spencer1, J. Shelton2, R. Hanel2. 1College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO., 2College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO., 3Abbott Laboratories, Abbots Park, IL., 4Taylor Monroe, Gravel Head Farm, Little Downham, UK.

The objective of this multi-center, parallel group, randomized, masked, placebo-controlled study was to evaluate the efficacy and safety of a proprietary formulation of buprenorphine hydrochloride for injection in the control of post-operative pain. After meeting protocol-specified criteria, eligible client-owned cats were randomized to buprenorphine 0.24 mg/kg (n = 109) or matching placebo (n = 112) administered subcutaneously (SC) every 24 hours for 3 days. Protocol-specified premedication and anesthesia procedures were limited to non-analgesic agents. Trained professionals assessed cats at baseline and post-recovery using a rating system comprised of the following: behavior from a distance and behavior during social interaction (1 = comfortable to 4 = severe), sedation and opioid excitation (0 = absent, 1 = present), palpation response (1 = normal to 4 = severe), and overall pain assessment (1 = mild) vs. 3 being severe). Clinical and behavioral observations, cardiovascular events during anesthesia, and other safety parameters were also monitored. Cats were removed from the study (rescued) if thought to have inadequate pain control based on clinical evaluations and observations. The primary efficacy endpoint was the rate of treatment success, defined as cats not requiring additional analgesia during the 72-hour period. Most surgeries were ≤ 1 hour and the types of surgery were similar among the 2 groups. Total enrollment was the criteria for efficacy analyses (93 buprenorphine and 102 placebo). Sixty-six buprenorphine-treated cats (71%) were treatment successes compared with 45 placebo-treated cats (44%; p = 0.005). The proportion of cats rated as having well-controlled pain was greater in the buprenorphine group compared with placebo at the 30 minute (66% vs. 46%), 1 hour (77% vs. 59%), 2 hour (81% vs. 68%), and 3 hour (84% vs. 78%) post-recovery time points. Ninety-four of 109 buprenorphine-treated cats (86%) and 82/112 cats (73%) in the placebo group experienced adverse events. The following events occurred in buprenorphine-treated cats at a
frequency at least 5% higher than placebo: tachycardia (42% bu- norephrine vs. 30% placebo), hyperthermia (37% vs. 17%), and hypertension (25% vs. 11%). Rates of sedation and excitation were low, the majority of cases occurring in the first 4 hours after recovery. No notable changes in other safety findings were observed.

When administered once daily SC for 3 days, this formulation of buprenorphine provided effective analgesia with an acceptable adverse event profile in cats undergoing soft tissue surgery.

OT-9
RISK FACTORS AND PREVALENCE OF HAEMOPLASMA SPECIES INFECTION IN CATS IN SOUTHERN GERMANY
B. Stuetzer 1, T. Englert 1, J.R. Hawley 2, M.R. Lappin 2, C.J. Cupp, D.P. Laflamme. Nestle Purina
K. Hartmann 1. 1Clinic of Small Animal Medicine, LMU University of Munich, Germany. 2Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523, USA.

Data on risk factors and frequency of hemotropic Myco- plasma spp. (hemoplasmas) infections in cats in Southern Germany are limited. The aim of this study was to determine the prevalence of haemoplasma infections in cats in Southern Germany and to assess for risk factors associated with infection.

DNA of the feline haemoplasmas were amplified by use of a previously reported PCR assay from total DNA previously extracted from blood samples of 479 cats presented to different veterinary hospitals in Bavaria for various reasons. Direct sequencing was used to confirm all purified amplicons and com- pared to homoplasma sequences reported in GenBank.

The overall haemoplasma prevalence rate was 9.4% (45 of 479). Candidatus Mycoplasma haemominutum DNA was ampli- fied from 42 samples, M. haemofelis from 2 samples, and M. haemophilus from 1 sample. Cats from multi-cat households and cats with outdoor access had a significantly higher risk to be infected with a haemoplasma. Haemoplasma DNA was amplified from 11.6% (44 of 378 cats) of the samples from cats from multi-cat households. In contrast, only 1.5% (1 of 67 cats) of the samples from cats in single cat households were positive. In cats with out- door access, 16.2% (37 of 229 cats) of the samples were positive for DNA of the haemoplasmas, whereas only 2.0% (4 of 203 cats) of indoor cats were positive.

Candidatus Mycoplasma haemominutum infection is common in cats in Southern Germany. Increased prevalence in multi-cat households may reflect the potential for direct transmission amongst cats. Outdoor access is an additional risk factor that may relate to exposure to infected cats or vectors.

OT-10
CANINE AND FELINE DERMATOLOGY AT ANKARA UNIVERSITY: 663 CASES (2010-2012)
A.A. Sancak, B. Demir, S. Duzlu. Ankara University, Faculty of Veterinary Medicine, Department of Medicine, Ankara, Turkey.

The purpose of this study is to determine the risk factors, prevalence and distribution of dermatological disorders in dogs and cats examined at the internal medicine clinic, Ankara Uni- versity from 2010 to 2012.

A total of 6613 dogs and 3298 cats were assessed during study period. Of 6613 dogs and 3298 cats, 518 (7.8%) and 145 (4.4%) were diagnosed with dermatological disorders, respectively. Cases with disorders were mainly young animals (1-3 years old; 44% in cats; 38.6% in dogs). Dermatological disorders were observed mostly in mixed breed dogs (38.4%) and domestic cats (78.2%).

The most common clinical symptoms were pruritis and alope- cia in both dogs (70.9% and 13.2%, respectively) and cats (66% and 17.7%, respectively). The most common primary final diag- nosis in cases with dermatological disorders was Ectoparasites related diseases (31.8% dogs; 7.5% cats) and atopic dermatitis (27.3% dogs; 19.7% cats). The most common dermatological dis- orders in pure breed dogs were Ectoparasites (34.1%), endocrine (30%), and bacterial (31.3%) dermatitis.

Atopic dermatitis was ¼ of all dermatological cases in dogs and cats. Therefore, cases with atopic dermatitis should be evalu- ated with further detailed dermatological examination by a board certified dermatologist.

OT-11
IMPACT OF A 15-MINUTE PETTING SESSION ON SHELTER DOG WELL-BEING
R.T.S. McGowan, C. Bolte. Nestle Purina Research Center, St. Louis, MO.

It is well established that human interaction has positive effects on shelter dogs. However, the majority of work supporting this impact involves repeated sessions mixing play, petting, grooming and training. Few studies have examined the impact of petting spe- cifically and even fewer have examined the impact of a single session on dog well-being. Thus, we set out to answer the question: “Does one 15-minute petting session make a difference for shelter dogs?”

Fifty-five dogs were subject to one 15-minute petting session with an unfamiliar volunteer, in an observation room at a county animal shelter. Volunteers were instructed to interact with dogs in a controlled manner. Sessions were video recorded for later analysis of dog behavior; saliva was collected before and after the session to assess change in cortisol; cardiac activity was moni- tored throughout the session.

There was a great deal of variation in how dogs responded, pos- sibly reflecting differences in age, temperament, coping styles, time spent in the shelter and past experiences with people. We found no significant change in salivary cortisol (p > 0.05) from beginning to end of the session. However, when comparing cardiac activity and behavior from the first two minutes to the last two minutes of the session, dogs had a decrease in heart rate (p < 0.0001), an increase in heart rate variability (HF: p < 0.05, pNN50: p < 0.05) and changes in behavior (p < 0.05) associated with a positive state of relaxation. As a result of this study we can say: “Yes, 15 minutes does make a difference” for many shelter dogs.

OT-12
EFFECT OF NEUTERING ON LIFE EXPECTANCY IN ADULT CATS
C.J. Cupp, D.P. Laflamme. Nestle Purina Research Center, St. Louis, MO.

Research in dogs showed that neutering increased life expec- tancy by 14% to 26% in males and females, respectively (Hoff- man 2013). Similar data has not been published for cats. The goal of this project was to determine the effect of gender and neuter status on lifespan, using data from 2 pet nutrition centers.

Medical records from cats that died of natural causes between 1989 and 2003 were reviewed. Cats were initially included in the dataset if a specific date of birth and date of death and gender were recorded. Only cats that died at 4 years of age or older were included. Data were analyzed to determine age at death, and to evaluate any differences based on gender and neuter status using analysis of variance. The database from one center did not have comprehensive records regarding neutering, so these cats were excluded from the evaluation on effect of neutering. Signifi- cance was set at p < 0.05.

Records from 1120 cats were included in the final dataset. The average age at death was 11.7 ± 3.7 years. The oldest cat died at 28.4 years of age. Female cats lived 14.6% longer than male cats (12.5 ± 3.5 vs 10.9 ± 3.6 years, respectively; p < 0.001). Spay/ neuter data was available for 446 cats: neutered cats of both gen- ders lived approximately 2 years longer than intact cats (12.7 ± 3.0 vs 10.6 ± 3.8, respectively; p < 0.001).

Similar to dogs, neutering significantly extends life expectancy of male and female cats.
OT-13 COMPARISON OF DIFFERENT METHODS AND LOCATIONS OF TEMPERATURE MEASUREMENT IN HEALTHY DOGS. F. Juscel, A.M. Gimenes, C.N. Duarte, J.R. Castro, M.M. Mantovani, D.S. Schwartz, School of Veterinary Medicine and Animal Science, University of Sao Paulo (USP), Sao Paulo, SP – Brazil. Department of Internal Medicine.

The difference between body and peripheral temperature (PT), or temperature gradient has been proposed as having a prognostic value for intensive care patients, but there is a lack of information on the normal temperature gradient in dogs when obtained by different methods, and in different regions of the body. This study aimed to assess the difference, correlation and agreement of rectal temperature, obtained by digital and mercury-in-glass (Hg) thermometer, with temperatures obtained in different regions of body surface (gum, anus area, internal aspect of thoracic and pelvic limbs and interdigital space of all four limbs) by a infrared thermometer (IRT). The study population included 173 dogs. (100 males; 73 females), of several breeds and sizes. The measurements were obtained in a random sequence and compared with Wilcoxon, Friedman and Bland & Altman tests. There was a good agreement between digital and Hg, and with IRT at anus area. There was positive intermediate correlation between rectal and gum temperatures (R = 0.53; R = 0.45 respectively). There was no correlation between rectal and other areas of surface temperature measured by IRT. The average difference between rectal and interdigital temperature was 4.12 ± 0.17; CI95[3.85-4.39], range (0.2-15.67). These results imply that IRT could be used to assess temperature gradient between anus region and interdigital space, but there is large variation. Further controlled clinical studies are required before it can be applied to intensive care patients.


Early diagnosis and appropriate treatment of pyometra is essential for a favorable outcome. The disease may have deadly consequences if sepsis develops. Sepsis, defined as systemic inflammatory response syndrome, SIRS, is caused by infection and is associated with increased morbidity and mortality rates in both veterinary and human medicine. Early diagnosis and treatment is crucial for survival. In female dogs with pyometra, the majority fulfil clinical criteria for sepsis. However, sepsis is difficult to diagnose since clinical findings are diffuse and bacterial blood culture results may take 24 hours and are not always reliable (e.g. to contamination or no growth because of antimicrobial therapy). There is an urgent need of a rapid, sensitive and specific diagnostic test for early identification of sepsis. The aim of the present study was to evaluate concentrations of the inflammatory markers Serum amyloid A (SAA), C-reactive protein (CRP) and Insulin-like growth factor-I (IGF-I), and their use as possible markers for sepsis in a group of female dogs with pyometra.

Blood samples were obtained before ovariohysterectomy (OHE) from 31 female dogs with pyometra, whereof 23 with sepsis and 8 SIRS-negative and 8 without sepsis (SIRS-negative). After centrifugation, serum concentrations of SAA and CRP were measured with canine ELISA tests (Tridelta Development Limited, Kildare, Ireland) and concentrations of IGF-I with an IGF-I E20 IgGBP-blocked ELISA (Mediagnost, Reutlingen, Germany), all validated for use in dogs. Student’s t-test and ANOVA were used to test for differences between dogs with and without sepsis. P < 0.05 was considered significant.

Concentrations of SAA (mean ± SE) were significantly higher in the female dogs with sepsis (131 ± 8 mg/L) compared to those without sepsis (88 ± 21 mg/mL) (p = 0.006). Mean ± SE concentrations of CRP were not different in dogs with sepsis (226 ± 18 mg/L) compared to dogs without sepsis (176 ± 41 mg/L) (p = 0.1). Concentrations of IGF-I were not different in dogs with sepsis compared to those without sepsis (mean ± SE, 224.3 ± 24.7 ng/mL, and 212.2 ± 53.7 ng/mL, respectively) (p = 0.8).

Presence of sepsis was associated with increased serum concentrations of SAA. We conclude that analysis of acute phase protein SAA may have clinical value as a marker for sepsis in dogs. The concentrations of CRP and IGF-I were not as useful in the detection of sepsis in this study.


The pathogenesis of osteoarthritis (OA) has been attributed to up-regulation of pro-inflammatory molecule expression in chondrocytes and other joint tissues. These molecules include prostaglandin E2 (PGE2), cytokines, and chemokines. Expression of these genes is regulated by the transcription factor nuclear factor-kappa B (NF-kB). Inhibition of their up-regulation is a major objective in the management of OA. In this study, we transfected canine chondrocytes with the NF-kB-p65 subunit and measured the production of inflammatory mediators. We hypothesized that NF-kB transfection enhances PGE2, interleukin-8 (IL-8), and macrophage chemotactic protein-1 (MCP-1) production. We also evaluated whether we could modulate NF-kB-p65 transfection effects with the NSAID Carprofen, or the combination of avocado/soybean unsaponifiables (ASU), glucosamine hydrochloride (GLU), and chondroitin sulfate (CS).

Canine primary chondrocytes (5x10^5 cells/well) were transiently transfected (X-tremeGENE HP, Roche) with a plasmid containing the NF-kB-p65 subunit (Invivogen). Cells were incubated for 24 hours with: control media, Carprofen (40 μg/mL), or the ASU (NMX1000®; 8.3 μg/mL)+GLU (FCHG49®; 11 μg/mL)+CS (TRH122®; 20 μg/mL) combination. PGE2, IL-8, and MCP-1 production were measured by ELISA and analyzed by one-way ANOVA. Tukey post-hoc test, with p < 0.05 significance. Intracellular localization of NF-kB and cyclooxygenase-2 (COX-2) were determined by immunofluorescence.

Canine chondrocytes were successfully transfected with NF-kB-p65 with low cytotoxicity and with ≥11-35% efficiency. Transfected chondrocytes demonstrated an increase in NF-kB and COX-2 nuclear staining compared to non-transfected controls. Non-transfected chondrocytes produced low levels of PGE2, IL-8, and MCP-1 compared to transfected cells which showed a 4- to 8-fold increase. Pretreatment of transfected chondrocytes with Carprofen or ASU+GLU+CS showed a significant decrease in pro-inflammatory molecule production.

The observation that NF-kB-transfected chondrocytes increase production of PGE2, IL-8, and MCP-1 indicates that our transfected canine chondrocyte model may facilitate identification of compounds that could effectively inhibit NF-kB activity. (Research supported by Nutramax Laboratories, Inc.)

OT-16 SAFETY OF THE EP4 RECEPTOR ANTAGONIST, GRAPP grunt, ADMINISTERED DAILY TO BEAGLE DOGS FOR 9- MONTHS AT 1, 6 AND 50 MG/KG. L. Rausch-Derra, L. Rhodes. Aratana Therapeutics, Inc., Kansas City, KS.

Grapprant is a selective antagonist of the EP4 receptor, whose physiological ligand is prostaglandin E2 (PGE2). The EP4 recepto...
Grapiprant is under development for use in humans and dogs for the control of pain and inflammation associated with osteoarthritis. The study described here was undertaken to evaluate the potential toxicity and systemic exposure of grapiprant in Beagle dogs and also to assess the reversibility of any observed changes.

Grapiprant was administered orally by gavage, once daily, for 13 months to Beagle dogs at doses of 0 (0.5% methylcellulose), 1, 6, and 50 mg/kg/day in a dose volume of 5 mL/kg. Four animals/sex were used in each dose group and 2 additional animals/sex were used in the 50 mg/kg dose group for recovery purposes in the study. Clinical signs and food consumption were assessed daily. Body weight was recorded weekly. Ophthalmologic examinations, electrocardiograms, and clinical pathology analysis and urinalysis were conducted at several time points during the dosing and recovery phases. Serum drug concentrations were dose proportional from 1 to 6 mg/kg and decreased by 50% at 13 months. The only histopathology of note was a mild mucosal regeneration of the ileum in one dog at 50 mg/kg.

Grapiprant at doses given daily up to 50 mg/kg (more than 10X the anticipated dose for dogs with osteoarthritis) for 9 months resulted in minimal toxicity, with no mortality, or effects on body weight, food consumption, ophthalmic exams, electrocardiograms, hematology, coagulation, organ weights, or gross pathology. Treatment was associated with mild gastrointestinal signs such as soft formed stools, stool with mucus, and occasional blood seen in the stool. Emesis was seen sporadically and watery stool was seen sporadically with prolonged use. Treatment was also associated with mild and reversible decreases in total protein and albumin over time, with incidence increasing as dose increased. Calcium decreases were also seen but were considered secondary to diet and low albumin levels. Serum drug concentrations were dose proportional from 1 to 6 mg/kg and more than dose proportional from 6 to 50 mg/kg. The only histopathology of note was a mild mucosal regeneration of the ileum in one dog at 50 mg/kg.

Treatment with grapiprant was well-tolerated when given at doses up to 50 mg/kg for 9 months.

Dogs, like humans, experience eye changes with aging: hardening and clouding of the lens and accumulated oxidative damage from UV sunlight. Whether such changes could be affecting the visual function of dogs has been debated. The objective of this study was to determine if auto-refractometry could be used as a surrogate measure of visual function in dogs.

Eye evaluations were completed on 10 beagle dogs (ages 1 to 13 years old) by a veterinary ophthalmologist. Spherical Equivalent Refractive Error was measured by hand-held auto-refractor (WelchAllyn SureSight) under both indirect and direct lighting conditions with 5 measurements per condition, per eye. Measures were repeated on 3 different days for each dog within 6 weeks. Non-parametric statistics was used to detect differences among lighting conditions and test days, and between eyes. Spearman correlation assessed the visual measurement outcomes’ association with age.

There was no difference for day to day or intra-eye measurements. Significantly, beagles without cataracts showed a myopic shift with aging (average spherical equivalent ranged from plano to -3.00 diopters), suggesting that the older the dog, the more near-sighted (r = -0.48 and -0.73 under direct and indirect lights; P < 0.05 both). Younger dogs were able to make larger accommodation changes from indirect light to direct light conditions, indicating a more flexible lens (r = 0.09 and 0.05).

This pilot study shows the clinical hand-held portable human auto-refractor technique to be applicable to dogs, and sensitive to light conditions. The non-cataractous myopic refractive shift observed could be expected to compromise dogs’ visual functions with aging.

The objective of this study was to assess the safety of a proprietary formulation of buprenorphine hydrochloride administered subcutaneously (SC) to young domestic shorthair cats under exaggerated use.

Four cohorts were created, each consisting of 8 cats ≥24 months of age with equal numbers of males and females. Treatment groups were 0.24, 0.72, and 1.20 mg/kg/day buprenorphine SC (representing 1X, 3X, and 5X of the therapeutic dose) or saline (control) for 9 consecutive days. Clinical observations, behavioral responses to injection, adverse event (AE) monitoring, food and water consumption, urination/defecation, and injection site inspections were conducted at least once per day. In addition, physical examinations, body weights, bleeding times, clinical pathology of blood and urine samples, vital signs, and electrocardiograms (ECGs) were assessed at regular time points. After 9 days of dosing all cats were euthanized and a complete necropsy was performed. Selected organs were weighed and protocol-specified tissues were examined microscopically from all cats.

All cats survived to the scheduled necropsy. Four AEs were noted in buprenorphine-treated cats during study days 0-4; 2 of these AEs were not considered treatment related. The other 2 AEs consisted of hyperactivity, difficulty in handling, gastrointestinal agitation, and dilated pupils that occurred in one 0.24 mg/kg/day group male on day 2 and one 0.72 mg/kg/day group male on day 2; both were considered possibly related to study drug. There were no drug-related effects on survival, behavioral response to injection, injection site inspections, body weight, food or water consumption, bleeding time determination, urinalysis, respiration rate, heart rate, ECGs, blood pressures, body temperatures, macroscopic examinations, or organ weights. Nonadverse treatment-related findings included difficulty in handling, lower evidence of urination, dilated pupils, higher percent basophils and fibrinogen, lower bile acids in females, higher creatine kinase,
lower triglycerides and blood urea nitrogen levels, and minimal to moderate subacute inflammation at the injection sites. Once daily SC administrations of a proprietary formulation of buprenorphine at doses of 0.24, 0.72, and 1.20 mg/kg/day for 9 days were systemically well-tolerated in young domestic cats.

P-1
IN VITRO EFFECTS OF YUNNAN BA YAO (YB) ON COAGULATION. K. Loyd1, L.A. Cohn1, S.A. Smith2. 1University of Missouri-College of Veterinary Medicine, Columbia, MO., 2University of Illinois Department of Biochemistry, Urbana, IL.

YB is a complex herbal remedy used in veterinary medicine to promote hemostasis, despite a lack of published evidence of efficacy. The purpose of this study was to determine if YB is procoagulant in vitro, and if so, to determine a mechanism of action. YB was suspended in purified H2O, 50 mM Tris pH 5.0, 50 mM Tris pH 8.0, or 100% EtOH at 25 mg/mL. YB was then further diluted in 50 mM Tris pH 7.4, 100 mM NaCl, 0.1% BSA, 100 uM phospholipid and added at concentrations of 0.002 to 50 mg/mL to coagulation assays employing a trigger of tissue factor, factor (F) Xa, or YB (figure). Coagulation was evaluated using pooled normal canine or human plasma, or factor X- or factor XII-deficient human plasma. Coagulant factor inhibitor or tissue factor pathway inhibitor were added to some assays. Calibrated automated thrombography was also evaluated using YB and human plasma deficient in FV, FVIII, FIX, FXI, or protein S, or heterozygous for FV II.

In standard coagulation assays YB decreased time to clot formation regardless of activation trigger, species of plasma used, deficiency of bypassable factors, or replacement of zymogen factors with enzymes. YB activated the contact pathway, but also was procoagulant when either the contact pathway or the extrinsic pathway were eliminated. The effect was not eliminated by removal of water, acid, or base soluble components, heating, or removal of metals. Addition of YB increased both peak thrombin generated and endogenous thrombin potential. YB is procoagulant in vitro, with the effect limited to the common pathway at the level of prothrombinase complex.

P-2
ALTERNATE DAY DOsing OF ITRACONAZOLE IN HEALTHY CATS. S.M Middleton1, L. Dirikolu1, M.G. Papich1, S.I. Rubin1. 1University of Illinois College of Veterinary Medicine, Urbana, IL, 2Veterinary Specialty Center, Melbourne, FL, 3North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Chronic administration of oral medications to cats can be challenging and lead to poor compliance. Systemic fungal disease in cats requires long-term treatment, and itraconazole is often the drug of choice. Although a dose of 10 mg/kg/day has been recommended for the treatment of systemic fungal disease in cats, this dose is difficult to administer accurately because the capsules are of a fixed strength (100 mg) and the oral solution has poor palatability. Compounded formulations have documented poor oral absorption in other species. The purpose of this study was to evaluate serum concentrations and safety of a 100 mg itraconazole capsule given orally every 48 hours in healthy adult cats. Ten adult cats (average 5.3 kg, range 3.8-8.1 kg) received this regimen for 8 weeks. Peak and trough serum concentrations of itraconazole were measured weekly using high performance liquid chromatography (HPLC) and yielded the following results (mean ± standard deviation):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Geriatric CKD</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (μg/mL)</td>
<td>3.44 ± 0.95</td>
<td>0.74 ± 0.62</td>
</tr>
<tr>
<td>Clearance (L/hr/kg)</td>
<td>1.80 ± 0.25</td>
<td>0.79 ± 0.42</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>1.34 ± 0.25</td>
<td>0.70 ± 0.70</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>57 ± 24</td>
<td>55 ± 26</td>
</tr>
<tr>
<td>Total bilirubin mg/dL</td>
<td>0.00 - 0.1</td>
<td>0.1 - 0.1</td>
</tr>
</tbody>
</table>

A 100 mg per cat every other day oral dosing regimen for itraconazole produced consistent serum concentrations. In people trough serum itraconazole concentrations above 0.5 μg/mL have been associated with therapeutic success. A mean value above this concentration was achieved by week 3 in this study, but there was wide variability. The protocol yielded no adverse effects in 8 of the 10 study cats. The two cats who developed adverse effects recovered fully with discontinuation of the drug. The adverse drug events in these cats were likely an idiosyncratic reaction because itraconazole concentrations in these cats were in the same range as the others tested.

P-3

Ondansetron is an effective anti-emetic in cats. The purpose of this study was to compare the pharmacokinetics of subcutaneous ondansetron in geriatric cats to cats with chronic kidney disease (CKD) and liver disease using a limited sampling strategy.

12 geriatric cats, 16 CKD cats and 8 liver disease cats were enrolled. Based on limited sample modeling, blood was drawn 30 minutes and 2 hours following 2 mg subcutaneous ondansetron. Ondansetron concentrations were measured by liquid chromatography coupled to tandem mass spectrometry. Drug exposure (AUC) was predicted using a limited sampling approach based on multiple linear regression analysis of previous full sampling studies and clearance estimated using non-compartmental methods.

One-way ANOVA demonstrated no statistically significant difference between groups although a subjective decrease was seen in clearance for liver cats. A subset of cats (10 CKD, 5 liver) was analyzed for increased statistical power.

There was no significant difference in the clearance of subcutaneous ondansetron in CKD or liver disease cats when compared to geriatric cats. Additional cats with liver disease should be added to the study in future studies.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Geriatric CKD</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (μg/mL)</td>
<td>3.44 ± 0.95</td>
<td>0.74 ± 0.62</td>
</tr>
<tr>
<td>Clearance (L/hr/kg)</td>
<td>1.80 ± 0.25</td>
<td>0.79 ± 0.42</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>1.34 ± 0.25</td>
<td>0.70 ± 0.70</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>57 ± 24</td>
<td>55 ± 26</td>
</tr>
<tr>
<td>Total bilirubin mg/dL</td>
<td>0.00 - 0.1</td>
<td>0.1 - 0.1</td>
</tr>
</tbody>
</table>

Mirtazapine is commonly used in veterinary medicine as an appetite stimulant at 1.87 or 3.75 mg dose. The objectives of this study were to determine the most common adverse effects reported and the dose associated with these signs.

Records of cats with mirtazapine exposure (2006–2011) were obtained from the Animal Poison Control Center. The following parameters were recorded: signalment, weight, outcome, agent ingested, amount ingested, route of exposure, and clinical signs observed.

A total of 104 records of cats with mirtazapine ingestion were available. Twenty-six were excluded: no monitoring or follow-up (22), signs not related to mirtazapine (3), no development of signs (1). The most commonly observed adverse effects in the remaining 78 cats included: vocalization (57.7%), agitation (33.5%), vomiting (28.2%), ataxia (16.7%), restlessness (15.4%), tremors/trembling (14.1%), hyperactivity (14.1%), hypersalivation (14.1%), taquihypea (12.8%), anorexia (9.0%), lethargy (11.5%), anorexia (9.0%), anorexia (2.65 mg/kg), and disorientation (9.0%; 2.34 mg/kg). The dose most frequently reported with signs of toxicity was 15 mg (40) followed by 3.75 mg (25), 7.5 mg (4), 30 mg (1), 18.75 mg (1), 11.25 mg (1), 5.8 mg (1), 1.87 mg (1).

The greater prevalence of adverse effects at 3.75 mg rather than 1.87 mg advocates the latter is a more appropriate starting dose for stimulating appetite while limiting toxicity. The benefit of compounding mirtazapine is implied given the likelihood of accidental administration of a full tablet (15 mg) and resulting toxicity.

Pharmacokinetics of minocycline in domestic cats, B. E. Tynan1, L. A. Cohn1, M. E. Kerl1, M. G. Papich2. University of Missouri – College of Veterinary Medicine, Columbia, MO. 1North Carolina State University – College of Veterinary Medicine, Raleigh, NC.

Minocycline hydrochloride may be considered as an alternative to doxycycline if there are shortages in availability. Minocycline is more lipophilic, less protein bound, and has equivalent or superior activity against bacteria compared to doxycycline. However, no studies are available to guide dosing for cats. The purpose of this study was to determine the pharmacokinetics of minocycline in domestic cats in order to facilitate dosage decisions for the use of this doxycycline-alternative drug for the treatment of infectious disease.

A single dose of minocycline hydrochloride was administered to healthy, laboratory raised domestic cats either by an intravenous route (5 mg/kg IV infused over 10 minutes; n = 4), or by mouth (50 mg/cat PO; n = 6). The oral dose was administered as a single, intact capsule. Blood was collected from each cat into lithium heparin-containing tubes at frequent intervals up to 24 hours after drug administration. Samples were placed on ice until plasma separation and kept frozen until the time of assay. Plasma protein binding also was measured by fortifying blank plasma from untreated healthy cats with minocycline at two concentrations. After incubating the drug with plasma, an ultracentrifugation method was used to determine protein binding. Minocycline in all samples was measured using High Pressure Liquid Chromatography (HPLC) with ultraviolet detection. A one-compartment pharmacokinetic model was fit to the oral data and a two-compartment model fit to the IV data by use of a computer program.

Minor adverse events were observed. Two cats became lethargic and taquihypea during IV drug infusion but normalized within 20 minutes. One cat vomited 6 hr after IV infusion, and two cats vomited either 1.5 hr or ~5 hr after oral drug administration. None of these cats were removed from the analysis because their pharmacokinetic values were within the range of all the other cats. The mean oral dose administered was 13.9 mg/kg ±0.47. Protein binding was 60% at 2 µg/mL. After IV administration, the elimination half-life (T1/2), apparent volume of distribution at steady-state, and systemic clearance was 657 hours (14.4% CV), 1.5 L/kg (34.5% CV), and 2.9 mL/kg/min (40.8% CV), respectively. After oral administration the terminal T1/2 and peak concentration (C MAX) was 6.3 hr (9% CV) and 4.77 µg/mL (36% CV), respectively. Oral absorption in these cats was approximately 62%. Because most bacteria will have a minimum inhibitory concentration of 0.5 µg/mL or less, an oral once daily dose of 8.8 mg/kg would be adequate to meet pharmacokinetic-pharmacodynamic (PK-PD) targets, after adjusting for protein binding. Although some gastrointestinal upset may occur with higher doses, a single 50 mg capsule orally once daily would provide appropriate dosing for most cats.

Pharmacogenetics is the study of how variations in the genome influence drug pharmacokinetics (the bodies affect on the drug) and pharmacodynamics (the drugs affect on the body). The MDR1 gene codes for a membrane-bound drug transporter, P-glycoprotein (P-gp) that transports drugs across the cell membrane using an energy dependant mechanism. Anecdotal reports in the literature suggested that dogs with a mutation in the MDR1 gene (mdr1-l/delta) showed increased sensitivity to routinely used veterinary sedatives such as acepromazine, resulting in increased duration and depth of sedation. The purpose of this study was to investigate the effect of acepromazine on MDR1 genotyped rough coated collies. Genotyping for the mdr1-l/delta mutation was performed in 29 rough coated collies. Dogs were considered healthy based on a physical examination, CBC, serum chemistry and urinalysis. After administration of acepromazine (0.04 mg/kg, IV) sedation scoring was performed at 0, 30 min, 60 min, 90 min, 2 hr, 2.5 hr, 3 hr, 4 hr and 6 hr by an observer blinded to the results of the MDR1 genotype. Homozygous mutant collies (MDR1–/–) (n = 10) reached a greater level of sedation and remained sedated for longer when given acepromazine compared to heterozygous mutants (MDR1 +/−) (n = 9) and wild-type collies (MDR1 +/+ ) (n = 10). This effect approached but did not reach statistical significance when analyzed with a linear mixed-effect model (p = 0.086). The trend for increased sedation in MDR1–/– collies might be clinically significant, especially when larger doses of acepromazine or opioid combinations are used.

Phenylpropanolamine (PPA) is a commonly used sympathomimetic amine for the treatment of urinary sphincter hypertonus in dogs. This study reviewed medical records pertaining to 109 incidents of PPA toxicity in Labrador Retrievers from the toxicology database of the ASPCA Animal Poison Control Center (Urbana, IL, USA) between 2006 and 2011.

Median age of dogs was 4 year sold (range 0.2–14) and median weight was 30.4 kg (range 8.1–52.5). Median dose was 43.3 mg/kg (range 2.2–342), and median number of clinical signs was 3 (range 0–11). Conditions included neurological, cardiovascular, GI, respiratory, ocular, renal, and cutaneous signs. The following
P-8  
ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF CLINICAL ISOLATES OF E. COLI ISOLATED FROM DOGS AND CATS IN THE UNITED STATES. K. Thungrat1, D.M. Boothe1, Stuart.B. Price1, D. Mark Carpenter2. 1College of Veterinary Medicine, 2College of Science and Mathematics, Auburn University.

Escherichia coli is among the most common bacterial pathogen in dogs and cats. The lack of a national monitoring program limits its evidence-based empirical antimicrobial choices. This study describes antimicrobial susceptibility patterns for presumed E. coli pathogens in dogs (n = 2254) or cats (n = 745) collected from six geographic regions in the United States between May 2008 and January 2012. Minimum inhibitory concentrations (MIC) were determined to 15 drugs representing 6 drug classes. Urinary tract isolates were most common (71%). Population MIC distributions were generally bimodal with the second mode above the resistant breakpoint for all drugs except efotaxime, gentamicin, and meropenem. The MIC90 exceeded the resistant breakpoint for ampicillin, amoxicillin-clavulanic acid and cephalothin (model adjusted as "moderate" (69%) than "mild" (31%) in juvenile animals compared to adult/senior animals (54% moderate vs 46% "moderate").

Premature Ventricular Contractions (PVCs), severe potential side effects of PAPA toxicsis, were reported in 7 cases (4.1%). Dogs with PVCs displayed significantly more clinical signs (median of 4; range 2-7) than those without PVC (median of 3; range 0-11). All dogs with PVCs were categorized in the "major" illness levels (100%) compared to only 8 among dogs without PVC (5.6%). Interestingly, PVC dogs received higher doses (median 75 mg/kg; range 17.3-300) compared to dogs without PVCs (median 43.1 mg/kg; range 2-342).

P-9  
EVALUATION OF THE PHARMACOKINETICS OF AMMONIUM TETRATHIOMOLYBDATE AND ITS EFFECTS ON SERUM COPPER CONCENTRATIONS IN HEALTHY DOGS. C.M. Chan, D.K. Langlem2, J.P. Buehler3, A.F. Lehner2, N.B. Oliver4, T.H. Herdt1, M.B. Baille1, W.D. Schall1. 1College of Veterinary Medicine, 2Diagnostic Center for Population and Animal Health, 3Pharmacology and Toxicology; Michigan State University, East Lansing, MI.

Current therapies for canine copper-associated hepatitis (CAH) have numerous side effects and variable therapeutic efficacy. Ammonium tetrathiomolybdate (TTM) is a copper chelator in ovine and human copper storage diseases but has not been investigated in CAH. Only rodent and ovine pharmacokinetics are described previously. The objective was to describe TTM’s pharmacokinetics in dogs. We hypothesized TTM administration would increase serum copper.

Eight dogs received one dose (1 mg/kg) of TTM orally and intravenously in randomized cross-over design. Blood was collected at set time points 0-72 hours post-administration. Serum molybdenum (TTM’s surrogate marker) and copper concentrations were measured using inductively coupled plasma mass spectrometry. Pharmacokinetics were best described using a noncompartmental model, and serum copper concentrations were analyzed for significance using two-tailed RMANOVA and one-tailed Dunnett’s.

For intravenous administration, the mean half-life, time to maximum concentration, maximum concentration, clearance rate, volume of distribution, and area under the curve were 27.7 hr, 0.05, 4913.7 ng/mL-hr, 32.6 L/kg-hr, 1.0 L/kg, and 30702.55 ng/mL-hr, respectively. For oral administration, the mean half-life, time to maximum concentration, maximum concentration, clearance rate, volume of distribution, and area under the curve were 26.8 hr, 3.8 hr, 223.5 ng/mL, 154.8 L/kg-hr, 6.0 L/kg, and 6458.74 ng/mL-hr, respectively. Oral bioavailability was 21%. Serum copper increased significantly (P < 0.05) after intravenous and oral dosing.

Pharmacokinetics for TTM in normal dogs are described. Oral TTM absorption was fair overall. Increases in serum copper suggest that TTM mobilizes tissue copper. Further studies are needed to evaluate TTM’s therapeutic potential in CAH.

P-10  
THE PHARMACOKINETICS OF INTRAVENOUS FENOLDOPAM IN HEALTHY AWAKE CATS. K.E. O’Neill, M.A. Labato. Tufts Cummings School of Veterinary Medicine, North Grafton, MA.

Fenoldopam is a selective dopamine-I receptor agonist that can improve diuresis by increasing renal blood flow, perfusion and causing peripheral vasodilation. Fenoldopam has been shown to induce diuresis and be well-tolerated in healthy cats. It has been used clinically in cats with oliguric kidney injury at doses extrapolated from human medicine and canine studies. The pharmacokinetics in healthy beagle dogs has been reported; however, pharmacokinetic data in cats is lacking.

The goal of this study was to determine pharmacokinetic data for healthy, awake cats receiving an infusion of fenoldopam. Six healthy, awake client-owned cats ages 2-6 years old were utilized. Each cat was administered a 120-min fenoldopam constant rate infusion at 0.8 ug/kg per minute followed by a 20-min washout period. Citrated blood was collected during and after the infusion for the measurement of plasma fenoldopam concentration by HPLC with mass spectrometry.

Preliminary data show that steady-state plasma fenoldopam concentration of 17.6 ng/mL within 45 minutes of starting the infusion. Area under the plasma-concentration-time curve was 463 ng/mL min and half-life was 4.19 min. No adverse events were noted during infusion. Fenoldopam at a constant-rate infusion of 0.8 ug/kg per minute was well tolerated in healthy cats. Based on the results of this study, further evaluation of fenoldopam in cats with kidney disease is recommended.

P-11  
PHARMACOKINETICS AND PHARMACODYNAMICS OF LEFLUNOMIDE AND ITS METABOLITE, TERIFLUNOMIDE (A77-1726), IN DOGS. J. Sofge, H. Bunn, C. Cruz-Espindola, H.D. Gossett, D.M. Boothe. Auburn University College of Veterinary Medicine, Auburn, AL.

Leflunomide is a human approved immunomodulatory drug that is commonly used in dogs for a variety of immune-mediated
diseases. Leflunomide is a prodrug with its activity solely dependent on rapid and complete conversion to the active metabolite, teriflunomide (A77-1726). No studies appear to have described the pharmacokinetics of leflunomide or its metabolite in dogs. The purpose of this study was to describe the disposition of teriflunomide in dogs after single oral dosing (pharmacokinetics) and then to determine a therapeutic range for teriflunomide in dogs with spontaneous immune-mediated disease (pharmacodynamics).

A total of 192 canine patients were monitored as part of the Auburn University Clinical Pharmacology Laboratory Therapeutic Drug Monitoring (TDM) service. These samples included samples received between January 2012 and October 2013. Concentrations were compared among animals whose disease was considered controlled versus not controlled using ANOVA.

Half-life was calculated from peak (C1) in canine serum using their respective ARK Diagnostic (Sunnyvale, CA) immunoassay. Half-life was calculated from peak (C1, t½) and trough (C2, t) serum concentrations where t½ = 0.693/ [ln (C1/C2)] (t1-t2)]. Variables were evaluated by multiple linear regression for their effects on the t½ of an AED. The purpose of this study was to retroactively assess the impact of PB on the disposition of AED.

The study population comprised 10 healthy beagles. A single IV TA dose (20, 30, 40, and 50 mg/kg) was administered in ascending dose order with a washout period of 1 week, until each dog had vomiting. In a separate experiment, blood was collected 1 h before and 20 min, 3 h, and 24 h after TA administration (50 mg/kg, IV). Change in antifibrinolytic potency was assessed using a lysis index, which is a representative parameter used to determine fibrinolytic potency using rotational thromboelastometer.

TA induced vomiting in a dose-dependent manner, with all dogs vomiting after administration of ≤50 mg/kg. The lysis index increased significantly 20 min after TA administration (50 mg/kg, IV), indicating that TA exhibited antifibrinolytic potency. However, the lysis index decreased to normal 3 h and 24 h after administration. These results reveal the dose-dependent efficacy of IV TA. TA exhibits antifibrinolytic activity for less than 3 h after administration. Further studies are warranted to investigate the emetic action as well as the side effects of TA in various dog breeds and at different ages.
remodeling was evaluated via thoracic CT scans at month 8 and 12 using a scoring system for lung attenuation (LA) and bronchial wall thickening (BWT). All variables were assessed statistically using a two-way repeated measures ANOVA except CT data which were assessed with a t-test or Mann-Whitney test; p < 0.05 was considered significant.

No difference was noted in airway cosinophilia, AHR, and immunologic assays. Lung attenuation and bronchial wall thickening scores were significantly lower in MSC-treated compared to placebo-treated cats at month 8 (LA p = 0.031; BWT p = 0.0489), but not month 12 (LA p = 0.406; BWT p = 0.077). We concluded that therapy slows airway remodeling in chronic asthma; however, the effect is not sustained long term. Further study of MSC therapy is warranted in cats with naturally occurring disease.

R-2

The purpose of this report is to evaluate short- and long-term outcomes in dogs and cats after balloon dilation (BD), placement of a non-covered metallic stent (MS) or covered metallic stent (CMS) for the treatment of nasopharyngeal stenosis (NPS).

Medical records of patients that underwent treatment of NPS in the authors’ practice with BD, MS or CMS were retrospectively reviewed. Data on signalment, history, clinical signs, NPS location, degree of attenuation, treatment approach, and short- and long-term outcomes were recorded.

Fifteen dogs and 27 cats with NPS were included. The median age was 3.9 years (0.5-18). Twenty-seven had BD, with 3 (15%) having long-term success (median 1 procedure). Thirty-three had a stent placed after failed BD (n = 19) or as a naive procedure (n = 14). Twenty-two were treated with a MS and 11 with a CMS. Overall, 58% of stent patients had 1 or more complications including tissue in-growth (24%), chronic infection (33%) and development of an oronasal fistula (21%), stent fracture (<1%), stent irritation (<1%), and stent migration (<1%). Despite requiring multiple procedures, long-term outcome was considered good-excellent in 69% of patients.

NPS can be successfully treated with BD, MS and/or CMS. Although BD alone has fewer complications, there was poor long-term success in this series. Success increased with MS and CMS, however there are more complications, of which, most were easily manageable.

R-3
1Faculty of Veterinary Science, University of Melbourne, Werribee, VIC, Australia, 2Faculty of Pharmacy, University of Sydney, Camperdown, NSW, Australia.

Aerosolized medications are increasingly being used to treat respiratory diseases in dogs by delivery from metered dose inhalers (MDI) and nebulizers. Scintigraphic assessment of lung deposition of radiolabelled aerosols is routinely performed in people. The aim of our study was to assess and compare lung deposition of an inhalant corticosteroid (fluticasone) delivered from a MDI and a nebulizer in healthy dogs.

Ten healthy foxhounds were recruited (age 2-6 yrs, BW 28-37 kg); study approval was granted by The University of Melbourne animal ethics committee. Initial inhalation (MDI vs nebulizer) was randomly assigned and then crossed over after a seven-day wash-out period. Acetzolamide (0.05 mg/kg) was necessary to perform the scintigraphies. Fluticasone was labelled with 99mTc for both devices. The dogs were imaged using 2D planar scintigraphy, with lung deposition quantified by manual Region-Of-Interest analysis. Lung deposition in counts per minute (cpm) was calculated as a percentage of the delivered dose of radiolabelled aerosol (cpm). An ANOVA with effects of sequence, dog within sequence, period and treatment was conducted with statistical significance set at P < 0.05. Six of the 10 dogs were then randomly selected and reassessed without sedation after additional training (three imaged from the MDI, three from the nebulizer). A paired t-test was used to assess differences in lung deposition in these six dogs with and without sedation (whilst inhaling from the same device); significance was set at P < 0.05.

Treatment had a significant effect on lung deposition (P = 0.027). Higher deposition was achieved by nebulization with mean deposition of 4.2% (SD 1.4%, range 1.9-6.1%), whilst MDI therapy achieved a mean of 2.3% (SD 1.4%, range 0.2-4.2%). Nebulization achieved higher lung deposition in 7/10 dogs. No statistical difference (P = 0.68) was found between mean lung deposition achieved by the six unsedated dogs (3.8%, SD 1.5%) and deposition achieved by the same dogs with sedation (3.6%, SD 1.7%). A significant correlation was found between delivered dose (cpm) and lung deposition (cpm) for nebulization (r = 0.71; 95%CI 0.14 to 0.92; P = 0.022); the correlation was not significant for MDI therapy (r = 0.26; 95%CI -0.44 to 0.77; P = 0.46).

In conclusion, this study confirms lung deposition of inhalant medications delivered from a MDI and a nebulizer in healthy, tidally breathing dogs with and without sedation. The results suggest that nebulization achieves more reliable lung deposition than MDI therapy. Lung deposition in this group of dogs was compared to reported deposition in adult humans but is on par with reported deposition in pediatric patients less than 4-5 years old.

R-4
1Department of Veterinary Medicine, University of Cambridge, Cambridge, UK, 2Veterinary Epidemiology Consulting, Suffolk, UK.

Brachycephalic canine breeds often develop upper airway obstructions due to malformation of the skull, resulting in the so-called brachycephalic obstructive airway syndrome (BOAS). BOAS-affected dogs suffer life-long respiratory problems as a result of stenotic nares, oversized soft palate, intranasal obstructions, and laryngeal collapse. Clinical signs range from stertorous breathing and exercise intolerance to life-threatening conditions such as cyanosis and syncope. Many brachycephalic dogs exhibit some respiratory signs and the diagnosis of BOAS is subjective. Unrestrained whole-body barometric plethysmography (WBBP) is a method of objectively assessing respiratory function that has been introduced to companion animals recently.

This study investigated the feasibility of using WBBP to discriminate clinically affected French bulldogs from healthy French bulldogs. Forty-two unsedated French bulldogs and twenty non-brachycephalic control dogs underwent a 30-min WBBP test. An established clinical grading system of BOAS was used to classify each dog as experiencing either absent/minimal or moderate/severe BOAS. The ratio of expiratory time to inspiratory time (Te/Ti), the ratio of peak expiratory flow to peak inspiratory flow (PEF/PIF), and the minute ventilation divided by body weight (MV/BW) were considered to be potential indicators of upper airway obstructions. Quadratic discriminant analysis (QDA) was used as a classification tool and followed by a permutation test using a Monte Carlo method. Non-brachycephalic control, absent/minimal BOAS French bulldog, and moderate/severe BOAS French Bulldog groups were well discriminated from each other and the probability of an individual being classified into the BOAS-affected group (moderate/severe BOAS) was provided.

Sensitivity, specificity & classification accuracy of the classifier.
Lung inflammation associated with acute necrotizing pancreatitis in dogs. V. Vrolyk, B. Wobeser, A. Carr, B. Singh. Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada.

There is some clinical evidence that dogs suffering from acute necrotizing pancreatitis (ANP) can develop clinical signs of respiratory disease. However, there are no data describing the lung histopathology in cases of ANP in dogs. Also, there has been some evidence that pulmonary intravascular macrophages (PIMs), a pro-inflammatory cell normally found in ruminants, can be induced in species that normally don’t have PIMs. Because of the established role of PIMs in lung injury, the recruitment of PIMs in ANP dogs may let's express very little vWF. Because of the established role of PIMs in lung injury, the recruitment of PIMs in ANP dogs may predispose them to more lung injury.

R-7
SIMILARITIES IN LUNG REMODELING AS ASSESSED BY COMPUTED TOMOGRAPHY BETWEEN EXPERIMENTAL AND SPONTANEOUS FELINE ASTHMA. A. Banuelos1, I. Masseau1, C. Reingro2,1. Department of Veterinary Medicine and Surgery and, Comparative Internal Medicine Laboratory, College of Veterinary Medicine, University Missouri, Columbia, MO.

Airway remodeling (permanent architectural change) is a prominent feature of asthma. Computed tomography (CT) has appeal as a minimally-invasive diagnostic. The purpose of this study was to compare indices of airway remodeling between experimentally-induced and spontaneously asthmatic cats and healthy cats using CT. We hypothesized that experimental and spontaneous feline asthma would have similar remodeling changes assessed by CT and would be significantly different from healthy cats. Scans were performed using a 64-detector row CT scanner in awake and unrestrained (using a Vet Mouse Trap) experimentally asthmatic cats and healthy cats using CT. We hypothesized that experimental and spontaneous feline asthma would have similar remodeling changes assessed by CT and would be significantly different from healthy cats. Scans were performed using a 64-detector row CT scanner in awake and unrestrained (using a Vet Mouse Trap) experimentally asthmatic cats and healthy cats using CT.

R-8

Arterial blood gas (ABG) analysis is the cornerstone of evaluating respiratory disease severity. Ageing in people is associated with a decline in the arterial partial pressure of oxygen (PaO2) and an increase in the alveolar-arterial oxygen gradient (P(A-a)O2). Obesity and gender are other factors known to affect PaO2.
and P(A-a)O2. The primary objectives were to establish normal reference intervals for PaO2 in healthy, lean geriatric dogs, and to determine whether dogs experience a decrease in PaO2 and increase in P(A-a)O2 with ageing, similar to humans. We also investigated whether sex, obesity and age-related pulmonary changes seen on thoracic radiographs have any effect on PaO2 and P(A-a)O2 values in geriatric dogs.

Thirty-eight lean 1-4 years old control (C), 28 lean geriatric (LG) and 28 obese geriatric (OG) dogs were enrolled; groups were matched for sex and breed-size distribution. Dogs were excluded if they had evidence of respiratory or cardiac disease, systemic hypertension or pathologic arrhythmias. Thoracic radiographs were reviewed for age-related pulmonary changes using a standardized grading system by a single board-certified radiologist, blinded to clinical data. ABG samples were collected from the dorsal pedal (n = 47 dogs) or femoral artery (n = 47 dogs) while unsedated dogs were restrained in lateral recumbency, and analyzed on RapidLab® 1265 blood gas analyzer within 15 minutes of collection. P(A-a)O2 was calculated with a respiratory quotient of 0.9. Samples from LG group were used to determine reference intervals for geriatric dogs.

Obese geriatric dogs had a significantly (p = 0.0023) lower PaO2 (median 80.8 mmHg; minimum 62.8, maximum 95.6 mmHg) than LG (85.4 mmHg; 77.8, 94.2 mmHg), while PaO2 of LG dogs (85.6 mmHg; 66.7, 102.5 mmHg) did not differ significantly from C and OG groups. P(A-a)O2 differed significantly between all groups (p < 0.0001), and increased from C (17.6 mmHg; 10.3, 29.3 mmHg) to LG (19.6 mmHg; 12.5, 39.9 mmHg) and OG (25.9 mmHg; 11.7, 40.5 mmHg). There was a significant difference in P(A-a)O2 between LG (30.5 mmHg; 21.6, 39.3 mmHg) and C (31.8 mmHg; 26.1, 37.7 mmHg) (p = 0.0483), and between OG (29.9 mmHg; 25.7, 37.1 mmHg) and C (p = 0.003), but no significant difference between the LG and OG. No significant difference in PaO2, PaCO2 and P(A-a)O2 was detected between male and female dogs. Most age-related radiographic changes were not associated with PaO2 or P(A-a)O2. However, dogs with moderate intrathoracic fat deposition (IFD) had significantly lower PaO2 (p = 0.001), and those with mild and moderate IFD had significantly higher P(A-a)O2 (p < 0.001). Obese geriatric dogs had greater IFD than LG (p = 0.0017) and C (p < 0.0001) dogs.

Results suggest that obesity, resulting in IFD, together with ageing, is associated with a decrease in PaO2 and an increase in P(A-a)O2 in dogs.

E-2 CORRELATES BETWEEN POST-MORTEM AND ECHO-CARDIOGRAPHIC MEASUREMENTS OF THE RIGHT VENTRICLE IN HORSES. H.J. Howard, I.M. Bowen, G.D. Hallowell. School of Veterinary Medicine and Science, University of Nottingham, United Kingdom.

Reliable measures of right ventricle (RV) dimensions in horses is limited and those available have not been compared with right-sided dimensions obtained at post-mortem. Repeatability of published RV dimensions have been found to be acceptable but generally poorer than left ventricle parameters and reproducibility of measurements have not been reported in the horse. The objectives of this study were to determine the reliability and validity of RV parameters measured using echocardiography in horses and to compare with post-mortem measurements. Reliable measures were then applied to echocardiograms from horses with known right-sided enlargement. Seven adult Thoroughbred or cross horses were recruited and examined. Echocardiographic images were obtained from the right parasternal position using standard and modified 2-D and M-mode views. The parameters measured included RV diameter, height and area and moderator band length and width. Post-mortem measurements included RV height, volume and weight. Correlations between parameters measured using echocardiography and post-mortem were assessed using Pearson correlation coefficients. Data are displayed in Table 1. Moderate to excellent repeatability was found for all echocardiographic measurements (ICC=0.43) apart from RV area measured in systole. Reproducibility was found to be better for M-mode parameters (ICC=0.80-0.98) and excellent for moderator band length (ICC=0.92-0.94) but poor for other 2-D parameters. The measurements of RV diameter and moderator band length were found to be most reliable. Significant differences were found between normal horses and those with known right-sided enlargement using these parameters. As such, these measurements may be useful in the evaluation of RV enlargement in horses with clinical cardiac disease.

Table 1. Correlation between measurements taken using echocardiography and post-mortem.

| Echocardiographic measurement | Post-mortem measurement | Intra-class correlation coefficient | P value | R²  

| MB length | RV length | 0.55 | 0.33 | 0.31  

| MB length | RV length along FW | 0.98 | 0.05 | 0.99  

| MB length | RV length along IVS | 0.90 | 0.40 | 0.5  

| MB length | RV Volume | 0.60 | 0.29 | 0.35  

| RVIdM M-mode | RV FW Volume | 0.62 | 0.36 | 0.53  

| RVIdMs M-mode | RV FW Volume | 0.75 | 0.15 | 0.56  

| RVIdMs M-mode | RV FW Volume | 0.65 | 0.24 | 0.42  

MB: Moderator band, RVIdD: RV internal diameter, diastole, RVIdMs: RV internal diameter, systole, IVS : Interventricular septum, FW: Free wall.

E-3 EVALUATION OF ACID-BASE AND ELECTROLYTE DISTURBANCES USING A FENCIL-STEWART APPROACH AND CORRELATES TO SURVIVAL IN HORSES WITH COLIC. K.A. Berry, I.M. Bowen, G.D. Hallowell. School of Veterinary Medicine and Science, University of Nottingham, United Kingdom.

Horses with abdominal pain have severe acid-base and electrolyte derangements. The purpose of this study was to adapt the quantitative Fencel-Stewart (F-S) approach for specific use in horses, to evaluate F-S derangements seen in horses with colic at admission and over time and to evaluate how findings at admis-
tion compared with those identified using a traditional approach. F-S effects, blood gas and electrolyte parameters were compared between survivors and non-survivors.

Normal blood gas values were used to adapt the F-S approach for horses. Basic history, major body system assessment, PCV, total solids and venous blood gas and electrolytes were evaluated at admission and at 12 hour intervals from 85 horses admitted with colic. Horses were grouped based on the type of colic.

The following formulae were used to calculate cortisol for the horse: free water effect (3.[Na+]–138), chloride effect (100.+[Cl]–[138]/[Na+]1), albumin effect (0.37[2.5–Alb]) and lactate effect (1.[lactate]). It was not possible to predict the complex acid-base derangements in horses with colic, even within sub-groups (Table 1); albumin, lactate, chloride and unmeasured anion effects contributed to the metabolic acid-base derangements. With abnormal albumin concentrations, traditional and quantitative approaches yield different interpretations of the acid-base abnormalities. There were differences in free water, chloride, lactate and unmeasured anion effects and calcium concentrations between survivors and non-survivors in horses with surgical lesions.

In conclusion, the F-S approach can be successfully applied to the horse and may allow therapy to be tailored to individual cases. Assessment of complex metabolic acid-base derangements in the horse should not be underestimated.

Table 1. Results (mean ± SD) displaying the relative effects calculated using the F-S approach using admission data for subgroups of horses presenting with colic.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Medical (n=30)</th>
<th>Surgical (n=55)</th>
<th>Intravascular changes</th>
<th>Surgical L1 lesions (n=16)</th>
<th>Surgical L1 displacements</th>
<th>Strangulating SI lesions (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>1.2 ± 1.5</td>
<td>1.2 (2.4 ± 0.2)</td>
<td>0.04 (1.4 ± 0.6)</td>
<td>1.9 ± 1.3</td>
<td>0.04 (2.4 ± 0.3)</td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>-1.5 (2.1 ± 0.8)</td>
<td>1.1 (3.1 ± 0.9)</td>
<td>0.5 (3.8 ± 1.3)</td>
<td>-1.5 (2.1 ± 0.9)</td>
<td>-2.5 (1.5 ± 0.8)</td>
<td></td>
</tr>
<tr>
<td>Free water</td>
<td>0.8 ± 0.9</td>
<td>0.2 ± 0.6</td>
<td>0.07 ± 0.6</td>
<td>0.2 ± 1.0</td>
<td>0.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>-2.6 ± 3.1</td>
<td>-1.4 ± 3.1</td>
<td>1.1 ± 3.4</td>
<td>0.7 ± 3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>-4.1 ± 4.2</td>
<td>4.9 ± 5.8</td>
<td>-1.9 ± 7.9</td>
<td>-2.7 ± 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmeasured anions</td>
<td>2.7 ± 13</td>
<td>3.8 ± 13.5</td>
<td>-2.9 ± 2.9</td>
<td>-4.4 ± 2.3</td>
<td>-1.4 ± 4.9</td>
<td></td>
</tr>
</tbody>
</table>

E-5
CHARACTERIZATION OF INSULIN RECEPTOR AND INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR IN THE DIGITAL LAMINAR TISSUE OF ADULT HORSES AND MIXED-BREED PONIES. A. Kullmann1, J. Bishop1, T.M. Roux1, P.S.D. Weber1, T.A. Burns1, L.J. McCutcheon1, J.K. Belknap2, R.J. Geor3, 1Michigan State University College of Veterinary Medicine, East Lansing, MI, 2The Ohio State University College of Veterinary Medicine, Columbus, OH.

Hyperinsulinaemia is implicated in the pathogenesis of endocrinopathic laminitis. Insulin binds to different isoforms: the insulin receptor (InsR), which has two isoforms (InsR-A and InsR-B), insulin-like growth factor-1 receptor (IGF-1R), and InsR/IGF-1R hybrid receptor (HR). The objectives of this study were to (1) characterize InsR-A, InsR-B, IGF-1R and HR in laminar tissue (LT) of healthy adult horses and (2) examine the effect of the level of dietary nonstructural carbohydrate (NSC) on gene expression of these receptors in LT of lean and overweight/pbolse ponies. RNA and protein lysates were prepared from LT from 8 horses and evaluated by qRT-PCR at the mRNA expression level and immunoblotting at protein level. Archived LT from lean (n = 11, body condition score [BCS] 5.8 ± 1.4) and overweight/pbolse (n = 11, BCS 7.6 ± 0.6) ponies fed either a low (<6% NSC as dry matter [DM]; n = 5 obese, 5 lean) or a high NSC diet (>42% NSC as DM; n = 6 obese, 6 lean) for 7 days was used to evaluate receptor expression by qRT-PCR. Statistical significance was set at P < 0.05. Equine LT samples were found to contain InsR, IGF-1R and HR with the expression of isoform InsR-A greater (P < 0.05) than InsR-B, and relative IGF-1R expression greater (P < 0.05) than either InsR isomor. In ponies, the LT mRNA expression of InsR-A, InsR-B and IGF-1R was not different between diets. The functional characteristics of these receptors and their role in endocrinopathic laminitis warrants further investigation.

E-4
GLUCOCORTICOID RECEPTOR DENSITY AND BINDING AFFINITY DETERMINATION IN EQUINE PERIPHERAL BLOOD MONONUCLEAR CELLS USING FLOW CYTOMETRY. C.J. Hoffman1, H.C. McKenzie IH, M.O. Furr1, A. Desrochers1, R.M. DuPont Scott Equine Medical Center, Virginia/Maryland Regional College of Veterinary Medicine, Leesburg, VA, 2Department of Large Animal Clinical Sciences, Virginia/Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

The purpose of this study was to determine glucocorticoid receptor (GR) density and binding affinity in peripheral blood mononuclear cells (PBMC’s) in the equine patient using flow cytometry. Eight healthy adult horses were used. An intravenous jugular catheter was aseptically placed, and 0.5 µg/kg of cosyntropin (synthetic ACTH) was administered to stimulate cortisol release. Peripheral blood was obtained from the catheter at baseline, and 4, 8 and 24 hours post-injection. Serum cortisol concentration was measured by an external laboratory. PBMC’s were isolated using Ficoll gradient centrifugation. Cells were labeled with PE-CD44 (a panleukocyte marker) and either purified mouse anti-glucocorticoid receptor with a FITC-labeled secondary antibody or FITC-dexamethasone to determine GR density and binding affinity via flow cytometry.

Peak cortisol concentration occurred 4 hours following cosyntropin administration (mean 5.9 ± 0.8 µg/dl). Mean GR density ranged from 16.16 to 31.63% of labeled PBMC’s, and mean GR binding affinity ranged from 33.49 to 51.12% over the 24-hour period. These values were poorly correlated with changes in cortisol concentrations (r=0.11) and r=0.028 for GR density and binding affinity respectively.

GR density and binding affinity can be measured in equine PBMC’s. While cortisol concentrations were poorly correlated with GR density and binding affinity in these normal horses, there is evidence to suggest that dysregulation of the cellular response to cortisol in horses with critical illness-related corticosteroid insufficiency (CIRCI) occurs at the level of the GR. This technique can be used in future studies to examine the GR in horses with CIRCI.

E-6
PRE-ANALYTICAL STABILITY OF ADRENOCORTICOTROPIC HORMONE IN BLOOD FROM HORSES WITH AND WITHOUT PITUITARY PARS INTERMEDIA DYSFUNCTION. J. Prutton, P. Kass, J. Watson, N. Pusterla. School of Veterinary Medicine, University of California, Davis.

Equine pituitary pars intermedia dysfunction (PPID) is a disease of aged horses that is most frequently diagnosed using endogenous adrenocorticotropic hormone (ACTH) in blood alongside clinical signs. The stability of equine ACTH in collected blood samples has not been fully elucidated. The goal of this study was to address this and to demonstrate which, if any, of the following storage conditions would affect ACTH recovery: time, temperature or storage as whole blood or plasma.

Equine horses were blood sampled and ACTH concentrations were assessed using a chemiluminescence assay after storage at 4°C, 21°C, -20°C and -80°C for up to 30 days either as whole blood or plasma. Concentrations of ACTH were similar between whole blood and plasma. Time affected ACTH concentrations with storage between 24-48 hours and up to 72 hours reducing ACTH recovery. Freezing at both -20°C and -80°C did not deacrete ACTH concentration for at least 30 days. ACTH measurements in the equine patient were subject to degradation but appreciable changes were only seen at 48 hours and longer in samples stored at 21°C or 4°C. Storage as whole blood or plasma had no appreciable effect.
This information allows practitioners to store samples without centrifugation for at least 24 hours and freeze plasma samples at -20°C for 30 days with fewer concerns about ACTH depression.

E-7

There are several methods currently employed to determine insulin resistance status in the horse. Whilst the frequently sampled intravenous glucose tolerance test and the euglycaemic-hyperinsulinaemic clamp methods are considered to be the gold standard techniques, they are not practical in the field situation. Instead the oral glucose test and the oral sugar test have been advocated; however the two tests have not been directly compared previously. The purpose of the study was to compare the insulin resistance status in the horse. Whilst the frequently sampled glucose test (OGT) and the oral sugar test (OST) in eight ponies of unknown insulin sensitivity.

Animals were fasted overnight for eight hours before and throughout testing. At 8am animals were either fed a handful of grass with 1 g/kg glucose powder (OGT) or given 0.15 ml/kg Karo in a sugar solution (OST) in a randomised crossover design study with 48 hours between treatments. Blood samples were obtained at 0, 30, 60, 75, 90, 120 and 180 minutes. Insulin concentrations were measured using a previously validated radioimmunoassay. Maximum insulin concentration (Cmax), time to maximum insulin concentration (Tmax) and area under the curve of insulin concentration over time (AUC) for the two tests were compared using a paired t-test. P < 0.05 was considered significant.

OGT Cmax, (221 ± 98 μU/l), Tmax, (142 ± 53 minutes) and AUC (21397 ± 7489) were significantly (p < 0.05) greater compared to OST Cmax, (93 ± 62 μU/l), Tmax, (60 ± 10 minutes) and AUC (7153 ± 4926). When ranked by AUC, the animals were ranked in the same order for the OGT and the OST. Using previously defined (but not validated) criteria of hyperinsulinaemia the OGT identified 7/8 animals as hyperinsulinemic whereas the OST identified 5/8 animals as hyperinsulinemic.

In conclusion the OGT and the OST show agreement in the identification of insulin resistant ponies in 75% of subjects.

E-8
ACTH RELEASE FOLLOWING TRH STIMULATION IN THRIFTY HORSES COMPARED TO METABOLICALLY NORMAL HORSES. J. Fredrick1, D. McFarlane2, F. Yang1. 1Center for Veterinary Care, Millbrook, NY, 2Oklahoma State University.

Horses with a thrifty phenotype are at risk for obesity, hyperinsulinemia, and laminitis; a collection of clinical signs known as equine metabolic syndrome (EMS). It is suspected that horses with EMS are at greater risk for developing PPID, although the exact mechanism by which EMS may lead to PPID is of yet unexplored. We hypothesized that genetically thrifty horses have a decreased pituitary and adrenal response to the stress hormone ACTH.

Seven horses were selected: four thrifty and three normal with a mean age of 11.25 yrs. Six had a history of laminitis. The normal group was comprised of thoroughbred horses (n = 9) with a median BCS of 5, mean age of 10.4 yrs, and no history of laminitis or normal hair coats. Oral sugar testing revealed a greater insulin response in the thrifty group (mean serum insulin @ 75 mins: 70.9 ± 21.9 μU/ml vs 37.2 ± 17 μU/ml, P < 0.01), as well as a difference in baseline insulin (34.2 ± 3.6 μU/ml vs 17 ± 4.3 μU/ml, P < 0.01). TRH stimulation testing was performed in July and October, with ACTH measured at 0, 10 and 30 minutes after TRH. One thrifty horse had an ACTH markedly above the reference range on all 6 samples; results from this animal were excluded from analyses. ACTH response was analyzed using two way repeated measures ANOVA with animal as the subject, and breed and time as factors. When compared to normal horses, thrifty horses had greater ACTH release following TRH stimulation when tested in October (P = 0.03) but not in July (P = 0.09). Thrifty horses may have a greater PI response to stimulation, particularly in the fall. Further studies are needed to determine if chronic hyperactivity of the PI leads to PPID.

E-9
PHARMACOKINETICS AND PHARMACODYNAMICS OF PERGOLIDE MESYLATE AFTER CHRONIC ORAL ADMINISTRATION IN HORSES WITH PPID. D. McFarlane, H.E. Banse, F. Yang, L.K. Maxwell. Oklahoma State University, Center for Veterinary Health Sciences, Stillwater, OK.

Equine pituitary pars intermedia dysfunction (PPID) is a common, debilitating condition of aged horses. Pergolide mesylate is the drug of choice for PPID. Previous pharmacologic research has focused on the kinetic properties of pergolide following a single oral dose to young, healthy horses. However in clinical practice, pergolide is chronically administered to aged horses with PPID. This study investigated the pharmacokinetic and pharmacodynamic properties of pergolide mesylate in horses with PPID after 6 months of oral administration. Six horses with confirmed PPID were administered pergolide from July through January at 0.002 μg/kg po q24 hr for 2 months, followed by 0.004 μg/kg po q24 hr for an additional 4 months. Pergolide concentrations were assessed using liquid chromatography/mass spectrometry and preliminary pharmacokinetic analysis was performed by noncompartmental analysis on samples drawn over 4 weeks after the last dose of pergolide was administered. Plasma ACTH concentration was determined by chemiluminescent immunoassay. Pergolide was rapidly absorbed (Tmax=0.5 ± 0.3 h) with a Cmax of 0.6 ± 0.8 ng/ml, and a terminal elimination half-life of 24 ± 10 h. Pergolide was detectable in the serum for a maximum of 14 days (range: 5-14 d). Plasma ACTH concentration remained unchanged for 6 days compared to ACTH concentration on the last day of treatment. Plasma pergolide concentration and ACTH suppressing effects persist for several days after cessation of long-term pergolide administration in horses with PPID.

E-10
SUSPECTED ACORN TOXICITY IN NINE HORSES. S. Smith1, R.J. Naylor1, E. Knowles1, T. Mair1, B. Dunkel1. 1The Royal Veterinary College, London, UK., 2Bell Equine Veterinary Hospital, Maidstone, UK.

Acorn toxicity is anecdotally reported to cause potentially fatal colitis and colic in horses; reports in the scientific literature are sparse. This study describes diagnosis, treatment, and clinical outcome of nine cases of gastrointestinal disease attributed to acorn toxicity seen at two UK referral hospitals. Case records were reviewed from 2004-13 and cases that met three of the following four criteria were included: acorn exposure, clinical and laboratory data suggesting alimentary or renal dysfunction, acorn husks in feces and necropsy findings consistent with acorn toxicity. Compatible necropsy findings were gastrointestinal and mesenteric edema, ulcerative enterocolitis, acorn husks within the gastrointestinal lumen and nephrosis. Where possible bacterial infection, clostridial toxicosis, parasitic damage and displacement of the colon causing vascular compromise were excluded. Data collected included signalment, presentation, clinicopathological data, ultrasonographic findings, progression, and necropsy findings.

Nine horses met the inclusion criteria, four in 2011 and five in 2013. Five cases presented with hemorrhagic diarrhea and an additional four cases showed predominant signs of colonic non-hemorrhagic diarrhea. Most cases demonstrated marked hypovolemia (mean packed cell volume 71.1 ± 12.4%, median
EFFECTS OF METRONIDAZOLE AND FLUNIXIN MEGLUMINE ON THE MUCOSAL BIOLICAL TISSUE OF THE RIGHT DORSAL COLONIC MUCOSA.

Metronidazole (MZ) and flunixin meglumine (FM) are commonly used drugs for treating equine colitis. While the potential of FM to contribute to the pathophysiology of colitis has been known for a while (Simmons 1990), effects of MZ on the intestinal mucosa are controversial (Bjarnason 1992, Wlodarska 2011), and have not been studied in horses yet. The aim of this study was to investigate the effect of FM, MZ and a combination of these drugs on the equine colonic mucosa.

Equine right dorsal colonic tissue of 6 horses was studied in Ussing chambers. Tissue was treated with therapeutic dosages of MZ, FM, combined MZ and FM, or no medication (negative control). Transepithelial resistance (TER) as an indicator of tissue permeability, and direct measurement of mucosal to serosal fluxes with fluorescein labeled LPS were recorded. Further, histological tissue evaluation was performed.

Results showed a decrease in TER after applying FM to the mucosal side of the colon tissue, indicating increased tissue permeability. Application of a combination of MZ and FM lead to milder decrease in TER, and application of just MZ did not change TER, similar to the negative control. Histology revealed thinning of the mucous layer and mucosal ulceration with FM application.

Results of this study indicate that MZ does not increase tissue permeability of the right dorsal colonic mucosa in horses and may have a protective effect. Increased tissue permeability due to FM application can be decreased with a combined treatment with MZ suggesting a benefit to using FM alone.


Mitigating nosocomial outbreaks in veterinary teaching hospitals is important as these occurrences are economically devastating and disruptive to the normal operations of these hospitals. The purpose of this study was to compare the efficacy of two disinfectant solutions [5.8% accelerated hydrogen peroxide (AHP) and single and double applications of 2% peroxygen] for decontamination of a veterinary hospital environment. We hypothesized that mist applications of these solutions have similar efficacies for reducing bacterial contamination.

Transparencies (n = 78) were inoculated with known concentrations of Staphylococcus aureus, Salmonella enterica and Pseudomonas aeruginosa (26 transparencies per organism). Five transparencies each served as positive and negative controls. After cleaning and disinfection of the hospital environment, all surfaces were allowed to dry overnight and transparencies were then fastened on vertical surfaces in 25 locations, chosen at random. One at a time, each disinfectant was applied, a contact time of 30-minutes was observed, transparencies were collected, individually placed into 25 mL Dey-Engley broth, and transported to the laboratory for processing. Six ten-fold dilutions of each sample were plated onto tryptic soy blood agar for bacterial enumeration. Bacterial counts from the control transparencies were compared to results from transparencies exposed to disinfectant to quantify the percent bacterial reduction. Through regression analysis, a significant reduction was observed when applying a 2% peroxygen once and twice for all organisms evaluated; there was no significant reduction in colony count detected when using the AHP product. This study indicates that for the organisms evaluated, a 2% virkon solution effectively reduced the colony counts in a veterinary hospital environment.
E-14 EXPERIMENTAL TRANSMISSION OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS IN HORSES BY HOUSE FLIES. M. Barba1, A.J. Stewart1, T. Passler1, X.P. Hu2, M. Chmielorski1, K. Cuttle1, T. Hathcock1, J.A. Houghton1, A.A. Woolridge1, 1College of Veterinary Medicine, Auburn University, Auburn AL, 2College of Agriculture, Auburn University, Auburn, AL., USDA/ARS/CMAVE, Gainesville, FL.

The route of infection of pigeon fever remains undetermined. The purpose of this study was to investigate house flies (Musca domestica L.) as vectors of Corynebacterium pseudotuberculosis in horses.

Eight ponies were used in a randomized, controlled, blinded experimental study. Ten wounds were created in the pectoral region where cages for flies were attached. Three ponies were directly inoculated with C. pseudotuberculosis. Four ponies were exposed for 24 hours to 20 C. pseudotuberculosis-inoculated flies. One negative control pony was exposed to non-inoculated flies. Ponies were examined daily for swelling, heat, pain and drainage from the inoculation site. Blood was collected weekly for complete blood cell count and fibrinogen analysis. Serum was collected twice weekly for synergistic hemolysis inhibition titers.

Data were analyzed using linear regression or repeated measures ANOVA analysis. Significance was assumed at P < 0.05.

Clinical signs of local infection and positive cultures were observed in 7/7 ponies exposed to C. pseudotuberculosis and were absent in negative control. In exposed ponies, peak serologic titers (1:512 to 1:2048) were obtained between days 17 and 21. There was no difference in the linear increase in titer between the exposed groups. The titer increase was greater in both exposed groups compared to the negative control group (P = 0.0002). Fibrinogen concentrations were significantly greater on day 7 and 21, and neutrophil counts were significantly greater on day 3 in both exposed groups compared to the negative control.

House flies are confirmed as mechanical vectors of C. pseudotuberculosis and can transmit the bacteria to naive ponies.


Theileria equi is a tick-borne apicomplexan parasite and an agent of equine piroplasmosis. Although the United States was declared free of this disease in 1988, multiple recent U.S. outbreaks emphasize the ineffectiveness of current control programs and need for efficacious chemotherapeutic strategies for parasite clearance. Evolving drug resistance is commonly observed in many apicomplexans, including the human malarial agent Plasmodium falciparum, in which specific proteins of the ATP-binding cassette (ABC) transporter family have been identified as contributors to resistance. In T. equi, the recently annotated genome has revealed 45 ABC transporter family members, more than any many apicomplexans, including the human malarial Plasmodium falciparum, in which specific proteins of the ATP-binding cassette (ABC) transporter family have been identified as contributors to resistance.

In conclusion, adverse reactions occurred in approximately one-third of the horses that underwent general anesthesia and myelography. The types of adverse reactions varied and ranged in severity from minor problems to conditions requiring euthanasia. The adverse reactions that were attributed to the contrast material itself (idiosyncratic and nonidiosyncratic) were mostly minor and self-limiting. Increased frequency of adverse reactions due to any cause was associated with increased contrast volume and anesthesia time.

E-16 ACUTE AND DELAYED ADVERSE REACTIONS IN HORSES THAT UNDERWENT GENERAL ANESTHESIA AND CERVICAL MYELOGRAPHY. K.R. Mullen1, C. Furgess2, A.L. Johnson3, T.E. Norman4, K.A. Hart5, A.J. Burton6, R.C. Bicahi7, D.M. Ainsworth8, M. Thompson9, P.V. Sretnik10, 1Cornell University College of Veterinary Medicine, Ithaca, NY., 2Ontario Veterinary College, Guelph University, Guelph, ON., 3University of Pennsylvania College of Veterinary Medicine, Kennett Square, PA., 4Texas A&M College of Veterinary Medicine, College Station, TX., 5University of Georgia College of Veterinary Medicine, Athens, GA.

A perceived increased frequency of myelographic complications prompted this multi-institutional, retrospective study. The sample population consisted of horses (n = 278) which underwent myelography at Cornell University (87), Ontario Veterinary College (68), University of Pennsylvania (65), Texas A&M (46) and University of Georgia (12) between 2000-2012. Multivariate analysis was performed with significance set at 5%. An adverse reaction was observed in 95/278 (34%) of horses and was significantly associated with general anesthesia time and contrast volume (P = 0.041, 0.042, respectively). Acute idiosyncratic adverse reactions exemplified by hypotension following injection of contrast were observed in 11/246 (4%) of horses and were significantly associated with general anesthesia time and age (P = 0.027, 0.034, respectively). Delayed nonidiosyncratic adverse reactions exemplified by hyperthermia post-myelography were observed in 25/278 (9%) of horses and were not associated with duration of anesthesia, volume of contrast or neurologic grade. The neurologic grade remained unchanged post-myelography in 72/102 (71%) of cases, but increased one or two grades in 25/102 (25%) of cases.

E-17 CENTRAL NERVOUS SYSTEM TRANSCRIPTOME PROFILING IN EQUINE NEUROAXONAL DYSTROPHY. C.J. Finno1, S.J. Valberg1, A. Armien1, J. Mickelson1, 1University of Minnesota College of Veterinary Medicine, St. Paul, MN.

Equine neuroaxonal dystrophy (NAD) is an inherited neurodegenerative disease affecting young horses between 6-12 months of age. Definitive diagnosis requires a full neurologic examination and histologic evaluation of the brainstem and spinal cord upon necropsy. Dystrophic axons, which are visualized in the lateral accessory cuneate nucleus of the brainstem are observed in NAD; however, the cell body for this neuron is thought to originate in the spinal sensory ganglion and the axon traverses through the spinal cord to synapse in the brainstem. To assess regional differences in gene expression as it relates to NAD, total RNA was prepared from the brainstem (obex) and cervical spinal cord (C1; directly caudal to the brainstem region) of four affected NAD yearlings and four age-matched control horses. Ribosomal RNA was removed and a strand-specific library was created (Illumina TruSeq). RNA-Seq was performed on rRNA-depleted RNA.
using an Illumina HiSeq at a targeted 20 million reads/horse. Quality control measures were implemented and sequences aligned to the genome (~90% mapped). Transcript abundance was calculated as fragments per kilobase per million mapped reads (FPKM) and compared using Cuffdiff. Principle component plots revealed primary segregation (PC1) of samples based on anatomic region, but not disease phenotype. Within the brainstem, individuals did not segregate based on phenotype, whereas segregation by phenotype was observed in the spinal cord (PC2). Within the spinal cord, differential expression (>2 fold) was observed for 341 genes. This study highlights the importance of tissue selection and standardization for RNA-Seq experiments within the central nervous system.

E-19 A HIGH GLYCEMIC DIET ALTERS ADIPOSE TISSUE GENE EXPRESSION OF ADIPOKINES AND PRO-INFLAMMATORY MARKERS IN LEAN AND OBESE PONIES.

S.J. Valberg 1, K.L. Hepworth 1, D.E. Gomez 1, N.M. Biermann 1, L.C. Lewis 1, D. Wilhite 3, C.J. Finno 1, R. Reardon 4, P.S.D. Weber 1, K.N. Schermerhorn 1, L.J. McCutcheon 1, B. Norby 1, T.A. Burns 1, J.K. Belknap 1, R.J. Geor 1, 1University of Minnesota, College of Veterinary Medicine, St. Paul, Minnesota, 2Oregon State University, College of Veterinary Medicine, Corvallis, OR, 3Wilhite and Frees Equine Hospital, Peculiar MO, 4West End Veterinary Services, Delano MN.

Obesity is associated with chronic low-grade inflammation in human subjects and this inflammatory state is exacerbated by high glycemic diets. It is not known if a similar inflammatory state occurs in overweight/obese equids, nor whether diet affects inflammatory signaling in adipose tissue (AT). This study investigated the effect of a high glycemic diet on AT expression of adipokines, chemokines and cytokines in lean (body condition score [BCS] 5.8 ± 1.4) and overweight/obese (BCS 7.6 ± 0.6) ponies. Twenty-two ponies were fed either a low glycemic diet (low nonstructural carbohydrate [NSC]: -6% of dry matter [DM]; n = 5 lean, 5 overweight/obese) or high glycemic diet (high-NSC: -42% of DM; n = 6 lean, 6 overweight/obese) for 7 days. In AT samples collected from five depots (nuchal, tailhead, mesenteric, omental and retroperitoneal), total RNA was extracted, transcribed into cDNA and analyzed by RT-qPCR to quantify gene expression of macrophage activation marker (CD68), monocyte chemotactic protein-1 (MCP-1), interleukin-8 (IL-8), plasminogen activator inhibitor-1 (PAI-1), adiponectin, leptin and dipetidyl peptidase-4 (DPP4). Data were analyzed using linear mixed model approach and significance was set at P ≤ 0.05. No significant body condition by diet interactions were observed, indicating that these factors may influence AT inflammation independently. Expression of CD68 expression was not different between diets but was significantly higher in AT from all depots except nuchal in overweight/obese when compared to lean ponies. In high-NSC, AT expression of MCP-1 and leptin was significantly higher and expression of adiponectin and DPP4 was significantly lower while IL-8 and PAI-1 expression was significantly higher in a depot-specific manner. Diets that elicit a high glycemic response may alter AT inflammatory status, contributing to AT dysfunction in lean and overweight/obese equids.

E-20 COMPARISON OF ANION GAP AND STRONG ION GAP AS PREDICTORS OF PLASMA L-LACTATE CONCENTRATION AND THEIR ASSOCIATION WITH OUTCOME IN 81 HOSPITALIZED FOALS.

D.E. Gomez 2, N.M. Bierrmann 1, L.C. Sanchez 2, 1University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, 2College of Veterinary Medicine, University of Florida, Gainesville, FL, USA.

Plasma anion gap (AG) and strong ion gap (SIG) are used to assess unmeasured anions. The study objectives were to retrospectively assess associations between AG/SIG and severe hyperlactatemia ([L-lac] > 7 mmol/L) and between [L-lac], AG and SIG and non-survival in hospitalized foals.

Eighty one foals ≤ 7 days presented to UF from 2004-2008 were included. Admission arterial blood gas and plasma biochemical profiles were used to calculate SIG [A- - AG; A- = 0.22 x (TP g/L) / (1 + 10(6.65-pH))] and AG = [Na+ + K+ - (Cl- + HCO3-)]. Linear (AG and [L-lac]) and logistic ([L-lac], AG, SIG and non-survival) regression analyses were used to evaluate associations. ROC curve analysis was used to identify optimal AG and SIG cut points to predict severe hyperlactatemia.

AG and SIG were significantly associated with plasma [L-lac] (P < 0.0001). The ROC AUC of AG and SIG for prediction of severe hyperlactatemia were 0.89 (95% CI, 0.83-0.95; P < 0.0001) and 0.9 (95% CI, 0.81-0.96; P < 0.0001), respectively. Severe hyperlactatemia was best predicted by AG > 27 mmol/L (sensitivity 80%, 95% CI, 56-94, specificity 85%, 95% CI, 73-93; P < 0.0001) and SIG < -15 mmol/L (sensitivity 90%, 95% CI, 68-98; specificity 80%; 95% CI, 68-90; P < 0.0001). Foals with [L-lac] > 7 mmol/L (OR=6.1, 95% CI, 1.8-21; P = 0.004) and SIG < -15 mmol/L (OR=4.2, CI, 1.6-14.2; P = 0.02) had significantly increased odds of non-survival.

AG and SIG predicted clinically significant hyperlactatemia. Increased [L-lac] and SIG were significantly associated with non-survival. These findings emphasize the importance of calculating SIG, especially if [L-lac] is unavailable.
povolemic shock. Controversy still remains as to whether colloidal or crystalloidal solutions are more effective in resuscitation of hypovolemic foals. The objectives of this study were to evaluate the safety of a novel hydroxethyl starch product (HES, VetStarch) in healthy neonatal foals, and to compare the effects of a colloid on colloid osmotic pressure (COP) to that of a crystalloid administered as a bolus.

Healthy neonatal foals (n = 7, study ongoing) between 3 and 5 days of age were randomly assigned to either the control group (20 ml/kg IV Plasmalyte) or the treatment group (20 ml/kg IV HES). Vital parameters, COP, serum electrolytes, coagulation parameters, urinalysis, glucose, and lactate were measured through the study period.

No adverse effects were noted in any foal throughout the study. Initial data suggest that colloids exerted a beneficial effect on COP, with the HES group having a mean increase in COP of +2.66 mmHg at 1 hour. Mean COP remained above baseline until 120 hours in the HES group. Foals in the control group exhibited a mean decrease in COP of -1.65 mmHg at 1 hour. Study is ongoing and will be completed by the final abstract deadline.

Preliminary results indicate that administration of a novel HES product is safe in healthy neonatal foals. Increased COP was noted after a bolus of HES, whereas a decrease occurred following an equivalent dose of a crystalloidal solution.

E-22 PHARMACOKINETICS OF KETOROLAC TROMETHAMINE, A POTENT NON-STEROIDAL ANTI-INFLAMMATORY DRUG, IN HEALTHY ADULT HORSES. A.W. Bianco1, P.D. Constable1, B.R. Cooper2, S.D. Taylor1. 1Purdue University College of Veterinary Medicine, West Lafayette, IN., 2Purdue University Bindley Bioscience Center, West Lafayette, IN.

Non-steroidal anti-inflammatory drugs are an integral component of equine medicine given their analgesic and anti-inflammatory properties. However, the currently available NSAIDs are limited in their ability to provide sufficient analgesia, resulting in prolonged NSAID and supplemental opiate use, practices with well-established adverse effects.

Ketorolac tromethamine (KT) is a non-specific COX inhibitor that is widely used in human medicine as a morphine-sparing analgesic. KT administered orally or parenterally has a prolonged half-life and has equivalent risk of adverse effects as other non-selective COX inhibitors, but with increased analgesic potency. The pharmacokinetic profile of KT has been evaluated in dogs, sheep, goats, and calves, but data on KT in horses is lacking. The purpose of this study was to evaluate the pharmacokinetic profile of KT in the healthy adult horse after intravenous (IV), intramuscular (IM), and oral (PO) administration.

Nine healthy horses received a single 0.5 mg/kg dose of KT via each route of administration in a randomized crossover design with a 2-week washout period between each trial. Plasma was collected periodically up to 72 hours post-administration and analyzed for KT concentration using high performance liquid chromatography and tandem mass spectrometry.

No adverse effects were observed in any of the horses. Pharmacokinetic analysis indicated a 2-compartment model for IV administration with mean (+/− s.d.) kinetic parameters as follows: plasma clearance (CLp) = 17.5 (9.2) mL/kg/min, apparent volume of distribution = 0.13 (0.07) L/kg, and elimination half-life (t1/2) = 29 (14) min−1. Pharmacokinetic analysis indicated IM and PO administration were best described using a noncompartmental model. Mean (+/− s.d.) maximum concentration (Cmax) after IM and PO administration, respectively, was 0.58 (0.39) and 0.31 (0.20) μg/mL. Median (range) time to reach maximum concentration (tmax) after IM and PO administration, respectively, was 20.0 (10-60) and 15.0 (10-31) minutes. Bioavailability (F) for both IM and PO administration appeared to be 100%.

The results of this study indicate that a single dose 0.5 mg/kg of KT in healthy adult horses results in rapid achievement of peak plasma concentration, is highly bioavailable, and causes no obvious adverse effects. More studies are needed to evaluate KT’s analgesic and anti-inflammatory properties.

E-23 PHARMACOKINETICS OF A SUB-THERAPEUTIC AND THERAPEUTIC DOSE OF DICLAZURIL ADMINISTERED ORALLY AS A PELLETED TOP DRESS IN ADULT HORSES. L.M. Hunyadi1, M. Papich2, N. Pusterla3. 1William R. Pritchard Veterinary Medical Teaching Hospital, UC Davis School of Veterinary Medicine, Davis, CA., 2College of Veterinary Medicine, North Carolina State University, Raleigh, NC., 3Department of Medicine and Epidemiology, UC Davis School of Veterinary Medicine, Davis, CA.

The purpose of this study was to determine the pharmacokinetics and safety profile of a sub-therapeutic and therapeutic dose of diclazuril in adult horses. During each research period, six healthy adult horses received 0.5 mg/kg of 1.56% diclazuril pellets (Protazil™). Merck Animal Health) as a sub-therapeutic dose or 1 mg/kg as a therapeutic dose orally once in two separate phases. Blood samples were collected at regular intervals for 168 hours. Multiple daily oral doses were then administered for 10 days. CSF was collected once. After the 10th oral dose, plasma samples were collected at regular intervals for 168 hours. Plasma and CSF samples were analyzed by high-pressure liquid chromatography. A one-compartment pharmacokinetic model with first-order oral absorption was fitted to the single administration data. Steady-state pharmacokinetics was performed using non-compartmental analysis for steady-state analysis. The mean concentration of diclazuril in CSF following the sub-therapeutic dose was 0.026 μg/mL (stdev 0.005 μg/mL) while CSF in the therapeutic dose was 0.023 μg/mL (stdev 0.004 μg/mL). CSF concentrations were only 5-6% of corresponding plasma concentrations for both the sub-therapeutic and therapeutic doses. Substantial accumulation in plasma occurred at steady-state after the 10th dose for both doses. The results of this study show that diclazuril pellets given at both a sub-therapeutic and therapeutic dose reach plasma and CSF levels known to inhibit Sarcocystis neurona in cell culture after steady-state levels have been reached.

E-24 PHARMACOKINETICS OF ORAL CHLORAMPHENICOL BASE IN ADULT HORSES AT 50 MG/KG DOSAGE. S. Cox, C. Sommardahl, E. McElligott. University of Tennessee College of Veterinary Medicine, Knoxville TN.

The purpose of this study was to determine the pharmacokinetics of chloramphenicol after intravenous and oral administration in adult horses. Chloramphenicol is a broad-spectrum antibiotic that is typically administered orally in horses; however there are very few studies that have looked at its pharmacokinetics in adult horses. It is unique in that it is one of the few antibiotics that can be administered by mouth to horses without serious side effects to the equine gastrointestinal tract. Chloramphenicol has significant human health concerns and is illegal in food animal species which horses may be considered in certain countries. Therefore, determining the pharmacokinetics of chloramphenicol as it is routinely administered in horses will provide more information for making appropriate antibiotic therapeutic decisions. In this study, five adult non-fasted horses were administered 50 mg/kg of chloramphenicol base via 60 cc catheter tip syringe and blood samples were collected at 0, 5, 10, 15, 30 min, 1, 2, 4, 8 and 12 hours. After at least a 2-5 day washout period, three adult non-fasted horses were given chloramphenicol Na succinate at 25 mg/kg IV and blood samples were collected at 0, 3, 5, 10, 15, 30, 45, 60 min, 2, 4, and 8 hours. Samples were analyzed using a validated high performance liquid chromatographic method. No adverse effects were observed for either oral or intravenous administration. The preliminary data revealed that chloramphenicol half-life, volume of distribution at steady state, clearance and AUC after intravenous administration ranged from 0.65-1.2 h, 507- 782 mL/kg, 779-1217 mL/h/kg, and 20.5-32.1 h·μg/mL respectively. The half-life, T1/2, Cmax, and AUC after oral administration ranged from 1.7 – 7.4 h, 0.25 – 2 h, 0.81 – 5.45 μg/mL, and 10.3 – 21.6 h·μg/mL. The minimum inhibitory concentrations for organisms susceptible to chloramphenicol are ≤ 8 μg/mL. Administration of chloramphenicol resulted in concentrations greater than 2 μg/mL for 45 minutes and greater than 0.5 μg/mL for 12 hours.

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E-25
INFLUENCE OF SHORT TRANSPORTATION ON TRACHEAL ASPIRATES AND BRONCHOALVEOLAR LAVAGES IN HORSES. M. Allano, O. Labrecque, E.R. Batista, C. Bédard, J.P. Lavoie, M. Lessire. Faculty of veterinary medicine, Université de Montréal, St-Hyacinthe, QC.

The purpose of this study is to evaluate the effect of a short transportation (2.5 hours) on tracheal aspirates and bronchoalveolar lavages (BAL) in order to identify potential bias in the diagnosis of inflammatory airway disease in referral centers.

Eight healthy adult horses were studied using a prospective crossover design. Mucous scores, tracheal aspirates (cytology, bacterial culture) and BAL (cytology) were obtained under three conditions with a 10-day interval between each sampling: 1- Without prior transportation, 2- Following transportation without hay and 3- Following transportation with hay. Data were analyzed with Friedman Tests and Spearman correlations.

There were no significant effects of transportation, with or without hay, on tracheal mucous scores, tracheal bacterial counts, total BAL cell counts, or mast cell and eosinophil percentages. However, five BAL had more than 5% neutrophils and surprisingly, neutrophil counts and percentages were significantly increased only when horses were transported without hay. The four horses with the highest neutrophils (7 to 13%) were transported on the two coldest days of the study and there was a strong correlation between ambient temperature and mean neutrophil percentages ($r > 0.9$, $p < 0.02$). Potential respiratory pathogens were isolated after transportation, but only once above 10^5 colony forming units/ml.

Transportation of short duration had no significant effect on tracheal mucous scores and bacterial count but caution should be taken in the interpretation of BAL cytology or the presence of potential pathogens in small amounts. Cold ambient temperature during transportation may induce bronchoalveolar neutrophilia.

E-26
MACROLIDE INDUCED HYPERTERMIA IN FOALS: ROLE OF IMPAIRED SWEAT RESPONSES. A.L. Steier, L.C. Sanchez, M.F. Mallicote, B.B. Martabano, R.J. McKay. University of Florida College of Veterinary Medicine, Gainesville, FL.

Rhodococcus equi is an important cause of pneumonia in foals. Treatment of choice has remained the combination of a macrolide (e.g., erythromycin) with rifampin. Side effects of erythromycin include diarrhea, which is often self-limiting, and hyperthermia, which can be fatal. The mechanism of hyperthermia is unknown. This study evaluated the sweat response in foals treated with erythromycin to test the hypothesis that hyperthermia was due to impaired sweating. Ten pony-cross foals (4 fillies, 6 colts) aged 2-3 months of age were administered erythromycin base (25 mg/kg orally, three times daily) or placebo (lactose powder) for ten days in a randomized, crossover design. Vital signs were recorded at each treatment time for 10 days and twice daily for 10 days after treatment. Quantitative intradermal terbutaline sweat tests (QITST) were performed by blinded investigators on days 1 (start), 3, 10 and 20. Terbutaline-induced sweating was significantly ($P < 0.05$) reduced from baseline in erythromycin-treated foals at all post-baseline time points. After day 1, erythromycin treated foals produced less sweat at all time points compared to placebo treated foals. Ten days after discontinuation of erythromycin treatment (day 20), mean sweat response was less than 50% of baseline. When allowed paddock turnout on hot, humid days foals frequently were tachypneic with rectal temperatures from 103-105°F for 3-5 days after erythromycin therapy ceased. This study provides an explanation for hyperthermia in erythromycin treated foals and identifies a risk period that extends several weeks beyond discontinuation of treatment.

E-27
BLOOD PRESSURE, PLASMA ENDOTHELIN-1 AND TROPONIN I CONCENTRATIONS IN HORSES WITH EXPERIMENTALLY INDUCED ENDOXEMIA. K. Nosella, A. Franzén, D. Brojer. Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, SLU, Sweden.

Endothelin-1 is a potent vasoconstrictor that is increased in several disease conditions, such as endotoxemia and sepsis. Increased levels of troponin I (cTnI), a marker for myocardial damage, have also been shown in various species with endotoxemia. The objective was to determine plasma concentrations of endothelin-1 and troponin I as well as blood pressure in horses with experimentally induced endotoxemia. 5 Standardbred horses received an intravenous infusion of endotoxin (total dose 500 ng/kg) for 6 h. All horses showed mild signs of endotoxemia with fever, depression, tachycardia, tachypnea. Mean arterial blood pressure (MAP) was measured and blood samples were collected before the start of the infusion, every 60 min during the infusion and the first 3 h post-infusion. Plasma concentration of cTnI were 2.9 ± 4.9 pg/ml and 0.013 ± 0.07 µg/L, respectively. Endotoxin infusion caused a simultaneous increase in endothelin and cTnI after 4 h of infusion, reaching a mean peak value of 26.0 ± 16.2 pg/ml for endothelin and 0.152 ± 0.067 µg/L for cTnI. MAP values increased from pre-infusion values of 89 ± 13 mmHg to a mean peak concentration of 137 ± 22 mmHg after 4 hours of infusion. In conclusion, this study indicates that endothelin-1 has a direct or indirect effect on cardiac myocytes in endotoxemia.

E-28
ULTRASONOGRAPHY STUDY OF PERIPHERAL VEINS AND LARGE ARTERIES DIAMETERS/CIRCUMFERENCES IN NORMAL HORSES. L. Meurer, N. Medrano-Lange, G. Beauchamp, Faculté de Médecine vétérinaire, Université de Montréal, St-Hyacinthe, Canada.

Clinical evaluation of hydration status in horses is based on parameters that objectivity stays to confirm. The addition of a more objective cardiovascular parameter could be an improvement of our clinical hydration evaluation in hospitalized patients and also in a farm practice context. The objective of the study was to evaluate the relation between the size of horses and the maximum diameter/circumference of the peripheral veins, carotid artery and cardiac large vessels by means of ultrasonography in normal horses.

The study population included 17 normal mix horses (12 females, 3 gelding and 2 stallions). The thoracic circumference, weight and height of horses were evaluated. The maximum cross-sectional diameter and circumferences of the peripheral veins (jugular, cephalic and lateral thoracic) and large arteries (the carotid and pulmonary arteries and the proximal part of the aorta) were measured with a multi-frequency linear transducer working at 7.5 MHz (peripheral veins and carotid arteries) and a multi-frequency sectorial transducer working at 3.0 MHz (for large cardiac vessels). The association between the size of the horses (thoracic circumference, weight and height) and the vessels diameters/circumferences was determined by linear regression analysis. The difference between right and left side measurements for all vein and arteries was determined by a mix linear model. There was a significant positive relation between the size of the jugular-cephalic veins and the carotid artery with the weight and the height of horses. There is no significant difference of diameters/circumferences of the peripheral veins and large arteries between the right and the left side.

In conclusion, the results of this study suggest that there is a correlation between the peripheral veins/carotid artery with the weight and the height in normal horses and we anticipate that the vessels diameter/circumferences could be used as an objective parameter of hydration status to detect hypovolemia in equine patient in an hospital and field practice contexts.
E-29 CARDIAC BEAT-TO-BEAT VARIATION AND ARRHYTHMIAS IN ENDURANCE HORSES, M. Flethøj, J.K. Kanters, L.H. Olsen, R. Buhl. Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Variations in cardiac rhythm are physiological; however, it still needs to be defined when the rhythm variation is large enough to be considered arrhythmic. The purpose of this study was: 1) to investigate normal beat-to-beat variation in order to make evidence based threshold levels of maximum acceptable RR deviation for equine ECG analysis and 2) on basis of the threshold levels to evaluate the frequency of premature beats in healthy endurance horses during rest and exercise.

ECG recordings were obtained from 11 endurance horses of Arabian breeds for 24 hours at rest and during a standardized exercise protocol. Beat-to-beat variation was assessed by the percentage deviation of successive RR intervals defined as RRdev(i) = (RR(i) - RR(i-1))/RR(i-1)100%. The RRdev(i) was plotted against RR(i) to visualize beat-to-beat variation in relation to the underlying heart rate (HR) in order to define a threshold model of maximum acceptable RR deviation. Subsequently, a two-step timing algorithm compared each RR interval to a local mean of the four most adjacent intervals against the defined threshold levels. Aberrant intervals were manually classified.

Beat-to-beat variation showed similar patterns in all horses. At resting HRs <60 bpm the RRdev(i) ranged from 12-22% showing considerably inter-horse variation, for HRs between 60-100 bpm RRdev(i) was <10%, and at exercising HRs ≥100 bpm the RRdev(i) was <5% and very consistent among horses. These observations defined the threshold model that was used in the two-step algorithm. Supraventricular premature beats (SVPC) were observed in nine horses with median frequencies of 7 (range 2-45) SVPCs at rest and 2 (range 1-23) SVPCs during exercise. Only two ventricular premature beats were observed in one horse at rest.

In conclusion, beat-to-beat variation varies with heart rate and threshold levels of maximum acceptable RR deviation should be set accordingly. At rest rather large beat-to-beat variations can be tolerated although great inter-horse variation suggests that threshold levels should be adjusted individually. In contrast only minor variations should be tolerated during exercise where premature beats are easily overlooked. SVPCs were seen in the majority of horses although their frequency varied greatly. The clinical significance remains to be clarified.

E-30 EFFECTS OF AEROBIC TRAINING IN SYSTOLIC, DIASTOLIC AND MEAN PRESSURE IN ARABIAN HORSES, M. Mirian1, M.L. Piffer1, P.M Bogossian2, W.R. Fernandes1. 1 São Paulo University, FMVZ-USP, São Paulo, SP, Brazil.

Exercise causes several physiological changes resulting from autonomic and hemodynamic adaptation in animals and in humans, but there are few reports about the behavior of blood pressure (BP) during physical activity in horses, as well as the effect of training.

9 untrained Arabian breed animals have undergone two evaluations (initial and after 90 days of aerobic training at 60% HR max) using 6% incline and speed increment every 3 minutes starting from 2.5 m/s to reach 12.5 m/s. Facial artery was cannulated with a 20G catheter after local anesthetic block. Values of systolic blood pressure (SBP), mean (MBP) and diastolic (DBP) were obtained through a multiparameter monitor GE Dash 3000 model. Obtained data were evaluated by two-way ANOVA for repeated measures and Bonferroni multiple comparison, P < 0.05 significance level.

During initial evaluation SBP increased in a manner gradual to 10 m/s (P = 0.008) and very consistent among horses. These observations defined the threshold model that was used in the two-step algorithm. Supraventricular premature beats (SVPC) were observed in nine horses with median frequencies of 7 (range 2-45) SVPCs at rest and 2 (range 1-23) SVPCs during exercise. Only two ventricular premature beats were observed in one horse at rest.

In conclusion, beat-to-beat variation varies with heart rate and threshold levels of maximum acceptable RR deviation should be set accordingly. At rest rather large beat-to-beat variations can be tolerated although great inter-horse variation suggests that threshold levels should be adjusted individually. In contrast only minor variations should be tolerated during exercise where premature beats are easily overlooked. SVPCs were seen in the majority of horses although their frequency varied greatly. The clinical significance remains to be clarified.

E-31 EVALUATION OF COAGULATION AND FIBRINOLYSIS IN HORSES WITH ATRIAL FIBRILLATION, C.N. de Solis1,2, V.B. Reed2, J. Slack3, E. Jose-Cunilleras4. 1Swiss Institute of Equine Medicine, Vetsuisse Faculté, University of Bern und Agroscope, Bern (Switzerland), 2New Bolton Center, School of Veterinary Medicine, University of PA. Kennett Square, PA, 3Facultad de Veterinaria, Universidad Autonoma de Barcelona, Barcelona, Spain.

The objective was to test the hypotheses that a hypercoagulable state is present in horses with atrial fibrillation (AF), as described in humans, and that the plasma concentration of D-dimers would be the best marker of a procoagulant state in these horses.

A group of horses with atrial fibrillation (AFG, n = 25) was compared to a control group (CG, n = 17) of horses without cardiovascular or systemic disease. Fibrinogen plasma concentration, D-dimer plasma concentration, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured using compact hemostasis testing equipment. Results were compared using a Wilcoxon (Mann-Whitney) rank-sum test. The proportion of horses with abnormal D-dimer concentration, abnormal coagulation profiles (2 abnormal results) and abnormal coagulation parameters in the AFG and CG were compared using a Chi-Square test. A p value of <0.05 was used to distinguish significant from non-significant associations.

No clinical signs of hypercoagulation or thromboembolism were detected in the study group. Antithrombin activity was lower in the AFG (198%[184-222] vs. 232%[202-256] median [IQR], p = 0.008) and no significant differences in fibrinogen, D-dimer, PT or aPTT were detected. The proportion of horses with abnormal D-dimer concentration, abnormal coagulation profiles (2 abnormal results) and abnormal coagulation parameters in the AFG and CG were compared using a Chi-Square test. A p value of <0.05 was used to distinguish significant from non-significant associations.

No clinical signs of hypercoagulation or thromboembolism were detected in the study group. Antithrombin activity was lower in the AFG (198%[184-222] vs. 232%[202-256] median [IQR], p = 0.008) and no significant differences in fibrinogen, D-dimer, PT or aPTT were detected. The proportion of horses with abnormal D-dimer concentration (40 vs. 12%, p = 0.047), abnormal coagulation profiles (72 vs. 35%, p = 0.018) and the proportion of abnormal coagulation tests (21 vs. 8%, p = 0.015) was larger in the AFG than in the CG.

The group of horses with AF had activation of the coagulation system but no signs of thromboembolic disease were detected. This should be considered when assessing horses with AF.

E-32 EFFECTS OF DIFFERENT HEART DIMENSIONS ON RACE PERFORMANCE IN THOROUGHBRED HORSES, O. Pınar1, A.A. Sancak2. 1Equine Hospital of the Jockey Club of Turkey, İstanbul, Turkey., 2Ankara University, Faculty of Veterinary Medicine, Department of Internal Medicine, Ankara, Turkey.

The purpose of this study was to evaluate the effects of intensive exercise of Thoroughbred horses on their race performance. Clinically healthy 12 mares and 8 stallions were included. Horses were assigned as a control and 3 study groups according to their ages. Control group was >2 to ≤3 year-old while study groups of A, B, and C were >3 to ≤4, >4 to ≤5, and >6 to ≤7 year-old, respectively. Intensive exercise was applied to horses except control group for 2 (A), 3 (B), and 4 (C) years.

Dimensions of heart in horses were assessed before and after intensive exercise by echocardiography. Specifically left ventricle (LV) assessment by diameter in systole and diastole (LVdd and LVds) and posterior wall thickness (LVPWd and LVPWd), interventricular septal thickness (IVSd and IVSs), ejection fraction (EF), fractional shortening (FS), and mass (LVM) were determined. ANOVA and turkey tests were used for statistical analysis.

It was possible to observe a decrease in SBP and MAP during initial testing and after 90 days of aerobic exercise (60% HR max), at the same speeds, showing a decreased cardiac effort for the maintenance of physical activity.
Mean dimensions of LVDd and LVDs were increased with age. LVDs was obtained as 5.96 ± 0.27 in control group, 5.80 ± 0.32 in group A, 6.74 ± 0.32 in group B, and 7.51 ± 0.32 in group C.

Significant differences of LVDd (p < 0.001) and LVDs (p < 0.001) was only determined in group C compared to control group. Significant differences in values of LVM (p < 0.001) was determined in group A and group C compared to control group.

Intriguing study indicated that eccentric left ventricle hypertrophy developed in Thoroughbred horses during years of racing, especially more than 4 years of intensive exercise.

E-33 DIGITAL LAMINAR WHOLE TRANSCRIPTOME SHOT- GUN SEQUENCING IN LEAN AND OBESE PONIES SUB- JECTED TO HIGH CARBOHYDRATE FEEDING. T.A. Burns1, M.R. Watts1, P.S. Weber2, R.J. Geor2, L.J. McCutchen2, J.K. Belknap1. 1College of Veterinary Medicine, The Ohio State University, Columbus, OH., 2College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Endocrinopathic laminitis, particularly equine metabolic syndrome-associated laminitis (EMSAL), is the most frequently diagnosed cause of equine laminitis in developed nations and currently associated with unacceptable morbidity and mortality. While several mechanisms have been proposed to be involved in the pathogenesis of the condition (including vascular dysfunction, glucose deprivation, inflammation, and extracellular matrix dysfunction), the cause(s) of the condition remain obscure. Knowledge of the comprehensive transcriptome catalog of digital laminar tissue during conditions considered to increase risk of laminitis (i.e., high carbohydrate feeding in an IR individual) would both improve understanding of the pathophysiology of EMSAL and potentially provide attractive, relevant therapeutic targets for treating the condition. The purpose of this study was to characterize RNA transcripts present in digital laminar tissue of ponies subjected to a dietary carbohydrate challenge designed to mimic abrupt exposure to pasture rich in nonstructural carbohydrate (NSC) using novel RNA-seq (RNA-sequencing, or whole transcriptome shotgun sequencing) technology. Following 4 weeks of conditioning consisting of a diet of hay chop (NSC ~6% on a DM basis), mixed-breed ponies (body weight 270.9 ± 74.4 kg) were assigned to groups based on body condition scoring (lean vs. obese). Ponies either remained on the conditioning diet (CON diet; n = 5 obese, n = 5 lean) or received the same diet supplemented with sweet feed and oligofructose (CHO diet; n = 6 obese, n = 6 lean) for a period of 7 days. At the end of the feeding protocol, dorsal digital laminar tissue samples were collected immediately following euthanasia (within 15 minutes); samples were snap-frozen in liquid nitrogen and stored at -80 °C until processing. RNA-seq was used to analyze the populations of digital laminar RNA present at the time of euthanasia and their relative quantity, with particular attention directed toward transcripts that were differentially regulated in response to diet. Multiple matrix metalloproteinase genes (including ADAMTS4, MMP1, and MMP13) were upregulated in response to high-carbohydrate feeding in the laminae of lean and obese ponies, as well as several microRNAs involved in regulation of cell proliferation and survival (including MIR885 and MIR675). Multiple genes encoding extracellular matrix and cytoskeletal components and regulators of energy metabolism were down-regulated in response to high carbohydrate feeding (including gelsolin, laminin-72, and PPARG). Collectively, the results of this study suggest significant effects of dietary carbohydrate content on regulation of the digital laminar transcriptome, particularly involving cytoskeleton and extracellular matrix components; further, molecular targets are identified for evaluation in future studies.

E-34 SEASONAL VARIATION IN BLOOD NITRITE CONCENTRATION IN PREVIOUSLY LAMINITIC AND NON-LAMI- NITIC PONIES. E.J.T. Findings1, S.M. Ghosh2, N.J. Menzies-Childs1, P.A. Harris1, T.J. Emmans1. 1Royal Veterinary College, London, UK., 2Queen Mary, University of London, London, UK., 3WALTHAM Centre for Pet Nutrition, Leicestershire, UK.

Laminitis incidence varies with season and predisposition to laminitis may be associated with hyperinsulinaemia. Hyperinsulinaemia can impair endothelial function, an effect which may play a role in the predisposition to laminitis. Impaired endothelial cell function results in decreased production of nitric oxide. Nitrite is a direct metabolite of nitric oxide and decreased blood nitrite concentration is associated with impaired endothelial function in human patients. As part of investigations into the relationships between season, laminitis predisposition and endothelial function in the horse, blood nitrite concentrations were measured as an indicator of endothelial nitric oxide production. Measuring nitrite content is a more specific reflection of endothelial nitric oxide production than the combined nitrite and nitrate content which has been measured previously in the horse.

Blood samples were collected from previously laminitic (PL, n = 6) and non-laminitic ponies (NL, n = 6) at 3 seasonal time points (spring, summer and winter). All ponies were assigned to the same management regimen with ad lib access to pasture and no supplemental feeding apart from hay in winter. Whole blood was collected into a ferricyanide based solution to preserve the nitrite content by preventing oxidation by haemoglobin to nitrate. Nitrite was measured using ozone chemiluminescence. This method was first validated by assessing intra- and inter-assay variability and dilutional parallelism. Data was reported as mean ± standard deviation and analysed using a repeated measures mixed effects model with season and laminitis as fixed effect and individual animal as a random effect.

Intra- and inter-assay variability (mean coefficients of variation = 13.1 and 16.8% respectively) and dilutional parallelism (r² = 0.9993, p > 0.01) were all acceptable. Blood nitrite concentrations varied significantly with season (p = 0.0005) with values higher in summer (1501 ± 686 nM, NL = 1672 ± 825 nM, PL = 1331 ± 539 nM) than winter (806 ± 679 nM, NL = 1004 ± 354 nM) or spring (474 ± 461 nM, NL = 646 ± 620 nM, PL = 303 ± 114 nM). There was no significant effect of laminitis predisposition.

This fully validated method has identified differences in nitric oxide production with season. These seasonal effects could be due to changes in ambient temperature, level of physical activity or diet. Nitric oxide production may reflect changes in endothelial nitric oxide synthase (NOS) activity and hence altered endothelial function. Alternatively, it may indicate increased nitric oxide production by inducible NOS as part of a pro-inflammatory state induced by summer pasture. Further investigations into endothelial nitric oxide production and endothelial function are warranted in the horse.

E-35 GLUCOSE AND INSULIN DYNAMICS DIFFER AMONG STANDARDBREDS, QUARTER HORSES AND THOR-oughbreds. S.J. Valberg1, L.A. Borgia1, A.K. Rendahl2, M.E. McCue3, R.C. Boston1, J. Pagan4, R.J. Geor3, 1College of Veterinary Medicine and, 2School of Statistics, University of Minnesota, St. Paul, MN., 3School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA., 4Kentucky Equine Research, Versailles, Kentucky, 5College of Veterinary Medicine Michigan State University, East Lansing, MI.

Although differences in insulin sensitivity/dynamics between horses and ponies were described over 30 years ago, only recently have differences in glucose and insulin dynamics among horse breeds been explored. Recent work has described differences between Standardbreds (STB), Andalusians and ponies, and STB and Icelandic horses using frequently sampled glucose insulin tolerance tests (FSGITT) and combined insulin and glucose tolerance tests, respectively. However, breed differences in insulin and glucose dynamic between the STB and other light horse breeds
developed for athletic performance, and characterized by large proportion of lean muscle mass have not been described. Our objective was to identify differences in glucose effectiveness (Sg), insulin sensitivity (SI) and glucose excursions below baseline across the STB, Quarter Horse (QH), and Thoroughbred (TB). A standard FSGITT protocol was performed in 15 STB, 13 QH and 27 TB horses; 19 geldings and 36 mares, between 3 and 17 years of age, with body condition scores (BCS) ranging from 4.5 to 7 out of 9. A large number of the horses in this study had a deflection of glucose values below baseline during the FSGITT. Therefore, 8 additional indices were calculated from insulin and glucose measurements during the FSGITT, including the area under the curve below baseline (HAUC). Multiple regression models were performed to identify significant breed differences, while controlling for confounding effects of age, gender, season, and BCS. STB had lower baseline insulin (Bi), Sg, disposition index (Di), acute insulin response to glucose (AIRg), peak insulin (Imax) and HAUC values, and higher minimum glucose (Gmin) values, when compared to TB and QH. QH had significantly higher peak insulin and higher peak glucose (Gmax) values than TB; and a greater glucose deflection below baseline (dGB) and greater glucose deflection below baseline at sampling end-point (dGe) than the TB. Other indices were not different between QHs and TBs. Age was negatively correlated with SI in Di and positively correlated with dGe. Bi, Di, AIRg, Gmax, and dGe were significantly lower, and SI significantly higher, in horses sampled in winter. STBs appear to maintain glucose homeostasis with lower insulin response to IV glucose challenge than QHs and TBs. In addition, STBs do not develop the notable hypoglycemia that occurred in QHs and TBs during the FSGITT. In conclusion, glucose and insulin dynamics during a FSGITT were significantly different among these three breeds despite similar lean body mass phenotype. Breed should be considered along with season, age, gender and BCS when interpreting FSGITT data. Future work is warranted to determine the factors underlying breed differences in glucose and insulin dynamics.

E-37 INSULIN DYNAMICS IN HORSES WITH PITUITARY PARS INTERMEDIA DYSFUNCTION BEFORE AND AFTER TREATMENT WITH PERGOLIDE. S.J. Jacob, R.J. Geor, L.J. McCutcheon, H.C. Schott II. Michigan State University College of Veterinary Medicine, East Lansing, MI.

Insulin resistance (IR) and hyperinsulinemia have been described in horses with pituitary pars intermedia dysfunction (PPID). Whereas improvement in clinical signs of PPID with treatment with the dopamine agonist pergolide is well documented, there are no reports of the effects of pergolide therapy on insulin dynamics. We tested the hypothesis that pergolide treatment would increase insulin sensitivity and/or alleviate hyperinsulinemia in PPID-affected horses. Twenty aged (25 ± 3 years; mean ± SD) mixed breed horses were assigned to PPID treated (n = 12), PPID untreated (n = 4), or aged controls (n = 4). A diagnosis of PPID was based on clinical signs of PPID (hypertrichosis) and results of an overnight dexamethasone suppression test. Fasting insulin concentrations and groups in model parameters (insulin sensitivity [SI] and acute insulin response to glucose [AIRg]) from analysis of an intravenous glucose tolerance test were obtained in January and August, between which the PPID treated group received pergolide at 0.002 mg/kg BW/day for 6 months. Data were analyzed by a linear mixed-model with significance set at P < 0.05.

In January, fasting insulin concentrations were significantly higher in PPID-affected horses than in aged controls. Although all horses showed improvement in clinical signs of PPID in response to pergolide treatment, the difference in insulin concentration between PPID-affected horses and aged controls was also evident in August. Based on an insulin cutoff value of 20 μU/L, 8 PPID-affected horses were hyperinsulinemic in January (6 treated, 2 untreated) and 7 of 8 remained hyperinsulinemic in August. Mean SI and AIRg did not differ among groups in January and were unchanged in August. Two of the PPID treated group horses developed clinical laminitis in April (all horses were housed at pasture); these horses had a history of prior bouts of laminitis, were hyperinsulinemic, and had the highest values for AIRg in both January and August.

E-36 COMPARISON OF RESTING AND DYNAMIC ADRENOCORTICOTROPIC HORMONE FOLLOWING ADMINISTRATION OF THYROTROPIN-RELEASING HORMONE DURING AUTUMN AND NON-AUTUMN SEASONS IN HORSES. J.C. Haffner, R.M. Hoffman, H.S. Spooner, S.G. Grubbs. Middle Tennessee State University.

Circannual variation in equine adrenocorticotropic hormone (ACTH) creates challenges in predicting early signs of Pituitary Pars Intermedia Dysfunction (PPID) without established autumn reference values. The goal was to establish predictive ACTH concentrations for early signs of PPID during Autumn based on established Non-Autumn predictors using a thyrotropin-releasing hormone (TRH) test. ACTH concentrations in 32 horses aged 1 to 27 yrs were evaluated before and after administration of TRH during June (Non-Autumn) and October (Autumn). Venous samples were collected into EDTA-treated tubes before (PRE) 1 mg TRH administered i.v. and 10 (T10) and 30 (T30) min post-TRH. Blood was chilled and centrifuged within 2 h, and plasma stored at -80°C pending ACTH analysis using a sequential immunometric assay. Horses were scored for muscle wasting, hirsutism, sweating and abnormal fat deposits. Frequency histograms of the Autumn ACTH data indicated two populations at each sample time, and confidence intervals were used to designate the break between these populations. The 95% confidence breakpoint indices for PPID “Positive” at ACTH greater than 80, 620 μg/mL, and “Negative” at ACTH less than 60, 340 and 190 μg/mL, at PRE, T10 and T30 respectively. Chi-square measures of association indicated no difference in Non-Autumn versus Autumn predictions of PPID at PRE, T10, or T30 (P > 0.27). Age positively correlated with PRE (R² = 0.37; P = 0.04) and T10 (R² = 0.43; P = 0.01). Muscle wasting scores positively correlated with age (R² = 0.50; P = 0.004) and PRE (R² = 0.63; P < 0.001). Hirsutism scores positively correlated with T10 (R² = 0.46; P = 0.01), T30 (R² = 0.42; P = 0.015), and sweating scores (R² = 0.46; P = 0.01).

In these aged horses, minimal model analysis of SI did not differ between PPID-affected and control animals. In contrast, hyperinsulinemia was a prominent feature, suggesting dissociation between fasting insulin concentration and insulin sensitivity in PPID-affected horses. Neither SI nor basal insulin concentration were affected by pergolide treatment despite clinical improvement.

E-38 EVALUATION OF AN ARGinine STIMULATION TEST FOR ASSESSMENT OF ACUTE INSULIN RESPONSE IN ADULT HORSES. J.M. Manfred, P.S.D. Weber, L.J. McCutcheon, R.J. Geor. Michigan State University College of Veterinary Medicine, East Lansing, MI.

Insulin dysregulation is a feature of the equine metabolic syndrome and there is a need for clinically practical tests for evaluation of insulin dynamics. Previously utilized in humans, camels and foals, the arginine stimulation test (AST) may be a useful tool to assess insulin dynamics in equids but to date it has not been evaluated in adult horses. Therefore, the aims of this study were to compare, in adult horses, the acute insulin response (AIR): (1) in a standardized AST protocol using different dosages of intravenous (IV) arginine, and (2) in the AST when compared to an IV glucose test (IVGT; 300 mg/kg IV dextrose). The AST was administered to 6 horses at two dosages (70 mg/kg IV and...
100 mg/kg IV) with one day between tests. Within the first minute following arginine administration, insulin concentrations increased from a mean (± SD) baseline value of 11 ± 3 uIU/ml to 25 ± 13 uIU/ml and remained significantly greater than baseline, with a mean of 23 ± 3 uIU/ml at all time points measured during the first 7 min post arginine. There was no difference of the AIR between doses (ANOVA with Bonferroni correction, P ≤ 0.005) or area under the insulin curve (AUCins 0-5). There were strong associations between the two dosages, the lower dosage was used subsequently in comparing the AST to the IVGT in 20 Arabian horses (2-20 years of age). Spearman correlations were used to determine associations between several indices of AIR derived from the AST and IVGT, including insulin concentrations at 2 and 5 min (ASTins 2 min, IVGTins 2 min, ASTins 5 min and IVGTins 5 min), the overall highest peak insulin concentration from 0-15 minutes in the AST (AST peak) and from 0-19 minutes in the IVGT (IVGT peak), and the AUCins between 0 and 5 min in each test (AST AUCins 0-5 and IVGT AUCins 0-5). There were strong associations (r) between AIR indices from the AST and IVGT, specifically: ASTins 2 min vs. IVGTins 2 min (rho = 0.7), ASTins 5 min vs. IVGTins 5 min, the overall highest peak insulin concentration from 0-15 minutes in the AST (AST peak) and from 0-19 minutes in the IVGT (IVGT peak), and the AUCins between 0 and 5 min in each test (AST AUCins 0-5 and IVGT AUCins 0-5). There were strong associations between the indices of AIR in the two tests.


Obesity is associated with insulin resistance (IR), and both are risk factors for laminitis. Improved understanding of these relationships may identify new targets for prevention of laminitis. In other species, the myokine myostatin is upregulated in obesity and may contribute to obesity-associated IR. We hypothesized that myostatin expression in muscle and adipose tissue (AT) would be higher in overweight/obese versus lean ponies. Groups of lean (n = 11; body condition score [BCS] 5.8 ± 1.4) and overweight/obese (n = 11; BCS 7.6 ± 0.6) were formed by body condition score (BCS) (5.8 ± 1.4) and overweight/obese (n = 11; BCS 7.6 ± 0.6) were further stratified into those designated to receive either a low (42% nonstructural carbohydrate [NSC] as dry matter [DM]) or high (6% NSC as DM) diet for 7 days. On day 7, muscle and adipose tissue samples were obtained for determination of myostatin mRNA (RT-qPCR) and protein (muscle only). Insulin sensitivity (SI) was assessed by minimal model analysis of a frequently sampled intravenous glucose tolerance test performed on day 0. Data were analyzed by a 2-fixed factor ANOVA and unpaired t-tests, with significance accepted at P ≤ 0.05. A Spearman correlation was used to determine association between myostatin mRNA and SI. Muscle myostatin mRNA was higher in overweight/obese versus lean ponies but not different between diets. Tailhead AT myostatin expression was higher in lean-low NSC versus lean- or obese-high NSC, with obese-low NSC having higher expression than lean- or obese-high NSC. Mesenteric AT myostatin expression was higher in lean-low NSC versus lean-high NSC. No differences in myostatin protein expression were detected. There was a negative association between muscle myostatin mRNA and SI (rho = -0.623). Our findings of higher myostatin mRNA expression in muscle of overweight/obese ponies, and an inverse relationship between myostatin expression and SI, are consistent with previous observations in humans. Higher AT myostatin mRNA in ponies fed the low NSC diet suggests dietary influence on myostatin.

E-40 ADRENOCORTICOTROPIN AND CORTISOL DYNAMICS IN RESPONSE TO DEXAMETHASONE ADMINISTRATION TO AGED HORSES WITH AND WITHOUT PITUITARY PARS INTERMEDIA DYSFUNCTION. H. Schott II, K. Rel-sal. Michigan State University, East Lansing, MI.

Lack of suppression of endogenous cortisol concentration 20-24 h following dexamethasone (DEX) administration has been well documented in equations with pituitary pars intermedia dys- function (PPID). In contrast, response of adrenocorticotropic (ACTH) to DEX administration has not been described in either normal or PPID-affected horses. Consequently, plasma ACTH and cortisol concentrations were measured in 8 PPID-affected horses and 4 aged controls (Cortisol Coat-A-Count RIA Kit, Siemens, Inc.) Changes in hormone concentrations between groups and over time were analyzed by repeated measures analysis of variance. 

Mean pre-DEX ACTH concentration in PPID-affected horses was 231±85 pg/ml (range 40-801 pg/ml) and values did not change over time. In contrast, mean pre-DEX cortisol concentration in PPID-affected horses was 5.5±1.0 µg/dl and decreased (p<0.01) from 2-8 h after DEX administration to a low value of 1.7±0.5 µg/dl at 6 h but returned to 4.1±0.4 µg/dl at 18 h. In aged control horses mean pre-DEX ACTH concentration was 22.8±3.5 pg/ml (range 15-32 pg/ml, p=0.01 as compared to PPID-affected equids) and values progressively decreased (p<0.01) from 2 h to a low value of 12.3±1.5 pg/ml 18 h after DEX administration. Similarly, mean pre-DEX cortisol concentration in aged control horses was 5.7±1.1 µg/dl (p=0.99 as compared to PPID-affected equids) and decreased (p<0.01) at all time points after DEX administration (lowest value of 0.07±0.03 µg/ dl 8 h after DEX administration and 0.17±0.09 µg/dl after 18 h).

These data are consistent with previous work documenting a blunted response of the hypothalamic-pituitary-adrenal axis (attenuation of suppression of endogenous cortisol concentration) to DEX administration in PPID-affected horses. The absence of suppression of plasma ACTH concentration in PPID-affected horses supports that the majority of ACTH (or immunoreactive ACTH and ACTH fragments) in plasma of PPID-affected horses arises from melanotropes in the expanded PI, rather than corticotropic in the pars distalis (PD). Further, the transient suppression of plasma ACTH concentration in PPID-affected horses was 5.7±1.1 µg/dl (p=0.99 as compared to PPID-affected equids) and decreased (p<0.01) at all time points after DEX administration (lowest value of 0.07±0.03 µg/dl 8 h after DEX administration and 0.17±0.09 µg/dl 18 h).

These data are consistent with previous work documenting a blunted response of the hypothalamic-pituitary-adrenal axis (attenuation of suppression of endogenous cortisol concentration) to DEX administration in PPID-affected horses. The absence of suppression of plasma ACTH concentration in PPID-affected horses supports that the majority of ACTH (or immunoreactive ACTH and ACTH fragments) in plasma of PPID-affected horses arises from melanotropes in the expanded PI, rather than corticotropic in the pars distalis (PD). Further, the transient suppression of endogenous cortisol concentration following DEX administration to PPID-affected horses provides additional support that PI-derived immunoreactive ACTH is less bioactive than ACTH released by PD corticotropes.

E-41 COMPARISON OF MAGNETIC RESONANCE IMAGING AND HISTOLOGICAL SCORES FOR ASSESSING PITUITARY PARS INTERMEDIA ENLARGEMENT IN HORSES WITH PITUITARY PARS INTERMEDIA DYSFUNCTION, A. Pease1, J. Patterson1, E. Howey1, D. McFarlane2, H.van der Marel3, P.S.D. Weber, L.J. McCutcheon, R.J. Geor. Michigan State University College of Veterinary Medicine, East Lansing, MI, 1Oklahoma State University, Stillwater, OK. 2Euregio Laboratory Services, Maastricht, The Netherlands.

Progressive enlargement of the pituitary pars intermedia (PI) due to hyperplasia and subclinical macroadenomas (<5 mm) and macroadenomas (>5 mm) formation is the pathologic lesion of pituitary pars intermedia dysfunction (PPID). The purpose of this study was to assess the utility of magnetic resonance imaging (MRI) to provide information about subgross changes in PI morphology in PPID-affected horses. The morphometric PI grading system developed by Miller et al. (2008) was used to grade T2.
E-43 FACTORS ASSOCIATED WITH LEPTIN AND ADIPOSE CELL DYNAMICS IN A LARGE ACROSS BREED COHORT OF HORSES AND PONIES. N.E. Schultz¹, R.J. Geor², J. Manfredi³, M.E. McCue³. College of Veterinary Medicine, University of Minnesota, St. Paul, MN, ¹College of Veterinary Medicine Michigan State University, East Lansing, MI.

The adipokines leptin and adiponectin have been suggested as potential diagnostic markers for equine metabolic syndrome (EMS), a clustering of clinical signs associated with increased laminitis risk. This suggestion stems from work in human metabolic syndrome (MetS) where adipokine dysregulation has been shown to play a role in pathophysiology, making leptin and adiponectin useful biomarkers for MetS and its co-morbidities. While MetS shares several salient features with EMS including adiposity, insulin resistance and dyslipidemia, it is unclear the role adipokines play in EMS. Our objective was to assess the correlation between circulating leptin (LEP) and high molecular weight adiponectin (APN) concentrations and other EMS phenotypic measures and to identify factors associated with variation in leptin and adiponectin concentrations. Phenotypic measurements were collected from 610 horses/ponies from 166 farms. Measures included: neck- (NH) and girth- (GH) to height ratio, obesity status (body condition score BCS ≥ 7), laminitis status, fasting blood glucose (GLU mg/dl), insulin (INS uU/ml), adrenocorticotropic hormone (ACTH pg/ml), triglyceride (TG mg/dl), nonesterified fatty acids (NEFA mmol/L), LEP (ng/ml) concentrations, and post oral sugar test insulin (INS OST) and glucose (GLU OST). Age, breed, sex, month, diet and exercise measures were also collected. Due to the complex nature of the data, multivariate, multilevel, multiple regression analysis was performed with explanatory variables both at the farm and individual level. LEP concentrations were positively correlated with NH, GH, GLU, INS, GLU OST, and INS OST. LEP varied significantly with season being lower in summer and highest in October. LEP concentrations were higher in mares than geldings, and were also different between breeds, being highest in the Morgan and lowest in the Quarter Horse. APN was negatively correlated with NH, INS, INS OST, and TG, but did not differ by gender or breed. When the study population was parsed into 4 clinical groups based on obesity and laminitis status, LEP concentration were significantly higher in obese horses both with (O-L) and without (O-NL) a prior history of laminitis, than in non-obese horses with or (O-NL) or without a history of laminitis (NO-NL). In contrast, APN levels were significantly lower in NO-L and O-L horses when compared to O-NL and NO-NL horses. Neither LEP nor APN varied with age, dietary composition or amount of exercise. In conclusion, LEP concentration was correlated to measures of generalized (BCS, GH) and localized (NH) adiposity, but did not differ in horses with and without a history of laminitis. APN levels were significantly lower in horses with a history of laminitis and correlated to localized adiposity (NH) but not generalized obesity, and were not confounded by breed, age, gender or season; thus APN may be a more specific biomarker of an abnormal “unhealthy” metabolic profile and therefore provide increased utility as a diagnostic marker for EMS.
E-44 TRANSABDOMINAL ULTRASOUND OF ADRENAL GLANDS IN HORSES. M. Holland, A. Homm, A.J. Stewart. College of Veterinary Medicine, Auburn University, Auburn, AL.

Previous reports have identified adrenal glands in horses using both transrectal and laparoscopic procedures. A pilot study in 2009 at Auburn University, using four different ultrasonographers, examined the feasibility of identifying the adrenal glands via transabdominal ultrasound in 11 horses. In the previous study both adrenal glands were identified and measured in 10/11 horses using a transabdominal ultrasound technique. The purpose of this study was to refine the technique for localization and measurement of the adrenal glands in 10 horses using one ultrasonographer.

Ten adult horses were used without fasting prior to the examination. The hair was clipped then alcohol and ultrasound gel was applied to the skin. Minimal restraint and sedation as needed were used during the ultrasound examination. Transabdominal ultrasound was performed using a C4-2 curvilinear probe or a S3-1 sector array probe. A sagittal imaging plane was used for evaluation of all adrenal glands imaging just cranial to the renal artery as it leaves the hilus of the kidney. The right kidney was located beginning at the 16th intercostal space, the right adrenal gland was located cranial and medial to the kidney and lateral to the caudal vena cava. The left kidney lies medial to the spleen beginning at the 17th intercostal space, the left adrenal gland was located craniomedial to the kidney.

Both right and left adrenal glands were identified in all 10 horses. In three horses the ultrasound scans had to be repeated 4-6 hours later due to intestinal gas obscuring visualization of the kidneys and adrenal glands. The adrenal glands appeared hypoechoic relative to the kidney and surrounding fat. The scanning depth for location of the right adrenal gland was 15.5 ± 1.42 cm. The right adrenal gland length was 4.84 ± .75 cm and the width 1.45 ± .23 cm. The scanning depth for the left adrenal gland was 19.4 ± 2.2 cm. The left adrenal length was 4.8 ± .81 cm and width 1.1 ± .26 cm.

Transabdominal ultrasound is a reliable method for evaluation of adrenal glands in adult horses. Repeat imaging in 4-6 hours may be needed if the patient has not been fasted prior to the ultrasound examination. (Presented at 2013 ACVIR Annual Scientific Conference.)

| SI ≤ 0.0 | PP-Dglu > 45 min | 78.9 | 78.6 | 83.3 | 73.3 |
| SI > 0.5 | PP-Dglu > 45 min | 35.0 | 34.0 | 38.8 | 80.6 |


The intestinal microbiota has been shown to have an enormous impact on maintenance of health. The presence of different environmental filters, like low pH, presence of digestive enzymes and anaerobic conditions in the intestinal tract selects unique bacterial species in each of the intestinal compartment. The objectives of this study were to use high throughput sequencing to characterize and to compare bacterial profiles from different intestinal compartments of eleven healthy horses euthanized due to gastro-intestinal related problems.

Content from the stomach, duodenum, ileum, cecum, large colon, small colon, rectum and feces were collected from 11 horses without gastrointestinal disease immediately after euthanasia. DNA was extracted and the V4 region of the 16S rRNA gene was amplified by PCR and sequenced using the MiSeq Illumina technology. A total of 6,536,523 sequences passed all quality control filters. High richness was present. Marked differences in relative abundance and population structure were present between compartments. Firmicutes comprised the main bacterial phylum in all compartments. Lactobacillus and Sarcinia were predominant genera in the stomach, with a marked increase of Streptococcus spp. in the duodenum. Actinobacillus spp. and Clostridium sensu stricto were the most abundant genera in the ileum and “5 genus incertae sedis”, a genus from the Subdivision S5 class of the Verrucomicrobia phylum predominated from the large colon through feces. Only a few genera were present in all compartments within the same horse, with four of the horses presenting no genera shared between all compartments. There was a significant increase in diversity towards the distal gut (P<0.001) and a stable bacterial composition at the class level was observed from cecum through feces. Samples clustered mainly by site collection, but intra-horse stability was observed as neighboring sites tended to cluster by animal.

The composition of the bacterial microbiota in the equine intestinal tract varies greatly among compartments, especially at lower taxonomic levels. Neighboring compartments are more similar among each other. The distal gut harbors a highly diverse and more complex microbiota then the proximal gut. The bacterial profiles found at higher taxonomic levels reveals that fecal
samples may be useful as representative of changes occurring in the distal compartments.

E-47 THE EFFECT OF PARASITE BURDEN ON FAECELLY EXCRETED ALBUMIN. N. Kerbyson, D. Knottenbelt, T.D.H. Parkin. School of Veterinary Medicine, University of Glasgow, Scotland, UK.

The aim of this study was to establish if faecally excreted albumin, measured using a commercially available test kit, was related to the parasite burden of otherwise healthy horses. Twenty horses were included in the study. They were aged from six to 26 years (mean age 15, median 16 years), there were six mares and fourteen geldings, breeds included draught breeds and ponies. The parasite management programme prior to the study included irregular faecal worm egg counts (FWECs) the last of which had been performed five months prior to the study.

A fresh (<1 hr old) faecal sample was collected from each horse and divided into two sub-samples. Half of the faecal sample was submitted for FWEC and half was tested using the commercial available qualitative faecal occult blood detection kit (Succeed®). This test uses lateral flow immunoassay technology to detect haemoglobin and albumin in equine faeces and expresses the result as a colour change, the result being qualitative rather than quantitative. Blood samples were collected for tapeworm ELISA from all horses. FWECs ranged from 0pg/100g to 10000 pg/100g. Faecal density of tapeworm ELISA ranged from 0.113 to 1.913 (mean 0.466, median 0.335). Anthelmintics were administered to the horses one week after sample collection; all horses received moxidectin as a larvicidal treatment due to the time of year, praziquantel was administered to those with a tapeworm optical density of >0.2.

The faecal occult blood test was repeated two weeks after anthelmintic administration. Of the 20 horses tested five were positive for albumin both prior to and following the administration of the anthelmintic; eleven were positive prior to anthelmintic use and negative after and three were negative on both occasions. With respect to haemoglobin: ten horses were positive on both occasions; two were positive and then negative; six were negative and then positive and two were negative on both occasions.

McNemar’s tests were conducted to identify significant changes in the proportion of albumin and haemoglobin positive horses prior to and following administration of the anthelmintic. Faecal albumin was significantly more likely to be detected prior to administration of the anthelmintic than after (P = 0.004). However, there was no statistically significant association between faecal haemoglobin status and time of testing (P = 0.16). Mann-Whitney tests failed to identify a significant differences in tapeworm ELISA optical density or FWEC between horses with different faecal haemoglobin (P = 0.30 and P = 0.34, respectively) or albumin status (P = 0.35 and P = 0.64) This work indicates that parasite burden is associated with the likelihood of being positive for faecal albumin using the faecal occult blood detection kit (Succeed®). It is likely that greater numbers of horses are required to identify associations between the specific parasite burden of an individual horse and the detection of faecal albumin.

E-48 EFFICACY OF A COMBINATION OF APOLECTOL, LIVE YEAST (CNCM I-1077) AND MAGNESIUM HYDROXIDE IN THE PREVENTION OF EGUS AND Fecal ACIDOSIS IN THOROUGHBRED RACEHORSES. B.W. Sykes1, K.M. Sykes2, G.D. Hallowell3. Upper Orara, NSW, 1University of Nottingham, Sutton Bonington, UK.

To date prevention of EGUS has relied upon management changes, which may be impractical, and pharmaceuticals. Faecal acidosis is associated with the feeding of high grain diets and may reduce fermentation efficiency and increase the risk of gastrointestinal disruptions in affected horses. The ability to identify a neutraceutical with preventative properties for both conditions would be advantageous. Thoroughbred racehorses in training without clinically significant gastric ulceration identified on gastroscopic examination. Squamous and glandular EGUS scores were recorded. Horses were randomised to receive either 95 grams Apolectol, 2 grams live yeast (CNCM I-1077) and 20 grams magnesium hydroxide or a placebo 1 – 4 hours prior to exercise. Faecal analysis was performed weekly and gastroscopy and EGUS scoring was repeated at 24 – 27 days. Data was normally distributed and analysed using Student’s T-test, Fisher’s exact test, logistic regression analysis and repeated measures ANOVA. Significance was assumed at p = 0.05.

Twenty-four horses met the inclusion criteria and were randomised into two equal groups. No differences between the groups at enrolment, or in the duration of therapy, were present. Faecal samples were collected from all horses at all time points while twenty-three horses were available for follow-up gastroscopy. Adequately visualisation of the squamous mucosa was possible in all gastroscopies, while visualisation was not possible for the glandular mucosa in one horse in the treatment group and four horses in the placebo group at both the initial and follow up examinations. The same five horses were affected on both occasions. Mean squamous ulcer grade in the treatment group did not significantly change over time, but mean squamous ulcer score in the placebo group significantly increased over time (p = 0.013). Horses in the placebo group were significantly more likely to have worsening of their squamous (p = 0.04) and glandular (p = 0.028) ulcer scores than horses in the treatment group. Mean faecal pH significantly decreased over time in the placebo (p = 0.04) but not the treatment group (p = 0.07). When compared with the treatment group, faecal pH was consistently lower in the placebo group with this effect statistically significant at days 14 (p = 0.046) and 21 (p = 0.0075). In conclusion, the combination of a Apolectol®, live yeast (CNCM I-1077) and magnesium hydroxide may be an effective prophylactic against EGUS and faecal acido- sis in horses in high intensity work.

E-49 PHARMACOKINETICS OF INTRAVENOUS AND ORAL ENTERIC COATED OMEPRAZOLE. B.W. Sykes, C. Underwood, P.C. Mills. School of Veterinary Science, University of Queensland, Gatton, 4343, QLD, Australia.

To date the pharmacokinetics of oral enteric-coated omeprazole (ECO) in fed and fasted horses have not been described. The objective of this study was to measure the pharmacokinetics of intravenous and oral ECO in fed and fasted animals. Twelve healthy Thoroughbred racehorses were enrolled a cross-over study. A commercially available ECO formulation was used. Horses were administered a single dose of either 0.5 mg/kg omeprazole IV (IV group), 4 mg/kg ECO PO with concurrent 16 hour fast (ECO fasted) or 4 mg/kg ECO PO with concurrent free choice access to hay (ECO fed). There was a minimum 7 day wash-out period between treatments; during which time the horses were kept at pasture with hay supplementation. Blood samples were collected before omeprazole administration and at 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 150, 180, 210, 240, 300, 360, 540 and 720 minutes after administration. The blood was separated by centrifugation within 30 minutes of collection and the plasma frozen at -20°C until analysed. Plasma omeprazole concentration was determined using a Nexera UHPLC coupled with a LCMS-8030 Triple quadruple mass spectrometer operating in positive electrospray ionization mode with a reverse phase C18 column. Non- compartmental analysis was performed using PK Solver.

One dose of IV omeprazole was inadvertently administered per osually. Consequently complete data sets were available for 11 horses. The following pharmacokinetic parameters (mean ± SD) were observed in the IV group (t1/2 54.6 ± 12.7 min, Tmax 2 ± 0 min, Cmax 1.7 ± 0.4 µg/mL and AUC0-inf 38.8 ± 7.8 µg/mL min), the ECO fasted group (t1/2 120.3 ± 40.4 min, Tmax 20 ± 24.5 min, Cmax 0.55 ± 0.47 µg/mL and AUC0-inf 61.3 ± 38.0 µg/mL min) and the ECO fed group (t1/2 72.3 ±
E-50
ULTRASONOGRAPHICALLY VISIBLE DIMENSIONS OF THE LIVER IN NORMAL HORSES. L. Johns, A. Miles. Equine Referral Hospital, Royal Veterinary College, London, UK.

Hepatic ultrasound is widely used for evaluating horses with suspected liver dysfunction. Although a change in size is considered suggestive of pathology, no clear guidelines exist to define the expected hepatic ultrasonographically visible dimensions (HUVD) in horses. The aim of the study was to describe the HUVD in clinically normal horses, and to determine whether this is altered by signalment, height, body weight and body condition score (BCS). Bilateral ultrasonographic evaluation was performed in 58 clinically normal horses with no history of hepatic disease. The most cranial/caudal intercostal spaces (ICS), the total number of ICS in which the liver was visualized and the ventral extent of the liver was recorded. Liver was visualized on the right in 56/58 (97%) of horses, on the left in 41/58 (71%) and on both sides in 39/58 (67%). The most cranial ICS was 5 (bilaterally) and the most caudal 16 (right) and 10 (left). Liver was visualized in 0-11 ICS (right) and 0-5 (left). The liver did not extend ventral to the CCJ in any horse. There was no significant effect of sex, breed, height, body weight or BCS on the HUVD. Liver was visualized on the right and the ventral extent of the liver is unlikely to be clinically relevant. Liver dimensions may be decreased in older horses.

E-51
SERUM HAPToglobIN AS A BIOMARKER FOR SIRS IN HORSES. P.J. Johnson, J.R. Amorim, J. Kramer, S.K. Reed, A. LaCarrubba, A.E. DeClue. University of Missouri College of Veterinary Medicine, Columbia, MO.

Haptoglobin is an acute phase reactant produced in response to physiologic stress. Serum haptoglobin concentrations have been successfully used to aid in the diagnosis of and predict clinical outcome in people with sepsis. Horses commonly develop SIRS and endotoxemia but there are few diagnostic or prognostic biomarkers for these conditions. The purpose of this pilot study was to determine if serum haptoglobin concentrations could be used as a biomarker for inflammation in the horse.

Horses that were presented to the MU VMTH with evidence of systemic inflammation were eligible for enrollment (SIRS group). Additionally, a control group of healthy horses were enrolled (healthy group). These horses had no history of illness for the preceding month, unremarkable physical examination, CBC, and plasma biochemical profile. Blood was collected from horses in the SIRS group on the first day of hospitalization and the healthy group on three consecutive days. Serum was harvested and frozen at -80°C for batch analysis. Serum haptoglobin was measured by a commercial laboratory (VDI Laboratory). Data were analyzed using a Mann-Whitney Rank Sum test or a RMANOVA on ranks with a P<0.05 considered significant.

Sixteen horses were enrolled in the SIRS and 12 horses were enrolled in the healthy group. The SIRS group (median, Q1, Q3; 66, 42, 159.5 mg/dL) had significantly greater serum haptoglobin concentration compared to the healthy horses (37.7, 27.9, 40 mg/dL) (P<0.002). Using a cut off of 43.5 mg/dL, sensitivity was 75%, specificity was 91.7% and AUC was 0.86 for differentiation of healthy and SIRS horses on day one of hospitalization. There was no significant difference in haptoglobin concentrations over the course of 3 days in the healthy horses (day 1: 37.7, 27.9, 40; day 2: 38.7, 31.1, 47.2; day 3: 38.5, 30.8, 50.8, mg/dL).

Serum haptoglobin is a promising biomarker for identifying systemic inflammation in the horse. Further evaluation in a larger cohort of horses to determine the clinical diagnostic and prognostic utility is warranted.

E-52
COMPARISON OF INFLAMMATORY AND ENDOCRINE MEASURES IN GERIATRIC HORSES. M.H. Siard, S.E. Reedy, K.E. McMurry, A.A. Adams. Gluck Equine Research Center, University of Kentucky, Lexington, KY.

Pituitary pars intermedia dysfunction (PPID, also known as equine Cushing’s disease), hyperinsulinemia, and insulin resistance are common endocrinopathies of geriatric horses and are risk factors to developing laminitis, an inflammatory condition of the hoof. Systemic low-grade chronic inflammation occurs with aging, termed inflamm-aging. To determine whether inflamm-aging, like laminitis, may be linked with these endocrinopathies, immune and endocrine measures were compared in geriatric horses. Thryrotrpin releasing hormone (TRH) stimulation testing and dexamethasone (dex) testing were conducted to determine PPID status, while oral sugar testing (OST) determined insulin resistance.

Forty-three old horses (mean 23.2±3.8 yrs) were used to measure immune and endocrine parameters. Heparinized blood was collected aseptically, and peripheral blood mononuclear cells (PBMCs) were isolated, purified, and antibody-stained intracellularly for interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα). Flow cytometry was performed to determine the percent of lymphocytes producing IFNγ and TNFα. TRH stimulation testing was also performed, in which adrenocorticotropic hormone (ACTH) levels were measured in plasma pre, 10 minutes post (T-10), and 30 minutes post (T-30) intravenous administration of TRH (1 mg/mL saline/horse). For dex testing, serum cortisol levels were analyzed prior to and 19-20 hours post intramuscular dex (0.04 mg/kg) injection. An OST was performed to measure serum insulin pre and 1 hour post administration of a bolus of light corn syrup (0.15 mL/kg).

To compare immune and endocrine parameters, Pearson correlation testing was performed. Basal cortisol and %TNFα showed a significant negative correlation (r = -0.316, p = 0.0290), while %IFNγ and %TNFα showed strong positive correlation (r = 0.850, p < 0.001). Trends were observed for basal cortisol and %IFNγ (r = -0.273, p = 0.0767), %IFNγ and T-30 (r = 0.263, p = 0.0884), %TNFα and T-30 (r = 0.284, p = 0.0647), and basal ACTH and %TNFα (r = 0.259, p = 0.0934). Among endocrine parameters, positive correlations were significant (p < 0.05) between basal ACTH and T-10, T-30, basal insulin, and post dex cortisol. Significance (p < 0.05) was also determined between T-10 and T-30; T-10 and post dex cortisol; T-30 and post dex cortisol; and basal insulin and post OST insulin. These results indicate correlations between basal cortisol and %TNFα, as well as alignment between diagnostics for PPID and a relationship between PPID and hyperinsulinemia. Further investigations of other inflammatory markers may be stronger correlates with endocrinopathies, indicating additional biomarkers for these conditions as well as elucidating underlying mechanisms.
The objectives of this study were to longitudinally evaluate the prevalence of *C. difficile* shedding by foals, characterize *C. difficile* isolates and evaluate the impact of *C. difficile* shedding by mares on colonization of their foals. Fecal samples were collected from foals from birth to four months of age on southern Ontario breeding farms. A questionnaire was applied at each farm to collect information about foaling practices, biosecurity, and disease management to assess risk factors associated with fecal shedding of pathogens and development of diarrhea.

Fecal samples (n=1830) were collected from 162 Thoroughbred and Standardbred foals and their dams at 2 week intervals from birth until approximately four months of age on eight breeding farms in southern Ontario. Foals that developed diarrhea had additional fecal samples (n=50) collected at the time of disease occurrence. Fecal samples were cultured for *C. difficile* using standard culture techniques and characterized by PCR ribotyping and detection of toxin genes.

Of the samples processed to date, 40 of 604 (7%) samples were positive for *C. difficile*, 30/350 (8.5%) from foals and 10/254 (4%) from mares. The cumulative prevalence was 30/470 (7.5%) in foals and 10/254 (5%) in mares. Both diarrheic (4/30) and clinically healthy foals (26/30) shed *C. difficile*, and 4 mare-foal pairs each shed *C. difficile* at different points throughout the study. A majority (21/30, 70%) of foals shedding *C. difficile* were neonates (less than one month of age), and neonates were significantly more likely to shed *C. difficile* than older foals (p=0.036).

Of 29 diarrheic foal samples processed, only 4 (14%) were *C. difficile* positive (compared to 26/321 or 8% of non-diarrheic foal samples positive for *C. difficile*), and shedding was not significantly correlated with diarrhea (p=0.31). All isolates were toxigenic and 21 different ribotypes were identified, including ribotypes 001 (n=5), 078 (n=2), 027 (n=2), and 014 (n=5), which have been reported in human *C. difficile* infections. All five 014 ribotypes were found on a single farm, and other ribotypes were found in multiple samples from the same farm. Mares and foals who both shed *C. difficile* did not carry the same ribotype in samples processed to date.

These results demonstrate that younger foals are significantly more likely to shed *C. difficile* and that shedding does not have an association with diarrheal disease, likely due to the large number of other potential pathogens causing foal diarrhea. Mares and foals may not necessarily shed the same strain of *C. difficile*, but ribotypes were partially clustered by farm, suggesting geographical variation in *C. difficile* strains. The presence of ribotypes previously identified as human pathogens has further implications for biosecurity practices and public health.

E-54  

Since 2010, Zoetis has been conducting an ongoing program to evaluate the susceptibility trends of frequently used antimicrobials against equine pathogens. The data are analyzed annually and the first three years of susceptibility data for cefotiofur, penicillin, enrofloxacin, tetracycline, and trimethoprim/sulfamethoxazole against *Streptococcus equi* subspecies zooepidemicus and *S. equi* subspecies equi isolates (S. *equi* subspecies *zooepidemicus* and *Streptococcus equi* subspecies *equi* (S. *equi* subspecies *equi*) are presented here. The MICs for all strains were tested using a broth microdilution system (Sensititre Division, Trek Diagnostic Systems, Inc., Cleveland, OH) that conforms to Clinical Laboratory Standards Institute (CLSI) guidelines. Bacterial strains isolated as the etiological agent in diseased horses were submitted to this monitoring program by 20 veterinary diagnostic laboratories throughout the United States (US) and Canada and analyzed for minimal inhibitory concentration (MIC) testing. A total of 1,104 isolates were analyzed from 2010 – 2012.

The MIC data collected during the first three years of this program showed that the MIC<sub>50</sub> and MIC<sub>90</sub> values remained stable (within one doubling dilution) for each bug/drug combinations for each year of surveillance. Ceftiofur showed good *in vitro* activity against *S. equi* subspecies *zooepidemicus* and *S. equi* subspecies *equi* isolates tested, with MIC<sub>50</sub> and MIC<sub>90</sub> values ranging from 0.06-0.12 µg/ml. Both the MIC<sub>50</sub> and MIC<sub>90</sub> values for penicillin against *S. equi* subspecies *zooepidemicus* and *S. equi* subspecies *equi* isolates remained the same (0.06 µg/ml). For enrofloxacin against *S. equi* subspecies *zooepidemicus* and *S. equi* subspecies *equi*, MIC<sub>50</sub> and MIC<sub>90</sub> values remained consistent, ranging from 1-2 µg/ml. The MIC<sub>50</sub> and MIC<sub>90</sub> values from 2010-2012 for tetracycline against *S. equi* subspecies *equi* isolates, 0.25-0.5 µg/ml and 1 µg/ml, respectively, were several dilutions lower than the MIC<sub>50</sub> and MIC<sub>90</sub> values for tetracycline against *S. equi* subspecies *zooepidemicus*, 8 µg/ml and >32 µg/ml, respectively. Values for trimethoprim/sulfamethoxazole against *S. equi* subspecies *zooepidemicus* and *S. equi* subspecies *equi* remained consistent, with MIC<sub>50</sub> values ranging from 0.12-0.25 µg/ml and MIC<sub>90</sub> values ranging from 0.25-0.5 µg/ml, for both *Streptococcus equi* subspecies tested.

Overall, susceptibility data analyzed in 2011 and 2012 were comparable to the data generated during the initial year of this program. Surveillance of the antimicrobial susceptibility of veterinary pathogens is an important component of good stewardship of veterinary antimicrobial agents.
E-56  DETERMINING PERSISTENCE OF MODIFIED-LIVE EQUINE INTRanasal VACCINE PATHOGENS IN NASAL SECRETIONS USING REAL-TIME PCR AND CONVENTIONAL CULTURE. N. Posterla, C. Harms, C. Rocha, N. Akana, S. Mapes, C. Wademan. School of Veterinary Medicine, University of California, Davis, CA.

The equine modified-live intranasal vaccines available for strangles (Streptococcus equi subsp. equi) and equine influenza virus (EIV) are commonly used in horses during outbreaks of respiratory disease due to their ability to induce a rapid onset of immunity. However, real-time PCR testing from a nasal swab does not distinguish between vaccine and wild-type pathogens. Thus, this study aimed to provide a timeline for how long after vaccination a horse will continue to shed modified-life vaccine pathogens and to assist with interpretation of nasal swab real-time PCR results originating from symptomatic but recently vaccinated horses.

Twenty-three adult and cohabiting horses were randomly assigned to one of two vaccine groups (12 horses in S. equi group and 11 horses in EIV group), with two unvaccinated horses per group serving as environmental sentinels. Both nostrils of all horses were swabbed daily for up to 10 days post-vaccination. The swabs were processed and analyzed via real-time PCR and culture (S. equi group only).

The S. equi group developed a transient mild serous nasal discharge following initial vaccine administration, along with mandibular lymphadenopathy primarily on the vaccinated side. Eight of twelve horses tested positive by real-time PCR for S. equi on day 1 post-vaccination, including one unvaccinated sentinel. Only one horse still tested real-time PCR positive for S. equi by day 2. Most positive samples originated from the unvaccinated nostril. Upon revaccination at 3 weeks, the S. equi group displayed less nasal discharge and mandibular lymphadenopathy compared to the first period. Three horses tested real-time PCR positive on day 1, all in the vaccinated nostril. No more positives were detected on following days during the remainder of the period. Cultures never tested positive for S. equi during either trial period, however, the vaccine strain itself was successfully cultured. The EIV group displayed no significant changes upon physical examination following vaccination. Seven of the 11 horses tested real-time PCR positive for EIV on day 1, live on day 2 and one on day 3. Only samples taken from vaccinated nostrils tested positive for EIV.

Our data indicates that a positive real-time PCR result for S. equi subsp. equi beyond day 2 post-vaccination or day 1 after a 3-week booster vaccination is likely due to a natural infection. Further, a positive PCR result for EIV beyond day 3 post-vaccination is likely unrelated to the vaccine EIV strain. The likelihood of a conventional microbiological culture testing positive from a recently administered S. equi subsp. equi modified-live vaccine is negligible.


Epizootic lymphangitis (EZL), a chronic contagious fungal disease, is a devastating welfare problem of horses, mules and donkeys. Epizootic lymphangitis is endemic in Ethiopia, with an average prevalence of 18% being reported within a population of more than eight million equids (6.2 million donkeys, 2.0 million horses and 0.38 million mules). Current treatment measures offer limited efficacy and there are no preventative measures in low-income countries where the disease is endemic.

The Society for the Protection of Animals Abroad (SPANA) provides treatment for horses with EZL in seven towns in central Ethiopia. Case histories and clinical records of 61,752 horses that presented at the SPANA-Ethiopia project over five years (July 2008 to June 2013) were analysed.

Data revealed that EZL cases presented throughout the year, but with a lower number of cases presenting between April and July. The prevalence of EZL in the towns was: Akaki (11.1%), Debre Zeit (7.7%), Modjo (6.5%), Adama (3.0%), Shashemene (2.9%) and Hawassa (1.9%). This showed significant reductions in the prevalence of EZL in the majority of these towns from data previously published. In 2011, five horses presented with EZL in Debre Brehan, a high land area (2750 meters above sea level), after movement from an endemic mid altitude area. All horses were successfully treated. No EZL cases were reported before or after this event in this town.

In conclusion, EZL is a major health and welfare problem of equids in Ethiopia. The prevalence reduction recorded by the SPANA-Ethiopia project could be attributed to owner awareness initiatives, the treatment of early, mild cases and the euthanasia of severely affected horses. Further research is needed, including a comprehensive investigation into EZL epidemiology and transmission in order to identify risk factors and develop preventative strategies to reduce the incidence of this disease within the equid population of Ethiopia.

E-58  CLINICOPATHOLOGIC FINDINGS ASSOCIATED WITH INTRATHECAL CATHETER USE IN HORSES. A.D. Ramos, A.J. Stewart, E.A. Spangler, T. Hathcock, S.H. Duram, J. Costello. College of Veterinary Medicine, Auburn University, AL.

Intrathecal catheters allow for experimental measurement of cerebrospinal fluid (CSF) drug concentrations and clinical administration of analgesics, antimicrobials and chemotherapeutics. Published reports on their utilization in horses is sparse. This study aimed to document the effects of indwelling intrathecal catheters in horses as evidenced by physical examination findings, CBCs, CSF analysis with cytology and bacterial cultures.

Six healthy adult horses had catheters placed into their lumbo-sacral space for a period of up to 12 days on two occasions. Physical examination was performed daily. CSF and blood samples were analyzed on day one, at the midpoint, and in conjunction with a CSF culture at the end of each catheterization. 9/11 catheterization periods were uneventful. All horses had a mild to marked pleocytosis on at least one occasion with or without the presence of extracellular bacteria. Bacterial cultures detected organisms considered as contaminants. One horse developed acute fever, tachypnea, anorexia and lethargy. Systemic neutrophilia, leukocytosis and CSF neutrophilic pleocytosis prompted immediate catheter removal and treatment with trimethoprim-sulfonamide for five days. Recovery was rapid. In another horse, inadvertent aspiration of air resulted in severe neurologic signs and tachypnea. Despite marked neutrophilic inflammation in the CSF, no fever or systemic CBC abnormalities were detected. Pneumococcal meningitis was suspected and the horse recovered without catheter removal or treatment.

Intrathecal catheters in horses may be a valuable research and clinical technique, but careful monitoring of all clinicopathologic findings should be performed. CSF neutrophilia may occur due to irritation from the catheter, bacterial infection, or pneumococcal meningitis.

E-59  EXPERIMENTAL ENDOTOXEMIA IN HORSES INDUCES INSULIN RESISTANCE AND ALTERATIONS IN LIPIDS AND LIPOPROTEINS. J. Broer, A. Edner, K. Nostell. Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, Sweden.

The time course and nature of the lipoprotein response to endotoxins varies depending on species but this subject has not been studied in horses. The objective was to evaluate the metabolism of lipids and lipoproteins in horses with experimentally induced endotoxemia. Endotoxemia was induced in eight healthy Standardbred horses (5 mares and 3 geldings; age 3 – 14 years)
by intravenous continuous rate infusion of 500 ng/kg endotoxin over 6 hours. Six baseline blood samples were collected over 24 hours before the start of the endotoxin infusion followed by sampling every 2 to 3 hours over the next 72 hours. All horses developed signs of endotoxemia including depression, pyrexia, tachycardia and tachypnea. A proxy index of insulin sensitivity, the reciprocal of the root square of insulin (RISQI), was calculated. The RISQI values were lowest between 8 and 21 hours post endotoxin infusion.

The serum triglyceride concentration started to increase at the end of the endotoxin infusion and, compared to baseline, the increase was approximately six-fold between 21 and 48 hours post endotoxin infusion. The endotoxin-induced hyperlipidemia resulted mainly from accumulation of very-low-density lipoprotein (VLDL) and to a minor extent of high-density lipoprotein (HDL) and low-density lipoprotein (LDL). The increase in VLDL and HDL preceded the increase in LDL. The serum concentration of total cholesterol did not change over time but VLDL-cholesterol increased and LDL-cholesterol decreased between 27 and 48 hours post endotoxin infusion. The lipid response to a bolus of endotoxins appears to have an unusually long duration in the horse.

E-60
BREED DIFFERENCES IN INSULINEMIC RESPONSE AFTER FEEDING HAYLAGE WITH DIFFERENT CONTENT OF WATER SOLUBLE CARBOHYDRATES.
S. Lindäse, K. Nostell, C. Müller, M. Jensen-Waern, J. Bröjer. 1Department of Clinical Sciences, 2Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The study aim was to compare the postprandial glycemic and insulinemic responses to haylage with different contents of water soluble carbohydrates (WSC), and whether these responses differed between a pony-related breed (Icelandic horses) and a light horse breed (Standardbreds). Sixteen healthy horses (8 Icelandic horses and 8 Standardbreds): age 13 ± 5 years, body condition score 5.6 ± 0.6, were included in the study. Horses were randomly assigned to one of three groups fed haylage containing 180 (HC), 130 (MC) or 40 (LC) g WSC/kg DM. Each haylage was fed for seven days in a cross-over design. Horses were fed 100% of their metabolizable energy requirement for maintenance daily, divided into 3 meals. The morning of the last day of each period (day 7), horses were fed 50% of their daily energy requirement. Blood samples were drawn before feeding and every 30 minutes post-feeding for 5 hours.

In Standardbreds, insulin concentrations at 120 min post-feed increased with increasing WSC-content of haylagets, whereas no differences were found for Icelandic horses. The area under the curve for insulin response (AUCins) was similar for HC, MC and LC haylagets respectively in Icelandic horses. In contrast, the AUCins was higher for HC and MC compared to LC haylage in Standardbreds. The glucose response (AUCglu) was higher for HC and MC compared to LC in both breeds. In conclusion, the study indicated that breed-related differences in insulinemic response occur after feeding the same haylagets; possible due to different insulin sensitivity and/or incretin effects.

E-61
DIET-INDUCED WEIGHT GAIN AND ACCESS TO PAS-TURE DO NOT DECREASE INSULIN SENSITIVITY.
S. Lindäse, K. Nostell, C. Müller, J. Söder, M. Jensen-Waern, J. Bröjer. 1Department of Clinical Sciences, 2Department of Animal Nutrition and Management, 3Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The effect of diet-induced weight gain based on a low-starch high-fat and fibre (LS-HFF) diet followed by access to pasture on insulin sensitivity (IS) was evaluated in nine Standardbred mares with intermediate insulin resistance as determined by euglycemic hyperinsulinemic clamp (mean rate of glucose metabolism: 2.5 ± 0.6 mg/kg/min). The horses were fed haylage supplemented with pelleted alfalfa and fat. During a period of 22 weeks, weight gain was achieved through a gradual increase in the feed ration using a protocol starting at 100% of the maintenance requirement of metabolizable energy and reaching a maximum of 250 ± 10% of the maintenance requirements. Horses were then turned out on pasture. Euglycemic hyperinsulinemic clamps (EHC) were performed before and after weight gain, and 3 weeks after pasture turnout. The body weight, body condition score (BCS) and plasma concentrations of insulin and triglycerides were measured regularly.

Body weight increased by 9% from 497 ± 44 kg to 542 ± 47 kg and BCS increased from 5.5 ± 0.6 to 7.1 ± 0.4 during the weight gain period. Plasma insulin increased over the weight gain period whereas plasma triglycerides increased during pasture. There was no difference in IS or metabolic clearance rate of insulin (MCR) over the weight gain period as determined by EHC, whereas 3 weeks turn out on pasture improved IS and MCR generally by 54% and 32% respectively. In conclusion, short term induced weight gain in intermediate insulin resistant horses using a LS-HFF diet did not decrease IS.

E-62
EQUINE ODONTOCLASTIC TOOTH RESORPTION AND HYPERCEMENTOSIS: A CAUSE OF WEIGHT LOSS IN AGED HORSES.

The objective was to describe the signalment, presenting complaint, clinical signs, radiographic findings, treatment, progression and histopathology of horses with equine odontoclastic tooth resorption and hypercementosis (EOTRH).

Medical records from horses presented to a university hospital or field service between January 2000 and January 2012, diagnosed with cementoma, hypercementosis, cementoblastoma, or cement-osseous dysplasia, were studied. Follow-up telephone surveys were conducted to determine long-term outcomes.

Fourteen cases were identified. There were 13 geldings and one stallion, with no breed predilection. The age was (mean ±standard deviation) 23 ± 5.5 years. The presenting complaints were: weight loss (n = 5), gingivitis (n = 4), oral ulceration (n = 4), fractured teeth (n = 3), lost teeth (n = 3), decreased appetite (n = 1), and nasal discharge (n = 1). Clinical signs included: gingival swelling and ulceration (n = 8), periodontal pockets (n = 9), firm swelling over tooth roots (n = 6), and abnormal dentition (n = 2). Radiographic changes included: lysis/resorption of teeth (n = 11), soft tissue and bony proliferation at tooth roots (n = 11), and fractures of teeth and alveolar bone (n = 10). Many teeth had radiographic but no clinical disease (n = 8). Teeth extraction resolved clinical signs in 8/9 cases with available follow-up. One horse had progression of disease in oral clinical and radiographic evaluation 3 years apart; most horses (n = 6) had no remaining incisors due to extractions or loss. Histopathologic diagnoses (n = 6) were: benign dental cementoma (n = 4), EOTRH (n = 1), hypercementosis (n = 3), and apical periodontitis (n = 1).

EOTRH is an uncommon, slowly progressing disease, primarily affecting older geldings. EOTRH can be diagnosed clinically, however radiographic evaluation is more sensitive. EOTRH has a good prognosis with incisor removal.

E-63
USE OF A GENERALIZED BOOSTED REGRESSION MODELING (GBM) TO DEVELOP A SURVIVAL SCORING SYS-TEM FOR HOSPITALIZED FOALS.
K.A. Dembeck1, S.D. Hurcombe1, N.M. Slovis2, P.R. Morresey3, R.E. Toribio1. 1The Ohio State University, College of Veterinary Medicine; Columbus, OH, USA, 2Hagyard Equine Medical Institute, Lexington, KY, USA, 3Rood and Riddle Equine Hospital, Lexington, KY, USA.

Diagnosis and treatment of critically ill foals is often expensive and labor intensive, and the survival rate for septic foals is
around 50%. Predicting the odds of foal survival using basic clinical information could facilitate the decision-making process for owners and clinicians. Numerous prognostic indicators and mathematical models to predict the release of live foals from equine hospitals have been published; however, to our knowledge, a scoring method to predict survival in sick newborn foals has not been reported. The goal of this study was to develop and validate a scoring system that can be used by clinicians to predict survival shortly after admission.

### Table 1. Survival score in hospitalized neonatal foals

<table>
<thead>
<tr>
<th>Survival score in hospitalized neonatal foals</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold extremities no</td>
<td>yes</td>
</tr>
<tr>
<td>Prematurity no</td>
<td>yes</td>
</tr>
<tr>
<td>≥2 infection/inflammation sites</td>
<td>0</td>
</tr>
<tr>
<td>IgG (mg/dL) ≤400</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Glucose (mg/dL) &lt;80</td>
<td>&gt;80</td>
</tr>
<tr>
<td>WBC (10^3/μL) ≤4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>TOTAL SCORE</td>
<td></td>
</tr>
</tbody>
</table>

Medical records from 339 hospitalized foals admitted to 3 equine hospitals, ≤4 days (median age 12 h) old were included to develop the model (retrospective study). Thirty seven variables including historical information, physical examination and laboratory findings were entered into GBM to determine which one would be included in the survival score. Of these, six variables (prematurity, cold extremities, ≥2 infection/inflammation sites, white blood count, IgG and glucose concentrations) were retained in the final model for the survival score (Table 1). The weight (individual score) for each variable was calculated using generalized linear model and subsequently the probability of survival for each total score (range 0-7) was calculated. The highest (7) and the lowest (0) scores represented 97% and 3% probability of survival, respectively. Accuracy of this survival score was validated in the prospective study on data from 283 hospitalized foals. Sensitivity, specificity, positive and negative predictive values of the survival score were 96%, 71%, 91%, and 85% respectively, in the prospective population.

The survival score developed in our study is easy to apply using data available in most equine hospitals and validated in a large number of foals with a wide range of diseases. Further evaluation and validation of this scoring system in field conditions will strengthen its use in clinical practice.

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E-64

**LAMINITIS INDUCTION IN THOROUGHBRED HORSES USING A PROLONGED EUGLYCEMIC HYPERINSULINEMIC CLAMP TECHNIQUE (P-EHC) AND THE EFFECTS OF PENTOXIFYLLINE ON LAMENESS ASSOCIATED WITH THIS MODEL: PRELIMINARY RESULTS.** L. A. Fuqier, S. C. Eades, A. M. Chapman, L. M. Riggs, P. Camacho. Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

A p-EHC has been used previously to induce laminitis in horses and Standardbred horses, but has not been evaluated in Thoroughbreds. Pentoxifylline reduces lameness associated with carbohydrate overload-induced equine laminitis and has been shown to inhibit insulin resistance in rats. Therefore, we hypothesized that laminitis could be induced in Thoroughbred horses using a p-EHC, and that pentoxifylline would reduce/prevent lameness associated with this model.

Eighteen Thoroughbred horses (n=6 control, n=6 p-EHC, and n=6 p-EHC + pentoxifylline) are being used in this study. P-EHC horses receive a continuous infusion of insulin and dextrose until the development of Obel grade 2 laminitis. Treated horses receive pentoxifylline (8.5 mg/kg) in 1 L saline solution over 30 minutes, IV q 12 hours) in addition to the p-EHC. Control horses receive LRS. Blood is collected and clinical parameters are evaluated until Obel grade 2 laminitis develops or for 48 hours. Serum samples are analyzed for insulin resistance, insulin sensitivity, and glucose metabolism.

Pentoxifylline treated horses have developed Obel grade 2 laminitis within 45 hours, and 3/4 pentoxifylline treated horses have not developed lameness within 48 hours. More data is forthcoming.

Preliminary data suggests that this p-EHC model will induce laminitis in Thoroughbred horses. Subsequent data will determine if pentoxifylline is useful in treating/preventing lameness associated with metabolic laminitis.

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E-65


In equine sports medicine, thermoregulation is widely investigated due to several metabolic and musculoskeletal disorders. The aim of this study was to assess, by thermography, temperature changes in thoracic and pelvic regions of Quarter Horses practicing team roping.

For this purpose, six trained sound Quarter Horses (three heading and three heeling horses) performed three sequential ropes each pair. Images were taken from left thoracic fetlock, shoulder and croup areas before (PRE), immediately after the last steer (POST) and ten minutes after exercise (recovery time - REC). Images were captured using a Flir T300® thermal imaging camera and analyzed with the software Flir Quick Report®. Data were analyzed using a statistical program. In order to determine if there were significant differences between moments ANOVA was used followed by Tukey’s test. The program has adopted the significance level of 5%.

Minimum temperatures of shoulder and croup increased immediately after exercise, returning to pre-exercise values after the recovery time (PRE 33.1 ± 0.5°C and 33.3 ± 0.1°C; POS 34.4 ± 0.8°C and 34.7 ± 0.9°C; REC 34.0 ± 0.95°C and 34.4 ± 0.87°C, respectively), but minimum temperatures of thoracic fetlock increased only ten minutes after exercise (PRE 31.0 ± 0.87°C; POS 31.4 ± 1.2°C; REC 32.6 ± 0.72°C).

We can conclude that exercise increases surface temperature of body and limbs in horses submitted to team roping and that thermoregulation is more effective in the body. This data reinforce the importance of cooling down limbs after exercise, aiming not only tendons but also other structures of locomotor apparatus.

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E-66

**POST-MORTEM ‘FAT SCORES’ FOR REGIONAL ADIPOSE TISSUE DEPOTS AND THEIR ASSOCIATION WITH BODY CONDITION SCORE IN EQUIDAE.** P. Morrison, P. Harris, C. Maltin, D. Grove-White, C. M. Argo. 1University of Liverpool, UK., 2WALTHAM Centre for Pet Nutrition, Leicester, Leicestershire, UK.

Body condition score (BCS) is a poor predictor of body fat in obese Equidae and fails to account for internal fat deposition. Over 50% of UK leisure horses/ponies are overweight/obese. In man, internal adipose depots are associated with increased disease risk. Study One, developed post-mortem scores for 6 regional adipose depots. Study Two, evaluated associations between ante-mortem BCS and post-mortem ‘fat scores’ (FS) in horses/ponies euthanased for non-research purposes.
Study One: Photographic images of anatomically-defined, omental, mesenteric, epididymal and rump adipose depots (40 animals), across the full range of BCS, were ranked and deposited specific, FS descriptors were developed. Depths of nuchal-crest and ventro-abdominal-retroperitoneal adipose depots were recorded. The range of excessive fat present). Depths of nuchal-crest and ventro-abdominal, mesenteric, epididymal, rump, nuchal-crest and ventro-abdominal-retroperitoneal FS [1-5] and carcass weight.

Multi-variable, random effect linear regression models were fitted with breed as random effect and BCS as the outcome variable. All phenotypic and FS data were offered to the initial model as explanatory variables. Model fit was assessed and compared using Likelihood-ratio tests. The final model had crest, retroanterioral, and rumus FS, height, and carcass weight as explanatory variables for BCS. Neither omental, mesenteric nor epididymal FS were retained. Similarly, a model was built using FS data only to predict BCS. Again, neither omental, mesenteric nor epididymal FS were associated with BCS. There appeared to be an underlying trend of phenotypic data on rumus FS (increase in magnitude of its coefficient), whilst values for nuchal-crest and ventro-abdominal-retroperitoneal FS remained largely similar to those in the full model.

Clear differences in relationships between BCS and the 6 discrete FS suggest that the specific adipose tissues evaluated may have different roles in terms of function and obesity development.

E-67 POST-MORTEM STABILITY OF RNA AND TISSUE-SPECIFIC EXPRESSION OF MYOSTATIN, ACTIVIN RECEPTOR IIB, FOLLISTATIN AND PERILIPIN IN THE HORSE, P. Morrison1, P. Harris1, C. Bing1, C. Maltin1, D. Grove-White1, C.M. Argo1, 1University of Liverpool, UK., 2WALTHAM Centre for Pet Nutrition, Leicester, UK.

Myostatin (MSTN) and perilipin (PLIN) are respectively considered as key regulators of muscle mass and stored-fat lipolysis. Both proteins are implicated in the development of human and murine obesity. An understanding of 1) post-mortem time-contrasts for the extraction of quality RNA from tissues and 2) the anatomical distribution of expression are pre-requisites for further studies in horses.

Objectives were addressed in 2 studies using tissues from healthy Thoroughbreds (6-20 yrs), euthanased for non-research purposes. Study One: Maseter muscle and peri-renal adipose tissues were collected post-mortem from 3 horses. Sub-samples of each tissue were snap-frozen in liquid nitrogen at nine time points (5 minutes to 6 hours post-mortem). The purity and integrity of RNA extracted from each sample were respectively evaluated by spectrophotometry and agarose-gel electrophoresis. Study Two; characterised tissue variation in the expression of MSTN, activin receptor IIB (ActRIIB; MSTN receptor), follistatin (inhibits MSTN:receptor binding) and PLIN in a further 4 horses. Protein and RNA expression of each tissue were examined across 7 age groups and 6 cell types.

Study One: Overall, good quality, intact RNA was extracted for up to 30 minutes post-mortem from adipose tissue and up to 2 hours from skeletal-muscle in each horse. Study Two: MSTN and ActRIIB genes and proteins were almost exclusively expressed by skeletal muscles. Follistatin (gene) was widely expressed across many tissues. PLIN gene and protein expression were predominantly adipose-specific.

E-68 CHARACTERIZATION OF LATE OUTGROWTH ENDOTHELIAL COLONY FORMING CELLS IN ADULT HORES, M.M. Salt1, L.C. Sanchez2. 1University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, 2College of Veterinary Medicine, University of Florida, Gainesville, FL, USA.

Endothelial colony forming cells (ECFCs) circulate in peripheral blood and function in vascular formation and repair. The number and function of ECFCs are emerging biomarkers for metabolic and cardiovascular disease in humans. ECFCs are also used for treatment of ischemia and vascularization of engineered tissues. ECFCs have not been characterized in horses and may have important roles in regenerative therapies and in endothelial dysfunction associated with metabolic disease or sepsis.

The purpose of this study was to culture ECFCs from equine peripheral blood and characterize these cells as true ECFCs using functional assays and marker expression. Heparinized blood (5 mL) was collected from 24 adult horses, cultured with endothelial media, and evaluated for colony formation. Two-dimensional vascular tube formation in Matrigel and uptake of Dil-acetylated low density lipoprotein (Dil-Ac-LDL) were assessed at increasing cell passage. Expression of cell markers was evaluated using indirect immunofluorescence.

Three of 24 horses produced colonies at 12 ± 2.45 days with 3.50 ± 1.50 colonies/mL blood. Cells displayed single layer cobblestone morphology and significant outgrowth upon expansion. Equine ECFCs formed vascular tubes and 85% of the cells took up Dil-Ac-LDL. Functional activity was gone at passage 16 to ±1.15. Equine ECFCs were positive for endothelial markers vWF, CD34, and CD105 and negative for the late hematopoietic marker CD14.

ECFCs can be cultured from peripheral blood samples of horses and share functional and cell marker characteristics of ECFCs from other species. ECFCs have potential therapeutic use in diseases associated with ischemia or delayed vascularization in the horse.

E-69 PHYSICOCHEMICAL INTERPRETATION OF ACID-BASE DISORDERS IN 793 HOSPITALIZED FOALS, D.E. Gomez1, N.M. Biermann1, L.C. Sanchez2. 1University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada, 2College of Veterinary Medicine, University of Florida, Gainesville, FL, USA.

Physicochemical approach offers an alternative analysis of acid base disorders, quantifying the contributions of strong ions, pCO2 and plasma concentrations of major strong acids in plasma pH. The study objective was to determine and characterize the mechanisms of acid-base abnormalities in hospitalized foals.

The study included 793 foals ≤ 7 days of age, admitted to UF from 1982-2008. Arterial blood gases, plasma electrolytes Na+, K+ and Cl- and total plasma protein (TP, g/L, refractometer) were collected. Strong ion difference was calculated as SIDi = (Na+i+ K+) - (Cl-). Total charge of the plasma protein as A tot = 0.22 x (TP(g/L); strong ion gap as SIG, r 2 23%, P < 0.001; SIG, r 2 23%, P < 0.001. P=0.001). A forward stepwise logistic regression pattern was performed to determine the quantitative association between measured pH and physicochemical variables (SIDi, A tot and SIG).

The most common physicochemical acid-base events were weak acid (hypoproteinemic) alkalosis (672 foals, 84.7%) and SIG acidosis (616 foals, 77.6%). Weak acid alkalosis and SIG acidosis were concomitantly present in 536 (67%) foals. Changes in plasma pH resulted primarily from alteration of strong ions (SIDi, r=30%, P=0.001; SIG, r=23%, P=0.001; P=0.001) and [A tot (r=28%) and [A tot (r=8%) also contributed (P=0.001). The explanatory power of the model was 89%.

These results emphasize the importance of plasma proteins and strong ions in determining acid-base balance. Further characterization of physicochemical acid-base disorders of foals with specific diseases is warranted.
E-70


Laminitis incidence varies with season and predisposition to laminitis may be associated with hyperinsulinaemia. Hyperinsulinaemia can impair endothelial function, an effect which may play a role in the predisposition to laminitis. The gold standard method for non-invasive assessment of endothelial function in humans is flow mediated vasodilation (FMD). Measurement of arterial diameter via ultrasonography before and after induced reactive hyperaemia allows assessment of endothelium dependent vasodilation. An alternative method for detecting this induced vasodilation is measuring the change in pulse wave velocity (PWV) along the arterial segment. As part of investigations into the relationships between season, laminitis predisposition and endothelial function in the horse, FMD and PWV were measured to assess endothelial function.

Flow mediated vasodilation was measured in previously laminitic (PL, n=6) and non-laminitic ponies (NL, n=6) at 4 seasonal time points (spring, summer, autumn and winter). The median artery diameter obtained by automated edge detection software was plotted against time to give a graphical representation of FMD. The numerical value obtained for FMD (percentage increase in arterial diameter following reactive hyperaemia) was compared to visual inspection of the graphical representation of median artery diameter. Each pony was obtained as judged as either compatible with diastolic diameter occurring in the median artery, or an artefact generated by movement of the ultrasound transducer relative to the artery. Pulse wave velocity was measured in PL and NL ponies on one occasion and in one pony on six occasions by two observers to evaluate intra-pony within and intra- and inter-observer agreement. Coefficients of variation (CV) and intra-class correlation coefficients (ICC) were reported as appropriate. All ponies were kept under the same management regimen with ad lib access to pasture and no supplemental feeding apart from hay in winter.

The calculated value for FMD was considered accurate in 3/48 occasions, on other occasions FMD was considered more likely due to an artefact (32/48) or the FMD value was higher (11/48) or lower (2/48) than suggested by visual examination of the graphical trace record. Measurement of PWV was highly variable with CVs ranging from 35.9% to 94.6%. The ICCs were low for repeated measurements between (0.08) or within observers (0.0).

Neither FMD nor PWV proved to be suitable methods for assessing endothelial function in horses due to lack of accuracy or precision. This reflects the difficulty in obtaining reproducible ultrasound images in the weight-bearing equine limb, in contrast to the supine human subject. Alternative methods for assessing endothelial function in the horse should be pursued.

E-71


The relationship between the occurrence of colic and laminitis manifestation has been studied by numerous researchers. Some studies with experimental induced laminitis demonstrated involvement of the inflammatory response in the development of the disease. Therefore, the aim of this study was to investigate the inflammatory response in the horse’s hoof dermal and epidermal tissues by real time PCR and the histological changes after intraluminal small colon obstruction.

For this purpose, eight healthy adult mixed-breed horses were subjected to a retro-umbilical celiotomy to induce intraluminal distension of the small colon, associated with hoof dorsal transmural biopsies. Ethical approval: Comissão de Ética e Bem Estar Animal (CEBEA) - Protocol no 007568-09). The pedal dermal and epidermal tissue were collected before intestinal obstruction (M0), after 4 hours of obstruction (M4 - when the ball was deflated and removed) and 72 hours after decompression (M72). Data were submitted to a normality test and then analysis between moments was performed by using One-Way ANOVA and Duncan’s post hoc test.

This model induced alterations in tumor necrosis factor-α (TNF-α) in the hoof, the means at M72 was significantly higher when compared to M0. Nevertheless the variables interleukin (IL) -1ß and IL-6 showed no significant difference. Mean of TNF-α (+ standard error) was M0 = 0.733 ± 0.160; M4 = 2.336 ± 0.979 and M72 = 7.577 ± 2.258. IL-1ß was M0 = 0.170 ± 0.141; M4 = 0.003 ± 0.001 and M72 = 0.002 ± 0.002. IL-6 was M0 = 0.206 ± 0.161; M4 = 1.107 ± 0.322 and M72 = 0.002 ± 0.521. Histologically was observed in M72 increase distance from tip of secondary dermal laminae to keratinized axis of primary epidermal laminae, the basal cells lost their normal shape and presented pyknotic nuclei. When stained with periodic acid Schiff the basement membrane showed thickening.

The implementation of the latex ball was effective to mimic an intestinal non-strangulating intraluminal obstruction, leading to changes in hoof dermal and epidermal tissue, suggestive of laminitis.

E-72


Although gentamicin is highly active against Rhodococcus equi, the clinical efficacy has been limited presumably due to poor cellular uptake. We previously demonstrated that administration of a single dose of liposome-encapsulated gentamicin results in significantly higher drug concentrations in bronchoalveolar cells compared with free gentamicin. The objective of this study was to compare the pharmacokinetics and tolerability of liposomal and free gentamicin in foals after repeated administration.

E-73


Although gentamicin is highly active against Rhodococcus equi in vitro, its clinical efficacy has been limited presumably due to poor cellular uptake. We previously demonstrated that administration of a single dose of liposome-encapsulated gentamicin results in significantly higher drug concentrations in bronchoalveolar cells compared with free gentamicin. The objective of this study was to compare the pharmacokinetics and tolerability of liposomal and free gentamicin in foals after repeated administration.
Twelve healthy 4- to 6-week-old foals were administered either liposomal or free gentamicin at 6.6 mg/kg IV q 24 h for 7 doses. Concentrations of gentamicin were measured in plasma, urine and bronchoalveolar cells using HPLC-MS. Urinary protein, creatinine, γ-glutamyltransferase, and electrolytes were measured on days 0, 3 and 7 to quantify renal injury. Data were analyzed using a two-way repeated measure ANOVA.

Liposomal gentamicin was well tolerated by all foals and indicates of renal injury were not significantly different from those of foals administered free gentamicin. Mean (± SD) peak gentamicin concentrations in bronchoalveolar lavage cells were significantly \( P = 0.015 \) higher for liposomal gentamicin \((12.1 ± 5.2 \mu g/mL)\) compared with free gentamicin \((6.7 ± 1.9 \mu g/mL)\). Plasma elimination half-life was significantly \( P = 0.012 \) longer for liposomal gentamicin \((7.9 ± 3.4 \text{ h})\) than for free gentamicin \((4.7 ± 1.2 \text{ h})\). There was no evidence of drug accumulation over the 7 day period.

Administration of IV liposomal gentamicin once daily for 7 days is well tolerated and results in significantly higher gentamicin concentrations in bronchoalveolar cells compared with free gentamicin.

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**E-74**

**SERUM AND CEREBROSPINAL FLUID CONCENTRATIONS OF GENERIC LEVETIRACETAM AFTER MULTIPLE ORAL DOSING IN HEALTHY ADULT HORSES.** J. Costello, A.J. Stewart, D.M. Booth, S.H. Duran, A.D. Ramos, F.B. Cesar. College of Veterinary Medicine, Auburn University, AL.

Levetiracetam (LEV) is a novel anti-epileptic drug that prevents neurotransmitter release by interacting with synaptic vesicle proteins. Our purpose was to determine changes in serum and CSF concentrations over 9-days of LEV administration.

Six healthy adult horses were administered 30 mg/kg q12 hr of a generic LEV for 9 days. Serum and CSF samples were collected at predetermined intervals and analyzed via an immunosassay validated in horses.

Serum maximum concentration (Cmax) after the first dose was 20.1 ± 5.6 μg/mL after 9.0 ± 5.2 hr. Cmax for CSF was >110 ± 30 μg/mL at >13.3 ± 5.5 hrs, but still rising in some horses with the final sample collection. The predicted area under the curve to infinity (AUC) in serum was 338 ± 21 hr*μg/mL.

Serum concentrations 12 hrs after the 1st, 8th and 18th doses were 12.9 ± 2.6, 28.8 ± 5.8 and 31.4 ± 10.5 μg/mL, and CSF concentrations were 10.1 ± 3.3, 33.7 ± 7.8 and 36.8 ± 10.6 μg/mL, respectively. From day 1 to 8, the 12 hr serum and CSF concentrations increased 2.2 fold (P=0.004) and 3.3 fold (P=0.002), respectively, and from days 8 to 18 by 1.1 fold in CSF (P=0.01).

The therapeutic range for humans is 5-45 μg/mL, with midpoint of 25 μg/mL. LEV concentrations persisted in serum and CSF for 12 and 18 hrs, and for 30 and 42 hrs above 5 μg/mL, respectively after the last dose. Levetiracetam has favorable pharmacokinetics in serum and CSF for horses and appears to accumulate over a 9-day dosing period.

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**E-75**

**THE EFFECT OF CHRONIC, LOW-DOSE CLENBUTEROL ADMINISTRATION ON PERCENTAGE BODY FAT IN WORKING ADULT HORSES.** C. Moore, R.C. Boston, L.R. Soma, R.D. Nolen-Walston. Department of Clinical Studies - New Bolton, University of Pennsylvania, Kennett Square, PA.

Clenbuterol is an oral β2 agonist used as a bronchodilator in horses, but it has also been shown to cause an increase in muscle-directed protein deposition with concurrent reduction in total body fat (re-partitioning) at high doses in horses, humans, and food animals. We hypothesized that clenbuterol, even at low doses, has re-partitioning effects. This study was a placebo-controlled, blinded clinical trial using 47 polo ponies in work. Horses were randomized to receive either clenbuterol (0.8 μg/kg, PO q12 h) or placebo (corn syrup) for 21 days, and then were followed for a 21 day wash-out period. Body fat was estimated using the formula: %body fat = 2.47 + (5.47 x rump fat cm) cm, with rump fat measured sonographically twice weekly throughout the study.

Mean bodyfat% at baseline was 6.2%. Using mixed effects multiple regression, a significant treatment effect was seen by day 3 \( p=0.04 \) which peaked on the last day of clenbuterol, with a mean change from baseline% bodyfat of -0.80% \( SD=0.54 \) versus the control group at -0.32% \( SD=0.68 \); \( p=0.001 \).

During the wash-out period, the clenbuterol group reversed their reduction in%bodyfat, and from the 11th day of wash-out onward, there was no statistical difference between groups \( p=0.14-0.25 \). No interactions were seen between change in%bodyfat with weight, work level, sex, age, or breed.

In conclusion, clenbuterol at 0.8 μg/kg twice daily resulted in significant decreases in body fat, suggesting muscle/fat re-partitioning. This has importance regarding the potential abuse of low-dose clenbuterol as an anabolic compound in show, race, and performance horses.

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**E-76**

**THE DISPOSITION OF LIDOCAINE DURING A 6-HOUR INTRAVENOUS INFUSION TO YOUNG FOALS.** C.M. Ohmes, E.G. Davis, B. Kukanich, B.J. White. Kansas State University, College of Veterinary Medicine, Manhattan, KS.

Differences in pharmacokinetic parameters exist between young and adult animals which become especially important for drugs with a narrow therapeutic index. Therefore, while the pharmacokinetics of intravenous lidocaine have been studied in adult horses, determination of the disposition in foals is necessary before appropriate clinical use can be determined. We examined the disposition of lidocaine during a 6-hour intravenous infusion to young foals. Our hypothesis was that healthy foals would require a higher dosage compared to adults. Six healthy 8-10 week old foals were used. One catheter was placed in each jugular vein for plasma sampling and determination of accuracy in using one double lumen catheter for infusion and sampling for a subsequent clinical trial. A standard bolus (1.3 μg/kg) of lidocaine was administered followed by a 50 μg/kg/min infusion for 6 hours. Plasma lidocaine and monoethylglycinexylidide concentrations were determined over the 10 hour study period. Data for each foal was analyzed using non-compartmental analysis. Compared to adult horses, total body clearance of lidocaine was faster at 72.2 ± 7.8 ml/min/kg, elimination half-life was slower at 26.3 ± 3.7 min. Cmax was lower at 0.79 ± 0.07 μg/ml and the volume of distribution was larger at 1.8 ± 0.4 L/kg in young foals. Despite reaching the target steady state concentration for lidocaine, the presence of metabolites may help lead to therapeutic benefits. It is likely an increased dose will be necessary in foals although further investigation is required in foals suffering from illness before comprehensive dosing recommendations can be made.

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**E-77**

**RISK FACTORS FOR EXERCISE INDUCED PULMONARY HEMORRHAGE IN THOROUGHBRED RACEHORSES IN WESTERN AUSTRALIA.** E.J. Crispe, G.D. Lester, C.J. Secombe, I.D. Robertson. Murdoch University, Western Australia, Australia.

Exercise-induced pulmonary hemorrhage (EIPH) is a highly prevalent condition of racehorses worldwide. Ambient temperature has been identified as a risk factor for EIPH. This study was designed to further investigate the effect of temperature and other climatic factors, along with a range of race and performance variables, on the prevalence and severity of EIPH in a population of Australian Thoroughbred racehorses.
Over a 12-month period tracheobronchoscopy was performed in 584 animals 30-220 minutes after racing. Examinations were recorded and graded blindly by 2 experienced veterinarians using an established grading system (0-4). Univariable analyses were performed, and variables with a p-value <0.25 were entered into a multivariable logistic regression model. The analyses were conducted twice using the presence of blood (EIPH 0 vs ≥1) and EIPH ≤1 vs ≥2 as dependent variables, respectively.

Blood was noted in 56.6% of observations. Lower ambient temperature and bar shoes were significantly associated with EIPH in both models. Horses with bar shoes were 6.2 times more likely to have EIPH≥1. Lifetime starts and race distance were also significantly associated with EIPH≥1 and EIPH≥2, respectively. We were unable demonstrate any significant effect of EIPH on performance in this population.

This supports previous studies that lower ambient temperatures are associated with an increased risk of EIPH. The relationship between bar shoes and EIPH is novel and warrants further investigation.

E-78 PREVALENCE AND INCIDENCE OF TONGUE TIE USE IN RACING THOROUGHBREDS AND THE EFFECT ON PERFORMANCE. A.L. Evans, T. Morris, M.J. Green, I.M. Bowen, A.L. Evans, T. Morris, M.J. Green, I.M. Bowen, G.D. Hallowell. School of Veterinary Medicine and Science, University of Nottingham, United Kingdom.

Tongue ties are frequently used as a form of conservative intervention in Thoroughbred racehorses to prevent dorsal displacement of the soft palate. The objectives of this study were to report the prevalence and incidence of tongue tie use in Thorougbred racehorses in the UK, to identify factors associated with tongue tie use and to report the effects of tongue tie use on racing performance.

Veterinary surveillance reports, provided by the British Horse Racing Authority (BHA), from 1st January 2001 until 31st July 2012, were used to identify racehorses that were raced with a tongue tie. The overall prevalence of tongue-tie use was 6.0% and incidence 14.3% obtained from 1,072,210 race starts by 90,516 Thoroughbred racehorses. An increased prevalence (p=0.001) and incidence (p=0.001) of tongue-tie use was identified over the decade evaluated. Prevalence of tongue-tie use varied according type of race. Based on prevalence, tongue-ties were most frequently used in horses that raced over steeplechase fences (10.4%), then over hurdle fences (8.6%) and were least commonly used in horses that raced on the flat (4.5%). Multivariable models were created following univariable analysis to identify factors associated with tongue tie use. Two control groups were created that contained a maximum of three racehorse performances from those that did not wear tongue-ties that a) were trained with the same age and gender, b) were trained with the same age and gender, c) were trained with the same age and gender, d) were trained with the same age and gender.

In the multivariable model, tongue tie use was most prevalent in older horses, particularly those aged between 5-7 years and were rarely worn by maidens and novices (p=0.0001). Mares and fillies were more likely to wear tongue ties than geldings or colts (p=0.0001). Horses were considerably more likely to wear a tongue tie when competing over shorter distances (p=0.0001) and when racing on all-weather tracks (p=0.0001). Additionally, country of registration, going of ground, season, trainer and weight carried were also factors significantly associated with tongue tie use.

A variety of performance indicators (BHA ratings, finishing position, length of finish, percentage of wins and winning places per race start) indicated that tongue ties did not enhance performance. A reduction in BHA ratings was recorded for racehorses which ran with a tongue tie (p=0.0001). Racehorses which ran with a tongue tie made significantly fewer wins per race starts and achieved fewer winning positions per race start in all race types evaluated.

In conclusion, the use of tongue ties appeared to have increased over time, their use is more common in older female animals and in those horses competing over fences. Their use did not enhance performance.

E-79 A RETROSPECTIVE SURVEY OF BRONCHOALVEOLAR LAVAGE FLUID CYTOLOGY OF HORSES IN WESTERN AUSTRALIA. G. Secombe, G. Lester, I. Robertson, A. Cullimore. Murdoch University, Western Australia, Australia.

There are a few bronchoalveolar lavage (BAL) cytological profiles that are consistent with inflammatory airway disease (IAD) in equids. This study aimed to characterise the cytological changes in equine BAL samples over multiple years to determine if the prevalence of a relative mast cell response is influenced by season. Medical records of 228 horses with clinical signs or poor performance where a BAL was performed were reviewed retrospectively. BAL fluid cytology and categorised variables were evaluated using a Chi square test to determine associations. The predominant signalment was the racing horse between 2-6 years of age, poor athletic performance was the most common complaint. Based on published criteria, 70% of horses had abnormal BAL cytology. The presence of nasal discharge was the only significant difference between horses with normal and abnormal cytology. The most common cytological derangement was a mixed cell response (25%), the majority of which comprised elevated percentages of neutrophils and mast cells. A solely neutrophilic response or mast cell response occurred with equal frequency (19% each), and an isolated eosinophilic response was noted in 7% of cases. Fifty nine percent of horses with cytology consistent with IAD had increases in the relative percentage of mast cells. Mast cell responses were significantly associated with spring, while eosinophilic and neutrophilic responses were significantly associated with summer. It is concluded that a relative mast cell increase is the most common IAD cytological profile in Western Australian horses and is associated with season.

E-80 PULMONARY FUNCTION TESTING, BRONCHOALVEOLAR FLUID CYTOLOGY AND MAST CELL TRYPSEASE IN A GROUP OF WESTERN AUSTRALIAN HORSES. C.J. Secombe, G.D. Lester, I.D. Robertson, A. Cullimore. Murdoch University, Western Australia, Australia.

An increase in the relative percentage of mast cells in equine bronchoalveolar lavage fluid (BALF) may reflect increased airway responsiveness. The commonly reported relative percentage of mast cells in BALF >2% in normal horses. This study examined the relationship between airway responsiveness, relative mast and eosinophil cell percentage, total mast and eosinophil cell concentration, and mast cell tryptase (MCT) concentration in BALF collected from 25 clinically normal horses. Pulmonary function testing (PFT) and histamine provocation was performed using a commercial plethysmography system. BALF was collected 16 hours after PFT and total cell count and cell differentials were performed on 400 cells. Equine MCT was measured from the BALF supernatant using a commercial ELISA. Fifteen horses had >2% mast cells. Ten horses had airway hyperresponsiveness. Neither the relative cell percentages nor the total numbers of mast or eosinophils cells were significantly correlated with airway responsiveness. The concentration of MCT increased with increasing airway responsiveness (p=0.05). MCT was not correlated with relative mast cell percentage or the total mast cell concentration. MCT was greater in horses with mast cell mixed inflammatory response compared to those with a single eosinophilic or neutrophilic cell response (p=0.05). It was concluded that in this population of horses the relative mast or eosinophil percentage was not indicative of airway responsiveness as cells may be quiescent in the respiratory tract. Airway responsiveness is caused production of cellular products including MCT and the presence of a mixed mast cell inflammatory response may indicate active mast cell degranulation.
New World Camellids (NWCs) have reduced insulin production and peripheral insulin sensitivity compared to other species. Sick NWCs commonly develop hyperglycemia, hyperketonemia, hypertriglyceridemia, and hepatic lipidosis, but little is known about pancreatic function in this state. Our aim was to use epinephrine to induce transient fat mobilization in healthy NWCs and then intravenous arginine stimulation testing (IVAST) to assess pancreatic alpha and beta-cell function. The hypothesis was that NWCs, in a state of epinephrine-induced fat mobilization, would have reduced insulin secretion and increased glucagon secretion as measured by IVAST.

Fourteen healthy lambs were used in a control design and intravenous catheters were placed. IVAST involved intravenous administration of 67 mg/kg 10% arginine HCl. Blood samples were collected at 0, 2, 3, 4, 5, 7, 10, 15, and 20 minutes post-arginine injection for plasma glucose, insulin and glucagon measurement. All 14 animals underwent a baseline IVAST. Thirty-six hours later feed was withheld for 12 hours and each animal received 1 mg/kg epinephrine IM (t=0). One hour later IVAST was repeated (t=60). Serum non-esterified fatty acids (NEFAs), ketones, and triglycerides were measured at t=60 and at t=0.

Epinephrine induced mild elevations in NEFAs, ketones, and triglycerides (p>0.0001). Baseline insulin (but not glucagon) was lower after treatment with epinephrine (p<0.01). During IVAST the AUC at 10 minutes was lower for [insulin] (p<0.05) and higher for [glucagon] (p<0.0001) after epinephrine treatment. The results of this study may reflect a direct effect of alpha-adrenergic stimulation on the pancreas. Our findings may provide insights into changes that occur in pancreatic function in sick NWCs.

Calves with diarrhea commonly develop strong ion metabolic acidosis. The mechanisms underlying acid-base disorders can be demonstrated using the physicochemical approach. The objectives of this study were to determine the quantitative contribution of D- (D-lac-) and L- lactate (L-lac-) to the strong ion gap (SIG) of this study were to determine the quantitative contribution of plasma protein concentrations in determining plasma [HCO3-] and [H+]. Plasma strong ion difference was calculated as SID = [Na+] + [K+] - [Cl-] - [L-lactate] - [D-lactate] - [HCO3-]. The C max of enrofloxacin in the plasma is 1.31 g/mL and occurred at the four hour sample. The C max of enrofloxacin was in the ileum and spiral colon of 12 Holstein steers. An ultrafiltration probe was also placed subcutaneously to collect interstitial fluid. The ultrafiltration probes were highly successful at extracting fluid from the ileum and spiral colon. Sufficient volume to perform HPLC to measure drug concentrations was achieved in 95% (114/120) of GI samples. Upon necropsy, no significant gross pathology resulting from GI implantation of the probes was noted.

Cefitofur sodium was found in higher concentrations in the ileum, however enrofloxacin was found in higher concentrations in the spiral colon. The Cmax of cefitofur sodium in the ileum is 7.39 µg/mL and occurred at the four hour sample. The Cmax of cefitofur sodium in the ileum is 6.12 µg/mL and occurred at six hours. The Cmax of enrofloxacin in the ileum is 1.31 µg/mL at 6 hours, whereas the Cmax of enrofloxacin in the spiral colon is 1.41 µg/mL, occurring at 24 hours. Next, model enteric bacteria will be exposed to the drug concentrations found in the GI tract to assess the risk of antimicrobial resistance development.
F-4
TOTAL PROTEIN AND IgG CONCENTRATION IN HOLSTEIN CALVES: A COMPARISON OF MEASUREMENT TOOLS, BLOOD COLLECTION TUBES, AND TIME TO ANALYSIS.
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Adequate passive transfer is recognized as a critical aspect of calf health. Ingestion of good quality colostrum, either maternal or a colostrum replacement product, within twelve hours of birth is an important step in infectious disease protection. Herd testing for adequate transfer of colostral immunity is performed on calves less than seven days of age. Measurement of serum total protein by refractometer provides an indirect assessment of passive transfer status. Measurement of immunoglobulins in the serum provides a more accurate assessment, however immediate analysis is not always feasible.

The purpose of the study was to evaluate the effect of time to analysis and type of blood collection tubes on serum total protein and IgG concentration using various measurement tools. Blood was collected into a red top tube and a serum separator tube from the jugular vein of Holstein heifer (n=41) and bull (n=9) calves less than seven days of age. Calves had received either maternal colostrum (n=47) or a colostrum replacement product (n=3) within twelve hours of birth. No clinical signs of dehydration or ill health were present. Serum from each tube was harvested within six hours of collection and evaluated for total protein and IgG concentration. The total protein was measured using a handheld refractometer (Kernco PUR, Japan) and a digital refractometer (Misco DD-3 Digital Dairy). The IgG concentration was measured by radial immunodiffusion (RID) assay (Triple J Farms Bovine IgG Test Kit) and the digital device. The same analyses were repeated 5 days later following storage at room temperature. Data were analyzed using paired t-tests with significance set at p < 0.05. Results revealed no statistical difference in total protein measurement according to type of blood collection tube used, measurement device utilized or time to analysis. However, there was a statistical difference noted between IgG values as measured by RID versus the digital device. No other differences in IgG measurements were observed within the data set. In conclusion, the use of different serum collection tubes did not affect serum total protein or IgG measurements. Serum stored for up to 5 days provided consistent serum total protein and IgG measurements. The use of the digital refractometer for measurement of total protein provided comparable results compared to the handheld refractometer, but the IgG concentrations by the digital refractometer were consistently lower than the results by RID. Development of a calf-side test would be valuable tool for monitoring of adequate passive transfer.

F-5
GENOTYPIC IDENTIFICATION OF STAPHYLOCOCCI FROM CASES OF SUBCLINICAL BOVINE MASTITIS PREVIOUSLY IDENTIFIED AS STAPHYLOCOCCUS HYicus.
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The goal of this project was to use gene based speciation methods to characterize a collection of non-Staph. aureus coagulase positive (CP) mastitis isolates from Pacific Northwest dairies that previously were phenotypically identified as Staph. hyicus using a biochemical speciation method. Previously banked isolates collected from cows' milk on 12 dairy farms in Washington and Idaho were grown on culture media and lysates produced. The rpoB and tuf genes were amplified using polymerase chain reaction and Sanger sequenced. Sequences were aligned with NCBI rpoB and tuf gene sequences from Staph. agnetis (a new species of CP staphylococci closely related to Staph. hyicus identified in 2012) and Staph. hyicus reference strains.

The median (range) prevalence of phenotypically identified Staph. hyicus isolates among coagulase positive staphylococcal isolates within herd was 31% (7-100%). A total of 43 isolates were genotypically characterized. The rpoB gene sequences were found to be ≥ 99% similar to Staph. hyicus and Staph. agnetis. However, when aligning rpoB genes with the reference strains, 42 of 43 of the isolates aligned closest with the Staph. agnetis reference strain. To date tuf gene alignment has been completed for 8 isolates, one isolate matched 100% with Staph. agnetis isolate, while the 7 other isolates aligned more closely with S. agnetis than Staph. hyicus. According to the sequence alignment completed to date, these isolates appear to be Staph. agnetis, rather than Staph. hyicus. Correct classification of isolates is necessary to understand true prevalence and impact of these organisms on udder health.

F-6
DECLINE OF MATERIAL ANTIBODIES TO CAPRINE ARTHRITIS ENCEPHALITIS VIRUS AS MEASURED BY COMPETITIVE ELISA. S.G. Genova1, R.N. Streeter2, L.J. Dawson2, S.J. Kapil1, D.R. Smith1, T.D. Taylor2. 1College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, 2Boren Veterinary Teaching Hospital, Oklahoma State University, Stillwater, OK.

Caprine arthritis encephalitis virus (CAEV), a non-oncogenic lentivirus in the retrovirus family, causes multi-systemic disease in goats. Infection with CAEV results in life-long persistence of infection. CAEV infection can result arthritis, leukoencephalomyelitis, pneumonia, mastitis, and chronic weight loss. Transmission occurs most commonly through the ingestion of colostrum and/or milk from CAEV-positive does, though transplacental and horizontal transmission does occur. Currently, detection of antibodies to CAEV, utilizing AGID and ELISA techniques, is the mainstay of diagnosis and control programs in goat herds.

We hypothesized that if goat kids remain uninfected after being fed colostrum from CAEV positive does then passively acquired CAEV antibodies would decline over time. Colostrum was harvested and frozen from 20 known CAEV-positive does. When 15 liters of colostrum had been collected all colostrum was thawed, pooled, and packaged into 100 ml aliquots and refrozen until use. Half of the pooled colostrum was treated with 9.8% formic acid to a pH of 4.2 to determine if this affected weight gain, kid health, and seroconversion.

Twenty goat kids were immediately separated from their dams at birth. All kids were CAEV-negative prior to ingestion when tested with a commercial cELISA kit. Kids were tube fed colostrum at a rate of 10% of their body weight within 8 hours of birth. Following colostrum feeding kids were maintained on a commercially available goat kid milk replacer until weaning. Kids were tested for CAEV antibodies by cELISA each month.

All 20 kids remained seronegative until 6 months of age. All kids became seronegative at 6 months of age and remained seronegative until the end of the project at 8 months. At the 6 month time point 2 of 20 kids had percent inhibitions near the validated cutoff of 35% for the test kit.

Based on this study we conclude maternal antibodies from uninfected goat kids fed treated or untreated colostrum from known CAEV-positive does declined below detection levels by 6 months of age and were well below the cut-off value by 7 months of age. False-positive cELISA results might be avoided by screening kids for the presence of antibodies at 7 months of age or later.

F-7
HYPERKETONEMIA AS AN EARLY DIAGNOSTIC TOOL TO PREDICT PREGNANCY TOXEMIA IN DAIRY GOATS. V. Doré, J. Dubuc, A.-M. Bélanger, S. Buczinski. Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada.

Measurement of ketone bodies concentration in blood is used in goats as a diagnostic tool for clinical pregnancy toxemia (PT).
Many authors have suggested different empirical concentration cutoffs to define sub-clinical and clinical forms of this disease. However, no cutoff has been determined based on predicting PT with the best possible accuracy. The objective of this study was to determine the optimal ketone bodies cutoffs for early prediction of PT in late gestation dairy goats. A prospective cohort study was performed on 1242 lactating goats from 10 commercial herds in Quebec, Canada. Each goat was followed weekly during the 5 last weeks of pregnancy or until parturition. During each farm visit all pregnant goats were sampled until more than 95% of the group had kidded. Blood samples from jugular vessel were collected and were analyzed directly on farm using an electronic on-farm test for the quantification of blood beta-hydroxybutyrate acid (BHBA) concentration (Precision Xtra, Abbott Diabetes Care, Saint-Laurent, Canada). Producers evaluated each goat for presence of PT based on a 4 degrees scale (absence, low, moderate, or strong suspicion of PT) using a standardized definition. Critical cutpoints for predicting PT diagnosis were determined based on the highest sum of sensitivity and specificity. Blood concentration above 0.3 mmol/L during week 5 before kidding (sensitivity (Se): 81%; specificity (Sp): 54%), 0.6 mmol/L during weeks 4 (Se: 37%; Sp: 91%), 3 (Se: 54%; Sp: 88%), and 2 (Se: 64%; Sp: 84%) before kidding, and 0.7 mmol/L during week 1 before kidding (Se: 61%; Sp: 90%) were selected as the optimal cutpoints in this study. In conclusion, hyperketonemia can be used to early predict goats at greater risk of PT during the last month of pregnancy.

F-9
EVALUATION OF A MODEL DEMONSTRATING MITIGATION OF NOCICEPTIVE RESPONSE TO OXYTETRACYCLINE INJECTION SITE INFLAMMATION BY FLUNIXIN MEGLUMINE IN DAIRY COWS. A. Lear1, A. Yager2, S. Byers1, J. Ahola2, R. Callan1. 1Colorado State University, Department of Clinical Sciences and Department of Animal Science, Fort Collins CO.

The purpose of this study was to determine if flunixin meglumine administration will mitigate nociceptive pain associated with oxytetracycline injection site inflammation as measured objectively by an algometer. Oxytetracycline (10 mg/kg) was administered intramuscularly at a single site in the semimembranosus/semitendinosus muscle group of the hind leg in 5 non-lactating, mature cull dairy cows. The opposite rear limb was used as the control site which received a sham injection. Cows were randomized receiving either flunixin meglumine (2.2 mg/kg) or equivalent volume of 0.9% saline IV at 24-hour intervals for 5 days. An algometer was used to measure the amount of pressure applied directly at the injection site required to elicit a nociceptive response at 1, 6 and 24 hours after treatments, daily. Statistical analysis was performed using standard ANOVA and pairwise comparisons. The oxytetracycline injection site demonstrated a more sensitive nociceptive response compared to site that received the sham injection. Cows treated with flunixin meglumine resulted in higher algometer pressure necessary to elicit a nociceptive response from 78-120 hours after oxytetracycline injection compared to control animals.

In conclusion, flunixin meglumine appeared to mitigate nociceptive response at the oxytetracycline injection site after 78 hours of treatment. Additional studies are needed to further refine this model for assessing the mitigation of inflammatory mediated pain by nonsteroidal anti-inflammatory drugs.
and a PO (median 1.3; range 0.7 to 15.6 mmol/L; P = 0.33) using blood cTnI concentration as independent variable and NO as dependent variable, the area under the curve for cTnI was 0.693. The maximal sum of sensitivity and specificity for predicting NO was found using a threshold of ≥ 0.70 ng/mL for cTnI with a sensitivity of 78.4% and a specificity of 54.1%.

This study is the first to report the potential benefit of using blood cTnI for predicting the outcome of downers cows in a field setting. Since this test is a relatively sensitive test to detect NO, it could be of practical interest for veterinarians to predict cows with a greater risk of NO, and therefore to recommend aggressive treatment (eg flotation tank and referral hospital) or stop to invest money (euthanasia) using higher thresholds with higher specificity.

F-11
OCULAR MANIFESTATIONS OF SEPSIS AS A PROGNOSTIC INDICATOR FOR NEONATAL CRIAS. W.F. Gilsenan, J.S. Smith, J.A. Angelos, J.D. Rowe, J.L. disease [15/19 (78.9%) vs. 101/136 (74.3%), p = 0.09]. There was no difference in survival for septic crias with or without ocular disease [15/19 (78.9%) vs. 101/136 (74.3%), p = 0.66]. Necropsy was performed in 5/15 (33.3%) of the surviving septic crias with ocular disease.

In septic neonatal crias, ocular disease is a common consequence of sepsis. Ocular disease is not associated with a decrease in survival. However, prognosis for retention of the affected globe is guarded.

F-12
CHONDROGENESIS OF EQUINE AND OVINE MESENCHYMAL STEM CELLS IN VITRO. I.G. Sancaş, S. Koch, J. Kuemmerle, L. Ettinger, B. Von Rechenberg. 1Department of Surgery, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey, 2Competence Center for Applied Biotechnology and Molecular Medicine (CABMM), Equine Hospital, Vetsuisse Faculty, University of Zurich, Switzerland.

The purpose of this study was to determine the isolation and differentiation of bone-marrow derived mesenchymal stem cells (MSCs) from animal species and also demonstrating the integration of cells in 2D and 3D cultures.

MSCs of sheep and equine MSCs were isolated from the bone marrow and sternal marrow, respectively. Isolated MSCs were expanded and directed for osteogenic, adipogenic and chondrogenic differentiation. Expression of cell surface antigens was confirmed by Alcian blue staining. Differentiation potential. Adipogenic and osteogenic differentiation was examined by using positive Von kossa staining. Directing MSCs into adipogenic lineage resulted in typical adipocyte morphology which was confirmed by positive Oil red O staining. Directing MSCs into chondrogenic lineage was assessed by micromass culture and the cells seeded in alginate beads. Chondrogenic lineage was confirmed by Alcian blue staining.

Present study determined that marrow derived MSCs from equine and sheep were capable of possessing the multi lineage differentiation with a rapid growth rate and of having the capacity to integrate into the 3-D cultures.

F-13
PHARMACOKINETICS OF FLUNIXIN MEGLUMINE IN PLASMA AND MILK OF DOMESTIC GOATS (CAPRA AEGAGRUS HIRSUS) FOLLOWING SINGLE SUBCUTANEOUS DOSING. J.S. Smith, J.A. Angelos, J.D. Rowe, J.L. Carlson, E.A. Lee, L.A. Tell. School of Veterinary Medicine, University of California, Davis, CA.

Flunixin Meglumine (FM) is a non-steroidal anti-inflammatory drug that is approved by the US Food and Drug Administration (FDA) for intravenous (IV) administration in cattle and intra-muscular (IM) administration in swine for the control of pyrexia associated with respiratory disease. There are several subcutaneous administration products that have FM combined with other active ingredients including two for cattle (FM + antibiotic). FM is commonly administered by the SC route in lactating goats, however pharmacokinetic data is lacking for this administration route in goats.

This study evaluated the single dose pharmacokinetics of FM in lactating goats (n = 8) dosed at 1.1 mg/kg body weight. Goats were administered FM by either IV or SC administration routes, using a two-way crossover study design with a 2 week washout period. Plasma concentrations of FM and the primary metabolite, flunixin 5-OH (5-OH), were measured using ultra performance liquid chromatography with mass spectrometric detection in samples collected at 0, 5, 10, 15, 30, and 45 minutes post-treatment as well as 1, 2, 4, 6, 8, 12, 18, 24, 30, 36, 48, 60, 72, 84, 96, 108, and 120 hours after administration. Preliminary non-compartmental pharmacokinetic parameters were then derived from the time versus 5-OH milk concentration data. Mean elimination half-life was 0.188 hours (range 0.104 to 0.256 hours), mean observed maximal concentration was 75.43 ng/mL (range 42.4 to 116.1 ng/mL). Flunixin 5-OH concentrations were above the assay’s limit of detection from 24-36 hours post injection (mean = 33 hours) in milk. In conclusion, based on these preliminary results, a withdrawal interval for healthy goats being treated subcutaneously with a single dose of FM at 1.1 mg/ kg could be as early as 36 hours post drug administration.
C. Cocquyt, S. Van Amstel, S. Cox, B. Rohrbach, T. Taylor 2. Ontario Veterinary College, Guelph, ON; 3. The Hospital for Sick Children, Toronto, ON.

The purpose of this study was to evaluate protein content in exhaled breath condensate (EBC) collected in order to identify potential biomarkers of airway inflammation. Holstein bull calves, 4-5 months of age, were randomly assigned to either Infected (n=6) or Control (n=5) groups. The animals were experimentally challenged with 25 mL of either sterile saline (Control) or 10^-9 cfu/mL Mannheimia haemolytica.

F-17 EVALUATION OF PROTEIN CONTENT IN EXHALED BREATH CONDENSATE OF HOLSTEIN CALVES AND THEIR POTENTIAL USE AS A MARKER FOR AIRWAY INFLAMMATION. R. Schubotz 4, 5, 6. Respiratory Research Inc, TX that was adapted for use in calves, in order to identify potential biomarkers of airway inflammation.

Holstein bull calves, 4-5 months of age, were randomly assigned to either Infected (n=6) or Control (n=5) groups. The animals were experimentally challenged with 25 mL of either sterile saline (Control) or 10^-9 cfu/mL Mannheimia haemolytica.
(Infected) that was instilled into the distal trachea endoscopically. EBC was collected at time points t = -72 h, -48 h, -24 h, 0 h, 6 h, 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, 84 h, 96 h, 108 h, and 120 h post-challenge. EBC samples were taken applying the device to the nose of the calves for 5 minutes.

Mass spectrometry (MS) analysis was performed on the EBC using a Q-Exactive hybrid mass spectrometer. Preliminary analysis of EBC samples that coincided with times of peak systemic inflammatory response, based on peak serum haptoglobin values, confirmed the presence of several proteins including Annexin A1 and Odorant-binding protein that have been shown in previous studies to be potential marker for airway inflammation.

This study demonstrated that EBC of calves does contain lung proteins, and that this sample may be used for further research to investigate changes in protein content of EBC in healthy and infected animals to identify potential protein biomarkers of lung inflammation.

F-18 ACUTE PHASE PROTEINS IN NATURALLY OCCURRING RESPIRATORY DISEASE OF FEEDLOT CATTLE: A NOVEL APPROACH TO DIAGNOSIS. I. Idoate, M. Heller, B. Vander Ley. University of Missouri College of Veterinary Medicine, Columbia, MO.

Bovine respiratory disease (BRD) is the most costly disease of feedlot cattle in the United States. Costs associated with BRD have been estimated from $13.90 to $15.57 per head with annual losses to the cattle industry exceeding $750 million. A presumptive diagnosis of BRD is usually based on clinical signs including elevated rectal temperatures. Physical exam alone lacks high sensitivity and specificity, leading to misclassification and unnecessary treatment or failure to treat true cases. More sophisticated diagnostic tests exist but are not practical in feedlot settings. The objective of this study was to evaluate the utility of three acute phase proteins as a method of improving BRD diagnosis in conjunction with a calf health scoring chart (CHSC, University of Wisconsin School of Veterinary Medicine). A study population of 77 beef calves was observed for signs of BRD. In total, 14 cases and pen matched controls were included in the initial data analysis. Cattle were grouped based on CHSC scores. BRD cases and pen matched controls were included in the initial data analysis. Cattle were grouped based on CHSC scores. BRD cases were defined as a score of ≥5, while controls were defined as a score ≤4. As expected mean CHSC score in cases was 6.9 which was significantly greater than controls 2.8 (P < 0.01). Mean plasma lipopolysaccharide binding protein and haptoglobin concentrations were significantly greater in cases than controls (P = 0.01). Likewise feed intake was significantly lower in cases than controls 2.8 (P = 0.01). Mean plasma lipopolysaccharide binding protein and haptoglobin concentrations were significantly greater in cases than controls (P = 0.01). Monitoring LBP, haptoglobin levels and performance variables, may be useful as prognostic tools and facilitate treatment decisions.

F-19 COMPARISON OF THORACIC ULTRASONOGRAPHY AND BRONCHOALVEOLAR LAVAGE FLUID ANALYSIS TO POST-MORTEM EXAMINATION FOR DETECTION OF SUBCLINICAL BRONCHOPNEUMONIA IN DAIRY CALVES. T.L. Ollivett1, D. Kelton1, D.V. Nydam, T. Duffield1, K.E. Leslie1, J. Hewson1, J. Caswell1, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada. Faculté de Médecine Vétérinaire, Université de Montréal, Montréal, Canada.

Diagnosis of central nervous system lesion in recumbent dairy cattle (RDC) is challenging since neurological examination is limited and medical imaging is often unrewarding. Cerebrospinal fluid (CSF) analysis is useful in the diagnosis of central nervous system disorders in cattle. However, its ability to detect spinal cord damage in RDC is unknown. We hypothesized that CSF analysis could discriminate RDC with or without spinal cord damage.

A medical record computer search for RDC of at least 20 months of age with CSF analysis and complete post-mortem evaluation was performed. RDC without any evidence of spinal cord damage macroscopically and microscopically at necropsy were considered as the control group (RDC-) whereas those with lesions to the spinal cord formed the affected group (RDC+). Medians of total proteins, leucocytes count and differential, and red blood cells (RBC) count were determined and compared between the groups (Mann–Whitney–Wilcoxon). Sensitivity, specificity, positive predictive value and negative predictive value were determined at selected cut-off values.

Of the 40 medical records retrieved, 21 matched the inclusion criteria of RDC+ group. Median (median [range]) total proteins and leucocytes count (0.33 [0.22–1.36] g/L; 3.85 [0–31.35] per μL) in the CSF of RDC+ cows were statistically significantly higher (p = 0.0141 and p = 0.0161 respectively) compared to RDC- cows (0.28 [0.17–0.38] g/L; 0.6 [0–4.4] per μL). No difference was observed for leucocytes differential or RBC count between the two groups. At a cut off value of 4.5 leucocytes per μL or 0.40 g/L of total protein, sensitivity, specificity, positive predictive value and negative predictive value (95% CI) for spinal cord damage were 71% [44–90], 100% [82–100], 100% [73–100] and 79% [58–93] respectively.

Based on this study, RDC with CSF total proteins above or equal to 0.40 g/L or CSF leucocytes count above or equal to 4.5 cells per μL, must be considered to have spinal cord damage. Additionally, CSF values for total proteins and leucocytes count in RDC without spinal cord damage are lower than previously published reference ranges. Prognosis associated with these findings remains to be determined.

F-20 CEREBROSPINAL FLUID ANALYSIS IN RECUMBENT DAIRY CATTLE WITH OR WITHOUT SPINAL CORD DAMAGE. Damien Achard, David Francoz, André Desrochers, Sylvain Niquet, Marie Babkine, Gilles Fecteau. Faculté de Médecine Vétérinaire, Université de Montréal, Montréal, Canada.

Thoracic ultrasonography (US) has not been validated for detecting lung consolidation associated with subclinical broncho-pneumonia (SBP) in dairy calves. The objective of this study was to evaluate the relationship between acute ultrasonographic lung consolidation, bronchoalveolar lavage fluid analysis, and post-mortem examination in apparently healthy dairy calves. Twenty-four Holstein bull calves, enrolled at 3 – 6 days of age, were assessed weekly for 12 weeks using respiratory scoring, and thoracic US with a portable 6.2 MHz variable frequency linear rectal transducer. Bull calves with no previous history of antimicrobial treatment, ultrasonographic lung consolidation, or elevated respiratory score were selected for bronchoalveolar lavage fluid (BALF) analysis and post-mortem exam (normal US, n = 5; comet-tailing, n = 4; consolidated, n = 15). BALF was collected for total nucleated cell count and differential cell counts via a flexible fiberoptic bronchoscope. Euthanasia and post-mortem exam followed BALF collection.

Using post mortem examination as the gold standard, sensitivity and specificity of US in detecting lung consolidation was 94% (95% CI: 69 – 100%) vs 80% (95% CI: 49 – 94%), respectively. Using a cut-off BALF neutrophil proportion (NP) of 4%, sensitivity and specificity of BALF was 81% (95% CI: 56 – 94%) and 75% (95% CI: 36 – 95%), respectively. Agreement between US and post-mortem examination was excellent (Kappa = 0.90). Sensitivity and specificity of US were greater in consolidated lungs (P < 0.01). Thoracic US is a non-invasive and reliable method of diagnosing SBP and can be performed using portable equipment on dairy farms in calves less than 3 months old. A NP of 4% or higher in BALF suggests SBP.

The aim of this study was to determine hypoxia and blood glucose levels during the first 48 hours of life in goat kids delivered by cesarean section. Ten newborn kids delivered by cesarean section after 149 days of pregnancy were used. For determination of blood lactate (mmol/L) and glucose (mg/dL), blood samples were obtained by venipuncture of the umbilical cord before it was cut (M0) and the jugular vein after the umbilical cord was cut at birth (M0), and at 15 minutes (M15), 30 minutes (M30), 60 minutes (M60), 24 hours (M24) and 48 hours (M48) after birth. Blood lactate and glucose levels were measured using the devices Accutrend Plus (Roche) and Accu-Check Active (Roche), respectively. Analyses of variance followed by Tukey post-hoc test was used to determine significant differences between times. Statistical significance was set at p<0.05.

Significant differences between times in both variables were found. In glucose levels (mean ± SD), significant differences occurred at M24 (105 ± 30) and M48 (118 ± 33) when compared to M0 (32 ± 5.1), M15 (30 ± 15), M30 (33 ± 11) and M60 (27 ± 23). Similarly, lactate levels demonstrated significant differences at M24 (7.9 ± 1.6) and M48 (6.5 ± 1.5) when compared to M0 (8.3 ± 1.8), M15 (9.5 ± 1.9), M30 (9.9 ± 3.1), and M60 (9.9 ± 2.2). The time to first feeding did not exceed two hours after birth.

The ingestion of colostrum by newborn kids up to one hour after birth is important in order to obtain an adequate blood glucose level and fulfill the animal’s metabolic requirement. Kids delivered by cesarean section present hypoxia up to one hour after birth and gradually adapt throughout the first 48 hours of life.

F.22 ACUTE PHASE PROTEINS IN HEALTHY GOATS; ESTABLISHMENT OF REFERENCE INTERVALS. M.C. Heller1, J.L. Johns2. 1Veterinary Medicine and Surgery, University of Missouri College of Veterinary Medicine, Columbia, MO., 2Comparative Medicine Department, Stanford University School of Medicine, Stanford, CA.

Acute inflammatory processes can trigger increased production of certain proteins termed acute-phase proteins (APPs) which can be useful biomarkers of inflammation. APPs are diverse and include proteins involved in coagulation, opsonization, iron regulation, and limitation of tissue injury. The predominant positive APPs can differ markedly by species, therefore species-specific reference intervals are necessary. Haptoglobin, serum amyloid A and alpha-1 acid glycoprotein in 54 clinically normal adult goats in Missouri representing a range of breeds, sexes, and pregnancy or lactation status. APPs were measured using goat specific commercial ELISA kits. Results were analyzed by one-way ANOVA to compare sexes and breeding status. Reference Values Advisor was used to calculate reference limits according to the IFCC-CLSI guidelines. Only 1 APP was found to vary in healthy animals; serum haptoglobin was elevated in lactating animals and decreased in pregnant does in their second trimester when compared to open, non-lactating does. No sex differences were seen for any of the APPs measured. We report normal reference intervals for four serum APPs which may be useful as disease markers, however haptoglobin should be interpreted with caution in animals with unknown pregnancy status. Further studies are needed to determine whether these APPs are useful bio markers for specific disease states.

F.23 EFFECTS OF METHYLSULFONYLMETHANE ON E. COLI O157:H7 AND SALMONELLA ENTERICA IN PURE CULTURE AND IN VITRO MIXED RUMINAL MICROORGANISMS. F. Delgado1, M. A. T. Estudillo2, F. M. G. F. Neves3, M. C. Heller1. 1Research Unit, Agricultural Research Service, USDA, College Station, TX, 2Food and Feed Safety Research Unit, Agricultural Research Service, USDA, College Station, TX, 3Univ Estadual Paulista, unesp, FMVA, Campus de Araçatuba, São Paulo, Brazil.

Pathogenic bacteria living in the intestinal tract of food animals can be transmitted to humans via the food supply or indirectly through animal or fecal contact. Recently, non-antibiotic compounds have been used to modify the gastrointestinal microbiota of ruminants to decrease populations of pathogenic bacteria. The aim of this study was to investigate whether 1) the addition of methylsulfonylmethane ( MSM) could inhibit the growth of two important foodborne pathogens, E. coli O157:H7 and Salmonella enterica in pure culture or in ruminal fluid and 2) whether the addition of MSM to ruminal fluid in vitro would alter the composition of the ruminal microbiota.

Pure cultures (3 x 10^8 CFU/ml) of Escherichia coli O157:H7 strain 9333 and four serotypes of Salmonella enterica (Typhimurium, Derby, Newport, and Enteridis) were added to tubes containing MSM at 0, 1, 2, 5, 10, and 20% (w/v; n=3) and were incubated at 39°C for 24 hours. E. coli O157:H7 and S. typhimurium were added (2 x 10^8 and 3 x 10^7 CFU/ml, respectively) to mixed ruminal microorganism fermentations containing MSM at concentrations of 0, 1, 2.5, 5, 10, and 15% (w/v; n=3) and were incubated at 39°C for 24 hours. Methane, acetate to propionate ratio, and pH were measured in the ruminal fluid as markers of normal rumen fermentation. After 24 hours, bacterial populations were quantified and DNA was extracted from the ruminal fluid via bead beating procedures and analyzed by 16S rRNA gene.

In the first experiment, MSM treatment reduced the growth rate and final populations of E. coli O157:H7 and all four Salmonella serotypes in pure culture at concentrations greater than 5% (p < 0.05). Final populations of all pathogens were reduced between 100- to 1000-fold by MSM concentrations ≥10%. In the ruminal fluid, the addition of MSM did not affect final populations of E. coli O157:H7 and S. typhimurium at any concentration. Methane production and acetate to propionate ratios from the in vitro fermentations were not changed by MSM treatment, but the final pH was increased by MSM ≥ 10% (p=0.06). There were no significant changes in the proportion or diversity of the bacterial communities of the rumen fluid at any percentage of MSM.

These results suggest that MSM can modify the growth of enteropathogenic bacteria in pure culture, but this response is modified by the more complex ruminal environment.

F.24 PHARMACOKINETICS OF INTRAVENOUS AND SUBCUTANEOUS CEFOVECIN IN ALPACAS. S. Cox, C. Sommardahl, R. Seddighi, R. Videila, J. Hayes, T. Doherty. University of Tennessee, College of Veterinary Medicine, Knoxville TN.

The purpose of this study was to determine the pharmacokinetics of cefovecin (8 mg/kg) after intravenous (IV) and subcutaneous (SQ) administration to alpacas.

New World camelds are popular as pets, and are used for packing, fiber, and as show animals. Bacterial infections requiring long-term antibiotic therapy, such as neonatal bacteremia, pneumonia, peritonitis, dental and urogenital infections are among significant causes of morbidity and mortality in these species. Currently there are no antibiotics approved by the FDA for use in New World camelds.

However, few antimicrobials have been evaluated and proven to have favorable pharmacokinetics for therapeutic use. The lack of pharmacological data currently hampers effective patient care and causes practitioners to empirically treat camelds on the basis of dosage extrapolation from other species. Currently there are no antibiotics approved by the FDA for use in New World camelds.
Cefovecin (Convenia™), a third-generation cephalosporin, developed by Pfizer Animal Health is approved for use in dogs and cats as an aqueous solution via subcutaneous injection. Its *in vitro* activity against most Gram-positive and Gram-negative pathogens has been demonstrated and it is more effective and has lower MIC values than first-generation cephalosporins. The properties of cefovecin may be advantageous for medical treatment of camelids because of the convenience of its broad spectrum, route of administration and long-duration of activity.

Alpacas were given 8 mg/kg IV and blood was collected at 0, 0.08, 0.33, 0.75, 1, 2, 4, 8, 12 and 24 h and 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, and 22 days. After a 20 day washout period the alpacas were given 8 mg/kg SQ and blood samples collected at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h and 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, and 22 days. Samples were analyzed using a validated high performance liquid chromatographic method. Preliminary data from two healthy adult male alpacas after intravenous administration provided the following results: half-life 12.4 ± 4.3 h, volume of distribution at steady state 107 ± 15 mL/kg, clearance 8.5 ± 0.4 mL/h/kg, and AUC 934 ± 43 h·µg/mL, respectively. After subcutaneous administration the bioavailability, half-life, C max and T max were 100%, 13.2 ± 2.4 h, 112 µg/mL, and 2.5 h, respectively. No adverse effects were observed. The MIC90 values of cefovecin against *Staphylococcus intermedius*, *Escherichia coli*, *Streptococcus*, and *Pasteurella multocida* in canine and feline isolates are 0.25 µg/mL, 1 µg/mL, 0.5 µg/mL and 0.06 µg/mL, respectively. Administration of cefovecin resulted in concentrations greater than 1 µg/mL for 3 days and greater than 0.1 µg/mL for 6 days.

**F-25** MEASUREMENT OF ACTIVE ANTIBIOTIC CONCENTRATIONS IN THE PULMONARY EPITHELIAL LINING FLUID AND INTERSTITIAL FLUID OF HEALTHY CALVES. L. Martin, C. Warren, M. Papich, M. Jacob, D. Foster. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Bovine respiratory disease is the biggest health challenge facing the beef cattle industry today, accounting for 75% of illnesses and 50% of deaths on feedlots. Therefore, improved treatment of bovine respiratory disease will reap tremendous health and economic benefits.

The purpose of this study is to determine the drug concentrations produced at the site of action, the pulmonary epithelial lining fluid (PELF), and the correlation between PELF and interstitial fluid (ISF) if calves are given an antibiotic. This data will be used to evaluate the *in vitro* killing of *Mannheimia hemolytica* and ultimately, which drug properties control antibiotic concentration in PELF.

Twelve 200 kg steers were administered either enrofloxacin (n=6) or cefitiofur crystalline free acid (n=6) per label instructions. PELF, ISF, and plasma were collected for at least 3 half-lives. PELF was collected via a novel naso-tracheal intubation method in which absorbent cotton was passed through a flexible tube to collect the sample in a minimally invasive manner. Using this method, we were able to collect a sufficient amount and quality of fluid to be analyzed via HPLC without causing any pathologic changes in the animal, thus eliminating the need for euthanasia. ISF was collected via an in vivo ultrafiltration method that allows the collection of free drug while excluding drug that is bound to protein. ISF and PELF concentrations along with in vitro assays of protein binding and lipophilicity will be compared to determine the impact of drug properties on drug diffusion into the airways.

Analysis via HPLC showed that cefitiofloxacin reached its maximum mean concentration in the PELF of 2.36 µg/mL at 48 hours. In addition, the concentrations remained above the documented minimum inhibitory concentration (MIC) for *Mannheimia hemolytica* (0.5 µg/ml) through the end of the 192 hour sampling window. Enrofloxacin and its active metabolite ciprofloxacin cumulatively reached peak concentration in the PELF of 0.269 µg/mL at 6 hours. The cumulative concentration of enrofloxacin and ciprofloxacin remained greater than the recorded MIC for *Mannheimia hemolytica* (0.03 µg/ml) through the end of the 48 hour study window.

**F-26** DISPOSITION OF AMPICILLIN TRIHYDRATE IN PLASMA, UTERINE TISSUE, AND LOCHIAL FLUID OF POST-PARTUM DAIRY CATTLE. B.C. Credille1, S. Giguere1, T.W. Vickroy2, H.J. Fishman3, A. Lee Jones1, M.E. Mason1, R.O. DiPietro1, D.T. Enslow3. 1University of Georgia, College of Veterinary Medicine, Athens, GA., 2University of Florida, College of Veterinary Medicine, Gainesville, FL., 3Boehringer-Ingelheim Vetmedica, Inc, St Joseph, MO.

The objective of this study was to determine the disposition of ampicillin in plasma, uterine tissue, lochial fluid, and milk of post-partum dairy cattle.

Ampicillin trihydrate was administered intramuscularly (IM) at a dose of 11 mg/kg of body weight every 24 h (n=6, total of 3 doses) or every 12 h (n=5 doses) for 3 days. Concentrations of ampicillin were measured in plasma, uterine tissue, lochial fluid, and milk using High Performance Liquid Chromatography (HPLC) with ultraviolet absorption.

Qualitative ampicillin concentrations were found in plasma, milk, and lochial fluid of all cattle within 30 min, 4 h, and 4 h of administration of ampicillin trihydrate, respectively. There was no significant effect of dosing interval (every 12 versus every 24 h) and no significant interactions between dosing interval and sampling site on the pharmacokinetic variables measured or calculated. Median peak ampicillin concentration at steady state was significantly higher in lochial fluid (5.27 µg/mL after q 24 h dosing) than in other sample types and significantly higher in plasma (3.11 µg/mL) than in milk (0.49 µg/mL) or endometrial tissue (1.55 µg/mL).

Ampicillin trihydrate administered once daily by the IM route at the label dose of 11 mg/kg BW achieves therapeutic concentrations in milk, lochial fluid, and endometrial tissue of healthy post-partum dairy cattle. Twice daily administration does not provide any advantages over once daily dosing.

**F-27** MATHEMATICS OF DRUG DISPOSITION IN THE BOVINE UDDER. A. Woodward, T. Whitten. Faculty of Veterinary Science, University of Melbourne, AUS.

Residues of antimicrobial drugs in milk are of public health importance, necessitating accurate prediction of the time course of milk drug concentrations. Efficacy of intramammary treatments for bacterial mastitis may also be related to the drug concentration-time relationship. Accurate pharmacokinetic modeling could simultaneously address these concerns. However, the udder does not conform to the assumptions of empirical pharmacokinetic models. A mathematical model was developed to describe the pharmacokinetics of intra-mammary antibiotics.

Accumulation of milk in the cisternal and alveolar compartments of each quarter was modelled using data donated from a previous study (S Davis et al 1998). Dissolution of drug formulation was modelled as first-order mass transfer of drug to the cistern. Drug movement between compartments occurs by both diffusion and milk flow. Systemic absorption of drug occurs from the alveolus. These processes were described by a system of differential-algebraic equations, and solved numerically using computer software (MATLAB).

The model was trained with a data set drawn from the literature (R. Jakeman et al 1991) describing bulk milk concentrations of penicillin G after intra-mammary administration of procaine penicillin to 15 cows, and corresponding volumes of production at each milking. Estimation of model parameters was achieved using nonlinear regression and simulated annealing, minimizing the residual sum-of-squares as the objective function, in a two-stage approach.
Local optima were encountered during optimization, presenting the challenge of finding and selecting a ‘correct’ solution. Validation of the model could be achieved with more detailed data for each compartment’s milk volumes and of drug concentrations of milk fractions. Data between milkings cannot be collected without disrupting drug movement; the resulting data sparsity is a challenge in model development. Nevertheless, individual cow data were successfully described. Model fit was poorer for one medium-producing (26.3L/day) and one low-producing (9.6L/day) cow. The formulation dispersion rate and drug diffusion rate were found to be positively correlated (R = 0.62). Udder residual volume was correlated with the maximum alveolar (R = 0.76) and cisternal (R = 0.65) volumes. Systemic absorption from the study quarter, and redistribution to milk, were identified in all but one cow. The diffusion rate constant for systemic drug transfer was not correlated with the diffusion rate between quarters (R = −0.16), and had a right-skewed distribution.

Milk drug residues and the duration of effective drug concentrations in the alveolus can be predicted with the parameterized model. Potential applications of this model include the design and simulation of pharmacokinetic studies, and prediction of milk discard times after off-label administration.