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2019 ACVIM Forum Research Abstract Program
Phoenix, Arizona, June 6 - 8, 2019
Index of Abstracts

| Time   | Presenting Author | Abstract Title                                                                                                                                                   |
|--------|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 4:15 PM| C01 Meghan Allen  | Hemodynamic, Echocardiographic, and Sedative Effects of Oral Gabapentin in Healthy Cats (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 4:30 PM| C02 Amelie Beaumier| Extracellular Vesicular Micromas as Potential Biomarker for Early Detection of Doxorubicin-Induced Cardiotoxicity in the Dog (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 4:45 PM| C03 Allison Gagnon| Phase I Clinical Trial of an Antithrombotic Drug Protocol Combining Apixaban and Clopidogrel in Dogs (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 5:00 PM| C04 Allison Gagnon| Thoracocentesis in the Chronic Management of Congestive Heart Failure in Cats: 34 cases (2002-2016) (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 5:15 PM| C05 Tamilselvam Gunasekaran | Short-Term Electrocardiographic Recordings From 24-Hour Holter To Assess Heart Rate in Dogs with Atrial Fibrillation (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 5:30 PM| C06 Weihow Hsue    | Reliability of Measuring of Left Atrial Size in Dogs with Subclinical Myxomatous Mitral Valve Disease (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 5:45 PM| C07 Maureen Oldach | Acute Pharmacodynamic Effects of Pimobendan in 22 Client-Owned Cats with Hypertrophic Cardiomyopathy (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
### NEUROLOGY

| Time       | Session | Presenters          | Title                                                                                           |
|------------|---------|---------------------|-----------------------------------------------------------------------------------------------|
| 10:15 AM   | N01     | Ashley Hechler      | Mechanical Quantitative Sensory Testing in Cavalier King Charles Spaniels with and without Syringomyelia (ACVIM Resident Research Award Eligible) |
| 10:30 AM   | N02     | Rachel Lampe        | Comparison of Cerebellomedullary and Lumbar Cerebrospinal Fluid Analysis in Dogs with Neurological Disease (ACVIM Resident Research Award Eligible) |
| 10:45 AM   | N03     | Rachel Lampe        | Reliability of Dynamic Magnetic Resonance Imaging in the Evaluation of the Canine Lumbosacral Spine (ACVIM Resident Research Award Eligible) |
| 11:00 AM   | N04     | Vishal Murthy       | Evaluation of Phosphorylated Neurofilament as a Prognostic Biomarker in Dogs with Thoracolumbar Intervertebral Disc Herniation (ACVIM Resident Research Award Eligible) |
| 11:15 AM   | N05     | Vishal Murthy       | Evaluation of Horner's Syndrome in Dogs with Cervical Myelopathy (ACVIM Resident Research Award Eligible) |
| 11:30 AM   | N06     | Tessa Smith         | Incidence of Acute Hepatopathy in Dogs Receiving Zonisamide (ACVIM Resident Research Award Eligible) |
| 11:45 AM   | N07     | Izumi Toyoda        | Clinicopathological Characteristics of Central Nervous System Histiocytic Sarcoma in Dogs (ACVIM Resident Research Award Eligible) |
| 1:45 PM    | N08     | Daniella Vansteenkiste | MicroRNA Expression in the Cerebrospinal Fluid of Great Danes with and without Osseous-Associated Cervical Spondylomyelopathy (ACVIM Resident Research Award Eligible) |
| 2:00 PM    | N09     | Samantha Vitale     | Comparison of Serum Trace Nutrient Concentration in Dogs with Idiopathic Epilepsy Compared to Healthy Dogs (ACVIM Resident Research Award Eligible) |
| 2:15 PM    | N10     | Lauren Downey Koos  | Multiple System Degeneration in Cats with Gene Mutation in Serine Active Site Containing 1 |
| 2:30 PM    | N11     | Chai-Fei Li         | Qualitative Assessment of Canine Ambulatory Electroencephalography Recordings over 48 Hours |
| 2:45 PM    | N12     | Daniel Webb         | Retrospective Evaluation of Risk Factors Associated with Clinical Relapse of Meningoencephalitis of Unknown Origin (ACVIM Resident Research Award Eligible) |
| 3:00 PM    | N13     | Taesik Yun          | Temporal and Anatomical Distribution of 18F-flutemetamol Uptake in Canine Brain Using Positron Emission Tomography |
| 3:15 PM    | N14     | Cecilia Rohdin      | Pathological Characteristics of 30 Pugs with a Chronic Thoracolumbar Myelopathy |
| 4:15 PM    | N15     | Sarah Sterling      | Long-Term Outcome of Niemann-Pick Disease Type C1 Cats Treated with Cyclodextrin |
| 4:30 PM    | N16     | Thomas Parmentier   | Effect of Prior General Anesthesia or Sedation on the Diagnostic Utility of Wireless Video Electroencephalography |

### SMALL ANIMAL INTERNAL MEDICINE - ENDOCRINOLGY

| Time       | Session | Presenters          | Title                                                                                           |
|------------|---------|---------------------|-----------------------------------------------------------------------------------------------|
| 10:15 AM   | EN01    | Ellen Heinrich      | Comparison of a Novel Continuous Insulin Protocol to Standard of Care Treatment for Diabetic Ketoacidosis (ACVIM Resident Research Award Eligible) |
10:30 AM EN02 Carole Schuppisser Comparison of Continuous Glucose Monitoring Profiles and Glycemic Variability During Day and Night in Healthy and Diabetic Cats (ESVE Award Winner)

10:45 AM EN03 Lisa Stammleer Determination of Thyroid Function in 131I Treated Hyperthyroid Cats: Serum Thyroid Variables Compared to Scintigraphy

11:00 AM EN04 Chen Gilor Comparison of Pharmacodynamics Between Insulin Glargine 100U/mL and Insulin Glargine 300U/mL in Healthy Cats

11:15 AM EN05 Tanner Slead Evaluation of Hematologic Abnormalities of Dogs with Diabetes Mellitus

11:30 AM EN07 Matthew Lechner The Utility of Serum, Plasma and Whole Blood Glucose Measurement On a Point-of-Care Glucometer

1:45 PM EN08 Linda Fleeman An Epidemiological Study of Dogs with Diabetes Mellitus Attending Primary Care Veterinary Clinics in Australia

2:00 PM EN09 Cynthia Ward Field Efficacy and Safety of ProZinc® Insulin evaluated in 276 Dogs with Diabetes Mellitus

2:15 PM EN10 Olga Norris Mean Cell Volume Difference (dMCV) is a Marker for Serum Hypertonicity in Diabetic Dogs (ACVIM Resident Research Award Eligible)

2:30 PM EN11 Mary Lester Prevalence of Anti-Insulin Antibodies in Diabetic Dogs Receiving Recombinant Human Insulin (ACVIM Resident Research Award Eligible)

3:00 PM EN13 Stephen Cai Heritability and Complex Segregation Analysis of Diabetes Mellitus in American Eskimo Dogs

3:15 PM EN15 Imogen Schofield Application and Findings of a Novel Quality-of-Life Tool for Dogs with Hyperadrenocorticism

SMALL ANIMAL INTERNAL MEDICINE - GASTROENTEROLOGY

10:15 AM GI01 Elle Donnini Familial Protein Losing Enteropathy in Gordon Setters: A Genome Wide Association Study (ACVIM Resident Research Award Eligible)

10:30 AM GI02 Barry Hedgespeth Association of Feline Tritrichomonas foetus Fecal PCR Results with Sample Collection Technique and Treatment History (ACVIM Resident Research Award Eligible)

10:45 AM GI03 Susan Mehain Pain Scores in Cats with Pancreatitis Compared to Control Cats (ACVIM Resident Research Award Eligible)

11:00 AM GI04 Susan Mehain Pain Assessment in Feline Pancreatitis at Diagnosis Compared to Recheck Evaluation (ACVIM Resident Research Award Eligible)

11:15 AM GI05 Elizabeth Dwyer Effect Of Fecal Microbiota Transplantation On The Fecal Microbiome Of Healthy Dogs Treated With Antibiotics.

11:30 AM GI06 Aarti Kathrani IL-13 and IL-33 mRNA are under-expressed in German shepherd dogs with inflammatory bowel disease

11:45 AM GI07 Aarti Kathrani Cytokine Production Following Stimulation of Ex-Vivo Whole Blood with Diet in Dogs with Chronic Enteropathy

1:45 PM GI08 Kristen Maxwell Fat-Soluble Vitamin Deficiencies in Canine Chronic Enteropathy
| Time   | Session | Speaker       | Title                                                                 |
|--------|---------|---------------|----------------------------------------------------------------------|
| 2:00 PM| GI09    | Maho Nakazawa | Duodenal Expression of Antimicrobial Peptides in Dogs with Idiopathic Inflammatory Bowel Disease and Intestinal Lymphoma |
| 2:15 PM| GI10    | Patricia Ishii| Fecal Microrna 29a is Increased in Dogs with Chronic Enteropathy    |
| 2:30 PM| GI11    | Alison Manchester | Tylosin Causes Dysbiosis Associated with Altered Fecal Unconjugated Bile Acids in Healthy Dogs |
| 2:45 PM| GI12    | Rachel Pilla  | Evaluation of the Recovery of the Fecal Microbiome and Metabolome of Dogs Following Acute Diarrhea |
| 3:00 PM| GI13    | Sara Wennogle | Lymphatic Endothelial Cell Immunohistochemistry for Evaluation of Intestinal Lymphatics in Dogs with Chronic Inflammatory Enteropathy |
| 3:15 PM| GI14    | Annalis Cigarroa | Comparison of the Fecal Dysbiosis Index Between Growing Puppies and Adult Dogs |

**SMALL ANIMAL INTERNAL MEDICINE - HEPATOLOGY**

| Time   | Session | Speaker       | Title                                                                 |
|--------|---------|---------------|----------------------------------------------------------------------|
| 5:15 PM| HP01    | Ashley Wilkinson | Platelet Function in Dogs with Chronic Liver Disease (ACVIM Resident Research Award Eligible) |
| 5:30 PM| HP02    | Vaclav Ceplecha | Evaluation of AST/ALT Ratio and AST to Platelet Ratio Index in Dogs with Hepatobiliary Disease |
| 5:45 PM| HP03    | Yuri Lawrence  | Untargeted Metabolomic Profiling of Serum from Dogs with Chronic Hepatic Disease |

**SMALL ANIMAL INTERNAL MEDICINE - PHARMACOLOGY**

| Time   | Session | Speaker       | Title                                                                 |
|--------|---------|---------------|----------------------------------------------------------------------|
| 4:15 PM| P01     | Brittany Southern | The Effects of Grapiprant on Acute Pain and Inflammation Following Ovariohysterectomy in Dogs |
| 4:30 PM| P02     | Jennifer Slovak | In Vitro Investigation of Feline Transdermal Gabapentin |
| 4:45 PM| P03     | Butch KuKanich | Evaluation of Multiple Doses of a Long-Acting Oral Opioid Containing an Abuse Deterrent in Dogs |

**SMALL ANIMAL INTERNAL MEDICINE - RESPIRATORY**

| Time   | Session | Speaker       | Title                                                                 |
|--------|---------|---------------|----------------------------------------------------------------------|
| 5:00 PM| R01     | Carrie Cavett | Pharmacokinetics of a Modified, Compounded Theophylline Product in Dogs (ACVIM Resident Research Award Eligible) |

**EQUINE**

| Time   | Session | Speaker       | Title                                                                 |
|--------|---------|---------------|----------------------------------------------------------------------|
| 10:15 AM| E01    | Nicolas Herteman | Effect of Exercise On Electrical Impedance Tomography Derived Flow Variables in Horses with Equine Asthma (ACVIM Resident Research Award Eligible) |
| 10:30 AM| E02    | Nicolas Herteman | Distribution of Ventilation in Equine Pulmonary Diseases Measured by Electrical Impedance Tomography: A Case-Series (ACVIM Resident Research Award Eligible) |
| 10:45 AM| E03    | Sophie Mainguy-Seers | Does Azithromycin Potentiate the Anti-Remodeling Effects of Fluticasone in Equine Asthma? (ACVIM Resident Research Award Eligible) |
| 11:00 AM| E04    | Tamara Sierra-Rodriguez | Plasma, Pulmonary Epithelial Lining Fluid, and Nasopharyngeal Wash Concentrations of Voriconazole after Nebulization in Horses (ACVIM Resident Research Award Eligible) |
| 11:15 AM| E05    | Sarah Thomas  | Risk Factors for the Development of Equine Asthma (ACVIM Resident Research Award Eligible) |
| 11:30 AM| E06    | Shannon Darby  | Plasma l-Indospicine and 3-Nitropropionic Acid in Ponies Fed Creeping Indigo (ACVIM Resident Research Award Eligible) |
| Time       | Session | Presenter       | Title                                                                 |
|------------|---------|----------------|----------------------------------------------------------------------|
| 11:45 AM   | E07     | Anna Bohlin    | Assessment of an American Neonatal Foal Survival Scoring System in a Danish-Swedish Population (ECEIM Award Winner) |
| 1:45 PM    | E08     | Jacob Swink    | Changes in Cardiac Troponin I Concentrations in Healthy and Critically Ill Neonatal Foals (ACVIM Resident Research Award Eligible) |
| 2:00 PM    | E09     | Melissa Fenn   | Agreement of an Equine Stall-Side and a Laboratory Major Crossmatch Test in Healthy Horses (ACVIM Resident Research Award Eligible) |
| 2:15 PM    | E10     | Emily Schaefer | Comparison of White and Red Blood Cell Counts Between Warmbloods and Other Breeds of Horses (ACVIM Resident Research Award Eligible) |
| 2:30 PM    | E11     | Erica McKenzie | Biochemical Characteristics of a Commercial Plasma Product and Response to Transfusion of Clinically Diseased Horses |
| 2:45 PM    | E12     | Linda Paul     | Comparison of the gastric microbiome in horses with and without Equine Glandular Gastric Disease (ACVIM Resident Research Award Eligible) |
| 3:00 PM    | E13     | Lynn Martin    | Effect of Lidocaine On Stimulated Leukocyte Proinflammatory Cytokine Production in Horses (ACVIM Resident Research Award Eligible) |
| 3:15 PM    | E14     | Bruno Karam    | Performance of Single Versus Dual Dose Equine Influenza Vaccination in Immunized Horses After Changing Manufactures (ACVIM Resident Research Award Eligible) |
| 3:45 PM    | E15     | Lisa Edwards   | Phosphorylated Neurofilament Heavy Subunits as an Antemortem Biomarker in Equine Neurodegenerative Diseases (ACVIM Resident Research Award Eligible) |
| 4:15 PM    | E16     | Elizabeth Hodge| Effect of the Thyrotropin Releasing Hormone Stimulation testing on the Oral Sugar Test in Horses (ACVIM Resident Research Award Eligible) |
| 4:45 PM    | E17     | Jacob Swink    | Sex Steroids in Healthy and Hospitalized Neonatal Foals (ACVIM Resident Research Award Eligible) |
| 5:00 PM    | E18     | Tobias Warnken | Selection of Assay for Quantification of Equine Insulin Affects Results of Oral Glucose Test and Combined Glucose-Insulin Test in Horses (ECEIM Award Winner) |
| 5:15 PM    | E19     | Lauren Bookbinder| Effect of a-Tocopherol Deficiency and Repletion on Skeletal Muscle Morphology in Horses (ACVIM Resident Research Award Eligible) |
| 5:30 PM    | E20     | Mariya Pitel   | Owner Supplementation Practices and Their Influence on Selenium and Vitamin E Status in Horses (ACVIM Resident Research Award Eligible) |
| 5:45 PM    | E21     | Emily Berryhill| Pharmacokinetics of Maropitant Citrate After Oral Administration of Multiple Doses in Healthy Adult Horses |

**FOOD ANIMAL**

| Time       | Session | Presenter       | Title                                                                 |
|------------|---------|----------------|----------------------------------------------------------------------|
| 10:15 AM   | F01     | Ryan Breuer    | Agreement Between 3 Different Tests for Colostrum Quality in Beef Cattle with High Risk Pregnancies (ACVIM Resident Research Award Eligible) |
| 10:30 AM   | F02     | Ryan Breuer    | Agreement Between Four Different Tests for Assessment of Passive Transfer in High-Risk Beef Calves (ACVIM Resident Research Award Eligible) |
| 10:45 AM   | F03     | Maria Puerto-Parada | Cerebrospinal Fluid Protein Concentration, Nucleated Cells and Red Blood Cells Counts From Recumbent Cows (ACVIM Resident Research Award Eligible) |
| 11:00 AM   | F04     | Heather Bornheim| Priming Antibody Responses Against BRSV Induced in Beef Calves Through Early Vaccination (ACVIM Resident Research Award Eligible) |
11:15 AM  F05  Suzanne Clergue  Impact of Conservation Methods On the Quality of Rumen Juice Before Transfaunation in Dairy Cattle (ACVIM Resident Research Award Eligible)

11:30 AM  F06  Evelyn MacKay  Retrospective Study of Pet Pigs Presenting for Lameness (ACVIM Resident Research Award Eligible)

11:45 AM  F07  Mireille Meylan  Antimicrobial Treatment in Veal Calves and Association with Antimicrobial Resistance in Pasteurellaceae and E. coli

ORAL PRESENTATIONS - FRIDAY, JUNE 7

| Time      | #    | Presenting Author | Abstract Title                                                                 |
|-----------|------|-------------------|-------------------------------------------------------------------------------|
| 1:45 PM   | C08  | Derek Matthews    | Comprehensive Cardiac Evaluation Including Magnetic Resonance Imaging in Naturally-Infected Dogs Seropositive for Chagas Disease (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 2:00 PM   | C09  | Shane Murphy      | Diagnostic Utility of Point-of-Care Lung Ultrasound for Monitoring Congestive Heart Failure in Dogs (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 2:15 PM   | C10  | Saki Kadotani     | Single Dose Pharmacokinetics of Two Oral Formulations of Ranolazine in Healthy Cats (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 2:30 PM   | C11  | Christina Plante  | Effect of Concurrent Omeprazole-Clopidogrel Administration on Platelet Function and Clopidogrel Metabolism in Healthy Cats (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 2:45 PM   | C12  | Megan Poad        | Utility of Radiographic Parameters To Predict Echocardiographic Cardiac Enlargement in Dogs with Mitral Valve Disease (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 3:00 PM   | C13  | Michelle Rohrbaugh| Detection of Congestive Heart Failure by Doppler Echocardiography in Cats with Hypertrophic Cardiomyopathy (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 3:15 PM   | C14  | Joonbum Seo       | Biomarker Changes with Systolic Anterior Motion of the Mitral Valve in Cats with Hypertrophic Cardiomyopathy (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 4:15 PM   | C15  | Brienne Williams  | Transcatheter Mitral Valve Placement in Experimental Purpose-Bred Dogs (ACVIM Resident Research Award Eligible & Cardiology Research Abstract Award Eligible) |
| 4:30 PM   | C16  | Marlos Gonçalves Sousa | Assessment of Right Ventricular Systolic Function by Tissue Motion Annular Displacement in Healthy Dogs |
| 4:45 PM   | C17  | Katherine Scollan  | Evaluation of the NuCLEUS-X Balloon Valvuloplasty Catheter for Severe Pulmonic Stenosis in Dogs |
| 5:00 PM   | C18  | Ana Paula Sarraff Lopes | Assessment of Atrial Function Using Tissue Mitral Annular Displacement in Healthy Dogs |
| 5:15 PM   | C19  | Kazuki Takamura   | Related Factors for Residual Coughing in Dogs After Mitral Valve Repair |
| 5:30 PM   | C20  | Wan-Ching Cheng   | Disorganization of β-Catenin in Cats with Hypertrophic Cardiomyopathy |
5:45 PM  C21  Kursten Pierce  Radiation Dose During Interventional Cardiology Procedures

**ONCOLOGY**

8:00 AM  O01  Catherine Chan  Phase I Dose Escalating Study of Oral Cyclophosphamide in Tumor-Bearing Cats (ACVIM Resident Research Award Eligible & Early Career Clinical Oncology Research Award Eligible)

8:15 AM  O02  Megan Duckett  Adrenergic Receptor Antagonists Decrease Bone Sarcoma Cell Viability and May Enhance Radiosensitivity with Sustained Treatment (ACVIM Resident Research Award Eligible)

8:30 AM  O03  Erin Lashnits  Molecular Prevalence of Bartonella, Babesia, and Hemotropic Mycoplasma Species in Dogs with Hemangiosarcoma

8:45 AM  O04  Kenjiro Kaji  Protease-Activated Receptor-2 is Associated with Adverse Outcomes in Canine Mammary Carcinoma

9:00 AM  O05  Masanao Ichimata  Prognostic Factors in Dogs with Pulmonary Carcinoma Treated with Surgery plus or minus Adjuvant Chemotherapy

9:15 AM  O06  Katherine Hansen  Outcomes with Conventional Fractionated and Stereotactic Radiotherapy for Suspected Heart-Base Tumors in Dogs

9:30 AM  O07  Kristine Walters  Identification and Monitoring of Doxorubicin-Induced DNA Modifications as Biomarkers for Improving Veterinary Chemotherapy (ACVIM Resident Research Award Eligible)

10:30 AM  O08  Dana Connell  Upregulation of the Hedgehog Pathway and Associated Anti-Apoptotic Factors Can Be Inhibited by Itraconazole in Canine Osteosarcoma Cell Lines (VCS Award Winner)

10:45 AM  O09  Pierre Boyé  A Randomized Double-Blind Trial Investigating the Efficacy and Safety of a New Polyamine-Vectorized Anticancer Drug (F14512) for Treatment of P-Glycoprotein Overexpressing Non-Hodgkin Lymphoma in Dogs (VCS Award Winner)

11:00 AM  O10  Mona Uchida  Expression and Genetic Variation of Apoptosis Inhibitor of Macrophage in Canine Histiocytic Sarcoma

**SMALL ANIMAL INTERNAL MEDICINE - HEMATOLOGY**

8:00 AM  HM01  Melissa Gettinger  Retrospective Evaluation of Splenectomy in the Treatment of Canine Primary Immune Thrombocytopenia (ACVIM Resident Research Award Eligible)

8:15 AM  HM02  Sara Ravicini  Cold Storage of Canine Platelet Concentrates in Additive Solutions: An In Vitro Assessment (ACVIM Resident Research Award Eligible)

8:30 AM  HM03  Verena Steiner  Impact of Sampling Method on Thromboelastography Variables

8:45 AM  HM04  Akiyoshi Tani  Gene Mutations Detected in Miniature Dachshunds Diagnosed with Myelodysplastic Syndrome in Japan

9:00 AM  HM05  Matthew Kornya  Effects of Signalment and Phlebotomy Technique on Platelet Clumping in Cats

9:15 AM  HM06  Leigh Howard  Thrombocytosis in 158 cats (2011-2018)

**SMALL ANIMAL INTERNAL MEDICINE - INFECTIOUS DISEASE**

1:45 PM  ID01  Rebecca Rittenberg  Retrospective Case Review of Canine Influenza Virus Outbreak in Jefferson County, Kentucky (2017) (ACVIM Resident Research Award Eligible)
2:00 PM  ID02  Casey Sleznikow  Evaluation of Sample Sources for Point of Care Cytologic Diagnosis of Cytauxzoon Felis for Practitioners (ACVIM Resident Research Award Eligible)

2:15 PM  ID03  Allison Wilson  Clinicopathologic Findings, Treatment, and Outcomes in 21 Dogs with Hepatozoon Americanum (ACVIM Resident Research Award Eligible)

2:30 PM  ID04  Sarah Sweet  Using Big Data to Investigate Intestinal Parasite Prevalence by Geographic Region and Age

2:45 PM  ID05  Alexis Seguin  A Novel Parvovirus in an Unexplained GI Outbreak in Dogs from Colorado

3:00 PM  ID06  Erin Lashnits  Bartonella Infection in a Population of Chronically Ill People with Self-reported Neuropsychiatric Symptoms

3:15 PM  ID07  Jennifer Ogeer  Investigating Association Between Exposure To Ehrlichia and Risk of Developing Chronic Kidney Disease in Dogs

SMALL ANIMAL INTERNAL MEDICINE - NEPHROLOGY / UROLOGY

8:00 AM  NU01  Caroline Aldridge  Urinary Biomarkers as a Tool in the Diagnosis of Asymptomatic Bacteriuria in Dogs (ACVIM Resident Research Award Eligible)

8:15 AM  NU02  Millie Grimes  Retrospective Evaluation of Characteristics Associated with Urinary Bacterial Growth in Proteinuric Dogs (ACVIM Resident Research Award Eligible)

8:30 AM  NU03  Laura Rayhel  Outcomes, Clinicopathologic and Histopathologic Characteristics of Feline Proteinuric Kidney Disease: 61 Cases (ACVIM Resident Research Award Eligible)

8:45 AM  NU04  Phillip Watson  An Algorithm Based On Clinical Data Predicts Feline Chronic Kidney Disease Two Years Before Diagnosis

9:00 AM  NU05  Beasley Mason  Open-Label Pilot Efficacy Study of Recombinant Feline Erythropoietin in Client-Owned Cats with Non-Regenerative Anemia

9:15 AM  NU06  Beasley Mason  Double-Blind, Placebo-Controlled-Study To Evaluate Mirtazapine-Transdermal-Ointment in Cats Experiencing Unintended-Weight-Loss: Analysis of Cats with Suspected Renal-Disease

9:30 AM  NU07  C. Guillermo Couto  Serum Symmetric Dimethylarginine Concentrations in Greyhound Puppies – Evidence for Breed Specific Physiologic Differences

10:30 AM  NU08  Devyn Schultz  Analysis of Survival Among Different Categories of Chronic Kidney Disease in Dogs

10:45 AM  NU09  Carrie Palm  Efficacy of Hemoperfusion and Hemodialysis for Treatment of Carprofen and Ibuprofen Toxicosis in Dogs

11:00 AM  NU10  Sheri Ross  Application of Active Kidney Injury Biomarkers To Predict Kidney Involvement in Canine Urinary Tract Infection

11:15 AM  NU11  Matthew Kornya  Dissolution of Feline Struvite Uroliths with a Urinary Calming Diet

11:30 AM  NU12  Demetria Vasilatis  Comparison of IDEXX SediVue Dx® with Manual Microscopy for Detection of casts in Canine Urine

11:45 AM  NU13  Yann Quéau  The Effect of Dietary Sodium on Urinary Calcium and Calcium Oxalate Relative Supersaturation in Cats

4:15 PM  NU14  Harmeet Aulakh  Stability of Urine Specimens Stored with Preservatives at Room Temperature or without Preservatives Under Refrigeration

4:30 PM  NU15  Felipe dos Santos Muniz  Evaluation of Serum Symmetric Dimethylarginine (SDMA) in Dogs Naturally Infected by Leishmania infantum in Brazil
4:45 PM NU16 Valerie Parker Effects of Calcifediol Supplementation on Markers of Chronic Kidney Disease-Mineral Bone Disorder in Dogs

5:00 PM NU17 Adam Rudinsky Characterization and In Vitro Susceptibility of Canine UPEC Isolates to a Novel E. coli Probiotic

5:15 PM NU18 Vincent Leynaud Smartphone-Based Colorimetric Method for At-Home Urinalysis Dipsticks Reading in Cats: A Pilot Study

5:30 PM NU19 Tony Blanco Analysis of Bias Between the Sedivue Dx (TM) and Manual Microscopy in Detecting Urine Sediment Cells

5:45 PM NU20 Briana Staten-Hunkler Urinary Tract Infection Escherichia Coli’s Potential to Produce Biofilm and the Effect on Antimicrobial Resistance

SMALL ANIMAL INTERNAL MEDICINE - NUTRITION / METABOLISM

10:30 AM NM01 Taylor Foster Evaluation of Whisker Stress and Eating Habits in Healthy Cats

10:45 AM NM02 Dan Su Impact of Feeding Method On Overall Activity of Indoor, Client-Owned Dogs

11:00 AM NM03 Arnon Gal Less is More? Ultra-Low Carbohydrate Diet and Working Dogs Performance

11:15 AM NM04 Jennifer MacLeay Food with Novel Fiber Blend Improves Clinical Outcomes and Changes Gastrointestinal Microbiome Metabolism in Dogs

11:30 AM NM05 Brian Zanghi Hydration Measures in Cats During Brief Anesthesia: Intravenous Fluids Versus Pre-Procedure Water Supplement Ingestion

11:45 AM NM06 Itsuma Nagao Evaluation of Visceral Fat Mass in Dogs by Computed Tomography

1:45 PM NM07 David Griffin A Novel New S-Adenosylmethionine Salt with Increased Bioavailability

2:00 PM NM08 Dennis Jewell Cats with a Specific AGXT2 Genotype Differentially Respond To Dietary Intervention for Calcium-Oxalate Stone Risk

2:15 PM NM09 Emma Wils-Plotz Nutrient-Enriched Water Supplements Nutritively Support Hydration in the Domestic Cat

2:30 PM NM10 Stacy Ownby Pharmacodynamic and Pharmacokinetic Analysis of an Oral Sulforaphane Source in Beagle Dogs

SMALL ANIMAL INTERNAL MEDICINE - OTHER

9:30 AM OT01 Jared Jaffey Molecular Genetically Defined Canine Ehlers Danlos Syndromes

2:45 PM OT02 Julie Spears Measurement of Volatile Sulfur Compounds in Canine Saliva Using Gas Chromatography

POSTER PRESENTATIONS - THURSDAY, JUNE 6

Time # Presenting Author Abstract Title

CARDIOLOGY

12:15 PM C22 Lyndsay Kong Thymidine Kinase-1 and C-Reactive Protein Concentrations in Dogs with Neoplastic and Nonneoplastic Pericardial Effusion

12:30 PM C23 Guilherme Goldfeder Standardization of Echocardiographic Values of Left Atrial Measurement in Yorkshire Terrier Dogs

12:45 PM C24 Christopher Whipp Pulmonary Artery Denervation as a Treatment of Refractory Pulmonary Hypertension

1:00 PM C25 Ashley Bava Worsening Renal Function in Dogs during Treatment of Congestive Heart Failure: 2008-2018
| Time     | Code | Presenter          | Title                                                                                   |
|----------|------|--------------------|-----------------------------------------------------------------------------------------|
| 1:15 PM  | C26  | Beatrice Besche    | Efficacy of Torasemide in Degenerative Mitral Valve Disease Dogs with New Onset CHF     |
| 1:15 PM  | C27  | Jin-Ok Ahn         | Effect of Sodium Nitroprusside, Furosemide and Dobutamine in Dogs with Cardiogenic PE   |
| 1:15 PM  | C28  | Matthew Boothe     | Oral Pharmacokinetics of Slow-Release Metoprolol in Dogs                                 |
| 1:15 PM  | C29  | Sarah Cavanaugh    | Amino Acid Concentrations and Echocardiographic Findings in Dogs Fed a Commercial Diet  |
| 1:15 PM  | C30  | I Ping Chan        | Left Atrial Size and Function Predicting The Risk of CHF in Small-breed Dogs             |
| 1:15 PM  | C31  | Ayaka Chen         | CT Angiography in 5 Dogs with Atrial Septal Defect and Partial Anomalous Venous Connection |
| 1:15 PM  | C32  | Liselotte Christiansen | Pharmacokinetic Analysis after Multiple-dose Administration of Coenzyme Q10 in Mitral Valve Disease |
| 1:15 PM  | C33  | Marlos Sousa       | Assessment of Heart Rate Turbulence in Dogs with Myxomatous Mitral Valve Disease        |
| 1:15 PM  | C34  | Laetitia Duler     | Identification of Neutrophil Extracellular Traps in Feline Cardiogenic Arterial Thromboembolism: A Pilot Study |
| 1:15 PM  | C35  | Kate Creevy        | Reasons for Exclusion of Apparently Healthy Dogs from a Phase II Rapamycin Clinical Trial |
| 1:15 PM  | C36  | India Gill         | Indirect Doppler Systolic Blood Pressure Measurements Taken With and Without Headphones |
| 1:15 PM  | C37  | Marlos Sousa       | Assessment of Systolic Function Using Tissue Motion Displacement in Mitral Valve Disease |
| 1:15 PM  | C38  | Marlos Sousa       | Congenital Heart Disease in Dogs: A Retrospective Study of 95 Cases                      |
| 1:15 PM  | C39  | Marlos Sousa       | Computed Radiology as a Screening Test for Identification of Congenital Heart Disease in Dogs |
| 1:15 PM  | C40  | Catherine Gunther-Harrington | Where There's Smoke There's Fire: Echocardiographic Findings in 51 Cats with Burns from California Wildfires |
| 1:15 PM  | C41  | Chorok Jeong       | Application of Coagulation Function by Thromboelastography in Mitral Valve Insufficiency |
| 1:15 PM  | C42  | Nobuyuki Kanno     | Echocardiographic Assessment After Mitral Valve Plasty in Dogs                          |
| 1:15 PM  | C43  | Kathleen Kelly     | The Causes of Canine Myocarditis and Myocardial Fibrosis Are Elusive by Targeted Molecular Testing |
| 1:15 PM  | C44  | Jessica Ward       | Canine Myocarditis: Clinical Presentation, Cardiovascular Findings, Etiology, and Outcome |
| 1:15 PM  | C45  | Stacey Leach       | The Prevalence of the RNA-Binding Motif 20 Mutation in Genotyped Dogs                   |
| 1:15 PM  | C46  | Ga-Won Lee         | The Prevalence and Prognostic Effects of Non-thyroidal Illness in Mitral Valve Disease  |
| 1:15 PM  | C47  | Qinghong Li        | Compositional and Functional Changes in Gut Microbiome in Mitral Valve Disease          |
| 1:15 PM  | C50  | Ta-Li Lu           | Point-of-care NT-proBNP Assay to Screen Apparently Healthy Cats for Cardiac Disease in General Practice |
| Time     | Speaker       | Title                                                                 |
|----------|---------------|----------------------------------------------------------------------|
| 1:15 PM  | Ruthnea Muzzi | Systolic Dysfunction Caused by Parvovirus In Dogs: An Echocardiography Feature Tracking Assessment |
| 12:15 PM | Ruthnea Muzzi | Assessment of Myocardial Deformation by Feature Tracking Echocardiography in Cats with Ventricular Septal Defect |
| 12:30 PM | Ruthnea Muzzi | Left Atrial Function by Bidimensional Feature Tracking Echocardiography in Dogs with Asymptomatic Mitral Valve Disease |
| 12:45 PM | Dmitrii Oleynikov | Endomyocardial Biopsy-Proven Myocarditis in Cats. A Clinical Case Series |
| 1:00 PM  | Naoko Oshima  | Residual Severe Mitral Regurgitation After Mitral Valve Plasty in 6 Dogs with Mitral Valve Disease |
| 1:15 PM  | Tatsuyuki Osuga | Echocardiographic Estimation of Left Ventricular-Arterial Coupling in Canine Myxomatous Mitral Valve Disease |
| 12:15 PM | Acácio Pacheco | Electrocardiographic Evaluation in Dogs Treated with Imidocarb and Atropine/Nacl 0,9% |
| 12:30 PM | Guilherme Goldfeder | Total Atrial Conduction Time Evaluated with Tissue Doppler Imaging in Dogs with Mitral Valve Disease |
| 12:45 PM | Andres Pun Garcia | Echocardiographic and Radiographic Assessment of Left Atrial Enlargement in Dogs with Myxomatous Mitral Valve Degeneration |
| 1:00 PM  | Brian Scansen  | Transcatheter Closure of Patent Arterial Duct in Small Animals After Unsuccessful Surgical Ligation |
| 1:15 PM  | Marlos Sousa   | Assessment of Longitudinal Systolic Function Using Tissue Motion Annular Displacement in Healthy Cats |
| 12:15 PM | Simon Swift    | Assessment of Major Vascular Flow Patterns in the American Alligator (Alligator Mississipiensis) |
| 12:30 PM | Hasan Albasan  | Comparison of M Mode and Speckle Tracking Data in Turkish Kangal Dogs with DCM |
| 1:00 PM  | Rebecca Tinklenberg | Dose-Responsive Effects of Oral Short-Term Prednisone Therapy on Clinicopathologic and Hemodynamic Variables in Healthy Dogs |
| 1:00 PM  | Takuya Uehara  | High Energy Loss in the Left Ventricle of Small Dogs Detected by Vector Flow Mapping |
| 12:15 PM | Rachel Van Zile | Evaluation of Cardiac Cycle Length and Stroke Volume in Dogs with Atrial Fibrillation |
| 12:30 PM | Leticia Yonezawa | Echocardiographic Evaluation of Obese Dogs Submitted to an Exercise Routine |
| 12:45 PM | Philip Fox     | Long-term Incidence/Risk of Noncardiovascular and All-cause Mortality in Apparently Healthy and Preclinical Hypertrophic Cardiomyopathy Cats |
| 1:00 PM  | Leticia Yonezawa | Pre- and Post-Exercise Cardiovascular Evaluation in Obese Dogs Submitted to an Exercise Routine |
| 1:15 PM  | Chorong Yoo    | A Retrospective Assessment of Echocardiography and Clinical Efficacy of Ramipril in Dogs with Heart Disease |

**SMALL ANIMAL INTERNAL MEDICINE - ENDOCRINOLGY**

| Time     | Speaker       | Title                                                                 |
|----------|---------------|----------------------------------------------------------------------|
| 3:35 PM  | Alysha Vincent | Low-Dose DOCP Treatment of Hypoadrenocorticism in Dogs: A Randomized Controlled Clinical Trial |
3:35 PM EN17 Joana Aguiar Long-Term Effect of Neutering on Plasma Luteinizing Hormone Concentrations in Cats
3:50 PM EN18 Joana Aguiar Does Neutering Drive the Development of Hyperthyroidism in Cats? An Epidemiologic Study
3:35 PM EN19 Viviani De Marco Adrenal Incidentaloma: Clinical and Histopathological Characteristics
3:50 PM EN20 Viviani De Marco Investigation of Hyperlipidemia in Healthy Schnauzers in Brazil
3:35 PM EN21 Arnon Gal Thinking Big of a Small Tumor: Glucagon Stimulation Test for Insulinomas
3:35 PM EN22 Hakhyun Kim Effect of Levothyroxine Administration on Serum NT-proBNP and cTnI Concentrations in Hypothyroid Dogs
3:50 PM EN23 Agostino Buono The Effect of Oral Dexamethasone on Urine Corticoid: Creatinine Ratios in Healthy Dogs
3:50 PM EN24 Noriyuki Nagata Subclinical Pituitary-Dependent Hypercortisolism in Dogs: Comparison of Clinical Findings and Outcomes with Overt Hypercortisolism
12:15 PM EN25 Alan Pöppl Risk-Factors for Feline Hyperthyroidism in Southern Brazil: A Case-Control Study
12:30 PM EN26 Alan Pöppl Selegiline and Trilostane Association for Canine Pituitary-Dependent Hyperadrenocorticism: A Randomized Clinical Trial
12:45 PM EN27 Alan Pöppl Urinary Tract Infection in Canine Hyperadrenocorticism
3:50 PM EN28 Fabio Teixeira Leptin, IL-6 and Glucagon Differ in Diabetic vs Healthy Dogs But Diet Influences Only Leptin

SMALL ANIMAL INTERNAL MEDICINE - GASTROENTEROLOGY

6:00 PM GI16 Su-Jin An A Prospective Study of Effects of Fat Ingestion on the Intestinal Mucosa in Normal Dogs
6:00 PM GI17 Sandra Bermudez Sanchez Prevalence of Methanogens in Fecal Samples of Dogs with Chronic Enteropathy
6:00 PM GI18 Bradley Bishop Biomechanical Comparison of Two Percutaneous Gastropexy Techniques for Percutaneous Endoscopic Gastrostomy Tubes
6:15 PM GI19 Amanda Blake Quantification of Fecal Bile Acids and Clostridial Species in Puppies
6:00 PM GI20 Amanda Blake Comparison of Plasma, Serum, and Whole Blood Amino Acid Concentrations in Healthy Dogs
6:30 PM GI21 Julien Dandrieux Serum Cytokines before and after Treatment in a Cohort of Dogs with Chronic Enteropathy
6:15 PM GI22 Ricardo Duarte Chronic Enteropathies and Pruritus in Dogs
6:15 PM GI23 Alexander Gallagher Survey of Endoscopic Techniques for Esophageal and Gastric Foreign Body Removal in Dogs and Cats
6:15 PM GI24 Katherine Hedges Evaluation of the Effect of a Famotidine Continuous Rate Infusion on Intragastric pH in Dogs
6:15 PM GI25 Romy Heilmann Correlation between Mucosal and Fecal S100A12 Levels and Histologic Changes in Canine Chronic Inflammatory Enteropathy
6:00 PM GI26 Amanda Kuhl Comparative Analysis of the Effect of Intravenously Administered Acid Suppressants on Gastric pH in Dogs
6:30 PM GI27 Alison Manchester Conjugated and Unconjugated Bile Acids in Feces From Dogs with Chronic Inflammatory Enteropathy
6:30 PM GI28 Ko Nakashima Ultrasonographic Features of Intestinal Large Cell Lymphoma, Small Cell Lymphoma and Chronic Enteritis in Dogs
6:30 PM GI29 Gayoung Noh Effect of Oral Superoxide Dismutase Supplementation on the Clinical Improvement of Canine Inflammatory Bowel Disease
6:30 PM GI30 Hiroshi Ohta Genome-Wide Quantitative DNA Methylation Analysis in Canine Intestinal Lymphoma
6:30 PM GI31 Chul Park Thromboelastographic Evaluation in Dogs with Acute Pancreatitis
6:30 PM GI32 Jennifer Slovak Evaluation of PTHrP as a Biomarker in Feline Pancreatitis
6:45 PM GI33 Kate Spies Canned Gastrointestinal Diets and Feline Fecal Occult Blood Testing
6:45 PM GI34 Sara Wennogle Clinical, Clinicopathologic, and Histologic Variables in Dogs with Chronic Enteropathy and Low or Normal 25(OH)D
6:45 PM GI35 Melanie Werner Amoxicillin-Clavulanate in Dogs with Acute Diarrhea: Minimal Alteration of the Intestinal Microbiome Composition

SMALL ANIMAL INTERNAL MEDICINE - HEMATOLOGY
3:35 PM HM07 Melanie Dickinson Assessment of Extended Sample Storage for Delayed Platelet Function Testing in Normal Dogs
3:35 PM HM08 Braden Ishler StablePlate RX Canine Promotes In Vitro Thrombin Generation and Thrombus Formation Under High Shear
3:50 PM HM09 Lucy Kopecny Application of Therapeutic Plasma Exchange in Dogs with Immune-Mediated Thrombocytopenia
3:50 PM HM10 Hayden Marshall Evaluating Crossmatch Incompatibility and the Accuracy of a Point-of-Care Crossmatching Method in Cats
3:50 PM HM11 Hayden Marshall Evaluating the Incidence of Crossmatch Incompatibility and the Accuracy of Point-of-Care Crossmatching Methods in Dogs
3:50 PM HM12 Takuro Nagahara Effects of Budesonide Administration on Coagulation Variables in Dogs
3:50 PM HM13 Verena Steiner Method Comparison Between the Conventional Thromboelastograph 5000 and the New Thromboelastograph S6

SMALL ANIMAL INTERNAL MEDICINE - HEPATOLOGY
6:45 PM HP04 Adam Miller Metabolomic Investigation of Plasma Samples from Dogs with Hepatocutaneous Syndrome
6:45 PM HP05 Ayne Murata Hayashi Fibrinogen Levels as a Factor for Surgical Decision-Making in Dogs with Portosystemic Shunt
6:45 PM HP06 Punyamanee Yamkate Assessment of Copper Concentrations in Archived Cat Liver Specimens

SMALL ANIMAL INTERNAL MEDICINE - IMMUNOLOGY
9:35 AM IM01 Harry Cridge Effects of Cyclosporine on Feline Lymphocytes Activated In Vitro
9:35 AM IM02 Kenjiro Fukushima A Retrospective Study on Use of Mycophenolate Mofetil in Dogs with Immune-Mediated Diseases
9:50 AM IM03 Priscila Furtado Evaluation of Interleukin-6, Interleukin-8 and TNF-α as Prognostic Markers in Dogs in Critical Conditions
9:50 AM IM04 John Loftus Vitamin D Metabolites and Serum CXCL10 in Dogs with Immune Mediated Disease
SMALL ANIMAL INTERNAL MEDICINE - INFECTIOUS DISEASE

6:00 PM  ID08  Emilia Bourassi  Serologic and Urinary Survey of Exposure to Leptospirosis in a Feral Cat Population of Prince Edward Island

6:00 PM  ID09  Linda Okonkowski  Detection of Adenovirus-2, Parainfluenzavirus-2, and Bordetella bronchiseptica following Intranasal Vaccination of Dogs in a Shelter

6:00 PM  ID10  Melissa Beall  Stage of Feline Leukemia Virus Infection Impacts Diagnostic Test Results: A Prospective Study

6:00 PM  ID11  Pierce Chan  Pilot Study Assessing the Role of Babesia vogeli in Feline Hemolytic Anemia in the USA

6:00 PM  ID12  Chandra Chandrashekar  Field Performance of Two In-clinic Tests for Lyme

6:15 PM  ID13  Erica Chavez-Peon  Ultrasonographic Findings of Gastrointestinal Histoplasmosis in Dogs

6:15 PM  ID14  Nida Chornarm  Associations Among Feline Hemoplasmas and Select Variables in Domestic Cats in the USA

6:15 PM  ID15  Michelle Evasion  Vector-Borne Pathogen and Leptospira spp. antibodies in Atlantic Canada dogs: The Canadian K9 Lifetime Study

6:15 PM  ID16  Katrin Hartmann  Comparison of Different Point-of-Care Tests to Detect Antibodies Against Canine Distemper Virus

6:15 PM  ID17  Emmelyn Hsieh  Point Prevalence Survey of Antibiotic Use in a Veterinary Teaching Hospital

6:15 PM  ID18  Yikyeong Jeong  Serum Procalcitonin and Heparin Binding Protein Levels as Biomarkers of Bacterial Infection in Cats

6:15 PM  ID19  Kate KuKanich  Development of Feline and Canine Urinary Escherichia coli Antibiograms to Improve Antimicrobial Stewardship in Kansas

6:15 PM  ID20  Michael Lappin  Clinical Effects Induced by 1 Dose of H3N2 Vaccine in a 7 Day Challenge Model

6:30 PM  ID21  Susan Little  Prevalence of Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia, and Anaplasma in dogs: 2013 – 2017

6:30 PM  ID22  Laura Motschenbacher  A Retrospective Comparison of Blastomycosis Urine Antigen Concentration and Radiographic Findings as Predictors of Survival

6:30 PM  ID23  Heemyung Park  Epidemiological Relatedness of Methicillin-Resistant Staphylococcus aureus ST72-Sccmec IV Strains Among Companion Animals and Owners

6:30 PM  ID24  Junghoon Park  An Outbreak of Feline Calicivirus Associated Virulent Systemic Disease in Korea

6:30 PM  ID25  Kryste Reagan  Evaluation of the Clinical Performance of Two Point-of-Care Cryptococcal Antigen Tests in Dogs and Cats

6:30 PM  ID26  Stacie Summers  Bartonella Henselae Seroprevalence in Domestic Cats in the United States and Associations with Urinary Abnormalities

6:30 PM  ID27  Maggie Williams  Anti-Platelet Antibody Development and Thrombocytopenia in a Dog Passively Exposed to Canine Influenza (H3N2)

SMALL ANIMAL INTERNAL MEDICINE - NEPHROLOGY / UROLOGY

9:35 AM  NU21  Caitlin Johnson  Serum Aluminum Concentrations in Healthy Cats and Cats with Chronic Kidney Disease

9:50 AM  NU22  Kellyi Benson  Serum and Urine Concentration of Amoxicillin-Clavulanic Acid in Cats with and without Chronic Kidney Disease
9:35 AM NU23 William Cole  C-Reactive Protein in Hypertension and Immune-Complex Glomerular Disease
9:50 AM NU24 Laura Rayhel  Short-Term Efficacy of Epidural Pain Management in Dogs Undergoing Routine Cystoscopy
9:35 AM NU25 Giosi Farace  Changes in Creatinine and Symmetric Dimethylarginine During Pregnancy in the Dog
9:35 AM NU26 Lisa-Maria Grandt  Intravascular Fluid Volume Assessment of Dogs with Glomerular Disease Using Clinical, Laboratory and Imaging Parameters
9:35 AM NU27 Hakhyun Kim  Evaluation of Serum Erythropoietin, Cyanocobalamin, and Iron Concentrations in Dogs with Chronic Kidney Disease
9:35 AM NU28 Joonyoung Kim  Cystatin C as an Early Biomarker for Chronic Renal Failure in Dogs
9:35 AM NU29 Shannon McLeland  Histologic Assessment of the Aging Feline Kidney in Cats Without Kidney Disease
9:35 AM NU30 Felipe dos Santos Muniz  Bun to Creatinine Ratio: How could it Helps to Interpret Azotemia in Dogs
9:50 AM NU31 Felipe dos Santos Muniz  Evaluation of the Renal Function: A Quantitative and Multifactorial Index of Kidney Injury
9:35 AM NU32 Ran Nivy  Prospective Evaluation of 2 Treatment Protocols in Preventing Recurrence in 51 Cats with Obstructive FIC
9:50 AM NU33 Jennifer Ogeer  Identifying Common Dog Breeds At Risk for Kidney Dysfunction with Symmetric Dimethylarginine and Creatinine
9:50 AM NU34 Selena Tavener  Increase in Circulating Levels of Pro-Inflammatory Markers CCL16 and C5 in Canines with Kidney Dysfunction.
9:50 AM NU35 Kiran Panickar  Markers of Injury, PLD4 and Rho Gtpase-Activating Proteins, Are Increased in Kidney Tissue From Felines
9:50 AM NU36 Rosama Pusoonthornthum  Protective Effects of Antidesma acidum on Endothelial Nitric Oxide Synthase in Doxorubicin-Induced Feline Kidney Cells
9:50 AM NU37 Stacie Summers  Fecal Fatty Acids in Cats with Chronic Kidney Disease and Correlation to Gut-Derived Uremic Toxins

EQUINE
3:35 PM E52 Valentin Janvier  Equine Stereotactic Population Average Brain Atlas with Neuroanatomic Correlation
3:35 PM E53 Abigail McElroy  Evaluation of the Filum Terminale in an Equine Model of Ehlers-Danlos Syndromes

POSTER PRESENTATIONS - FRIDAY, JUNE 7

NEUROLOGY
9:50 AM N17 Hsuan-Ping Hong  Clinical Characteristics of Inflammatory Myopathy in the Boxer Dog and its Possible Association with Neoplasia
10:05 AM N18 Edouard Marchal  MiR-21, MiR-125b, MiR-146a and MiR-181c Expression in Exosomes from Canine Mesenchymal Stem Cells
9:50 AM N19 Jessica Reese  Evaluation of Hematologic Parameters in 40 Dogs Receiving Long-Term Cytarabine for Meningoencephalomyelitis of Unknown Etiology
10:05 AM N20 Benjamin Williams Prevalence of Neurologic Worsening Immediately Following Thoracolumbar Hemilaminectomy for Intervertebral Disc Extrusion

9:50 AM N21 Gibrann Castillo Diagnostic Utility of Inner Ear FLAIR MRI in Dogs with Vestibular Disease

10:05 AM N22 Yoonsoo Jeong A Retrospective Study of Canine Epilepsy: Etiological Distribution, Therapeutic Outcome, and Survival Time

9:50 AM N23 Dong-In Jung Evaluation of Treatment with Mycophenolate Mofetil and Prednisolone in Dogs with Meningoencephalomyelitis of Unknown Etiology

10:05 AM N24 Julia Luca Electrode Scalp Impedance Differences Between Two Electroencephalography Machines In Healthy Dogs

10:05 AM N26 Susana Monforte Effects of Radiotherapy on Seizure Freedom and Survival Time in Dogs with Brain Tumors

9:50 AM N27 Ayne Murata Hayashi Evaluation of Electroacupuncture Treatment in 38 Dogs Presenting Cervical and Cervico-Thoracic Neurological Syndrome

10:05 AM N28 Natasha Olby Silencing the Canine Superoxide Dismutase 1 Gene In Vivo Using U1 Adaptor Oligonucleotide

9:50 AM N29 Christine Toedebusch Transformation of Microglia Under the Influence of High-Grade Canine Glioma

10:05 AM N30 Natalia Zidan Long-term Postoperative Pain Evaluation in Dogs with Thoracolumbar Intervertebral Disc Herniation Following Hemilaminectomy.

ONCOLOGY

9:50 AM O11 Seokjin Choi Applicability of Canine Serum Thymidine Kinase 1 and C- Reactive Protein in Healthy and Solid Tumors

10:05 AM O12 Matt Dowling Overexpression of Prostate Specific Membrane Antigen by Canine Hemangiosarcoma Cells Allows for Microscopic Disease Detection in Blood with Polymerase Chain Reaction (VCS Award Winner)

9:50 AM O13 Jumpei Yamazaki Genome-Wide Quantitative DNA Methylation Analysis in Canine Melanoma

9:50 AM O15 Blake Marcum Biodynamic Imaging of Bone Marrow Aspirates from Tumor-Bearing Dogs as a Predictor of Chemotherapy-Induced Neutropenia: A Pilot Study (VCS Award Winner)

10:05 AM O16 Nina Milevoj Pharmacokinetics of Bleomycin in Dogs Treated with Electrochemotherapy

9:50 AM O17 Sookin Nam Expression of Hedgehog Signaling and Inhibition at the Level of Smoothened in Canine Osteosarcoma Cells

10:05 AM O18 Natasa Tozon Health-Related Quality of Life of Dogs Treated with Electrochemotherapy and/Or Interleukin-12 Gene Electrotransfer

SMALL ANIMAL INTERNAL MEDICINE - NUTRITION / METABOLISM

3:35 PM NM11 Laura Rayhel Inter- and Intra-Rater Reliability of Computed Tomographic Measurement of Feline Epaxial Muscle Area

3:35 PM NM12 Marcio Brunetto Canine Obesity: A Prevalence Study in the City of Sao Paulo, Brazil

3:50 PM NM13 Marcio Brunetto Frequency and Factors Associated with Canine Overweight and Obesity in a Hospital in Lavras, Brazil

3:50 PM NM14 Michael Lappin Effect of 2 Urinary Diets on Hematuria in Shelter Cats with Suspected Interstitial Cystitis
SMALL ANIMAL INTERNAL MEDICINE - OTHER

3:35 PM OT03 Nevra Keskin
Morphological Changes in the Round Window Membrane and Cochlea in Chinchillas with Otitis Media

3:50 PM OT04 Lisa Murphy
The Effect of Client Complaints on Veterinary Support Staff

SMALL ANIMAL INTERNAL MEDICINE - PHARMACOLOGY

3:35 PM P04 Sehoon Kim
Comparison of the Pharmacokinetics of Two High Doses of Intravenous Ascorbic Acid in Healthy Dogs

SMALL ANIMAL INTERNAL MEDICINE - RESPIRATORY

1:20 PM R02 Lucy Kopecny
Use of Doxycycline with or without Famciclovir in Kittens with Acute Upper Respiratory Tract Disease

1:20 PM R03 Elizabeth Lee
Categorization of Inflammatory Airway Disease in Cats

1:20 PM R04 Yukihito Shirosita
Feline Bronchorrhrea: a Retrospective Study of 18 Cases (2012–2017)

EQUINE

12:20 PM E22 Ryan Fries
Quantitative Assessment of Two-Dimensional Transthoracic and Transesophageal Echocardiography with Magnetic Resonance Imaging in Normal Foals

12:20 PM E23 Cristobal Navas de Solis
Non-Invasive Blood Pressure From Pulse Decomposition Analysis of the Digital Arterial Pulse in Standing Horses

12:20 PM E24 François-René Bertin
Effect of Various Ambient Temperatures on the Determination of Immunoreactive ACTH Concentrations in Aged Horses

12:35 PM E25 François-René Bertin
Determination of Immunoreactive Insulin Stability in Horses At Risk of Equine Metabolic Syndrome

12:50 PM E26 François-René Bertin
Clinical Implications of the Use of Different Methods to Determine ACTH Reference Intervals in Horses

1:05 PM E27 François-René Bertin
Evaluation of a Continuous Indwelling Glucometer in Healthy Adult Horses

12:35 PM E28 Teresa Burns
Systemic and Local Regulation of Leptin in a Model of Endocrinopathic Laminitis

12:50 PM E29 Laura Dunbar
Ghrelin and Leptin Response to Fasting, Lactose, and Dextrose Administration in Healthy Neonatal Foals

12:20 PM E30 Steve Grubbs
The Probability of Clinical Signs in Hyperinsulinemic Horses With Pituitary Pars Intermedia Dysfunction

12:20 PM E31 Janice Kritchevsky
Effect of Levothyroxine Supplementation on Racehorses' Performance

12:35 PM E32 Janice Kritchevsky
Effect of Supra-Physiologic Levothyroxine Supplementation on Thyroid Hormone Concentrations and TRH-Stimulation-Test Response in the Horse

12:50 PM E33 Hannah Manning
Glucagon, Insulin, and Glucose Response to Fasting, Lactose, and Dextrose Administration in Healthy Neonatal Foals

12:20 PM E34 Ashton Miller
Effects of Pituitary Pars Intermedia Dysfunction and Prascend® Treatment on Endocrine and Immune Function

12:20 PM E35 Elaine Norton
Narrowing the Search for Equine Metabolic Syndrome Genes
12:35 PM E36 Lindsey Rings  Enteroinsular Axis Response of Healthy and Hospitalized Equine Neonates
12:35 PM E37 Allison Stewart  Effect of Sample Handling on ACTH Concentrations Following Thyrotropin-Releasing Hormone Stimulation in Horses
12:50 PM E38 Tobias Warnken  Evaluation of Glycemic Carbohydrate Formulations for Assessment of Insulin Dysregulation in Equines
1:05 PM E39 Heidi Banse  Relationship of Endoscopic Appearance, Macroscopic, and Histopathologic Findings in Equine Glandular Gastric Disease
1:05 PM E40 Rachel Gough  Trends in Antimicrobial Use For Exploratory Laparotomy
1:05 PM E41 Kate McGovern  Ultrasound following Small Intestinal Colic Surgery to Predict Post-Operative Ileus or Anastomotic Obstruction
1:20 PM E42 Panna Sandor  Evaluation of An Online Monitoring System to Detect Colic Motion Patterns in Horses
1:20 PM E43 Angelika Schoster  Clostridial Shedding and Association with Microbiota Composition in Swiss Horses with and without Gastrointestinal Disease
12:50 PM E44 Adeel Khan  Factors Associated with Positive Bacterial Cultures of Jugular Vein Catheters from Sick and Healthy Horses
12:50 PM E45 Katarzyna Dembek  The Blood Bacterial Microbiome in Healthy and Hospitalized Foals
12:35 PM E46 Kallie Hobbs  Plasma Neuraminidase Activity in Septic Adult Horses
12:35 PM E47 Clare Ryan  Ex Vivo Effects of Azithromycin on Lymphoproliferative Responses of Adult Horses
12:50 PM E48 Breanna Sheahan  Identification of Equine Neutrophil Extracellular Traps and Implications for Future Research and Clinical Applications
12:50 PM E49 Randolph Winter  Combined Endothelial Colony Forming Cell / Hydrogel Microsphere Scaffold Decreases Equine Distal Limb Wound Inflammation
1:20 PM E50 Rana Bozorgmanesh  Equine Neonatal Symmetric Dimethylarginine (SDMA): Results of Two Pilot Studies
1:05 PM E51 Sophie Boorman  Associations Between Clinical Parameters and Outcome in 62 Equids with Facial Nerve Paralysis
1:20 PM E54 Luiza Stachewski Zakia  Cerebrospinal Fluid Analysis in Horses, Cattle and Sheep Diagnosed with Rabies
1:05 PM E55 Diego Gomez-Nieto  Effect of Different Methods for Electrolyte Measurement on the Diagnosis of Acid-Base Disorders in Horses
1:05 PM E56 Camilo Jaramillo  Blood Gas Analysis, Electrolytes and Acid-Base Balance of Horses Living at Three Different Altitudes
1:20 PM E57 Carolyn Warner  Evaluation of Equine Biomarkers Following Oral Administration of a Proprietary Blend of Beta-Glucans in Horses
12:50 PM E59 Carlos Medina Torres  A Herbal Spray Reduces Insect Bite Hypersensitivity in Horses Compared to Placebo
12:50 PM E60 Sian Durward-Akhurst  Genetic Variation and the Frequency of Deleterious Variants (Genetic Burden) in Healthy Horses
1:05 PM E61 Kathleen Mullen  Environmental Surveillance and Investigation Neonatal Dysphagia in Foals Born Near Unconventional Natural Gas Development Activity
| Time     | Session  | Author      | Title                                                                 |
|----------|----------|-------------|----------------------------------------------------------------------|
| 1:05 PM  | E62      | James Prutton | Equine Amniotic Membrane Transplantation for Non-Healing Corneal Ulceration Performed Under Standing Sedation in 7 Horses |
| 12:35 PM | E63      | Sherry Cox   | Determination of Grapiprant (Galliprant®) Concentrations in Horses    |
| 12:35 PM | E64      | Hunter Greer | Antimicrobial Hydrogel Dressings for Chronic Wounds                   |
| 12:35 PM | E65      | Elsbeth Swain | Pharmacokinetics of Sulfadiazine and Trimethoprim in Neonatal Foals   |
| 12:20 PM | E66      | Rachel Lemcke | Utilization of Serum Amyloid A in Managing the Clinical Progression of Equine Bacterial Pneumonia |
| 12:20 PM | E67      | Andrew Willis | Streptococcus equi Subspecies equi Point-of-Care PCR Validation      |
| 12:20 PM | E68      | Jane Woodrow | Phenotyping of Airway Mast Cell Proteases and TNFα Concentrations in Healthy, Asthmatic, and Indeterminate Horses |
| 1:05 PM  | E69      | Estelle Manguin | Tracheal Bacterial Populations in Horses with Mild To Moderate Equine Asthma (ACVIM Resident Research Award Eligible) |

**FOOD ANIMAL**

| Time     | Session  | Author       | Title                                                                 |
|----------|----------|--------------|----------------------------------------------------------------------|
| 3:50 PM  | F11      | Mere Saito   | Erythrocyte Osmotic Fragility of Blood Samples of Laying Hens with Different Anticoagulants |
| 3:35 PM  | F12      | Maisie Dawes | Bovine Lactoferrin Modulates Lipopolysaccharide-Induced Nitric Oxide Production |
| 3:50 PM  | F13      | Lisa Gamsjaeger | Serum Total Protein, Immunoglobulin G, and Failed Transfer of Passive Immunity in Neonatal Beef Calves |
| 3:35 PM  | F14      | Diego Gomez-Nieto | Simplified Strong Ion Difference Analysis in Neonatal Beef Calves with Failed Transfer of Passive Immunity |
| 3:50 PM  | F15      | Hakhyun Kim  | Effect of Quercetin on Porcine Neutrophil Extracellular Trap Formation |
| 3:35 PM  | F16      | Ailbhe King  | Effect of Pooled and Non-Pooled Colostrum on Passive Transfer of Immunity and Health in Calves |
| 3:50 PM  | F17      | Matthew Scott | On-Arrival Blood Transcriptomes Identify Altered Immune Pathways in Stocker Cattle That Develop Bovine Respiratory Disease |
| 3:35 PM  | F18      | Laurence Leduc | The Role of Hypophosphatemia in Downer Cow Syndrome: Retrospective Study of 995 Cases |
| 3:35 PM  | F19      | Julie Berman | Comparison of Thoracic Ultrasonography and Thoracic Radiography to Detect Lung Lesions in Hospitalized Dairy Calves |
2019 ACVIM FORUM RESEARCH ABSTRACT PROGRAM

C01

Hemodynamic, Echocardiographic, and Sedative Effects of Oral Gabapentin in Healthy Cats
Meghan Allen – Oregon State University; Katherine Scollan – Oregon State University; Nicole LeBlanc – Oregon State University

Gabapentin is increasingly being administered to cats prior to veterinary visits to decrease anxiety. While gabapentin appears to be well tolerated in cats, its effects on echocardiographic and hemodynamic parameters are unknown. Most injectable sedative, dissociative, and analgesic drugs affect echocardiographic and hemodynamic parameters, which can confound the clinical assessment. The purpose of this study was to investigate the use of gabapentin as a sedative in healthy cats and determine its effects on blood pressure, heart rate, and echocardiographic measurements.

Ten healthy adult cats weighing between 3.0-7.0 kg were recruited from the veterinary community at Oregon State University for this double-blinded, placebo-controlled study. Cats were considered healthy based on history, physical exam (PE), M-mode and two-dimensional (2D) echocardiography, electrocardiogram (ECG), Doppler blood pressure (BP), complete blood count and blood chemistry. After obtaining baseline diagnostic tests, cats were randomized to receive either oral placebo or gabapentin capsule (100 mg for cats weighing 3.0-4.0 kg, and 150 mg for cats weighing 4.1-7.0 kg) on an empty stomach in the home environment approximately 30 minutes prior to presentation. A minimum wash-out period of 7 days was mandated between visits. The level of sedation was assessed by 2 veterinary investigators (attending cardiologist and resident) using a previously published reference ranges. A single dose of oral gabapentin may produce a modest decrease in systolic function in healthy cats, however these data should be validated by a larger study. Therefore, the results of this study suggest that a single, oral dose of gabapentin produces mild sedative effects in most cats within 120 minutes of administration and causes clinically insignificant changes to echocardiographic measurements.

C02

Extracellular Vesicular Micrornas as Potential Biomarker for Early Detection of Doxorubicin-Induced Cardiotoxicity in the Dog
Amelie Beaumier – Tufts Cummings School of veterinary medicine; Sally Robinson – Tufts Cummings School of veterinary medicine; Nicholas Robinson – Tufts Cummings School of veterinary medicine; Katherine Robinson – Tufts Cummings School of veterinary medicine; Sally Robinson – Tufts Cummings School of veterinary medicine

Of 24 cats screened, 10 cats met study enrollment criteria. All cats were mixed breeds with the exception of a single Siamese cat. There were 7 spayed females and 3 neutered males with a median age of 3 years (range 2-13 years). The mean weight was 4.5 kg (range 3.5-6.3 kg) and mean body condition score was 5.4/9 (range 4-6/9). The mean dosage of gabapentin was 27.9 mg/kg (range 23.9-32.6 mg/kg); no cats experienced adverse events during the study. Three cats did not show appreciable sedative effects after having received gabapentin. Of the 7 cats that exhibited sedative effects, 5 were sedated by 60 minutes and all cats appeared sedate by 120 minutes post gabapentin administration. All sedated cats appeared modestly affected with mild ataxia but retained the ability to spontaneously ambulate. Near-perfect interobserver agreement of sedation scoring was obtained (weighted kappa 0.84). No significant correlation was found for gabapentin dosage with sedation scores at any time point. No significant difference was found between baseline, placebo, or gabapentin for heart rate, respiratory rate, or systolic BP. Two-dimensional fractional shortening (FS) was significantly decreased with gabapentin (49.1 ± 9.8 %) compared to baseline (53.5 ± 11.0 %). M-mode left ventricular internal diameter in systole (LVIDs) was significantly increased with gabapentin (8.2 ± 1.8 mm) compared to baseline (7.6 ± 1.8 mm). Left atrial volumes were significantly increased with gabapentin (1.5 ± 0.4 ml) compared to baseline (1.3 ± 0.3 ml). No significant differences were noted between baseline, placebo, or gabapentin for the remaining echocardiographic parameters including linear left atrial size, left ventricular wall thicknesses and chamber dimensions, aortic and pulmonic outflow velocities and timing intervals, mitral inflow velocities and ratios, isovolumetric relaxation time, deceleration time, and tissue Doppler velocities. Despite statistically significant echocardiographic differences between gabapentin and baseline, affected measurements remained within established reference ranges. A single dose of oral gabapentin may produce a modest decrease in systolic function in healthy cats, however these data should be validated by a larger study. Therefore, the results of this study suggest that a single, oral dose of gabapentin produces mild sedative effects in most cats within 120 minutes of administration and causes clinically insignificant changes to echocardiographic measurements.
Long-term use of doxorubicin (DOX) is limited by cumulative dose-dependent cardiotoxicity. The objective of this pilot study was to identify plasma extracellular vesicle (EV)-associated miRNAs as a biomarker for cardiotoxicity in dogs by correlating changes with cardiac troponin I (cTnI), echocardiographic and histologic findings. In this prospective pilot study, a total of nine client-owned dogs diagnosed with sarcoma receiving DOX single-agent chemotherapy were included (five total DOX treatments). Dogs with significant metastatic disease, preexisting heart disease, or breeds predisposed to cardiomyopathy were excluded. cTnI were monitored before each treatment and 1 month after the treatment completion. Echocardiogram was performed prior to treatments 1, 3, and 5, and 1 month after completion. Linear mix model analysis for repeated measurements was used to evaluate the effect of DOX with subsequent Bonferroni post-hoc analysis. Seven dogs showed histologic changes consistent with cardiotoxicity. Changes in 14 miRNAs reached statistical significance after treatment completion. Of those, downregulation of miR-107 (p = 0.037) and miR-203 (p = 0.047) were noted before administering the third dose. cTnI changed significantly but only 1 month after treatment completion, and levels correlated with EF and LVIDd. To the authors' knowledge, this is the first study investigating EV-miRNAs as potential biomarker for early detection of DOX-induced cardiotoxicity. Downregulation of miR-107 and miR-203 were detected prior to significant changes in cTnI or echocardiographic parameters were noted. Further validation with larger sample size will be required.

**C03**

**Phase I Clinical Trial of an Antithrombotic Drug Protocol Combining Apixaban and Clopidogrel in Dogs**

Allison L. Gagnon – Colorado State University; Brian Scansen – Colorado State University; Christine Olver – Colorado State University; Sarah Shropshire – Colorado State University; E. Christopher Orton – Colorado State University

Combining an established antplatelet drug, clopidogrel, with the direct oral Factor Xa inhibitor, apixaban, could provide an effective prophylactic and therapeutic antithrombotic strategy in dogs. Thus, a limited 3+3 Phase I dose-escalation clinical trial in normal healthy dogs was undertaken to evaluate toxicity (primary end-point) and pharmacodynamic (PD) and pharmacokinetic (PK) parameters (secondary end-point).

Beagle dogs, median body weight 10.3 kg (10.0–10.9), were enrolled at 3 sequential dose levels (3 dogs/dose) for 8 days. Clopidogrel dose was fixed at 18.75 mg PO q24h with escalation of apixaban dose at 5 mg PO q12h, 5 mg PO q8h, and 10 mg PO q12h. Laboratory testing evaluated complete blood count, serum diagnostic profile, urinalysis, fecal occult blood, standard coagulation parameters, factor Xa activity, serum apixaban level, whole blood impedance platelet aggregometry, and thromboelastography.

Evidence of toxicity (bleeding) was not observed at any dose. Dose dependent changes in PD/PK parameters between baseline and peak effect were observed including a mean increase in prothrombin time of 3.2 sec (95% CI: 2.5–3.9 sec), a mean increase in activated partial thromboplastin time of 2.1 sec (1.6–2.6 sec), a mean reduction in factor Xa activity of 80% (76–85%), and a reduction in the mean area-under-the-curve platelet aggregometry of 88% (80–96%). The mean 3 hr peak serum apixaban level for all rounds was 620 ng/mL (570–669 ng/mL). The combination of apixaban at up to approximately 1 mg/kg PO q12h and clopidogrel at approximately 1.875 mg/kg PO q24h appears safe in healthy dogs. Clinically relevant changes in PD/PK data occur at all dose levels.

**C04**

**Thoracocentesis in the Chronic Management of Congestive Heart Failure in Cats: 34 cases (2002-2016)**

Allison L. Gagnon – Colorado State University; Teresa DeFrancesco – North Carolina State University; Sandra Tou – North Carolina State University; Bruce Keene – North Carolina State University

Pleural effusion is common in cats with congestive heart failure. Although medical management is possible, intermittent thoracocenteses are often required. We examined whether repeat thoracocentesis was safe and well-tolerated in cats with cardiogenic pleural effusion.

Medical records of cats that experienced at least three thoracocenteses at the North Carolina State University between 2002-2016 were reviewed to extract relevant clinical and laboratory data for cases that were confirmed to have cardiogenic pleural effusion based on echocardiographic or necropsy findings. Standard thoracocentesis procedure included mild sedation and the use of a fenestrated over-the-needle IV catheter.

A total of 34 cats were identified with cardiogenic pleural effusion that had at least 3 thoracocenteses. Diagnosed heart diseases included hypertrophic cardiomyopathy, restrictive cardiomyopathy, unclassified cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, arrhythmia-induced cardiomyopathy, AV valve dysplasia, double-chambered right ventricle, ventricular septal defect, pulmonary stenosis, thyrotrophic and acromegalic cardiomyopathy. The median number of thoracocenteses was 4 (range 3-36, total 222) and the average amount of fluid obtained per thoracocentesis was 199 mL (2-500 mL). No major complications were observed. Minor complications were noted in 9/34 cats, including radiographic pneumothorax (3), minor bleeding/bruising (2), post-tap syncope (1), transient respiratory signs after thoracocentesis (2), and difficulty performing thoracocentesis (1). No significant hypoalbuminemia or electrolyte disturbances were noted. The median survival after initial diagnosis of pleural effusion was 273 days (5-1050 days).

Recurrent thoracocentesis for cardiogenic pleural effusion can be a safe and effective adjunctive strategy to prolong survival for cats in refractory congestive heart failure.
C05

Short-Term Electrocardiographic Recordings From 24-Hour Holter To Assess Heart Rate in Dogs with Atrial Fibrillation
Tamilselvam Gunasekaran – Veterinary Teaching Hospital, Michigan State University; Nicholas Olivier – Michigan State University; Robert Sanders – Michigan State University

Accurate estimation of heart rate (HR) is critical in dogs with atrial fibrillation (AF) since HR is one of the most important parameters used in the management of AF. Currently, a 24-hour continuous ambulatory electrocardiographic recording (Holter) is recommended for assessment of HR in dogs with AF. However, Holter monitoring may not be routinely performed due to cost and the need for additional equipment. The primary objective of this study is to evaluate the accuracy of short-term ECG recordings from a 24-hour Holter to assess HR in dogs with AF. Dogs with AF based on an in-hospital ECG underwent Holter monitoring. Using modified Bland Altman limits of agreement plot, in-hospital ECG HR was compared to 24-hour mean HR. Mean HR values were calculated for consecutive sample durations of five minutes, 30 minutes, one hour, two hours and three hours using the RR interval data extracted from the Holter recording. Percentage of mean HR values were calculated for each sample duration that fell within 10%, 15% and 20% of 24-hour mean HR for each dog. Twenty dogs (8 females and 12 males) with AF underwent Holter monitoring. The median age was 5 years (Interquartile range (IQR) = 6-10 years). The median body weight was 39.6 kg (IQR = 29.5-58.1 kg). Ten out of 20 dogs were receiving therapy to control HR. In-hospital ECG overestimated 24-hour mean Holter HR by 19 beats per minute (bpm) with wide limits of agreement (-41bpm to +79bpm). Less than 75% of mean HR values (95% confidence interval (CI) = 50%-87%) were within 10% range of 24-hour mean HR, when sample durations of three hours or less were evaluated. With recording durations of two and three hours, nearly 100% of mean HR values (95% CI = 88%-100% and 92%-100% respectively) were within 15% and 20% range of 24-hour mean HR respectively.

In agreement with a previous study, our study confirmed the inadequacy of in-hospital ECG for evaluation of HR in dogs with AF. Short term ECG recordings of three hours or less at home do not provide mean HR values within 10% range of 24-hour mean HR in dogs with AF.

C06

Reliability of Measuring of Left Atrial Size in Dogs with Subclinical Myxomatous Mitral Valve Disease
Weihow Hsue – University of California, Davis; Lance Visser – University of California, Davis

We sought to evaluate the reliability of several 2D echocardiographic measurements of left atrial (LA) size in dogs with subclinical myxomatous mitral valve disease (MMVD). Nine dogs with MMVD were subject to repeated echocardiographic examinations by 2 operators during mornings and afternoons of 3 non-consecutive days within 1 week. Each dog was examined 12 times. LA size was quantified by normalized LA diameter in long-axis (LADn), LA to aortic valve diameter ratio in long-axis (LAD/AoD), LA to aortic root ratio in short-axis (LA/Ao), and monoplane Simpson’s LA volume from right parasternal long-axis (LAVRPLx) and left apical views (LAVLAP). Each operator/sonographer performed their own measurements and was blinded to previous measurements. Within-day, between-day, and between-operator variability were quantified with the coefficient of variation (CV) and 95% repeatability coefficients (RC).

The CVs were < 5.2% for LADn and LAD/AoD, 5.9 - 14.1% for LA/Ao, 9.8 - 14.8% for LAVRPLx, and 15.7 - 19.6% for LAVLAP. An ANOVA model identified significant (P < 0.05) effects of day and operator for LA/Ao and operator for LAVRPLx and LAVLAP. Within-operator RCs were as follows: LADn = 0.18 cm/kg0.309, LAD/AoD = 0.32, LA/Ao = 0.41, LAVRPLx = 0.66 mL/kg, and LAVLAP = 0.68 mL/kg.

C07

Acute Pharmacodynamic Effects of Pimobendan in 22 Client-Owned Cats with Hypertrophic Cardiomyopathy
Maureen S. Oldach – Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California Davis; Joshua Stern – School of Veterinary Medicine, University of California Davis; Yu Ueda – School of Veterinary Medicine, University of California Davis; Catherine Gunther-Harrington – School of Veterinary Medicine, University of California Davis; Lance Visser – School of Veterinary Medicine, University of California Davis

There is a paucity of pharmacodynamic data in the literature supporting or refuting the use of pimobendan in cats. This clinical trial evaluated the pharmacodynamic effects of a single dose of orally administered pimobendan in cats with hypertrophic cardiomyopathy (HCM). We hypothesized that pimobendan would not exacerbate left ventricular outflow tract (LVOT) obstructions, would improve left atrial function, and would be well tolerated.

An echocardiographic examination and Doppler blood pressure assessment were performed in 22 client-owned cats with HCM before (pre-treatment) and 90-minutes after (post-treatment) 1.25 mg of orally administered pimobendan (Vetmedin). Echocardiographic measures were performed by a single investigator blinded during off-line analyses. Heart rate, systolic blood pressure, left auricular blood flow velocity, left ventricular fractional shortening, and tissue Doppler peak diastolic velocity of the lateral mitral annulus were not significantly different between pre-treatment and post-treatment groups. The number of cats with LVOT obstructions was not significantly different between groups. No adverse effects were observed in this study.

Median (IQR) LVOT velocity was significantly higher following pimobendan [1.9 m/sec (1.5, 3.4) vs 2.6 m/sec (2.0, 4.0); p < 0.01]. Mean (± SD) left atrial fractional shortening was significantly higher following pimobendan (28% ± 6 vs. 32% ± 7; p = 0.02). Mean tissue Doppler peak early systolic velocity of the lateral mitral annulus was significantly higher following pimobendan (7.4 cm/s ± 1.5 vs. 8.5 cm/s ± 1.6).
In cats with HCM, pimobendan acutely increased echocardiographic measures of left ventricular function, left atrial function and LVOT velocity without causing adverse effects.

C08

Comprehensive Cardiac Evaluation Including Magnetic Resonance Imaging in Naturally-Infected Dogs Seropositive for Chagas Disease

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Chagas disease is a cause of myocarditis and an important emerging disease for which there is no approved treatment in dogs. The objective of this study was to perform a comprehensive cardiac evaluation in naturally-infected, asymptomatic dogs seropositive for Trypanosoma cruzi. Ten client-owned dogs > 15 kg of various breeds were prospectively enrolled into this pilot study. Based on analysis of human data, enrollment of 10 dogs had a high likelihood of yielding at least one with cardiac magnetic resonance imaging (CMR) abnormalities. Tests performed: T. cruzi immunofluorescent antibody, high-sensitivity cardiac troponin I (cTnI), biochemistry panel, 5-minute 6-lead ECG, Holter, complete echocardiogram, CMR. CMR studies evaluated cardiac function, delayed myocardial enhancement (DME), wall motion abnormalities (WMAs), changes to extra cellular volume (ECV), and apical aneurysms.

Labwork results: positive Chagas titer (median 1:640; range, 1:80 – 1:≥ 1280), normal biochemistry, and cTnI was detectable in all dogs (median 0.0675; range, 0.010-0.242ng/ml). On 5-minute ECG all dogs had a sinus rhythm with normal ECG complex abnormalities. Holter abnormalities included ventricular arrhythmias (N=4; modified Lown scores: 1.1,3.5), supraventricular premature beats (N=6), second-degree atrioventricular block (N=2). Echocardiographic findings included normal left atrial and ventricular size, decreased right ventricular systolic function based on established parameters (tricuspid annular plane systolic excursion (TAPSE) and S’) (N=5), and included degenerative mitral valve disease (N=2; stage B1). CMR abnormalities were documented in 7/10 (5 DME, 2 WMAs, 2 increased ECV, 1 apical aneurysm).

Compared to standard diagnostic tests, Holter and CMR provide useful information for future clinical investigation in the study of Chagas disease.

C09

Diagnostic Utility of Point-of-Care Lung Ultrasound for Monitoring Congestive Heart Failure in Dogs

Shane Murphy – Iowa State University; Teresa DeFrancisco – North Carolina State University; Melissa Tropf – Iowa State University; Jennifer Fowler – Iowa State University; Rebecca Walton – Iowa State University; Wendy Ware – Iowa State University; Jessica Ward – Iowa State University

Point-of-care lung ultrasound (LUS) is an effective tool for the diagnosis of left-sided congestive heart failure (CHF) in dogs via the detection of ultrasound artifacts (B-lines) caused by increased lung water. The objective of this study was to determine whether LUS can be used to monitor resolution of cardiogenic pulmonary edema in dogs. Twenty-four client-owned dogs with CHF were prospectively enrolled. Protocolized LUS was performed on hospital admission, at hospital discharge, and at subsequent recheck examinations. LUS findings were compared between timepoints and to other clinical measures of CHF, including respiratory rate, thoracic radiographs, and NTproBNP.

From time of hospital admission to discharge (mean 19.6 hours), median number of LUS sites strongly positive for B-lines (> 3 B-lines per site) decreased from 5 (range 1-8) to 1 (range 0-5; p < 0.001), and median total B-line score decreased from 39 (range 6-74) to 4.5 (range 0-32; p < 0.001). LUS indices continued to decrease significantly from hospital discharge to first recheck (p < 0.01) and remained low at subsequent rechecks. LUS indices were positively correlated with respiratory rate at both hospital admission (p = 0.0008) and discharge (p = 0.0003). Radiographic pulmonary edema score was correlated with LUS indices (p < 0.01) at hospital admission and decreased at subsequent timepoints (p < 0.03). NTproBNP decreased between hospital admission and discharge (p = 0.0004) but was not correlated with respiratory rate or LUS findings at any timepoint.

LUS is a useful diagnostic tool for monitoring improvement of pulmonary edema in dogs with CHF. B-lines decrease significantly within 24 hours of CHF treatment.

C10

Single Dose Pharmacokinetics of Two Oral Formulations of Ranolazine in Healthy Cats

Saki Kadotani – University of Illinois; Ryan Fries – University of Illinois; Jennifer Reinhart-Dungar – University of Illinois; Zhong Li – University of Illinois

Ranolazine, a selective late sodium channel blocker, improves diastolic function in people with hypertrophic cardiomyopathy (HCM). Ranolazine may therefore have similar effects for feline HCM. This study was designed to determine the pharmacokinetic properties of two oral formulations of ranolazine in healthy cats. Four purpose-bred cats (2 males and 2 females) were used. A single dose (25 mg/kg, PO) of immediate-release ranolazine (IR) and a single dose (125 mg/cat, PO) extended-release ranolazine (Ranexa®) were administered to each cat with a 60-day washout period between formulations. Plasma concentrations of ranolazine were measured using liquid chromatography-tandem mass spectrometry. Ranolazine was detected in plasma in all cats for both formulations. Pharmacokinetic properties were determined using noncompartamental analysis. Maximum concentrations ranged from 85.50 to 3730.00 ng/ml (median 908.4 ng/ml) at 1 hour for IR and 291.00 to 3670 ng/ml (median 839.5 ng/ml) at 2 hours for Ranexa®. For both formulations, plasma concentrations were higher in females than males. The elimination half-life ranged from 2.16 to 15.87 hours (median 7.56 hours) and 3.18 to 5.04 hours (median 4.12 hours) for IR and Ranexa® respectively. The area under the curve ranged from 242.45 to 5487.60 ng•hr/ml (median 2163.90 ng•hr/ml) for IR and 6173.81 to 20101.78 ng•hr/ml (median 8376.50 ng•hr/ml) for Ranexa®. Both formulations were well tolerated in this population.
of cats. However, given the high variability of plasma concentrations and potential sex-specific differences, chronic multiple-dosing pharmacokinetic and pharmacodynamics studies are needed to determine optimal dosing and formulation of ranolazine in cats.

C11

Effect of Concurrent Omeprazole-Clopidogrel Administration on Platelet Function and Clopidogrel Metabolism in Healthy Cats

Christina Plante – Washington State University; Pamela Lee – Washington State University; Jillian Haines – Washington State University; O. Lynne Nelson – Washington State University; Michael Court – Washington State University; Clark Kogan – Washington State University

The objective of this study was to determine whether the concurrent use of omeprazole with clopidogrel interferes with the metabolism and antiplatelet effects of clopidogrel in healthy cats. In this two-sequence, two-period, two-treatment randomized crossover study, healthy cats were randomly assigned to receive clopidogrel only (18.75mg PO q24h) or clopidogrel with omeprazole (1mg/kg PO q12h) for 10 days, followed by a 2 week washout period, and then the opposite treatment for another 10 days. Blood was collected via jugular venipuncture on day 0, 5, and 10. Clopidogrel metabolism was evaluated through quantification of clopidogrel active metabolite (CAM) using high performance liquid chromatography-tandem mass spectrometry. Platelet function was evaluated using Plateletworks, Multiplate analyzer using adenosine triphosphate agonist, and Platelet Function Analyzer-100 (PFA-100). Average plasma CAM concentrations and platelet function with standard deviation at day 0, 5, and 10 for each treatment group for the first 4 cats completing the study are demonstrated in table 1. Preliminary results show no difference in plasma CAM concentrations or platelet function at day 5 or 10 between the two treatment groups suggesting that the concurrent use of omeprazole with clopidogrel therapy does not alter clopidogrel metabolism or platelet function in healthy cats.

Table 1. Plasma clopidogrel active metabolite (CAM) concentrations and platelet function testing using Plateletworks, Multiplate impedance aggregometry, and Platelet Function Analyzer-100 (PFA-100) for baseline (day 0) and after 5 and 10 days of drug administration with clopidogrel 18.75mg PO q24h only (C) or concurrent clopidogrel with omeprazole 1mg/kg PO q12h (C+O) reported as average and standard deviation. AUCl, area under the curve.

| CAM (ng/ml) | Platelets (% aggregation) | Multiplate (AUC) | PFA-100 (seconds) |
|------------|---------------------------|-----------------|------------------|
| Day        | C  | C+O | C  | C+O | C  | C+O | C  | C+O |
| 0          | 96 ± 5 | 217 ± 100 | 78 ± 21 | 217 ± 100 |
| 5          | 45 ± 9 | 11.7 | 329 ± 72 | 137 ± 53 | 144 ± 105 |
| 10         | 20 ± 11 | 18 ± 10 | 256 ± 74 | 133 ± 132 | 111 ± 65 |

C12

Utility of Radiographic Parameters To Predict Echocardiographic Cardiac Enlargement in Dogs with Mitral Valve Disease

Megan H. Poad – University of Pennsylvania; Timothy Manzi – University of Pennsylvania; Mark Oyama – University of Pennsylvania; Anna Gelzer – University of Pennsylvania

Evaluation of left heart size helps determine disease severity in dogs with preclinical myxomatous mitral valve disease (MMVD). The combination of normalized echocardiographic diastolic left ventricular internal dimension (LVDDN) ≥1.7 and left atrial to aortic ratio (LA:Ao) ≥1.6 were previously identified as important criteria for echocardiographic left heart enlargement (LHE ECHO). Radiography is a widely available means to assess heart size, and we sought to determine the utility of vertebral heart size (VHS) and vertebral left atrial size (VLAS) for predicting LHE ECHO in dogs with preclinical MMVD. We performed a prospective study of 70 client-owned dogs with preclinical MMV D that underwent echocardiography and radiography on the same day. Receiver-operating characteristic curves were used to assess the ability of VHS, VLAS, and VHS+VLAS to discern dogs with and without LHE ECHO and to identify clinically useful cutoffs. The ability of VHS and VHS+VLAS to predict LHE ECHO was moderate (area under the curve (AUC)_VHS=0.851; 95% CI, 0.762-0.941; AUC_VHS+VLAS=0.865; 0.783-0.947), and performance of VLAS and VLAS+VHS was not significantly different from that of VHS alone. A VHS cutpoint of >10.8 had sensitivity=91.2% (76.3-98.1%), specificity=69.4% (51.9-83.7%), +predictive value (PPV)=73.8% (58.0-86.1%), and – predictive value (NPV)=89.3% (71.8-97.7%). A cutpoint of >11.7 had sensitivity=32.4% (17.4-50.5%), specificity=97.2% (85.5-99.9%), PPV=91.7% (61.5-99.8%), and NPV=60.3% (46.6-73.0%) for predicting LHE ECHO. VHS>11.7 detected dogs with LHE ECHO and VHS<10.8 excluded dogs with LHE ECHO with high predictive values. Thirty (42.9%) of 70 dogs had a VHS ≥10.9 and ≤11.7. A limitation of the method is the large percentage of dogs with VHS intermediate to these cutoffs.

C13

Detection of Congestive Heart Failure by Doppler Echocardiography in Cats with Hypertrophic Cardiomyopathy

Michelle Rohrbough – The Ohio State University; Karsten Schober – The Ohio State University; Jaylyn Rhinehart – The Ohio State University; John Bonagura – The Ohio State University; Amy Habling – The Ohio State University

Left-sided congestive heart failure (CHF) is characterized by elevated filling pressures and related Doppler echocardiography (DE) filling patterns. This study addresses the general hypothesis that DE can be used to predict CHF in cats with hypertrophic cardiomyopathy (HCM). Prospective clinical cohort study with client-owned cats. Cats underwent physical examination, thoracic radiography, analysis of NT-proBNP, and echocardiography and were divided into three age-matched groups: G-1 (control), G-2 (preclinical HCM), and G-3 (HCM and CHF). Measured and calculated variables included respiratory rate, DE estimates of filling pressure using transmitral, pulmonary venous, and tissue Doppler variables, serum NT-proBNP, and a radiographic CHF score. Cats were examined twice, at baseline and 5-14 days later. Groups were compared using ANOVA, and presence of CHF was predicted using receiver-operating characteristic curve (ROC) and multivariate and logistic regression analyses. A total of 44 cats were enrolled: G-1 (n=14), G-2 (n=16), and G-3 (n=14). The E/A ratio (AUC 1.00, diagnostic cut-off 1.75, P = 0.006), diastolic functional class (AUC 0.95, cut-off class 2, P = 0.004), left atrial diameter (AUC 0.90, cut-off 20 mm, P < 0.001), E:E’ (AUC 0.83, cut off 12.5, P = 0.013), and rate of respiration (AUC 0.83, cut-off 45/min, P = 0.002) predicted presence of CHF best. Summation of
diastolic filling waves in cats with CHF represents a relevant obstacle in the DE prediction of CHF.

Various DE variables can be used to predict CHF in cats with HCM. Determination of the clinical benefit of such variables in initiating treatments and assessing treatment success need further study.

C14

Biomarker Changes with Systolic Anterior Motion of the Mitral Valve in Cats with Hypertrophic Cardiomyopathy

Joonbum Seo – Royal Veterinary College, University of London; Jessie Payne – Langford Vets Small Animal Referral Hospital, University of Bristol; Jose Novo Matos – Royal Veterinary College; Wesley Fong – Royal Veterinary College; David Connolly – Royal Veterinary College; Virginia Luis Fuentes – Royal Veterinary College

Systolic anterior motion of the mitral valve (SAM) is a feature of hypertrophic cardiomyopathy (HCM) in cats. The influence of SAM on the circulating cardiac biomarkers N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin-I (cTnI) is currently unknown. We hypothesized that cats with HCM and SAM (HCM\textsuperscript{SAM+}) would have higher circulating cTnI and NT-proBNP concentrations than cats with HCM but no SAM (HCM\textsuperscript{SAM−}) and that this relationship would be independent of other factors when assessed in a mixed general model.

The subjects of this case to case study were 140 cats with HCM with available plasma NT-proBNP ± serum cTnI results and a concurrent echocardiographic study. Exclusion criteria were hyperthyroidism, diabetes mellitus, packed cell volume < 20%, dehydration, systolic arterial blood pressure (SABP) ≥ 160 mm Hg, creatinine ≥ 2.9 mg/dL or creatinine ≥ 1.6 mg/dL without SABP. Cats with HCM\textsuperscript{SAM+} were matched with cats in the HCM\textsuperscript{SAM−} group for sex, left atrial (LA) size, left ventricular (LV) diameters, LV fractional shortening FS%, and proportion of cats with arrhythmias, congestive heart failure and aortic thromboembolism, with 70 cats in each group.

Cats with HCM\textsuperscript{SAM+} were younger, more likely to have a murmur and had greater LV wall thickness. They also had higher median NT-proBNP concentrations (729 pmol/L [IQR 286-1458] versus 65 pmol/L [27-255] in HCM\textsuperscript{SAM−} cats; P < 0.001) and higher cTnI (0.27 ng/mL [0.10-0.63] versus 0.07 ng/mL [0.01-0.17]; P = 0.002). Multivariable analysis showed that both NT-proBNP and cTnI were higher in cats with SAM independent of other variables. Both NT-proBNP and cTnI are increased in the presence of SAM in cats with HCM, and presence of SAM should be considered when interpreting biomarker results in cats with HCM.

C15

Transcatheter Mitral Valve Placement in Experimental Purpose-Bred Dogs

Brienne Williams – Atlantic Coast Veterinary Specialist; George Kramer – Atlantic Coast Veterinary Specialists; Bradley Youngblood – QTest Labs;
C16
Assessment of Right Ventricular Systolic Function by Tissue Motion Annular Displacement in Healthy Dogs
Marlos Gonçalves Sousa – Federal University of Paraná; Vinicius Bentivóglio Costa Silva – Federal University of Paraná; Ana Paula Sarraff Lopes – Pontifical Catholic University of Paraná; Stephany Buba Lucina – Federal University of Paraná; Marcela Wolf – Federal University of Paraná; Marlos Gonçalves Sousa – Federal University of Paraná

The objectives of the present study were to evaluate the right ventricle (RV) longitudinal systolic function of healthy dogs by the tissue motion annular displacement (TMAD) and to investigate whether there is a correlation of this technique with longitudinal strain (LSt) and with structural and functional parameters of conventional echocardiography.

A prospective observational study was performed including 100 healthy dogs. The morphological evaluation of the RV involved the measurement of the basal and mean diameters and its length, while the function analysis consisted of the tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC%), longitudinal strain of the RV free wall basal, middle and apical segments, velocity of the tricuspid annulus systolic excursion (S'), E/A and E'/A' ratios.

TMAD values in both percentage (%) and millimeters (mm) varied according to the size of the animal (Table 1). Although no difference was found in the comparison of body weight quartiles for TMAD (%), there is a tendency to reduce the values according to the weight increment. On the other hand, when evaluated in mm, the heaviest patients showed results superior and there was a statistical difference in the comparison of the experimental groups. When indexing the TMAD values (mm) to the body surface area, this bias was eliminated, represented by higher values in the animals with lower weights (Table 1). There was no correlation of TMAD (%) with the other echocardiographic variables. TMAD (mm) correlated with all the structural and functional variables of RV. The mean time for TMAD execution was lower in comparison to LSt (8.11 and 37.3 seconds, respectively).

In the repeatability study, good results were found for TMAD (%), with lower values for intraobserver analysis (coefficient of variation: 8.7%) compared to interobserver (coefficient of variation: 18.2%). Therefore, TMAD seems to be a promising tool with good results in the evaluation of RV systolic function, alternately to other methodologies previously described. However, more studies are needed to understand the real clinical applicability of this technique in patients with heart diseases.

C17
Evaluation of the NuCLEUS-X Balloon Valvuloplasty Catheter for Severe Pulmonic Stenosis in Dogs
Katherine F. Scollan – Oregon State University; Nicole LeBlanc – Oregon State University; Courtney Smith – ACCESS; David Sisson – Oregon State University

Percutaneous balloon valvuloplasty (BVP) has become the standard of care for treatment of severe or symptomatic canine valvular pulmonic stenosis (PS). Achieving balloon stability centered at the pulmonic valve should lead to increased likelihood of successful relief of the stenosis. Balloon slippage and instability is a commonly encountered problem during BVP and may result in multiple balloon inflations or an unsuccessful procedure. The NuCLEUS-X™ has a unique barbell shape with an ordered pattern of inflation that expands both ends of the balloon first, stabilizing the balloon to span the valve annulus prior to expansion of the center of the balloon. This should allow inflation of the balloon centered at the pulmonic valve with increased balloon stability and result in successful valvuloplasty. The aim of this pilot study was to assess the feasibility of using the NuCLEUS-X™ BVP in dogs with severe PS. We hypothesized that BVP performed with NuCLEUS-X™ catheters would be feasible and result in successful reduction of the transpulmonic pressure gradient.
Post-procedural success was defined as a reduction in the trans-pulmonic pressure gradient by at least 50% of the pre-procedural PG or to less than 75mmHg one month post procedure.

Ten client-owned animals with severe valvular PS based on transthoracic echocardiography (TTE) were enrolled in the study. The BVP procedure was performed by standard technique with the use of a NuCLEUS-X™ BVP instead of a conventional BVP. Right-sided pressure and cardiac output measurements were made pre- and post-balloon valvuloplasty and effective valve orifice area was calculated. The NuCLEUS-X™ balloon size was selected to achieve a balloon-to-annulus ratio between 1.2-1.5 and hand inflated with an iodinated contrast solution until loss of the stenotic valve waist was observed using fluoroscopic guidance. Pre- and 1-month post TTE PG measurements were recorded with continuous wave Doppler. Descriptive data is listed as mean ± standard deviation for normally distributed data and median [IQR] for non-normal data. Paired t-tests were used to determine significant differences in continuous variables with significance set at 0.05.

The median body weight was 16.6 kg ± 5.3 kg and age at BVP was 9 months [3.6-21 months]. All 10 cases achieved balloon stability centered at the pulmonic valve on the first inflation. Nine dogs had two total procedural inflations and one dog had a single inflation due to pronounced systemic hypotension following balloon deflation that resolved with balloon catheter removal. The median TTE derived preoperative transpulmonic PG was 141 mmHg ± 41 mmHg and the 1-month post-operative PG was 83 mmHg ± 41 mmHg. Procedural success was achieved in 60% of patients and no significant complications were noted using the NuCLEUS-X™ catheter. One instance of resistance when removing the deflated catheter from the right ventricular outflow tract was encountered, but resolved following re-inflation and deflation of the BVP. Nine dogs had a single NuCLEUS-X™ catheter used; one dog required upsizing with a conventional BVP catheter after the NuCLEUS-X™ inflation resulted in no apparent catheter waist. Using the angiographic pulmonic valve annulus measurement, the median balloon to annulus ratio was 1.2 ± 0.1 for the balloon waist, and 1.4 ± 0.1 for the distal portion of the NuCLEUS-X™ balloon. There was a significant difference in invasive peak-to-peak PG post-BVP compared to pre-BVP (p = 0.028) and no significant difference between pre- and post- pulmonic valve orifice area (p = 0.1).

The results of our study indicate that use of the pediatric NuCLEUS-X™ catheter is feasible for BVP in dogs with severe PS. The unique balloon shape provided catheter stability on the first inflation in all dogs. The NuCLEUS-X™ may be particularly useful in cases when stabilization of a conventional BVP catheter cannot be achieved.

### Table 1 - Comparison of age, LA TMAD, LA Strain, LA Emptying and LA Ejection Fraction in accordance with body weight

| (n) | 1.25–7.90 kg | 7.91–11.00 kg | 11.01–20.00 kg | 20.01–61.60 kg | p |
|-----|-------------|-------------|-------------|-------------|---|
| Age (years) | 2.3 (0.9-5) | 2.5 (2.3-3.0) | 2.7 (2.0-3.5) | 3.0 (2.0-5.5) | 0.4927 |
| LA TMAD (mm) | | | | | |
| Global AP 2.4 | 4.24 (0.79) | 6.05 (1.06) | 6.06 (1.20) | 7.16 (1.36) | <0.0001 |
| Systolic AP 2.4 | 2.55 (2.41-3.27) | 2.97 (2.42-3.86) | 3.40 (2.65-4.10) | 3.75 (3.30-4.58) | 0.0004 |
| LA TMAD (mm/kg) | | | | | |
| Global AP 2.4 | 18.48 (11.43-21.30) | 13.51 (12.11-15.07) | 10.36 (8.65-11.85) | 7.49 (5.93-8.41) | <0.0001 |
| Systolic AP 2.4 | 10.47 (7.59-13.65) | 8.70 (5.31-8.75) | 5.93 (4.35-6.78) | 3.76 (2.82-4.99) | <0.0001 |
| LA TMAD (mm/kg/LAL) | | | | | |
| Global AP 2.4 | 0.29 (0.21-0.32) | 0.28 (0.25-0.3) | 0.24 (0.23-0.28) | 0.20 (0.18-0.25) | <0.0001 |
| Systolic AP 2.4 | 0.17 (0.13-0.20) | 0.13 (0.10-0.18) | 0.13 (0.12-0.17) | 0.11 (0.08-0.14) | 0.0037 |
| LA Strain (%) | 34.47 (7.13) | 33.76 (6.19) | 28.98 (6.54) | 21.40 (4.66) | <0.0001 |
| LA EJ Fr (%) | 63.43 (4.64) | 62.27 (6.01) | 60.19 (6.69) | 54.97 (7.10) | <0.0001 |
| LA EJ Fr (%) | 42.14 (36.15-49.98) | 38.13 (32.72-44.35) | 38.38 (32.23-40.77) | 35.39 (28.64-44.13) | 0.0320 |

(n), number of animals in quartile; LA, left atrium; TMAD, tissue motion annular displacement; AP4, apical 4-chamber; AP2, apical 2-chamber; AP 2.4, average of 2 and 4 chamber; kg, kilograms; LAL, left atrium length; EJ Fr, Emptying fraction; EJ Fr, Ejection fraction. Data are expressed as means (standard deviation) or medians (interquartile range) depending on the parameter attaining a normal distribution or not on the Shapiro-Wilk normality test. Values with different superscripted letters indicate statistically significant differences between groups.
aim of this study was to demonstrate that Tissue Mitral Annular Displacement (TMAD) by two-dimensional Speckle Tracking, can be an easy and fast method to evaluate the longitudinal left atrium function. In this prospective cross-sectional observational study, a hundred healthy dogs (1-13 y / 1-61 kg) underwent echocardiogram. Apical 4-chamber (AP4) and 2-chamber (AP2) images were obtained, which allowed the calculation of TMAD (global and systolic), Longitudinal Strain and LA volume measurements. TMAD and Strain were acquired from Speckle Tracking and LA volumes from area-length method. LA ejection and emptying fractions were calculated from the volumes obtained. Data underwent the Shapiro-Wilk test to check for a normal distribution. Both TMAD and LA Strain varied in accordance with the size of the animals (Table-1). Our results showed that strain was higher in smaller dogs, which contrasts with the greater displacement of the mitral annulus (in mm) in heavier dogs. However, when TMAD was indexed, positive correlations were found to exist. Global TMAD (mm) varied with regard to sex (p = 0.003), (female = 5.56 (1.50); male = 6.52 (1.38)), while systolic TMAD didn’t (p = 0.393). LA ejection and emptying fraction presented lower values in larger dogs (p < 0.05). Global Strain was strongly correlated with global TMAD (indexed) and moderate with systolic TMAD. Global and systolic TMAD was moderated correlated with LA emptying and ejection fractions. There was no correlation between global TMAD and echocardiographic indices of diastolic function except for isovolumetric relaxation time (TRIV) (negative correlation), whereas systolic TMAD showed a negative correlation with some (E, E/A, TRIV), TMAD by speckle tracking is a reliable and fast method for assessment of LA longitudinal function and is less dependent on image quality. Further studies are warranted to validate the clinical applicability of TMAD in animals with heart diseases.

C19

Related Factors for Residual Coughing in Dogs After Mitral Valve Repair
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Coughing is one of the related clinical signs of dogs with myxomatous mitral valve disease (MMVD). Previous study has reported left atrial enlargement were associated with coughing in dogs with MMVD. Mitral valve repair (MVR) leads to reduce the heart size. The objective of this study was whether the reverse remodeling after MVR affects appearance of coughing in dogs. We retrospectively reviewed cases that underwent MVR between January 2018 and November 2018. 133 dogs that were coughing pre-operatively were separated into two groups, those that continued to cough 1-month after MVR (Cough group, n = 50) and those in which the cough disappeared (non-Cough group, n = 83). We then analyzed the data concerning demographics, tracheal collapse, bronchomalacia, and pre- and 1-month post-operative vertebral heart size (VHS), vertebral left atrial size (VLAS), left atrial to aortic root rate (LA/Ao), left ventricular internal diameter in diastole normalized to bodyweight (kg) (LVIDDn), ACVIM heart failure classification was significantly different between the groups, and post-operative VHS, LA/Ao and LVIDDn was significantly smaller in the non-Cough group than in the Cough group. Multivariable analysis revealed that post-operative LA/Ao (OR : 11.831, 95%CI : 1.679 - 83.354, P = 0.013) was significantly related to post-operative coughing. Our study findings revealed that post-operative LA/Ao is a related factor for residual coughing in dogs after MVR. Therefore, if enough reverse remodeling of the left atrium occurs after MVR, the cough disappears.

C20

Disorganization of β-Catenin in Cats with Hypertrophic Cardiomyopathy
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β-catenin is a member of the catenin protein family, it is widely expressed in many tissues and exists in two forms, membrane associated or cytosolic. In cardiac muscle, membrane associated β-catenin localizes to adherens junction proteins such as cadherin in intercalated discs, which are critical for electrical and mechanical coupling between adjacent cardiomyocytes. Perturbation of strict segregation of cell-cell and cell-matrix contact has been observed in human hypertrophic cardiomyopathy (HCM) by detecting lately located membrane associated β-catenin in cardiomyocytes. Stabilization of β-catenin in the cytoplasm leads to increased cytosolic β-catenin which can functions as a transcriptional regulator that promotes cardiomyocyte growth in response to hypertrophic-associated stimuli. The objective of the study was to investigate the expression and localisation of β-catenin in the left ventricle of cats with HCM. The expression of β-catenin in the myocardium from 10 normal cats and 9 cats with HCM was investigated using Western blotting. The intensity of the bands was expressed as fold change after normalization with a housekeeping internal control, GAPDH. To further explore the expression and localization of β-catenin, hearts from 5 normal cats, 14 cats with HCM were fixed in formalin, processed, and embedded in paraffin for fluorescent immunohistochemistry. Final confirmation of HCM was based on histopathological examination by an experienced specialist veterinary pathologist. The slides were coded and triple stained with β-catenin, desmin for better visualisation of the boarder of each individual cardiomyocyte, and DAPI the nucleus dye. 10 images were acquired under 40x magnification per cat and the presence of lateral localization of β-catenin was noted. The signal detected in cytoplasm was scored (0-3) with the observer blinded to the disease status of the cat. The results were compared between groups using Fisher’s exact test and Mann Whitney test. Spearman’s test was used to detect correlation between cytosolic β-catenin and disease severity.

There was no difference in the age and sex of different groups of cats (p > 0.05). No difference was detected in the protein level of β-catenin between HCM and Control (p = 0.0789). Laterally distributed β-catenin was only observed in HCM group and the presence of this distribution was significant compared to controls (p = 0.0048). HCM showed more β-catenin in the cytoplasm compared to controls.
(p = 0.0161), and the degree of cytosolic β-catenin present moderately correlated with disease severity (Rho = 0.5057, p = 0.0272). The detection of lateral distribution of membrane associated β-catenin in left ventricular cardiomyocytes provides evidence for disruption of cell-cell and cell-matrix contact in feline HCM. This might have a negative impact on the mechanical coupling of the cardiomyocytes. The observation that cytosolic β-catenin increased with disease severity indicates either a causative or consequential association suggesting that further investigation may reveal mechanisms driving phenotypic changes associated with HCM.

C21

Radiation Dose During Interventional Cardiology Procedures
Kursten V. Pierce – Colorado State University; Brian Scansen – Colorado State University; Sangeeta Rao – Colorado State University

This study evaluated radiation dose exposure to the patient, operators, and staff during interventional cardiology procedures. Patient dose during procedures using a portable C-arm were retrospectively analyzed and compared to those performed in a contemporary interventional suite. Operator and staff exposure per case was collected prospectively using a real-time dosimetry monitoring system in a subset of cases. Fluoroscopy equipment, procedure type, operator, patient weight, fluoroscopy time, dose area product, and air kerma were recorded and statistically modeled using univariate and multivariable linear regression to evaluate the effect of each factor as well as adjusting for other factors on patient dose. Patient dose population (154 dogs) comprised 61 patent arterial duct (PDA) occlusions, 60 balloon pulmonary valvuloplasties (BPV), and 33 pacemaker implantations. Patient dose was significantly reduced in the interventional suite compared to the portable C-arm, was positively correlated with patient weight, and was highest during BPV compared to PDA or pacemaker (all P < 0.01). Operator and staff dose exposure (24 cases) was higher during BPV and lowest during pacemaker implantation (P < 0.01). Dose to faculty versus resident clinicians was not significantly different (P = 0.69), but both were greater than exposure to technical staff (P < 0.01). We documented a significant reduction in patient radiation dose using a contemporary fluoroscopy system as compared to a portable C-arm for interventional cardiology procedures in animals. Improved knowledge of patient radiation dose factors and exposure risk to operators and staff may promote better radiation safety protocols in veterinary interventional cardiology.

C22

Thymidine Kinase-1 and C-Reactive Protein Concentrations in Dogs with Neoplastic and Nonneoplastic Pericardial Effusion
Lyndsay R. Kong – University of Missouri; Stacey Leach – University of Missouri; Kim Selting – University of Illinois; Randy Ringold – Veterinary Diagnostic Institute, Inc.

Pericardial effusion (PCE) carries diverse prognostic implications depending on its underlying cause. Thymidine kinase-1 (TK1) and C-reactive protein (CRP) are biomarkers that may aid in the diagnosis of various neoplasms. This study aimed to compare concentrations of TK1 and CRP in serum and PCE fluid in dogs with neoplastic and nonneoplastic causes of PCE, and to compare concentrations of TK1 and cCRP in dogs with right atrial (RA) tumors and heart base (HB) tumors. Serum (n = 60) and PCE (n = 34) samples were collected prospectively from dogs presenting with PCE. Samples were categorized as neoplastic (serum n = 42, PCE n = 24) if a mass was diagnosed via echocardiography or histopathology; within this group, samples were further categorized as RA (n = 18) or HB (n = 6) tumors. Samples were categorized as nonneoplastic (serum n = 18, PCE n = 10) based on a diagnosis of noninfectious pericarditis on histopathology or left atrial tear on echocardiography, or if the patient was alive 1 year after presentation without recurrent effusion. There was no significant difference in TK1 or cCRP concentrations between neoplastic and nonneoplastic groups, nor between RA and HB groups, for either serum or PCE samples. However, ROC curve analysis demonstrated that a ratio of serum TK1 to PCE TK1 was able to distinguish neoplastic and nonneoplastic causes of PCE (p = 0.027, AUC 0.72, CI 0.52 - 0.92) with 91% sensitivity and 50% specificity using a cut-off point of 0.20. These findings suggest that a ratio of serum TK1 to PCE TK1 could potentially serve as a useful rule-out test for neoplastic causes of PCE.

C23

Standardization of Echocardiographic Values of Left Atrial Measurement in Yorkshire Terrier Dogs
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The Yorkshire Terrier is one of the breeds with a greater predisposition to congenital heart disease and, mainly, to acquired diseases such as myxomatous mitral valve disease (MMVD). There is only one study establishing echocardiographic reference values for this breed, including aortic (Ao) and left atrial (LA) measurements, using a single method. The objectives of this study were: to determine and standardize LA measurement values, using LA/Ao ratio and employing four two-dimensional echocardiographic methods reported in the literature in Yorkshire Terrier dogs. An observational and cross-sectional study was performed with 50 adult dogs (between 15 months and seven years) and clinically healthy Yorkshire Terrier dogs. The animals were submitted to measurement of blood pressure, physical examination, echocardiographic and electrocardiographic examination, laboratory tests and chest radiographs. Ao measurement was done by three different methods, described by the authors Rishniw, Hansson ("Swedish" method) and Chetboul, and by the septum-lateral width in the right paraesternal axis view ("longitudinal" method). Three determinations of each parameter were performed, evaluated in different phases of the cardiac cycle, considering the average of the obtained values. The LA/Ao ratios found were: "Rishniw" 1.55 ± 0.12, “Chetboul" 1.15 ± 0.10 and "longitudinal" 1.75 ± 0.15, which are similar to the values.
Pulmonary Artery Denervation as a Treatment of Refractory Pulmonary Hypertension

Christopher Whipp – University of Minnesota; Christopher Stauthammer – University of Minnesota; Alex Rothman – University of Sheffield

Pulmonary hypertension is a debilitating disease in veterinary medicine, with phosphodiesterase-5 inhibitors such as sildenafil representing the mainstay of medical therapy. However, there are limited therapeutic options for patients who are refractory to high dose sildenafil therapy. Pulmonary artery denervation via catheter ablation of the sympathetic nerves in the pulmonary trunk has been shown to be safe and efficacious in experimental animal models, and has demonstrated encouraging results in preliminary human clinical trials. The feasibility, safety, and efficacy of pulmonary artery denervation for the treatment of pulmonary hypertension in clinical canine patients has not yet been evaluated.

Canine patients with severe refractory pulmonary hypertension were prospectively enrolled in a pilot study to evaluate the feasibility and safety of pulmonary artery denervation. Patients were included if they had severe pulmonary hypertension (tricuspid regurgitation (TR) pressure gradient ≥ 75mmHg) and displayed clinical signs despite high-dose sildenafil therapy (≥3mg/kg TID). Patients were excluded if pulmonary hypertension was secondary to left-sided heart disease or if other significant co-morbid disease was present. Response to treatment was evaluated by assessing TR gradient, activity levels via accelerometer data, and clinical score via owner survey at 1, 3, and 6 months post-procedure. Right ventricular function was assessed via tissue Doppler S' and TAPSE. Medical therapy was not altered during the study period.

Two patients were enrolled and successfully underwent the denervation procedure with no significant complications. Progressive decreases in TR gradient were documented in all patients (Case 1: 77 mmHg pre-procedure, 47 mmHg at 6 months, 39 % reduction; Case 2: 80 mmHg pre-procedure, 59 mmHg at 3 months, 26 % reduction). Average activity levels pre- and post-procedure were comparable (Case 1: 2385 steps/day pre-procedure, 1946 steps/day 6 months post-procedure; Case 2: 2009 steps/day pre-procedure, 1831 steps/day 1 month post-procedure) though accelerometer data was negatively impacted by poor owner compliance. Both patients demonstrated improved clinical scores based on owner survey and are still alive at time of submission (Case 1: 12 months post-procedure, Case 2: 5 months post-procedure).

Pulmonary artery denervation is a safe and technically feasible procedure for canine patients with severe refractory pulmonary hypertension. In addition, promising reductions in TR gradient were observed. Based on these results, a larger clinical study is warranted to evaluate the efficacy of this procedure.

C25

Worsening Renal Function in Dogs during Treatment of Congestive Heart Failure: 2008-2018

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Risk of azotemia and renal injury has been suggested as a potential consequence of therapy for congestive heart failure (CHF); however, no canine study has validated this claim. In humans, worsening renal function (WRF) has been identified as a prognostic indicator for mortality and re-hospitalization rates in CHF, with controversy as to whether prognostic significance is dependent on severity of WRF versus persistence of congestion. The purpose of this study was to determine the incidence of WRF during treatment and resolution of acute fulminant CHF in canines and the effect of WRF on patient outcome as assessed by survival to hospital discharge, time to recurrence of CHF requiring hospitalization, and long-term all-cause mortality.

Electronic medical record review of all dogs hospitalized for CHF between January 2008 and October 2018, diagnosed on thoracic radiographs reviewed by a board-certified radiologist and/or echocardiogram completed by a board-certified cardiologist. Serum creatinine within 1 year prior to hospital admission, near/at the time of hospital admission, during hospitalization, and at follow-up following hospital discharge was collected. Patients were categorized as having WRF if they experienced a rise in serum creatinine ≥ 0.3 mg/dl between admission and discharge. The incidence of WRF was 33.3% (44/132), with only 34% (15/44) of these patients experiencing pseudo-worsening renal function defined by a change in creatinine ≥ 0.3 mg/dl from admission during hospitalization with a concurrent ≥ 5% increase in PCV, HCT, or TP suggestive of dehydration. Days until recurrence of CHF requiring hospitalization or all-cause mortality was significantly shorter in dogs that developed WRF (71 days versus 163 days, p = 0.01). These findings suggest that WRF commonly occurs in dogs treated for CHF and has long-term prognostic implications.
C26

Efficacy of Torasemide in Degenerative Mitral Valve Disease Dogs with New Onset Congestive Heart Failure

Beatrice Besche – Ceva Santé Animale; Emille Guillot – Ceva Santé Animale; Thomas Blondel – Ceva Santé Animale; Mark Oyama – University of Pennsylvania

Diuretics are a cornerstone treatment for congestive heart failure (CHF). Torasemide is a potent loop diuretic with potential to treat CHF in dogs.

Evaluate the efficacy and safety of torasemide compared to furosemide as a first line diuretic in dogs with new onset CHF due to degenerative mitral valve disease (DMVD).

A double-blinded randomized non-inferiority study of torasemide vs. furosemide in addition to standard CHF treatment was conducted on 319 dogs with CHF attributable to DMVD. The primary efficacy criterion was reduction of pulmonary edema and cough and no worsening of dyspnea or exercise tolerance at day 14. Secondary endpoints included clinical response at day 84 and time to death, euthanasia, or premature study termination due to cardiac causes.

Torasemide q24h (n=161) was non-inferior to furosemide q12h (n=158) at day 14. Percentages of dogs meeting primary efficacy criteria were similar between groups (torasemide, 74.5% [95% CI, 66.8%-81.0%] vs. furosemide, 73.5% [65.7%-80.4%]; P=.87). Clinical response was also non-inferior between treatments on day 84 (P=.60). Dogs receiving torasemide survived longer than dogs receiving furosemide (P=.044), indicating that dogs receiving torasemide were 54% less likely to die, be euthanized or prematurely withdrawn due to cardiac causes than dogs receiving furosemide at any time during the study.

Torasemide was non-inferior to furosemide as first line treatment for new onset CHF due to DMVD. Torasemide significantly reduced the risk of cardiac-related death or premature study termination compared to furosemide.

C27

Effect of Sodium Nitroprusside, Furosemide and Dobutamine in Dogs with Cardiogenic Pulmonary Edema

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Acute cardiogenic pulmonary edema is a major disorder seen in the emergency department. However, the efficacy of traditional therapies for management of this problem is limited. To overcome of the limitations of conservative therapies, we evaluated the efficacy of the vasodilator sodium nitroprusside (SNP), for treatment of acute cardiogenic pulmonary edema in dogs.

Twenty dogs were recruited for this study. If the recruited dogs had a respiratory rate more than 100 times per minute, as well as a vertebral heart score (VHS) of more than 10.5 and were diagnosed with acute congestive heart failure, they were categorized as the groups of treatment with SNP. Among them, 9 dogs were treated with SNP only and 7 dogs were treated with SNP, furosemide and dobutamine. The other 4 dogs, which had respiratory rate less than 100 times per minute, as well as a VHS of more than 10.5 and were diagnosed with acute congestive heart failure, were categorized as a group of non SNP treatment. The dosage of SNP was administered at 2 μg/kg/min in SNP only group, whereas 1 μg/kg/min in SNP, furosemide and dobutamine group. The period of treatment was 24 hours. Seven dogs, six dogs and 2 dogs were favorable responders in SNP only group, group with SNP, furosemide and dobutamine and non SNP treatment group, each. The respiratory rate was significantly decreased in all groups however the VHS and blood pressure (BP) were not changed significantly in favorable responders. The results of this study suggest that SNP can be an effective alternative therapy for dogs with acute cardiogenic pulmonary edema.

C28

Oral Pharmacokinetics of Slow-Release Metoprolol in Dogs Before and After Experimentally-Induced Mitral Regurgitation

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Beta blockers improve survival in humans with congestive heart failure (CHF) secondary to systolic dysfunction. Although beta blocker therapy for CHF has been investigated in dogs, data is limited. The purpose of this study was to investigate the pharmacokinetics of metoprolol succinate (100 mg by mouth q 12 hr; range 4.5-6.1 mg/kg) in dogs with and without experimentally-induced mitral regurgitation. Two pharmacokinetic studies were performed on hound mixes (n=6); once prior to and once after experimentally-induced mitral regurgitation. Blood samples were collected intermittently for 24 hrs and metoprolol was quantitated in plasma by high-performance liquid chromatography. Data was subjected to non-compartmental pharmacokinetic analysis and parameters were compared between studies using non-parametric methods. No adverse events were observed. Variability in metoprolol disposition within studies was profound. Pertinent parameters were (median [range]), for pre-induction group, Cmax (ng/ml) 78 (226), Tmax (min) 242 (226), and half-life (min) 318 (2346).

For post induction, Cmax was 56 (81), Tmax 210 (870), and half-life 203 (120). Although statistical differences were not detected for any parameter, numerically decreased Cmax post induction suggests decreased oral absorption. Peak and trough (12 hr) concentrations in both studies were numerically lower than those achieved in humans receiving metoprolol for treatment of CHF. This study shows that while q 12 hr dosing of metoprolol succinate results in minimal fluctuation in metoprolol concentrations, the oral dose may be too low. This was intended as a preliminary study to investigate the pharmacokinetic properties of metoprolol in dogs with and without mitral regurgitation. Further studies are warranted to more fully define both the
pharmacokinetic and pharmacodynamic profiles of metoprolol succinate in dogs with CHF associated with mitral regurgitation.

C29

Amino Acid Concentrations and Echocardiographic Findings in Dogs Fed a Commercial Plant-Based Diet

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Diet and nutrition studies in people have shown that plant-based diets are capable of preventing, arresting, and reversing heart disease and other chronic debilitating diseases. To date, there are no studies evaluating the effects of a plant-based diet on any naturally-occurring chronic disease in dogs. Moreover, studies evaluating the nutritional adequacy and safety of plant-based diets formulated for pets are sparse. This prospective study investigated whether differences in amino acid concentrations and left ventricular echocardiographic findings occur in dogs who are transitioned from a traditional diet to a plant-based diet.

Thirty-eight client-owned healthy adult dogs were enrolled; 34 dogs to receive a commercial plant-based diet (PB diet) and 4 dogs to remain on their regular diet. Amino acid analysis (including plasma and whole blood taurine levels) was performed in all dogs, and echocardiography was performed in 37 dogs at baseline. Thirty-four dogs were transitioned from their traditional diet (≥1 animal ingredient, commercially-available, non-prescription, non-grain-free) to the PB diet. Thirty-four of 38 dogs completed the study. Amino acid analysis was repeated in 34 dogs (30 PB diet, 4 comparison group) after 30 days, and echocardiography was repeated in 33 dogs (29 PB diet, 4 comparison group) after 90 days. Pre- and post PB diet data were analyzed using a paired t test or Wilcoxon’s signed rank test.

Nineteen of 28 amino acids showed statistically significant differences in pre- and post-diet values. There was a significant increase in arginine (P = .0031), asparagine (P = .0022), aspartic acid (P = .0170), cystathionine (P = .0027), glutamic acid (P < .0001), histidine (P < .0001), isoleucine (P = .0405), phenylalanine (P = .0185), taurine (P < .0001), threonine (P = .0005), tryptophan (P = .0114), tyrosine (P = .0002), valine (P = .0185), and whole blood taurine (P < .0001). There was a significant decrease in glutamine (P < .0001), glycine (P = .0017), 3-methylhistidine (P < .0001), methionine (P = .0209), and hydroxyproline (P = .0026). All post-diet amino acid values were within or above the established reference intervals except glutamine. There was no significant difference in normalized left ventricular internal systolic diameter (P = .2068) or fractional shortening (P = .4889) between pre- and post-diet. The normalized left ventricular internal diastolic diameter was higher post-diet (Mdn: 1.46) compared to pre-diet (Mdn: 1.41, P = .0006), but still within the reference interval (1.27-1.85). No significant changes were seen in the comparison group.

The results of this study suggest dogs transitioned from a traditional diet to a plant-based diet undergo changes to their amino acid profile. Further, three-fourths of the amino acids (including taurine) significantly increased after 30 days on a plant-based diet suggesting that meat/animal ingredients are not essential for amino acid homeostasis in dogs. Additional studies are needed to determine whether the significant changes in amino acid concentrations observed in this study are due to normal day-to-day variation or due to differences in type, quality, and/or quantity of nutrients in plant-based diets compared to traditional diets. After 90 days on a plant-based diet, no dogs had echocardiographic evidence of left ventricular systolic dysfunction or dilated cardiomyopathy.

C30

Left Atrial Size and Function Predicting The Risk of Congestive Heart Failure in Small-breed Dogs

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The left atrium (LA) plays an important role in modulating left ventricular filling. Left atrial enlargement and dysfunction are induced by
myxomatous mitral valve disease (MMVD). Both the size and function of the LA are recognized as valuable prognostic indicators in dogs with MMVD. The N-terminal pro-brain natriuretic peptide (NT-proBNP) is released from stretched cardiomyocytes, and its circulating concentration often reflects the volume and pressure loading status of the left ventricle. In this study, we aimed to evaluate the correlation between the size and functional variables of the LA and serum NT-proBNP concentration. We further aimed to evaluate the ability of left atrial size and functional variables to predict the risk of CHF development.

Seventy-four treatment-naive small-breed dogs (<10 kg) with MMVD were enrolled prospectively and underwent standard echocardiography and serum NT-proBNP measurements. Left atrial functional variables, including complete, active, and passive contraction, were assessed based on the left atrial volume without the pulmonary vein and calculated by echocardiographic analysis system using Simpson’s rule.

The results showed that the size of the LA increased, whereas the functional variables, especially reservoir and contractile function, decreased with increasing disease severity (p < 0.001). The left atrial size showed good correlation with the circulating NT-proBNP concentration. NT-proBNP levels were negatively correlated with the left atrial reservoir, conduit, and booster pump function. The circulating NT-proBNP level and size and functional variables of the LA could predict CHF development. Progressive enlargement and functional impairment of the LA are significantly correlated with NT-proBNP levels in dogs with naturally occurring MMVD. Hence, the left atrial size and functional assessment can estimate the risk of CHF development. These parameters can contribute to the management of dogs with MMVD.

C31

CT Angiography in 5 Dogs with Atrial Septal Defect and Partial Anomalous Pulmonary Venous Connection

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Sinus venosus atrial septal defect (ASD) is commonly associated with partial anomalous pulmonary venous connections (PAPVC) in humans. PAPVC can be diagnosed by two-dimensional transthoracic echocardiography, however some types are difficult to diagnose by that method. Computed tomography (CT) angiography is accurate in

| Parameter | OR (95% CI) |
|-----------|-------------|
| NT-proBNP | 1.10 (1.03-1.18) |
| LAmax     | 2.35 (1.31-4.22) |
| LAVmin    | 5.12 (1.70-15.4) |
| LAVpre    | 3.73 (1.55-8.97) |
| LATEV     | 2.48 (1.03-6.00) |
| LAPEV     | 3.14 (0.99-9.87) |
| LAAEV     | 1.94 (0.47-7.93) |
| LA-FA/Total | 0.88 (0.80-0.95) |
| LA-FA/Cross | 0.97 (0.88-1.06) |
| LA-FA/Cast | 0.89 (0.83-0.96) |
| LAV-TF     | 0.90 (0.84-0.96) |
| LAV-PF     | 0.94 (0.88-1.01) |
| LAV-AF     | 0.93 (0.88-0.98) |
| LAEI       | 0.26 (0.11-0.63) |
| LAPE       | 1.02 (0.98-1.07) |
| LAAE       | 0.98 (0.94-1.02) |
defining sinus venosus ASD and PAPVC in humans. This has not been reported in veterinary medicine.

Five dogs were referred to our hospital for evaluation of right heart enlargement. Two-dimensional transthoracic echocardiography revealed dilation of the right atrium and ventricle in each dog. A diagnosis of a sinus venosus ASD was made using echocardiography in 3 dogs. An ASD was not identified in the other 2 dogs. A CT angiogram was performed in each dog and revealed a defect in the interatrial septum located at the top of the atrial septum near the cranial vena cava in each case. Anomalous right cranial and middle lobar pulmonary veins converged to join the cranial vena cava where it joined the right atrium in 2 dogs. In the other 3 dogs, the right cranial and middle lobar pulmonary veins converged near the ASD, slightly to the right.

Sinus venosus ASDs can be associated with PAPVCs and anomalous pulmonary veins are difficult to detect using transthoracic echocardiography. CT angiography more consistently identifies a sinus venosus ASD in dogs when compared to echocardiography and allows the examiner to identify associated PAPVCs. Consequently, CT angiography is the preferred method for detecting and characterizing these defects in dogs.

C32

Pharmacokinetic Analysis after Multiple-dose Administration of Coenzyme Q10 in Dogs with Myxomatous Mitral Valve Disease

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Coenzyme Q10 (Q10) is a mitochondrial cofactor and antioxidant, available as dietary supplement and used in dogs with heart disease. Evidence for using Q10 in dogs is limited and dose size and dosing intervals are not well documented. The objective of this study was to obtain pharmacokinetic and tolerability data on a gelatine capsule formulation of Q10 administered to dogs with myxomatous mitral valve disease (MMVD). In a prospective, randomised, single-blinded, placebo-controlled crossover design, 19 Cavalier King Charles spaniels with MMVD ACVIM class B2 (n = 10) or C (n = 9) received 100 mg Q10 (ubiquinone) BID followed by placebo (or vice versa) for three weeks, separated by a 2-week washout period. Plasma concentrations of Q10 were analysed using high-performance liquid chromatography before and after each treatment period and, in five dogs, on five consecutive days following Q10 treatment. The average (±SD) plasma concentration of Q10 at baseline was 1.01 (± 0.36) μg/mL. After supplementation, the average (±SD) plasma concentration increased significantly to 4.42 (±2.76) μg/mL (P < 0.0001). The terminal elimination half-life was estimated to 3 days after the last dose. No dogs experienced adverse reactions or abnormalities on complete blood count and serum biochemistry following Q10 supplementation. In conclusion, Q10 dissolved in vegetable oil is well-tolerated and well-absorbed in dogs with heart disease and treated dogs reached higher plasma concentrations than previously reported in dogs administered with other Q10 formulations.

C33

Assessment of Heart Rate Turbulence in Dogs with Myxomatous Mitral Valve Disease

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Myxomatous mitral valve disease (MMVD) is the most prevalent heart disease in veterinary medicine affecting small dogs, it reduces the cardiac output and the response to that is the compensatory mechanisms activation, being the most important the activation of the sympathetic portion of the autonomous nervous system. This leads to a reduced heart rate variability (HRV), which is a very accurate analysis derived from R-R interval calculations. Recently in human medicine a new indicator was introduced known as heart rate turbulence (HRT). This new indicator can be divided into two other parameters,
turbulence slope (TS) and turbulence onset (TO) which can evaluate the response of the sinusual beating cycle after a premature ventricular contraction, and has also proven to be more efficient than HRV as an independent odd's death indicator. The purpose of the present study is to assess HRT in dogs with different degrees of myxomatous mitral valve disease.

In the current study the data of 37 dogs with various grades of myxomatous mitral valve varying from 2.2 to 25 kilograms was collected including electrocardiography, echocardiography and some patients also did holter monitoring. For analytical purposes the data was divided into groups according to the classification of Atkins et al, 2009. The HRT indicators, onset and slope, were calculated for each group and group results were compared.

The statistical analyses of TO and TS showed difference between grade B1 versus C and D, while B2 was considered to show similar results to B1 and C, D (TO, p = 0.0050; TS p = 0.0029). Interesting fact was the clear tendency of worsening results as the disease progressed. The ROC curve for TO differentiating among (B1/B2) from (C/D) showed a mean + standard deviation area under the curve of 0.88 + 0.060, while differentiating between normal hearts (B1) and remodelated hearts (B2/C/D) 0.80 + 0.087. The ROC curve for TS differentiating among (B1/B2) and (C/D) showed a mean + standard deviation area under the curve of 0.79 + 0.079. When it comes to differentiating among C and D grades the area under the ROC curve for TO was small, as showed by the mean + standard deviation area under the curve of 0.61 + 0.15, for TO and 0.55 + 0.179 for TS. The correlation between TO and TS with echocardiographic anatomical and doppler parameters showed moderate to weak correlation (p < 0.05), with TS showing more correlations with echocardiography than TO. Heart rate turbulence is a promising indicator, is ease to acquire using both electrocardiography and holter monitoring plus it is not influenced by the operator experience. This easeness to acquire and the fast response to hemodynamical changes showed in studies with humans makes HRT a strong indicator that could be used for advanced staging and therapy guidance for patients accordingly to their autonomic reflex.

C34

Identification of Neutrophil Extracellular Traps in Feline Cardiogenic Arterial Thromboembolism: A Pilot Study

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Neutrophil extracellular traps (NETs), composed of extracellular chromatin decorated with proteins like histones, are released from neutrophils secondary to systemic inflammation or pathogen exposure. NETs augment clot formation and inhibit fibrinolysis in humans and dogs. However, NETs in feline cardiogenic arterial thromboembolism (CATE) have never been described. Further understanding of NETosis in cats may provide future therapeutic targets for feline CATE. We aimed to describe a novel technique for immunodetection of NETs in paraffin-embedded clots from cats.

Paraffin-embedded thrombi from 8 cats with CATE (6 aortic bifurcation, 1 brachial artery and 1 left atrium) were sectioned at 3 μm and processed for antigen retrieval using a modified protocol. Samples were blocked with 5% goat serum, incubated with anti-human citrullinated histone H3 (citH3), and anti-cat myeloperoxidase (MPO) antibodies, followed by secondary antibodies conjugated to Alexa Fluor 555 and Dy-Light 755, respectively. DNA was stained with 300 nm 4′,6-diamidino-2-phenylindole. Fluorescence microscopy was used to identify NETs, defined as co-localization of MPO, citH3 and cfDNA, in 10 random views at 40x magnification. Neutrophils were identified based on nuclear morphology and cell size. Infiltration of neutrophils within all clots examined was identified. NETs surrounding these neutrophils consisted of extracellular citH3 and MPO. In addition, intracellular expression of citH3 was identified. Our data demonstrate the first identification of NETs in arterial thrombi from cats with CATE. Findings of this study offer new insights into the pathophysiology of this complex condition and support investigation into possible novel targeted therapies in the management of feline CATE cases.
C35

Reasons for Exclusion of Apparently Healthy Dogs from a Phase II Rapamycin Clinical Trial
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Recruitment of healthy dogs for a medical intervention is unusual; the majority of clinical trials recruit subjects with existing conditions. This study describes reasons for exclusion when a healthy canine population is sought. A Phase I randomized, controlled, double-blind clinical trial (RCT) of low-dose rapamycin, an mTOR-inhibitor, suggested improvement in diastolic and systolic function among treated versus placebo cases. A follow-up Phase II RCT is currently enrolling. Owners are invited to enroll healthy dogs who are 6 to 10 years of age, and weigh 40 to 80 pounds. Exclusion criteria were identified within Stage 1 (S1) – owner-provided survey information; Stage 2 (S2) – medical records review; and Stage 3 (S3) – screening examination. Subcategories identified Owner, Dog, or Other reasons for exclusion. Of 54 nominated dogs, 38 were excluded at S1 (n=18), S2 (n=6), and S3 (n=14). Dogs were excluded for Owner (n=4), Dog (n=27), Other (n=5), and concurrent (Owner + Dog; n=2) factors. The most common exclusion period was S1 (n=18), with weight outside the target range being the most common exclusion factor (n=10). Heart murmurs were the second most common exclusion factor (S3; n=5) while historical and current systemic illness were the third most common exclusion factor (S2; n=4). Among dogs who passed S1 and S2 screening (n=30), 14 dogs (46.67%) were excluded at S3, with heart murmur > grade II/VI (n=5), cardiac arrhythmias (n=2), and clinicopathologic abnormalities (n=2) representing more than one dog. Dogs whose owners seek enrollment in clinical trials for healthy dogs are likely to be excluded for size, previous diagnoses, and newly discovered cardiac abnormalities.

C36

Indirect Doppler Systolic Blood Pressure Measurements Taken With and Without Headphones in Privately-Owned, Conscious Dogs
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The purpose of this study was to assess the effects of headphone use and potential covariates on indirect radial Doppler flow systolic arterial blood pressure (BP) measurements in dogs. Between May and August of 2018, 100 privately-owned dogs were enrolled; 84 dogs completed the study. Blood pressure was measured in lateral recumbency per ACVIM guidelines, with and without headphones, using a randomized crossover design. Weight, BCS, MCS, anxiety score, and heart rate also were recorded. Mixed effects crossover analysis with corresponding ANCOVAs and Spearman rank correlation coefficients were performed to determine the impact of headphone use on the initial BP (BP1) and the mean of BP2-6, as well as potential covariates. P < 0.05 was considered significant. There was no difference in the number of dogs diagnosed as hypertensive between measurement types (19 vs 18), though only 7 dogs were categorized as hypertensive during both periods. BP1 and BP2-6 did not differ for measurements taken with (P = 0.8) or without (P = 0.1) headphones. Significant differences in BP1 measurements were found between measurement types (F [1,80] = 4.3, P = 0.04) due to higher results when measurements were taken without headphones. There was no significant association between BP2-6 and measurement type. Systolic BP was negatively correlated with weight and positively correlated with age and anxiety score at the start of BP collection. Use of headphones does not significantly affect BP results taken using the Doppler flow method when ACVIM guidelines are followed. Age, anxiety score, and weight significantly affected BP results.

C37

Assessment of Systolic Function Using Tissue Motion Annular Displacement in Dogs with Mitral Valve Disease
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Although mitral valve disease (MVD) is an essentially valve disorder, the myocardium undergoes structural changes with the progression of the disease, that may compromise left ventricular systolic function. Longitudinal systolic function, poorly explored in conventional echocardiography, may be affected before circumferential function. Tissue Motion Annular Displacement (TMAD) is a Speckle tracking technique that evaluates the function of longitudinal fibers by displacing the mitral annulus towards the apex during systole, which has never been studied in dogs with MVD.

One hundred and eight client-owned dogs with MVD of various stages and thirty-six healthy dogs as control group underwent physical examination, electrocardiography, systolic blood pressure (SBP) measurement, as well as a standard and speckle tracking echocardiography. Global Longitudinal Strain (GLS) and TMAD were used to assessment the longitudinal systolic function. These parameters were compared with the conventional echocardiography in the various stages of MVD, in order to evaluate the use of TMAD as a diagnostic method and the behavior of longitudinal function with the progression of MVD. The Global TMAD% values were higher in the animals in stage B2 than in the other groups. Although there is no significant reduction in TMAD values in the symptomatic group, there is a tendency of the median to be lower in this group (table 1). GLS, Global TMAD% and Global TMAD mm^2/m^2 were correlated with several parameters of conventional echocardiography, such as ejection fraction and fractional shortening. GLS was influenced by gender (P: 0.04) and SBP (P: 0.02; R: -0.17). Curiously, Global TMAD mm^2/m^2 (P: 0.002; R: 0.234) and Global TMAD% (P: 0.007; R: 0.205) presented a weak correlation with age. TMAD is a faster technique to perform than GLS (P: 0.0007) and presents good intra- and inter-observer repeatability in dogs with MVD. TMAD is repeatable and rapid technique for evaluation of longitudinal systolic
function in dogs with MVD. An evident impairment in longitudinal systolic function was not observed in this study by GLS and TMAD. However, future studies with a larger population of dogs refractory to the standard therapy may provide more information about TMAD as a prognostic and diagnostic evaluator and better elucidate the behavior of these variables in dogs in terminal stage of MVD.

C38

Congenital Heart Disease in Dogs: A Retrospective Study of 95 Cases

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The aim of the study was to determine the prevalence of congenital heart diseases in dogs attending two veterinary hospitals in southern Brazil and to identify possible associations between these conditions and epidemiological characteristics.

A retrospective study was carried out in the cardiology sections of a veterinary teaching hospital and a private veterinary hospital located in the city of Curitiba (Brazil) during a period of 70 months (from January 2012 to October 2017). Patients without definitive diagnosis obtained by Doppler echocardiography were excluded from the study. Fisher's exact test was performed to determine the Odds Ratio of the congenital heart disease with the gender, age and breed of the patients.

A total of 6,710 dogs attended in both veterinary hospitals, 115 congenital heart diseases were identified in 95 patients, representing a prevalence of 1.71%. The most commonly diagnosed conditions were sub-aortic stenosis (SAS) (23.28%) and pulmonic stenosis (PS) (21.55%). Furthermore, the prevalence of atrial septal defect (ASD) (7.6%) was higher and the prevalence of patent ductus arteriosus (PDA) (7.76%) lower when compared to the literature. The associations between the congenital heart disease and the epidemiological characteristics tested, along with the identification of what has already been cited and what has not yet been cited in the veterinary literature are described in Table 1. To the authors knowledge, this is the first retrospective study on congenital heart diseases in dogs conducted in Brazil. In summary, the prevalence of congenital heart disease was lower than that previously reported. Most of the findings were equivalent to those described in the literature. However, it is the
first time that an association of the Maltese dogs with ventricular septal defect (VSD) has been identified. Further retrospective studies of congenital heart disease in dogs in Brazil are needed to confirm the results of this study.

C39

**Computed Radiology as a Screening Test for the Identification of Congenital Heart Disease in Dogs**

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The objectives of this study were to evaluate the accuracy of thoracic radiology as a screening test for congenital heart diseases in dogs, to identify the main contributions and limitations of this modality, and to verify the reproducibility of the evaluations by three observers with different levels of training.

An interobserver, observational, retrospective and prospective study was carried out, including ninety dogs: thirty healthy animals, thirty with acquired heart diseases and thirty with congenital heart diseases, which all had thoracic radiographs and a confirmed echocardiographic diagnosis. The cases were separated and randomized by a mediator who did not participate in the reading of the radiographic examinations, and no evaluator had access to the patients' data. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of each observer were calculated in relation to the correct classification of dogs to groups of normal or acquired and congenital heart diseases, as well as identification of enlargement of the cardiac silhouette and large vessels of dogs with congenital heart diseases. Finally, the Kappa coefficient was obtained between the observers to verify the reproducibility of the radiological evaluations performed.

In general, sensitivity, PPV and accuracy were unsatisfactory (<70%), while the specificity and NPV were satisfactory (>70%), and the agreement ranged from poor to reasonable (between 0 and 0.39).

In conclusion, this modality was able only to identify healthy patients, and could not differentiate the individuals with different forms of heart disease or define the cardiac malformations. In addition, there was low reproducibility between observers, therefore, this technique should not be used as a sole screening method in dogs with suspected congenital heart diseases.

C40

**Where There's Smoke There's Fire: Echocardiographic Findings in 51 Cats with Burns from California Wildfires**

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Devastating wildfires have affected California resulting in severe burn injury, smoke inhalation, and death in humans and animals. The purpose of this study was to describe the echocardiographic findings from cats exposed to California wildfires in 2017 and 2018. We hypothesized that thermal burn injury and smoke inhalation would result in identifiable cardiovascular changes on echocardiogram in cats.

Cats presented from the Tubbs (Napa/Sonoma County, 2017) and Camp (Butte County, 2018) wildfires were prospectively enrolled. Cats with burn injuries of the thorax, and/or a temperament that precluded echocardiogram without sedation, were excluded. Echocardiography was performed in all cats, 1-4 days after presentation (initial exam) and 7-10 days after the initial exam.

Fifty-one cats were included in the study. Initial echocardiogram showed myocardial thickening (MT) in 18 cats (35%), spontaneous echo-contrast or thrombi (SEC/T) in 16 cats (31%), and congestive heart failure in 5 cats (10%). Median cardiac troponin-I from 19 cats measured 0.07ng/ml (range 0.00-2.11). Repeat echocardiograms from
38 cats showed MT in 12 cats (31%) and SEC/T in 4 cats (10%). Forty-two cats (82%) survived to hospital discharge, 7 died or were euthanized due to a cardiac cause, and 2 for non-cardiac reasons. Cardiovascular and coagulation effects of thermal burn and smoke inhalation injury are reported in humans and now for the first time in cats. High prevalence of MT not attributable to pseudohypertrophy was noted. Prevalence of SEC/T was reduced at second evaluation (p = 0.02). Future studies of cardiovascular effects in feline wildfire victims are warranted.

C41

Application of Coagulation Function by Thromboelastography in Dogs with Mitral Valve Insufficiency

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In veterinary medicine, a variety of diseases are known to cause coagulation abnormalities. Identification of these coagulation abnormality status has been relied on traditional plasma-based coagulation assays which fail to take overall hemostatic capacity into consideration and possess only a small part of the coagulation pathways. The value of TEG as an early predictor of coagulation state, especially hypercoagulability, has been published, associated with decreased R and K values, increased MA and α angle.

The objective of this study was to compare TEG results and those of traditional coagulation tests between dogs with mitral valve insufficiency and healthy control dogs.

Traditional coagulation test (prothrombin time, partial thromboplastin time, antithrombin activity, d-dimer concentration, and fibrinogen concentration) and TEG (R time, K time, alpha angle and MA) analyses of twenty patient dogs and eleven healthy dogs were performed. All of the traditional coagulation panel values were within the reference range except for fibrinogen value of patient group, but there were no statistical significant. R and MA values of patient group were outside the reference range and there was significant difference between MA values of normal and patient group (P < 0.05). To conclude, with the ability to detect hypercoagulability, TEG provides the clinician with the unique ability to identify dogs that are in the proinflammatory and hypercoagulable state and offer the novel possibility of clearly differentiating affected dogs from cardiovascular disease, and as such, TEG may potentially be useful for individualization of treatment.

C42

Echocardiographic Assessment After Mitral Valve Plasty in Dogs

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Mitral regurgitation causes left atrial and left ventricular dysfunction, dilatation, and remodeling. The purpose of this study was to assess changes in echocardiographic data after mitral valve plasty (MVP) under cardiopulmonary bypass in dogs with mitral valve disease (MVD).

85 dogs with mitral valve regurgitation, with a mean age of 10 (6-13) years and mean body weight of 4.05 (1.4-12.5) kg, underwent MVP.
with cardiopulmonary bypass between January 2016 and December 2017. Echocardiography was performed before, 1, 3, 6, and 12 months after surgery.

Left atrial-to-aortic root diameter ratio [2.54(1.42-4.36) vs 1.48 (1.04-3.1)], normalized left ventricular internal dimension in diastole [2.13(1.56-2.96) vs 1.56(1.15-2.36)], fractional shortening [47.1 (27.1-64.6) vs 33.8(15.0-53.4)], and peak early diastolic velocity of left ventricular inflow [1.40(0.64-2.11) vs 0.88 (0.55-1.46) m/sec] decreased a month after surgery. Fractional shortening improves gradually over 3 months after surgery. Peak atrial systolic velocity of left ventricular inflow [0.80(0.36-1.45) vs 1.09 (0.45-1.62) m/sec] increased 1 month after surgery. After MVP, relaxation filling pattern was shown in many cases.

These results suggest that MVP improves left atrial and ventricular dimensions and leads to reverse remodeling after surgery a month. Additional study is needed to clarify systolic and diastolic function after surgery in dogs with MVD.

C43

The Causes of Canine Myocarditis and Myocardial Fibrosis Are Elusive by Targeted Molecular Testing

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Complications of myocarditis vary from permanent heart damage to heart failure and death. Veterinarians know very little about the most frequent causes of canine myocarditis and there are few rewarding testing options for determining the cause due to the lack of epidemiological and etiopathologic data. We performed a retrospective case-control study using nucleic acid isolated from archived (2007-2015) tissues from dogs with myocarditis and controls. Based on a literature review and informed by the comparative epidemiology of human myocarditis, myocardial samples were tested with a panel of conventional and real-time PCR assays to screen for causes of canine myocarditis in pediatric/juvenile and adult dogs. Of the 66 cases screened, PCR was did not detect agents in 35 cases (53%; 95% confidence interval (CI) 41-65%) and was often negative in adults (21/26). Occasionally, PCR detected canine distemper virus, herpesvirus, adenovirus, respiratory coronavirus, parainfluenza virus, Mycoplasma haemocanis, and/or Neospora caninum but in both cases and controls thus PCR detection was not considered to indicate causation. It is unclear if the PCR detected agents are incidental or latent, or cardiopathogenic. We previously reported the continued association of canine parvovirus 2 (CPV-2) with myocarditis in pediatric/juvenile dogs despite widespread vaccination; in adult dogs, CPV-2 was detected in 2/26 cases and 4/22 controls. Borrelia spp., Bartonella spp., Rickettsia spp. and influenza A virus were not detected in any of the myocarditis cases and West Nile Virus was detected at the analytic limit in a single adult case. These data demonstrate the limitations of current targeted molecular diagnostic tests. More research and new approaches are needed to identify unrecognized, emerging, or novel pathogens of canine myocarditis in order to develop rewarding testing strategies.

C44

Canine Myocarditis: Clinical Presentation, Cardiovascular Findings, Etiology, and Outcome

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Myocarditis in dogs is a nonspecific disease with numerous potential etiologies. There is limited literature documenting the clinical features of canine myocarditis. The study objective was to describe presentation, cardiovascular abnormalities, etiology, and outcome of canine myocarditis in non-Chagas-endemic areas.

Medical records at two tertiary care facilities were searched retrospectively for dogs with a clinical or histopathological diagnosis of myocarditis. Myocarditis was diagnosed in 64 dogs (median age 7.9 years). Common examination findings included fever (13/61, 21%), heart murmur (12/64, 19%), and abnormal pulses (12/64, 19%). Median cardiac troponin I was 12.2 ng/mL (range 0.2-808.0 ng/mL), and troponin exceeded 1.0 ng/mL in 26/29 (90%) dogs. On ECG, 29/54 (54%) dogs had ventricular ectopy. Evidence of decreased left ventricular systolic function was the most common echocardiographic abnormality (31/43, 72%).

An infectious etiology was diagnosed in 27/64 (42%) dogs, while a non-infectious etiology was suspected in 37/64 (58%) cases. Confirmed infectious etiologies included bacterial sepsis (9) or endocarditis (3), toxoplasmosis (2), parvovirus (2), borreliosis (1), trypanosomiasis (1), leptospirosis (1), and fungal (1). Median survival time was 4 days (range 0 - 828 days) for all dogs, and 82 days for dogs who survived > 2 weeks after diagnosis. Presence of pericardial effusion and presence of azotemia were significant predictors of non-survival in multivariate analysis. Criteria for the antemortem diagnosis of canine myocarditis should be established.

C45

The Prevalence of the RNA-Binding Motif 20 Mutation in Genotyped Dogs

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Dilated cardiomyopathy (DCM) has been previously reported in standard schnauzers (SSNZ) due to an autosomal recessive mutation in the RBM20 gene and genotyping is commercially available. The purpose of this study is to investigate the genetic prevalence of RBM20 mutation in a large cohort of predominantly SSNZ.

The University of Missouri’s database was retrospectively reviewed for samples submitted and tested for RBM20 genotyping from 11/2013 to 5/2018. Data analyzed included: breed, mutation status (wild-type, heterozygous positive, homozygous positive), geographic origin of submission, and 3-generation pedigree and cardiac phenotype (when available).
A total of 2,140 sample results were identified, including 1,834 SSNZ and 270 giant schnauzers (GSNZ). Heterozygous or homozygous variants were only identified in SSNZ and GSNZ. A total of 389 SSNZ tested positive (21.2%) with 361 heterozygous (19.7%) and 28 homozygous (1.5%) dogs. Of the homozygous SSNZ, DCM was confirmed in 22/28 (78.5%), with the remaining either lost to follow-up (n = 3) or dying prior to echocardiographic evaluation (n = 3). Twenty-six GSNZ tested positive (9.6%) with 23 heterozygous (8.5%) and 3 homozygous (1.1%) dogs. Nine of these GSNZs belonged to one family, including the 3 homozygous dogs that also had a DCM phenotype. One unrelated GSNZ with DCM did not possess the RBM20 mutation, suggesting another rare cause for DCM exists in GSNZs.

Given the high prevalence of the mutation among tested SSNZ and GSNZ, genotyping is recommended prior to breeding, and exclusion of heterozygous carriers is not recommended as this could lead to a dramatic loss of genetic diversity.

C46

The Prevalence and Prognostic Effects of Non-thyroidal Illness in Dogs with Myxomatous Mitral Valve Disease

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Non-thyroidal illness (NTI) is a common finding in humans and dogs and is affected by serious clinical impairment or various drugs. Even though NTI is very common in patients with cardiovascular diseases, the data on the prevalence is lacking, and its prognostic role is also unclear in dogs with cardiovascular diseases. The aim of this study was to assess the prevalence and prognostic association of NTI and the serum NT-proBNP in dogs with myxomatous mitral valve disease (MMVD).

A total of 24 MMVD dogs were included and were divided by American College of Veterinary Internal Medicine (ACVIM) guidelines (class B2, n = 2; class C, n = 19; class D, n = 2) and Doppler echocardiography measured mitral regurgitation (MR) severity (mild MR, n = 5; moderate MR, n = 10; severe MR, n = 9). Healthy, age-matched dogs (n = 20) were included as controls. Routine physical, hematological examination, thoracic x-ray, electrocardiography and echocardiography were evaluated and thyroid hormones (TH) and NT-proBNP levels were examined. In this study, NTI was defined as low levels of circulating triiodothyronine (T3) or thyroxine (T4) in dogs with normal or slightly decreased thyroid stimulating hormone (TSH) concentrations. The prevalence of NTI in MMVD dogs was 20.8% (5/24). None of the dogs in control group showed TH abnormality. All NTI dogs were included in ACVIM stage C or D; However, the prevalence of NTI was not correlated with the MR severity. The prevalence of respiratory distress was significantly higher in NTI dogs compared to the non-NTI dogs with MMVD (p = 0.009). The presence of NTI had no significant effect on survival, but NT-proBNP has a significant effect on survival time in dogs with MMVD (p = 0.017).

In conclusion, the moderate to severe MMVD dogs (ACVIM stage C or D) have higher possibility to show low levels of circulating T3 or T4. TH treatment is not advised in NTI dogs. Thus, full TH panel should be considered for discriminating hypothyroidism from NTI in those dogs.

C47

Compositional and Functional Changes in Gut Microbiome in Dogs with Myxomatous Mitral Valve Disease

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Gut microbiota dysbiosis is associated with metabolic diseases. Changes in the gut microbiota are associated with heart failure in humans. Serotonin signaling is implicated in the pathogenesis of canine myxomatous mitral valve disease (MMVD) and over 95% of serotonin is manufactured in the gut. We hypothesized that gut dysbiosis is associated with MMVD.

Fecal samples were collected for metagenomic analysis from four groups of age, gender, and bodyweight balanced dogs with MMVD of ACVIM stages A (healthy), B1, B2, and C, and analyzed via 16S rRNA gene sequencing. The analysis for taxonomic sequences and metagenomic functions was performed using bioinformatics software UPARSE and PICRUSt respectively. Results showed a greater phylogenetic diversity in group A than other groups. No difference was found between B1, B2, and C groups. Group A also had more bacterial genera than other groups. Butyricoccus and Faecalibacterium, two beneficial short-chain fatty acid producing bacteria, were significantly more abundant in healthy dogs than dogs with MMVD. The abundances for these bacteria were inversely correlated to the key echocardiographic variables, left atrial-to-aortic root ratio (Butyricoccus) or left ventricular internal diameter. PICRUSt-estimated metagenomes showed overrepresentation of cardiolipin biosynthesis and valine degradation pathways but underrepresentation of tryptophan and glutamate/glutamine biosynthesis pathways in B2 dogs compared to healthy dogs. Tryptophan is the precursor of serotonin biosynthesis. Cardiolipin and glutamine play important roles in mitochondrial energy metabolism while defective branched-chain amino acid catabolism promotes heart failure in humans and animal models. Our data suggest a compensatory mechanism by gut microbiome in MMVD pathogenesis.

C50

Point-of-care NT-proBNP Assay to Screen Apparently Healthy Cats for Cardiac Disease in General Practice.

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The point-of-care (POC) NT-proBNP ELISA test is a convenient tool for screening cats for cardiac disease in general practice (GP). Previous studies have evaluated its sensitivity and specificity in teaching hospital populations, but investigations focused on cat populations from
Systolic Dysfunction Caused by Parvovirus In Dogs: An Echocardiography Feature Tracking Assessment

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Parvovirus infection has been suggested to be associated with myocarditis indicating endothelial cells of the myocardium could be a natural target for infection. Parvovirus myocarditis may also be secondary to sepsis. This study evaluated systolic dysfunction in dogs naturally infected with parvovirus using two-dimensional feature tracking echocardiograms and was under protocol 055/15 from the Ethics Committee on Animal Use of the Institution.

Thirty-seven dogs were divided into 3 groups: noninfected (n = 9), parvovirus infected without sepsis (n = 15) and parvovirus infected with sepsis (n = 13). Strain (St) and strain rate (StR) of global and in six myocardial segments in the radial, circumferential and longitudinal directions were obtained in the right parasternal transverse and four chambers apical view in the left ventricle. Circumferential and longitudinal directions were still obtained in the endocardial and epicardial planes.

In general, strain and strain rate were significantly higher in the noninfected group than in the infected groups (p < 0.05); in the sepsis group St and StR were significantly lower than in the non-sepsis group (p < 0.05). The segments commonly affected in both infected groups were mid-septal endocardial and basal-lateral and mid-lateral. There was no change in the conventional echocardiographic variables (p > 0.05). The intraobserver variability was low for all strain and strain rate variables (Intraclass correlation coefficient - ICC ≥ 0.75) and interobserver variability was low for circumferential (ICC ≥ 0.75), moderate for radial (0.75 < ICC ≥ 0.4), and high for longitudinal direction (ICC < 0.4).

Parvovirus infection in dogs causes systolic dysfunction, to a greater extent in septic patients, and with probable onset in the mid-septal endocardial and in basal-lateral and mid-lateral segments. Feature tracking echocardiography is more sensitive and early than conventional echocardiography in determining systolic dysfunction.

C52

Assessment of Myocardial Deformation by Feature Tracking Echocardiography in Cats with Ventricular Septal Defect

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The development of new tools, such as two-dimensional feature tracking (2D - FTI), allows early diagnosis of myocardial dysfunction in heart diseases including congenital heart disease. The ventricular septal defect (VSD) is the most frequently diagnosed congenital abnormality in cats, thus, more knowledge about cardiac dysfunction is necessary, especially in asymptomatic animals. The objective of this study was to evaluate the left ventricular myocardial deformation through 2D - FTI by the measurement of the radial, circumferential and longitudinal echocardiographic strain (St) and strain rate (StR) indexes in healthy and VSD diseased cats.

Twelve healthy cats (Group 1) and six cats with VSD (group 2) were evaluated. Animals with other cardiac diseases were excluded. The study was under protocol 052/11 from the Ethics Committee on Animal Use of the Institution. All the evaluated cats were male, and in the control group the breeds found were mongrel (10), Persian (1) and Siamese (1). In the VSD group the breeds were Persian (4) and mongrel (2). The age of the animals in the group 1 was 30.58 ± 19.32 months and in group 2 was 36.00 ± 26.29. The weight varied from 3.3 to 4.9 kg and the mean of the VSD diameter was 1.58 ± 0.79 mm. In conventional echocardiographic examination the animals showed...
atrial and ventricular chambers dimensions within normal limits. All cats were evaluated by two-dimensional feature tracking to obtain St and StR in six myocardial segments.

In the longitudinal direction, there was a statistical difference for the epimyocardial basal-septal (p = 0.0017), epimyocardial mid-septal (p < 0.0001), epimyocardial apical-septal (p = 0.0288), epimyocardial mid-lateral (p = 0.0327), endomyocardial mid-septal (p = 0.0035), endomyocardial mid-lateral (p = 0.0461) and St global (p = 0.0382) of the ventricular myocardium when comparing healthy and cats with VSD. There was also difference in the circumferential endomyocardial mid-lateral segment (p = 0.0248), radial mid-lateral (St: p = 0.0409; StR: p = 0.0166) and radial mid-posterior segments (p = 0.0369) between groups.

The study showed that even in asymptomatic animals with VSD there is a reduction in ventricular myocardial deformation mainly in the longitudinal direction, evidencing the fragility of these fibers and thus demonstrating that the lesions can possibly start on these segments.

C53

Left Atrial Function by Bidimensional Feature Tracking Echocardiography in Dogs with Asymptomatic Mitral Valve Disease

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The degenerative myxomatous mitral valve disease (DMVD) is the most prevalent acquired heart disease in dogs. The relationship between the left atrial (LA) diameter and the aorta (Ao) is considered a prognostic factor in the disease. Since LA is a three-dimensional structure, methods based on chamber volume measurement may be more accurate than linear methods. The study evaluated the atrial volume obtained through two-dimensional feature tracking (2D-FTI) echocardiography in healthy dogs and dogs with asymptomatic DMVD.

We evaluated 80 dogs into three different classes: group A (n = 21), group B1 (n = 30) and group B2 (n = 29 – Advanced B2). The values of atrial diastolic and systolic volumes, and atrial cardiac output were automatically provided by 2D-FTI using the four-chamber left apical view. These values were indexed to body weight and were referred as atrial diastolic (AdVi) and atrial systolic (AsVi) volumes indexes and atrial cardiac index (ACi), respectively. The atrial ejection fraction (AEF) was provided automatically by the 2D-FTI software. The normal distribution of the data was evaluated by the Shapiro-Wilk test and the data between the different classes were analyzed by the One-way ANOVA test, followed by the Tukey test (p < 0.05).

The AdVi was significantly higher in advanced B2 than in the control group (1.31 ± 0.95 x 0.96 ± 0.31, p = 0.038), as well as the ACi was also higher in advanced B2 when comparing to group A (102.38 ± 80.18 x 78.19 ± 33.38, p = 0.030). The increase of the AdVi and ACi indexes shows that their values increase even in asymptomatic animals due to the mitral regurgitation that increase with the progression of the DMVD.

The data demonstrate that 2D-FTI is a sensitive and early method for the detection of left atrial dysfunction and helps to better understand the role of LA in the pathophysiology of the disease.

C54

Endomyocardial Biopsy-Proven Myocarditis in Cats. A Clinical Case Series

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In cats, myocardial failure and rhythm disturbances secondary to myocarditis have been described in several reports, in which a final diagnosis had been reached through post-mortem histopathologic examination. In order to obtain an antemortem diagnosis and an etiologic characterization of myocardial inflammation, a technique for transvenous EMB of the right ventricular septum in cats has been proposed in 1990 in healthy cats. In this study, the procedure was well tolerated and was associated with low morbidity and no mortality, but nowadays this technique is still uncommon in the workup of unexplained myocardial and rhythm disorders in cats. The purpose of this case series is to report an in vivo endomyocardial biopsy diagnosis of myocarditis in cats from Saint-Petersburg, Russia.

The data were obtained between 2016 and 2018. Twenty-three cats of different breeds, age, and sex were included in this report. The diagnosis of myocarditis included anamnesis, physical findings, echocardiography, electrocardiography, endomyocardial biopsy with histopathological and PCR tests.

The age distribution was between 3 and 9 years and body weight was between 3 and 6 kg. The most common reason for owners visit were signs of respiratory distress and pulmonary edema (15/23), but in several cases, signs were associated with moderate appetite suppression and apathy (8/23). The common findings on EchoCG were: mild dilatation of left atria (19/23), slightly hypertrophied left ventricle walls (15/23), segmentary hypokinesia (12/23), hyperechogenic(10/23) or hypoechogenic(6/23) loci in the myocardium, systolic dysfunction(9/23). The common findings on ECG were: accelerated idioventricular rhythm (18/23), ventricle premature complexes (15/23), atrioventricular block and atrioventricular dissociation (10/23).

Biopsy findings included lymphocytic infiltration, cardiomyocyte loss, interstitial edema of different severity. In several cases (8/23) vasculitis was found with myocardial alteration concentrated in extravasal spaces, and one case was associated with microabscesses (1/23).

The PCR-tests of biopsies revealed several cases of Bartonella henselae infection (4/23), Toxoplasma gondii (2/23), and one case of a cat with lymphoma and associated FIV+FeLV infection (1/23).

We found that in several cases of myocarditis infection agents were well-known as myocarditis inducers, but also we didn’t obtain information about the cause of disease in all cases with vasculitis in histopathology study. We can assume that there are other causes of myocarditis in cats, including auto-immune reactions associated with vasculitis or chronic viral infection with low activity.
C55

Residual Severe Mitral Regurgitation After Mitral Valve Plasty in 6 Dogs with Mitral Valve Disease

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Severe mitral valve regurgitation (MR) remains in some dogs with mitral valve disease that had undergone mitral valve plasty (MVP) due to insufficient plasty. The purpose of this study was to investigate six dogs (8-13 years of age, ACVIM stage C: 4 patients, D: 2 patients) with post-operative severe MR, that had undergone MVP under cardiopulmonary bypass from 2015 to 2017. Cardiac examination was performed before, 1 month, and 1 year after surgery. VHS, LVIDDDN, LA/Ao, medication history and incidence of heart failure relapse were assessed.

Median VHS was 12.7 v (range 11.9-13.5 v), 12.9 v (range 11-13.4 v), 13.1 v (range 12.6-13.4 v), median LVIDDDN 2.23 (range 2.14-2.66), 2.29 (range 2.01-2.69), 2.31 (range 1.95-2.51), median LA/Ao 2.99 (range 2.54-3.1), 2.31 (range 1.4-3.25), 2.39 (range 1.6-3.56) before, 1 month, and 1 year after surgery, respectively.

Our results reveal, that in dogs with residual severe MR, there is limited evidence of reverse remodeling, postoperative diuretic is still necessary, and the risk of heart failure remains high. Reoperation may be effective in improving prognosis in these canine patients, as suggested in human literature.

C56

Echocardiographic Estimation of Left Ventricular-Arterial Coupling in Canine Myxomatous Mitral Valve Disease

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The interaction between left ventricle (LV) and arterial system, left ventricular-arterial coupling (VAC), has been conventionally evaluated via LV catheterization based on the ratio of effective arterial elastance (Ea), a measure of LV afterload, to LV end-systolic elastance (Ees), a measure of LV contractility. Recent studies demonstrate that worsened VAC indicated by the elevated Ea/Ees estimated using echocardiography is associated with poorer prognosis in human heart failure. This prospective cross-sectional study investigated the relationship between echocardiographically estimated Ea/Ees and disease severity in canine myxomatous mitral valve disease (MMVD).

Eighty-four MMVD dogs and 57 healthy dogs (Control group) were enrolled. These MMVD dogs were classified into 3 groups (25 in stage B1, 41 in stage B2, and 18 in stage C/D) according to ACVIM guidelines. Ea/Ees was echocardiographically estimated by the formula: 

$Ea/Ees = \frac{LV \text{ end-systolic volume}}{\text{forward stroke volume}}$

LV end-systolic volume was determined with the monoplane Simpson method of disc, while forward stroke volume was calculated by multiplying velocity-time integral of the aortic flow by aortic area.

Ea/Ees was significantly higher in stage B2 group than in Control group, and significantly higher in stage C/D group than in the 3 other groups. In multivariate logistic analysis, among Ea/Ees and conventional echocardiographic indices, Ea/Ees and the ratio of peak early diastolic transmitral velocity to isovolumic relaxation time were identified as the independent predictors of stage C/D.

Findings suggest that VAC is associated with disease severity and echocardiographically estimated Ea/Ees can be a useful severity marker in canine MMVD.

C57

Electrocardiographic Evaluation in Dogs Treated with Imidocarb and Atropine/Nacl 0,9%

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Hemoparasitosis are infectious diseases, transmitted by hematophagous vectors, and have worldwide distribution leading a non-specific condition, which may include anemia, thrombocytopenia, leucopenia and, in some cases, myocarditis. In view of several therapeutic options, a widely used protocol is an association of imidocarb with the previous application of atropine sulfate. The presente study aimed to evaluate the electrocardiographic variables related to the use of this protocol and the loading of atropine to reduce the effects of imidocarb. For this, 11 naturally infected dogs were diagnosed through suggestive blood count findings and/or hematozoal screening. Each dog was treated two times, with a physiological solution (NaCl) 0,9% (G1) and atropine (G2) at the dose of 0.04 mg.kg⁻¹, after 10 minutes, in both groups, was injected imidocarb (6mg.kg⁻¹). Respiratory rate, heart rate and arterial blood pressure were recorded as well as electrocardiographic evaluation of lead II, before administration of NaCl 0,9% or atropine (T0), after 10 minutes (T10) and re-evaluated after the application of imidocarb at 50 (T50) e 130 (T130) minutes. In G1, decrease of P wave amplitude (p=0.0001) and PR interval (p=0.0039) were statistically significant, while in G2, decrease of QT (p=0.0001) and PR (p=0.0009) intervals, increase of heart rate (p=0.0001) and decrease of QRS complex (p=0.0019) duration were observed. Although there were significant differences between the moments, the values remained within the physiological values. There was no significant difference in arterial blood pressure and respiratory rate between treatments. Ventricular extrasystoles were observed in one patient, that get worse after the application of atropine, however did not present any clinical sign. Thus, both protocols demonstrated clinical and electrocardiographic safety in patients who did not present
any significant rhythm disturbances before the treatment with the drugs.

**C58**

**Total Atrial Conduction Time Evaluated with Tissue Doppler Imaging in Dogs with Mitral Valve Disease**

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Conduction of electrical stimuli through the atria is related to Total Atrial Conduction Time (TACT). It is known that time measured between the beginning of the electrocardiogram P-wave to the peak of A-wave tissue Doppler graph (PA-TDI interval) represents an independent predictor of the onset of atrial fibrillation (AF) in people. Thus, it was hypothesized that PA-TDI interval might predict AF in dogs with myxomatous mitral valvopathy. In addition, it was proposed to investigate PA-TDI interval values in healthy dogs. The study was performed in a retrospective fashion, and a bank of echocardiographic images of dogs from Cardiology Service of HOVET/FMVZ-USP was used. PA-TDI interval was measured with tissue Doppler sample positioned on the lateral wall of the left atrium, just above the mitral valve annulus. For statistical analysis, variables were submitted to Shapiro-Wilk test and Pearson or Spearman correlation coefficient was estimated. Student’s t-test, ANOVA for non-repeated measurements and 2-factor ANOVA were used. A multiple Cox regression was performed considering time for the occurrence of atrial fibrillation. All statistical tests were considered significant when p < 0.05. 144 echocardiographic studies were selected out of 264 available. PA-TDI interval for small dogs were smaller than those of large or giant animals (p = 0.01). Gender, castration and obesity had no influence (p = 0.50, 0.24 and 0.98 respectively) on the values obtained. PA-TDI duration correlated with the duration of PR interval, weight, heart rate, and blood eosinophil concentration/mm³ in healthy animals. PA-TDI interval was significantly longer in patients with myxomatous mitral valvopathy stage B2 and C (55.4 ± 7.7 and 55.7 ± 9.2 ms, respectively). In addition, there was a 13% increase in chance of developing atrial fibrillation at each increased unit of PA-TDI interval (p < 0.01). Thus, it is possible that isolated atrial stretch and/or the advent of chronic heart failure may relate to conduction delays on the atria. Furthermore, TACT estimated by PA-TDI interval may predict the occurrence of atrial fibrillation secondary to myxomatous mitral valve disease in dogs. The low frequency of atrial fibrillation in the studied population may have masked more expressive results.

**C59**

**Echocardiographic and Radiographic Assessment of Left Atrial Enlargement in Dogs with Myxomatous Mitral Valve Degeneration**

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The purpose of this study was to evaluate the diagnostic value of left atrial (LA) radiographic assessment to predict the severity of echocardiographic LA enlargement. Transthoracic echocardiograms and thoracic radiographs were prospectively obtained on the same visit, on fifty dogs diagnosed with myxomatous mitral valve degeneration (MMVD), ACVIM stages B1 to C. Thoracic radiographs were reviewed by two radiologists. LA enlargement was evaluated on ventrodorsal or dorsoventral (VD/DV), left and right lateral views using a semi-quantitative score. A global radiographic LA enlargement score was also established. Echocardiographic LA size was assessed using the 2D LA-to-aortic root ratio (LA : Ao) by two sonographers. Spearman correlation coefficient between LA : Ao and radiographs was higher for VD/DV (0.77, p < 0.05) compared to left lateral (0.61) and right lateral (0.64) views and not statistically different from the global assessment score (0.73). Global radiographic score had higher sensitivity (0.65) and specificity (1) than any individual views to predict echocardiographic LA enlargement. LA : Ao values for patients with no evidence of LA enlargement on global radiographic score ranged from 1.25 to 2.46 (mean ± SD: 1.70 ± 0.3).

This study showed that semi-quantitative global radiographic score is a highly reliable indicator of echocardiographic LA enlargement in dogs with MMVD and that thoracic VD/DV images correlate best with echocardiographic findings compared to lateral images. However, LA enlargement can remain undetected using only radiographic evaluation.

**C60**

**Transcatheter Closure of Patent Arterial Duct in Small Animals After Unsuccessful Surgical Ligation**

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Surgical ligation remains a standard therapy for closure of a patent arterial duct (PDA), particularly in animals deemed too small for transcatheter closure. Hemorrhage during ductal dissection may prevent surgical completion, while residual flow can be seen after uncomplicated surgical PDA ligation. This study reviewed records of 9 animals (8 dogs, 1 cat) with aborted or incomplete surgical PDA ligation who then underwent attempted transcatheter closure. Average age at time of surgery was 10.5 months and at time of catheterization was 13.7 months with an average of 96 days between procedures (range, 13 to 256 days). Median weight at time of catheterization was 3.3 kg (2.0 to 17.2 kg). Reasons for failed surgery included hemorrhage in 5, residual flow in 3, and inability to locate the ductus in 1 case. Transcatheter closure was successfully performed with a canine duct occluder in 6 dogs, transarterial coil in 2 dogs, and retrograde transvenous coil in 1 cat. Metallic hemoclips partially obscured angiographic findings in 3 cases with prior surgical hemorrhage, but did not prevent transcatheter closure. Complete PDA closure was confirmed by 24-hour post-operative echocardiography in all animals. When surgical ligation is unsuccessful, either due to hemorrhage or residual flow,
transcatheter closure of PDA may be a viable option even in small patients.

**C61**

Assessment of Longitudinal Systolic Function Using Tissue Motion Annular Displacement in Healthy Cats

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The left ventricle systolic function is commonly evaluated using the shortening fraction obtained by standard echocardiography, which only verifies the transverse contraction of the cardiac muscle fibers. The longitudinal systolic function of the left ventricle can be evaluated using the M-mode echocardiography in an apical view (mitral annular plane systolic excursion – MAPSE), Longitudinal Strain (LSt) or Tissue Motion Annular Displacement (TMAD), a technique developed with speckle-tracking technology that follows the positions of the mitral ring during the systolic phase of the left ventricle. Comparing to LSt, TMAD have a reduced processing time and a lower requirement on echocardiographic image quality, which can be an excellent vantage in feline cardiology. TMAD, which has not been investigated in cats yet, is calculated as the dislodgement of a virtual point automatically determined between mitral’s septal and lateral annulus toward the left ventricular Apex (median point TMAD). In this prospective cross-sectional observational study thirty-nine cats (1-15 anos years/3-6.3 kg) underwent an echocardiogram to obtain an apical 4-chamber images, allowing the MAPSE obtention and the calculation of TMAD and LSt. After verifying the non-normality of the data using the Shapiro-Wilk test we opted for non-parametric test - Mann-Whitney test a- using the Statistica single user software version 13.2. Results are shown in table 1. No differences existed between males and females for MAPSE, TMAD and LSt. Further studies should be developed to determine TMAD values from 2-chamber view images, as well to clarify whether TMAD is a reliable substitute for LSt and MAPSE in the assessment of longitudinal systolic function in cats.

**C62**

Assessment of Major Vascular Flow Patterns in the American Alligator (Alligator Mississipiensis)

Simon T. Swift – University of Florida; Bonnie Gatson – University of Florida; Nicole Furst – University of Florida; James Wellehan – University of Florida; Darryl Heard – University of Florida

Crocodilians have a unique cardiovascular anatomy with variable shunting patterns. While they have a four chamber heart, they have two aortas and a main pulmonary artery. The right aorta is the single vessel leaving the left ventricle to supply oxygenated blood to the body, but the left aorta and main pulmonary artery leave the right ventricle containing deoxygenated blood. While the pulmonary artery supplies the lung, the left aorta feeds the proximal gastrointestinal tract. In addition, the two aortas are connected proximally by the foramen of Panizza which is under adrenergic control and can be covered by the open aortic valve. Flow is mainly right aorta to left aorta but can be bidirectional. There is a further connection dorsally. In addition, the entrance to the pulmonic valve is controlled by a cog valve which controls flow into the pulmonary artery. Echocardiographic maging of the cardiovascular system of alligators is challenging due to the thick dermis and scales and images are often suboptimal. During a study evaluating the response to

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**Table 1 - Shortening Fraction, Ejection Fraction, Tissue motion annular displacement, Mitral Annular Plane Systolic Excursion and Longitudinal Strain of the left ventricle in healthy cats**

| Variáveis                      | Sex          | Female (n=24) | Male (n=15) | Total (n=39) | P*  |
|-------------------------------|--------------|---------------|-------------|--------------|-----|
|                               |              | Mean ± Standard deviation | Mean ± Standard deviation | Mean ± Standard deviation |     |
| Shortening fraction           |              | 53,9 ± 7,6    | 53,3 ± 9,1   | 53,7 ± 8,1   | 0,6546 |
| Ejection fraction             |              | 86,9 ± 6,0    | 86,7 ± 7,2   | 86,8 ± 6,4   | 0,9425 |
| Free wall MAPSE              |              | 4,8 ± 1,0     | 5,0 ± 0,9    | 4,9 ± 1,0    | 0,6546 |
| LSt                           |              | 30,7 ± 5,9    | 29,5 ± 6,7   | 30,2 ± 6,1   | 0,4794 |
| Septum TMAD (mm)             |              | 3,7 ± 1,0     | 3,3 ± 1,2    | 3,5 ± 1,1    | 0,2664 |
| Free wall TMAD (mm)          |              | 3,8 ± 1,3     | 3,4 ± 1,3    | 3,6 ± 1,3    | 0,4025 |
| Medium point TMAD (mm)       |              | 3,9 ± 1,2     | 3,4 ± 1,2    | 3,7 ± 1,2    | 0,1572 |
| TMAD %                        |              | 17,9 ± 5,4    | 14,8 ± 5,5   | 16,7 ± 5,5   | 0,0006 |

* non-significant p-value by the Mann-Whitney test considering significance level of 5%. Free wall MAPSE: mitral annular plane systolic excursion measured at the free wall annulus; TMAD: tissue motion annular displacement; TMAD%: percent displacement of the mitral annulus towards the heart apex as compared with the left ventricular length; LSt: let ventricular longitudinal strain.
epinephrine under general anesthesia, ten alligators were evaluated using transthoracic echocardiography (TEE) prior to anesthesia. Transesophageal echocardiography (TEE) was then performed while they were anesthetized. Flow patterns into the three vessels could be clearly visualized using TEE. The foramen of Panizza and flow patterns across it could be seen as the blood was sufficiently echogenic. The action of the cog valve in the right ventricular outflow tract could be appreciated by the variable flow across the pulmonic valve. Flow began at the start of systole before terminating abruptly then restarting towards the end of systole. After administration of epinephrine, this pattern resolved to a more normal continual flow during systole.

C63

Comparison of M Mode and Speckle Tracking Data in Turkish Kangal Dogs with DCM
Osman Safa Terzi – Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ankara, Ankara, Turkey; Hasan Albaskan Veterinary Care Specialists – Milford, Mi; Merve Bensu Arikan – Ankara University

Systolic potential of left ventricle is evaluated both M-mode and speckle tracking echocardiography techniques. The aim of this study is to evaluate whether there is a correlation between the ejection fraction (EF) data and strain from the region of where M mode data is generated. This was done in healthy Turkish Kangals (TK) and in TK diagnosed with dilated cardiomyopathy (DCM). Sixty-two TK were included in the study: 49 healthy (HG) and 13 diagnosed with DCM (DCMG). Signalment and echocardiographic diagnosis were obtained from the medical records of TK assessed between 2017 and 2018 retrospectively. M mode EF and regional speckle tracking echocardiography analysis of radial strain (RSt) was performed at the levels of the papillary muscles (PM). Column analysis was performed to identify differences between groups. Correlation analysis was performed to identify relationship between EF and RSt of specific M mode region. Speckle tracking echocardiography and M mode variables about potential of ejection of left ventricle were lower in DCMG than in HG with statistical significance. Cardiac output was not different between the two groups, however, EF, FS, stroke volume (SV) and strain values from M mode measurement regions of interventricular septum (MAS) and posterior Wall (MP) were lower in DcmG (p < 0.001, p=0.004, p < 0.001, p < 0.001, p < 0.001 respectively). While there was a correlation between EF and FS p < 0.001 in healthy group, there was a correlation between SV, CO, FS and HR (p < 0.001, p < 0.001, p < 0.001, p=0.0092 respectively). There are significant differences in M mode and STE variables between HG and DcmG, which means that variables gathered from both techniques are sufficiently discriminatory for DCM diagnosis. However, the lack of correlation between EF and regional strain values showed that these two techniques could yield different results in cardiac muscle diseases other than DCM.

C64

Dose-Responsive Effects of Oral Short-Term Prednisone Therapy on Clinico-pathologic and Hemodynamic Variables in Healthy Dogs
Rebecca Tinklenberg – Iowa State University College of Veterinary Medicine; Shane Murphy – Iowa State University College of Veterinary Medicine; Jonathan Machel – Iowa State University College of Veterinary Medicine; Wendy Ware – Iowa State University College of Veterinary Medicine; Alyssa Mahaffey – Iowa State University College of Veterinary Medicine; Yuqi Yan – Iowa State University College of Veterinary Medicine; Jessica Ward – Iowa State University College of Veterinary Medicine

This clinical trial investigated a dose-response relationship for the effect of short-term prednisone therapy on clinico-pathologic and hemodynamic parameters in eight healthy purpose-bred beagle dogs. Dogs received 5-day courses of prednisone at various doses (0.5, 1.0, 2.0, and 4.0 mg/kg/day) followed by 9-day washout periods. Data collected pre- and post-prednisone courses included noninvasive blood pressure, complete blood count, serum biochemistry profile, fractional excretion of electrolytes, urine protein:creatinine ratio, glomerular filtration rate (GFR) estimated by iohexol clearance, NT-proBNP, plasma renin activity, and plasma cortisol. Compared to control condition (no treatment), oral prednisone administration at 4.0 mg/kg/day caused a significant increase in serum glucose (p = 0.018) and urine protein:creatinine ratio (p = 0.0015). GFR increased compared to control at doses of 2.0 and 4.0 mg/kg/day (p = 0.020 and p = 0.010, respectively), while fractional excretion of sodium decreased at doses of 0.5 and 1.0 mg/kg only (p = 0.024 and p = 0.044, respectively). Several expected changes in clinico-pathologic values also occurred with prednisone at all doses studied (decreased chloride, increased albumin, increased alkaline phosphatase and alanine aminotransferase activities, decreased total cortisol; p < 0.05 for all analyses). No significant changes occurred compared to control condition in blood pressure, NT-proBNP, or plasma renin activity. Administration of high-dose oral prednisone (2-4 mg/kg/day) can affect hemodynamics in systemically healthy dogs via dose-dependent hyperglycemia and increased GFR. Oral prednisone did not affect blood pressure, cardiac biomarkers, or activation of the renin-angiotensin-aldosterone system in this study. No clinically relevant effects were noted with administration of anti-inflammatory doses of oral prednisone at 1.0 mg/kg/day and below.

C65

High Energy Loss in the Left Ventricle of Small Dogs Detected by Vector Flow Mapping
Takuya Uehara – Azabu University; Minori Otomo – Azabu University; Mizuki Hasegawa – Azabu University; Yoko Fujii – Azabu University

Vector flow mapping (VFM), a novel echocardiographic technology developed in Japan, enables the analysis of blood flow vectors and velocity in the cardiac chambers using color-flow Doppler and speckle tracking data. Our previous study revealed that small dogs showed functional and morphological differences compared to large dogs. We also hypothesized that different body size influences intracardiac blood flow dynamics. The purpose of this study was to compare left ventricular (LV) blood flow characteristics in dogs of different body sizes using VFM. Clinically healthy adult dogs (n = 24) were divided into 2 groups on the basis of body weight, small dogs (<7 kg, SM) and medium and large dogs (>7 kg, ML). The following parameters were calculated using VFM: energy loss (EL) as an index of energy efficiency, circulation (Circ) as an index of vorticity in systole (sys) and diastole (dia), and intra-LV
pressure difference (IVPD) and gradient (IVPG). Heart rate, conventional M-mode index and E wave were also measured. EL was significantly higher in SM (median: sys 15.49, dia 17.40) than in ML (median: sys 9.88, dia 9.74). Circ was significantly lower in SM than in ML. IVPD was significantly lower in SM than in ML. EL in ML showed excellent correlations with Circ, IVPG, F5% and E wave, whereas in SM it showed a deviating distribution. The current findings reveal that small dogs have significantly high LV energy loss, and exhibit unique and inefficient blood flow characteristics, compared with larger dogs.

C67

Evaluation of Cardiac Cycle Length and Stroke Volume in Dogs with Atrial Fibrillation
Rachel Van Zile – The Ohio State University College of Veterinary Medicine; Jaylyn Rhinehart – The Ohio State University College of Veterinary Medicine; John Bonogura – North Carolina State University College of Veterinary Medicine

The optimal heart rate (HR) in atrial fibrillation (AF) remains unknown. In people with AF and heart failure, stroke volume (SV) variability might be more dependent on HR than ventricular contractile function. This study aimed to identify the influence of preceding cardiac cycle length on SV in dogs with spontaneous AF. We hypothesized that SV varies significantly with instantaneous HR and a “threshold” for optimal SV might be evident. Echocardiograms were retrospectively evaluated from 25 dogs associated with AF and cardiomyopathy (12), valvular disease (9), or structurally normal hearts (4). Preceding cycle length and SV were determined from five cardiac cycles per dog. Aortic cross-sectional area (CSA) was calculated from maximum systolic valve diameter and aortic velocity-time integral (VTI) was recorded. Stroke volume index (SVI) was calculated as CSA*VTI, indexed to bodyweight. Correlations between cycle length and SVI were determined by multiple linear regression accounting for repeated measurements. The HR associated with optimal SVI was evaluated by receiver operating characteristic curve (ROCC) analysis. SVI was significantly correlated with cycle length (p < 0.0005), with zero, partial- and part-correlations of 0.67, 0.87, 0.60. ROCC analysis (Youden) showed a HR< 145/minute was associated with a 70% sensitivity and 78% specificity for SVI> 1mL/beat/kg (AUC 0.77); additionally this “threshold” produced the highest average SVI/dog. Preceding cycle length explained ~40-50% of accounted unique variation in SVI. This study shows preceding cycle length impacts SVI in canine AF and is probably optimized by an instantaneous HR. Additional prospective studies are indicated.

C68

Echocardiographic Evaluation of Obese Dogs Submitted to an Exercise Routine
Letícia A. Yonezawa – Santa Catarina State University (UDESC); Willian Almeida – College of Agriculture and Veterinary (CAV), Santa Catarina State University (UDESC); Thais Santos – College of Agriculture and Veterinary (CAV), Santa Catarina State University (UDESC); Mere Saito – College of Agriculture and Veterinary (CAV), Santa Catarina State University (UDESC)

Obesity is an emerging disease in dogs which can cause cardiac remodeling due to increased systolic volume. Physical activity is one of the recommendations for the treatment of obesity, however, the consequences in the cardiovascular system of obese dogs are unclear. The objective of this study was to determine the echocardiographic changes in obese dogs submitted to three months of physical activity as a single treatment for the disease. Seven obese dogs underwent a 30-minute aerobic exercise routine on a treadmill, three times a week during a period of three months. Echocardiographic exam was performed in these animals in the B-mode, M-mode and Doppler evaluation, during rest, at pre-treatment time (M0) and monthly evaluations (M1, M2, M3) for three months. All animals had a final weight (median 19.30 kg) lower than the initial weight (median 20.07 kg), but there was no significant difference. All animals had hypertrophy of the left ventricle free wall in systole (median 13.10 mm) and diastole (10.37 ± 1.62 mm) at M0. There was a decrease in the distance of the E point septal separation (EPSS) in the M-mode evaluation at M1 (2.94 ± 1.03 mm) when compared to M0 (4.99 ± 1.03 mm), M2 (3.74 ± 1.27 mm) and M3 (3.37 ± 1.27 mm), however the EPSS remained within normal range at all times. None of the animals had changes in Doppler evaluation, signs of pulmonary hypertension or chronic mitral valve disease. Obesity caused hypertrophy of the left ventricle free wall in the studied dogs, and regular physical activity, as a single treatment for obesity, did not lead to significant weight loss or echocardiographic changes that could be considered harmful to these animals.

C69

Long-term Incidence/Risk of Noncardiovascular and All-cause Mortality in Apparently Healthy and Preclinical Hypertrophic Cardiomyopathy Cats
Philip R. Fox – Animal Medical Center; Bruce Keene – NCSU CVM; Kenneth Lamb – Lamb Consulting; Karsten Schoder – Ohio State University, CVM; Valerie Chetboul – Ecole Nationale Vétérinaire d’Alfort; Virginia Luis Fuentes – Royal Veterinary College; Gerard Wess – Ludwig Maximilians University; Jessie Rose Payne – Royal Veterinary College; Dan Hogan – Purdue University, CVM; Jens Haggstrom – Swedish University of Agricultural Sciences; Geoff Culshaw – Royal (Dick) SVS Hospital for Small Animals; Emilie Trehiou-Sechi – Ecole Nationale Vétérinaire d’Alfort; Deborah Fein-Ferreira – University of Missouri, CVM; Wendy Ware – Iowa State University, CVM; Manreet Singh – University of California, Davis, CVM; Sabine Riesen – University of Veterinary Medicine; Pamela Lee – Animal Medical Center; Reid Nakamura – Advanced Veterinary Care Center; Jonathan Abbott – Virginia – Maryland Regional CVM; Etienne Cote – Atlantic Veterinary College, University of Prince Edward Island; Michele Borgarelli – Kansas State University

Background: Few reports have detailed and compared major causes of death between apparently healthy cats (AH) and cats with preclinical hypertrophic cardiomyopathy (pHCM). Objectives: To identify and compare incidence rates, risk, and survival associated with noncardiovascular and all-cause mortality in AH and pHCM cats. Animals: 1730 client-owned cats (722 AH, 1008 pHCM) from 21 countries. Methods: Subanalysis from a retrospective, multicenter, longitudinal, cohort study (REVEAL). Long-term health data were recorded from medical records and owner/referring veterinarian interviews. Results: Noncardiovascular death occurred in 534 (30.9%) out of 1730 cats observed for up to 15.2 years. The proportion of noncardiovascular death did not differ significantly between AH or pHCM (P = .522). Most frequently recorded noncardiovascular-causes of death were cancer,
chronic kidney disease, and conditions characterized by chronic weight-loss-vomiting-diarrhea-anorexia, respectively. Incidence rates and risk of noncardiac death increased with age, although substantial mortality also occurred in middle-aged cats. Proportion of all-cause death was greater in pHCM vs AH (65 vs 40 percent, respectively; P < .001). Survival to noncardiovascular death did not differ significantly between AH vs pHCM (P = .105). However, all-cause survival was significantly shorter in pHCM (P < .0001). Conclusions: Cats that at study entry were AH or had pHCM did not differ significantly for survival to noncardiovascular death. However, all-cause mortality was significantly greater in pHCM cats due to increased cardiovascular death which was superimposed upon noncardiovascular mortalities.

C70

Pre- and Post-Exercise Cardiovascular Evaluation in Obese Dogs Submitted to an Exercise Routine

Letícia A. Yonezawa – Santa Catarina State University (UDESC); Willian Almeida – College of Agriculture and Veterinary (CAV), Santa Catarina State University (UDESC); Thais Santos – College of Agriculture and Veterinary (CAV), Santa Catarina State University (UDESC); Mere Saito – College of Agriculture and Veterinary (CAV), Santa Catarina State University (UDESC)

Physical activity is one of the recommendations for the treatment of obesity and causes increased cardiac output due to higher heart rate and systolic volume, which may have consequences for obese dogs. The purpose of this study was to determine the cardiovascular changes caused by exercise in obese dogs submitted to four weeks of physical activity as a single treatment. Nine obese dogs underwent an aerobic physical activity routine of 30 minutes on a treadmill, three times a week during four weeks, with electrocardiogram (ECG) evaluations before the treatment (M0) and weekly (M1, M2, M3 and M4) before and after exercise. Additionally, pre- and post-exercise arterial blood pressure was evaluated on first (M1) and last week of treatment (M4). At the end of four weeks of treatment, all animals had a final weight (median 17.55 kg) lower than the initial weight in M0 (median 18.99 kg), but there was no significant difference. No animal had low amplitude QRS complexes in the ECG at any moment and there was no significant change between pre- and post- exercise ECG parameters at all times. On the rhythm evaluation, two dogs had sinus tachycardia at M0, M1 and M2 and normal sinus rhythm at M3 and M4 before and after exercise, and the other seven animals had sinus arrhythmia and wandering pacemaker at all times. There was no significant difference in heart rate between pre- and post-exercise at all times (pre-exercise M0: 117.111 ± 32.994 bpm; M1:120.6 ± 36.7 bpm; M2: 115.3 ± 34.3 bpm; M3: 127.9 ± 34.8 bpm; M4: 109.1 ± 21.9 bpm), (post-exercise M1: 118.3 ± 30.3 bpm; M2: 122.0 ± 41.7 bpm; M3: 113.2 ± 30.7 bpm; M4: 108.3 ± 19.9 bpm) on the ECG, but all animals were truly tired at the end of exercise. No animal had arterial hypertension at rest before exercise (pre-exercise M1: 138.56 ± 10.97 mmHg; M4: 132.24 ± 21.33 mmHg), and the exercise did not cause significant changes in any of the times (post-exercise M1: 137.22 ± 26.02 mmHg; M4: 130.56 ± 21.57 mmHg). There was a slight decrease in blood pressure with the treatment, but with no significance. Four weeks of physical activity as a single treatment did not cause significant weight loss or changes in cardiovascular parameters in obese dogs.

C71

A Retrospective Assessment of Echocardiography and Clinical Efficacy of Ramipril in Dogs with Heart Disease

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The purpose of this study was to evaluate the clinical efficacy, adverse effects and echocardiographic findings after ramipril administration in dogs with acquired valvular and congenital heart disease. The medical records of 27 dogs that were diagnosed with heart disease and prescribed with ramipril were retrospectively reviewed. After admission, the dogs were prescribed with ramipril 0.125 mg/kg orally, once daily. The selected dogs were evaluated for general clinical signs, blood pressure, hematology, electrocardiography, and echocardiography including the tissue Doppler and speckle-tracking imaging techniques on day 0, 30 and/or 60. After the treatment with ramipril, clinical signs including coughing (P > 0.001), exercise tolerance, and respiratory effort were improved and the diastolic blood pressure in dogs with diastolic hypertension was decreased (P = 0.023). On echocardiography, the left ventricular internal diastolic diameter, end-diastolic volume index (p = 0.032), and left atrium-to-aorta ratio were decreased. Additionally, ramipril preserved myocardial systolic function without cardiac deformation, which was presented by fractional shortening, ejection fraction, global longitudinal strain, and segmental wall motion score. The hematologic evaluations indicated a tendency of increased plasma blood urea nitrogen with normal creatinine value, and azotemia was observed in 16.7 % dogs.

In conclusion, this study demonstrated that the treatment with ramipril improved clinical efficacy and decreased the blood pressure in dogs with acquired valvular and congenital heart diseases. The echocardiography revealed that ramipril decreased the cardiac volume overload and preserved the left ventricular systolic function. Meanwhile, prescribing ramipril to dogs with heart disease could cause a side effect of prerenal azotemia.

E01

Effect of Exercise On Electrical Impedance Tomography Derived Flow Variables in Horses with Equine Asthma

Nicolas Hertman – University of Zurich; Martina Mosing – Murdoch University; Klaudia Blaszczyk – Vetsuisse Bern; Claudia Graubner – Vetsuisse Bern; Simone Lanz – Vetsuisse Bern; Vinzenz Gerber – Vetsuisse Bern; Andreas Waldmann – Swisstom; Angelika Schoster – University of Zurich

Electrical impedance tomography (EIT) is a novel non-invasive imaging technique that calculates global and regional respiratory gas flow signals, based on impedance change during the breath cycle. Recently EIT has been shown to accurately diagnose bronchoconstriction in
healthy horses after histamine challenge. The aim of this study was to describe EIT parameters in horses affected by naturally occurring mild-to-moderate equine asthma (MEA). Thirteen horses diagnosed with MEA based on ACVIM consensus statement guidelines were prospectively included. Recording with EIT was performed before and after 15 minutes longing. After exercise, the recording began once the respiratory rate had reached the resting value. Tidal volume (TV_{EIT}), global peak inspiratory flow (PIF_{EIT}), peak expiratory flow (PEF_{EIT}), and distribution (ventral vs dorsal) were calculated. Data were compared between time points using a paired-t-test. At rest, mean global PIF_{EIT} was 0.089±0.030 AU (AU), ventral PIF_{EIT} 0.058±0.018 AU, and dorsal PIF_{EIT} 0.041±0.024 AU. After exercise, mean global PIF_{EIT} was 0.116±0.031 AU, ventral PIF_{EIT} 0.071±0.013 AU, and dorsal PIF_{EIT} 0.055±0.026 AU. Ventral and global PIF_{EIT} significantly increased after exercise (p = 0.03 and p = 0.0004, respectively). There were no significant differences in ventral or global PEF_{EIT} (p = 0.28 and p = 0.22, respectively) or TV_{EIT} (p = 0.71) before and after exercise.

Despite similar TV_{EIT} and respiratory rate, global and regional EIT gas flow changes were seen after exercise in horses with MEA. More pronounced flow changes were appreciated ventrally. EIT flow values can detect bronchoconstriction in horses with MEA before and after exercise.

**E02**

**Distribution of Ventilation in Equine Pulmonary Diseases Measured by Electrical Impedance Tomography: A Case-Series**

Nicolas Herteman – University of Zurich; Martina Mosing – Murdoch University; Klaudia Blaszczynk – Vetsuisse Bern; Claudia Grabner – Vetsuisse Bern; Simone Lanz – Vetsuisse Bern; Vinzent Gerber – Vetsuisse Bern; Andreas Waldmann – Swisstom; Angelika Schoster – University of Zurich

Electrical impedance tomography (EIT) is a novel non-invasive imaging technique to monitor the distribution of ventilation. This case series describes the EIT changes recorded in different equine pulmonary diseases.

Two healthy horses, 1 with exercise-induced pulmonary hemorrhage (EIPH), 2 with pleuroneumonia (one with left-sided and one with bilateral pleural effusion) and 1 with cardiological pulmonary edema (CPE) were included. EIT was recorded in standing non-sedated horses over 10 breath cycles. The distribution of the center of ventilation (CoV_{ventral-dorsal} and CoV_{right-left}) and poorly ventilated lung units (DSS and NSS) occurring with pulmonary pathology. Further studies with more horses are warranted to evaluate the diagnostic potential of EIT.

**E03**

**Does Azithromycin Potentiate the Anti-Remodeling Effects of Fluticasone in Equine Asthma?**

Sophie Mainguy-Seers – University of Montreal; Roxane Boivin – University of Montreal; Pierre Hélie – University of Montreal; James G. Martin – Meakins-Christie Laboratories; Jean-pierre Lavoie – University of Montreal

The airway remodeling present in severe equine asthma (SEA) is correlated with a decreased lung function and is only partially reversible with current therapies. The architectural changes are thought to be related to neutrophils-induced injuries and repairs, since neutrophilic mediators are upregulated in SEA. We therefore aimed to determine whether azithromycin, an immunomodulatory macrolide targeting neutrophils, potentiates corticosteroid’s effects on airway inflammation and remodeling.

Six severe asthmatic horses were administered inhaled fluticasone (2500 ug, q12h) for 5 months while 6 other horses received fluticasone combined with oral azithromycin (10 mg/kg, q48h). Impulse oscillometry, bronchoalveolar lavage and endobronchial biopsies were serially obtained (at baseline, after two weeks of therapy (T2), T4, T6, T8, T12, T16, T20). Peripheral lung biopsies were obtained thoracoscopically at baseline, T8 and T20. Lung biopsies were studied using histomorphometric technics. Statistical analyses were performed with two-way ANOVA and Dunnett’s tests.

The ratio of pulmonary resistance at 5 hertz and 10 hertz (R_{5}/R_{10}) improved overtime in both groups (p = 0.0002). Azithromycin combined with fluticasone significantly decreased airway neutrophilia from T4 to T20 while fluticasone alone decreased it only at T8 and T12. The endobronchial biopsy scores improved overtime (p = 0.03) in both groups. However, the airway smooth muscle and extracellular matrix masses in the peripheral airways remained unchanged. Adding a macrolide to fluticasone therapy reduced neutrophilic inflammation, without further improving lung function and airway remodeling. The results suggest that the treatments were not efficacious or too short a duration to reverse the peripheral structural changes.

**E04**

**Plasma, Pulmonary Epithelial Lining Fluid, and Nasopharyngeal Wash Concentrations of Voriconazole after Nebulization in Horses**

Tamara Sierra-Rodriguez – Auburn University; Anne Wooldridge – College of Veterinary Medicine, Auburn University; Erin Groover – College of Veterinary Medicine, Auburn University; Kara Lascola – College of Veterinary Medicine, Auburn University; Mariano Mora-Pereira – College of Veterinary Medicine, Auburn University; Yann-Huei Lee – Harrison School of Pharmacy, Auburn University; Sue Duran – College of Veterinary Medicine, Auburn University; William Ravis – Harrison School of Pharmacy, Auburn University; Elizabeth Spangler – College of Veterinary Medicine, Auburn University; Terri Hathcock – College of Veterinary Medicine, Auburn University
Risk Factors for the Development of Equine Asthma
Sarah Thomas – Texas A&M University; Michelle Coleman, Assistant Professor – Texas A&M University; Cristobal Navas de Solis, LV – Texas A&M University; Cannaan Whitfield-Cargile – Texas A&M University; Noah Cohen – Texas A&M University; Robert Chapkin – Texas A&M University; Nancy Ing – Texas A&M University; Susan Eades – TAMU; Ana Chamoun-Emamueli – TAMU

Early identification of horses at-risk for equine asthma is challenging due to undefined pathogenesis, clinical complexity, and lack of risk predictors. Early identification and intervention of horses at risk could ameliorate the severity and impact of disease. Thus, the objective of this study was to identify risk factors for development of equine asthma. A retrospective, case-control study was performed utilizing case records for horses admitted to (blinded) from 2014-2018. Only incident cases, with respiratory signs less than 6 months duration and cytologic confirmation of equine asthma were included. For each case, 2 control populations were identified including one seasonal control and one age-matched control presented within the same week of admission and one age-matched control presented within the same month, both of which presented for any reason other than respiratory disease. Data collected included geographic location, signalment, dietary and stable management, metabolic status, physical examination findings, and diagnostic testing. Conditional logistic regression was performed to identify risk factors for the development of disease, with P < 0.05 considered significant. A total of 36 cases, 36 seasonal controls, and 36 age-matched controls were identified. Horses with an obese body condition (i.e., body condition score ≥ 7) or a preexisting endocrinopathy were at increased odds of presenting with equine asthma. The present study identified risk factors for asthma that may assist not only in managing and preventing this disease, but also in guiding future research into its pathogenesis.

Assessment of an American Neonatal Foal Survival Scoring System in a Danish-Swedish Population (ECEIM Award Winner)
Anna Bohlin – Evidensia Equine Hospital; Anna Öhman – Evidensia Equine Hospital; Emma Karlsson – Large Animal Teaching Hospital, University of Copenhagen; Alexandra Sänge – Large Animal Teaching Hospital, University of Copenhagen; Katarina Nostell – University Equine Hospital, Swedish University of Agricultural Sciences; Inge Durie – Evidensia Equine
The aim of this study was to evaluate the performance of a previously published American Foal Survival Score (FSS) to predict survival in a Scandinavian foal population. The FSS included prematurity, cold extremities, ≥ 2 infectious or inflammatory sites, blood glucose, white blood cell counts and immunoglobulin G levels, with total score ranging from 0-7. Admission data was collected retrospectively from foals ≤ 14 days admitted to five Danish and Swedish hospitals. Foals euthanized for financial reasons were excluded. Score variables were compared with multivariate analysis. A receiver operator characteristics (ROC) curve determined optimal cut-off to predict survival, and test performance values and odds ratios were calculated with this cut-off value. All score variables, except immunoglobulin G levels, were strongly associated with non-survival. Optimal cut-off to predict survival was ≥ 6, resulting in sensitivity 78%, specificity 58%, positive predictive value 92% and negative predictive value 31%, and correct classification of 76% of foals. There was no difference in FSS test performance for 0-3 compared to 4-14 day-old foals. Compared to score 0-5, the odds ratio of survival was 3.5 (1.8-7.2) for score 6 and 6.6 (3.4-13.1) for score 7. The FSS performed relatively well in this different population, but had a lower sensitivity than in the original study. The age distribution was wider than in the original study, but did not influence the performance in this study. The FSS is easy to perform, is clinically relevant and may aid in determining prognosis in foals ≤ 14 days on hospital admission.

E08

Changes in Cardiac Troponin I Concentrations in Healthy and Critically Ill Neonatal Foals

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Sepsis, a major cause of foal mortality, can have detrimental effects on many body systems, including the heart. Despite cardiac troponin I (cTnI) concentrations showing an association with myocardial injury, recent studies have failed to relate sepsis and cTnI concentrations in neonatal foals. However, the dynamics of cTnI over time have not been evaluated. We hypothesize that cTnI concentrations in septic foals will remain high and be associated with survival. Blood samples were collected on admission (0) and at 24 and 48 hours from 15 healthy, 19 surviving septic, and 18 nonsurviving septic foals < 7 days of age (classified on sepsis score and survival to discharge). Serum cTnI concentrations were measured with an analytical system (ADVIA Centaur, Siemens).

At admission, cTnI concentrations were not different between the three groups (healthy, septic survivors, and septic nonsurvivors; P = 0.339). During hospitalization, cTnI concentrations decreased significantly in healthy (P = 0.015) and surviving septic foals (P = 0.011), but not in nonsurviving foals. The area under the curve of cTnI in healthy foals was higher during 0-24 hours compared to 24-48 hours (P = 0.021) while septic surviving and nonsurviving foals did not differ over the same periods.

As in the previously published study, cTnI concentrations were not different between septic and healthy foals at admission. cTnI concentrations decreased over time in healthy and septic surviving foals but remained elevated in nonsurviving foals. This study provides evidence that myocardial injury from sepsis is associated with outcome in critically ill neonates.

E09

Agreement of an Equine Stall-Side and a Laboratory Major Crossmatch Test in Healthy Horses

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Blood transfusions are needed to treat severe anemia in horses. Incompatible transfusions can cause life-threatening reactions. To avoid reactions, crossmatches are performed. The laboratory crossmatch procedure (LAB) is time consuming, costly, and requires technical expertise. A new equine stall-side gel crossmatch kit (Alvedia Veterinary Diagnostic Company, Limonest, France; KIT) has been developed. Our goal was to evaluate performance of the KIT with the LAB in horses with known and unknown blood types. Blood was collected and crossmatches performed on the same day by independent blinded observers. Expected positive (n=35) and negative (n=34) crossmatches were established using 21 blood typed and antibody screened horses (UC Davis Hematology Laboratory, Davis, CA). The sensitivity and specificity (95% confidence intervals) were 91 (77-98)% and 77 (59-89)% for KIT and 77 (60-90)% and 76 (59-89)% for LAB. The lowest agreement was found with anti-Aa (33%) then anti-Qab (60%) crossmatches, with 100% agreement for anti-Ca crossmatches. Agreement was 59% with expected negative reactions. To mimic field situations where unscreened horses may be transfused with untyped blood, reciprocal crossmatches were performed in 80 untyped horses. KIT and LAB agreed in 106/160 (66%) (Cohen’s k = 0.25 [0.10 to 0.40]) crossmatches. Our results showed that performance of both tests with expected reactions was blood type dependent and agreement was moderate to substantial. However, when comparing the tests directly with each other, agreement was only fair. Expected performance of both the KIT and LAB will depend on the prevalence of blood types within the local horse population.

E10

Comparison of White and Red Blood Cell Counts Between Warmbloods and Other Breeds of Horses

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Hematology is often used as a screening tool to determine health status in horses undergoing elective surgery. Clinical experience has led to our hypothesis that hematologic parameters, notably total nucleated cell (TNCC), neutrophil and lymphocyte counts, are lower in healthy Warmbloods as compared to other breeds, unnecessarily delaying elective procedures. Our aim was to compare hematologic parameters among different breeds.

In this prospective, observational cross-sectional study, 106 healthy horses were grouped based on breed (Thoroughbreds, Warmbloods, and “Other Breeds”). Horses were included based on normal physical examination findings, breed, having no illnesses in the preceding 30 days, and no current medications. A sample of EDTA-anticoagulated blood was collected through venipuncture. A complete blood count was performed within 6 hours of collection.

Data were tested for normality using the Kolmogorov-Smirnov test. One-way ANOVA or Kruskal-Wallis tests were used to compare hematologic parameters among groups. Warmbloods had a statistically significantly lower TNCC ($P = 0.0003$) and lymphocyte count ($p < 0.0001$) than the other two groups. Thoroughbreds had a statistically significantly higher red blood cell count ($p < 0.0001$), mean corpuscular volume ($P < 0.0001$), and hematocrit ($P = 0.0132$) than the other two groups. Mean cell hemoglobin concentration (MCHC) was higher in Warmbloods and Thoroughbreds as compared to the “Other Breeds” group ($P$).

Warmbloods have decreased TNCC and lymphocytes when compared to other breeds. Clinicians should take breed into consideration when interpreting hematologic values in systemically healthy horses.

**E11**

**Biochemical Characteristics of a Commercial Plasma Product and Response to Transfusion of Clinically Diseased Horses**

**Erica C. McKenzie – Carlson College of Veterinary Medicine, Oregon State University; Jennifer Johns – Carlson College of Veterinary Medicine, Oregon State University; Clarice Baumbach – Oregon State University; Madeleine Busby – Oregon State University**

Plasma transfusion is commonly performed to improve hypoproteinemia and colloid osmotic pressure (COP) in diseased horses. This study evaluated the biochemical characteristics of a common commercial plasma product and responses of transfused equine patients.

Biochemical characteristics of 66 liters of frozen commercial equine plasma (CP) were compared with 29 fresh plasma samples (FP) from healthy horses. Fourteen separate transfusion events in ten clinically diseased horses were prospectively evaluated by obtaining heparin plasma for biochemical analysis before and at 0, 1, 2, 4, 8 and 12 hours after transfusion with CP ($5.4 \pm 1.7$ ml/kg). Data were analyzed via multiple t-test, ANOVA and Spearman’s correlation ($P < 0.05$).

CP had significantly higher sodium than FP ($163 \pm 45$ vs. $136 \pm 2$; ref: $133 - 142$ mEq/L) with values ranging from 104 to 305 mEq/L. CP but not FP was below reference intervals with wide ranges for clinically relevant variables including albumin ($2.6 \pm 0.7$ vs. $3.3 \pm 0.3$, ref: $2.9 - 3.8$ g/dL), COP ($15 \pm 7$ vs. $19 \pm 2$, ref: $17 - 24$ mmHg) and chloride, though massive variability in CP denied significance. Product lot number denoted no biochemical similarity between individual CP liters. Transfusion of diseased horses with CP resulted in mild increases from baseline in TP ($3.6 \pm 1.2$ to $4.5 \pm 1.1$ g/dL at 2h peak), albumin ($1.5 \pm 0.5$ to $1.8 \pm 0.5$ at 2h peak), and COP ($9.9 \pm 2.5$ to $12.4 \pm 2.6$ at 1h peak) with significance lasting up to 8 hours.

Plasma transfusion resulted in small, transient changes in TP, albumin and COP in diseased horses. Unidentified factors appeared to result in substantial deviation from acceptable biochemical values in this commercial product, and lot number did not predict biochemical similarity between CP liters.

**E12**

**Comparison of the gastric microbiome in horses with and without Equine Glandular Gastric Disease**

**Linda J. Paul – Louisiana State University School of Veterinary Medicine; Heidi Banse – Louisiana State University, Veterinary Clinical Sciences, Equine Health Studies Program; Frank Andrews – Louisiana State University, Veterinary Clinical Sciences, Equine Health Studies Program; Michael Keowan – Louisiana State University, Veterinary Clinical Sciences, Equine Health Studies Program; Francisco Morales Yñiguez – Louisiana State University, Veterinary Clinical Sciences, Equine Health Studies Program; Aaron Ericsson – University of Missouri, Metagenomics Center, Equine Gut Group**

The pathophysiology of equine glandular gastric disease (EGGD), a common disorder of the equine stomach, remains poorly understood. Changes in the gastric microbiome have been associated with gastritis and gastric ulceration in people. The role of the gastric microbiome in development or persistence of EGGD remains to be investigated. The objective of this study was to characterize the glandular mucosal microbiome of horses with and without EGGD. It was hypothesized that significant differences in the microbiome of glandular gastric mucosa would be associated with the presence of EGGD. Twenty-four horses were enrolled in this study. For two weeks prior to the study, all horses were kept on the same pasture, with identical feeding protocols. Gastroscopy for each horse was performed within a one-week time period and EGGD scores recorded (grade 0, n = 6; grade 1, n = 8; grade 2, n = 2, n = 10). Gastric fluid and pinch biopsies of endoscopically healthy glandular mucosa and of any visible hyperemic or ulcerative lesions in the glandular mucosa were collected. Microbiome analysis using the 16S rRNA gene was performed on all glandular mucosal biopsies collected. Read counts and microbial populations were compared by EGGD grade as well as among EGGD lesions, healthy mucosa from horses with EGGD, and healthy mucosa of control horses (grade 0). A Kruskal-Wallis test demonstrated that apparently healthy mucosa of horses with (p = 0.004) and without EGGD (p = 0.02) had significantly less reads than biopsies from EGGD lesions. Biopsy samples with a read count of less than 1000 were excluded from the analysis. Principal coordinate analysis showed overlapping clusters of bacterial populations among groups. There was a distinctly tighter clustering for controls compared to biopsies from both healthy mucosa and lesions of horses with EGGD grade ≥ 1. This pattern was confirmed when the biopsies were grouped by the overall grade with grade 0 forming a tighter cluster compared to horses with grade 1 and grade ≥ 2 EGGD. Using a Random Forest analysis, the best performing classifier for grade 2 or more EGGD was the presence of Arcobacter cryaerophilus. For grade 0, the best classifier was
Nicoletella semolina. These findings suggest that EGGD is associated with changes in the glandular mucosal microbiome. Further study in a less controlled population of horses is warranted to confirm these findings.

E13
Effect of Lidocaine On Stimulated Leukocyte Proinflammatory Cytokine Production in Horses
Lynn M. Martin – University of Missouri; Philip Johnson – University of Missouri; Amy DeClue – University of Missouri

Administration of lidocaine (LIDO) to horses affected with endotoxicemia/systemic inflammation is partly justified by an anti-inflammatory effect. However, reports supporting anti-inflammatory actions for LIDO are conflicted. The effect of LIDO on proinflammatory cytokine production by equine leukocytes stimulated by Gram-negative and Gram-positive pathogen-associated molecular patterns (PAMPs) has not been investigated. Therefore, we evaluated in vitro effectiveness of LIDO on PAMP-stimulated cytokine production in whole blood culture (Cwb).

Whole blood was collected from six healthy adult horses. Tumor necrosis factor (TNF) and interleukin (IL)-1β bioactivities in Cwb were measured in the presence of phosphate buffered saline (PBS; control), lipopolysaccharide (LPS, endotoxin), lipoteichoic acid (LTA), peptidoglycan (PG) and three different concentrations of LIDO (0-2000 ng/mL). Parametric data were compared using a Repeated Measures ANOVA with post-hoc Fisher’s least significant difference test. Log-transformed nonparametric data were compared using Friedman Repeated Measures ANOVA on Ranks with post-hoc Student-Newman Keuls method (p < 0.05).

At all tested concentrations, LIDO increased TNF compared to PBS. PAMP stimulation (LPS, LTA, PG) increased TNF compared to PBS, despite treatment with LIDO. TNF was increased at 500-2000 ng/mL LIDO in LTA-stimulated Cwb compared to 0 ng/mL LIDO. LIDO did not increase IL-1β compared to PBS. LTA and PG stimulation increased IL-1β at all tested LIDO concentrations, while LPS stimulated IL-1β at 0 and 500 ng/mL LIDO compared to PBS. Cell viability was unaffected. LIDO may not inhibit TNF and IL-1β bioactivity in the face of systemic inflammation. These results contradict studies that have demonstrated anti-inflammatory properties for LIDO.

E14
Performance of Single Versus Dual Dose Equine Influenza Vaccination in Immunized Horses After Changing Manufactures
Bruno Karam – University of California - Davis; Nicola Pusterla – University of California - Davis; William Wilson – University of California - Davis; Thomas Chambers – Gluck Equine Research Center; Stephanie Reedy – Gluck Equine Research Center

Prospective study investigating humoral immune response to equine influenza virus (EIV) vaccine from different manufacturers when administered to previously immunized adult healthy horses. Sixty five previously immunized healthy, adult, horses were divided into 3 different groups and vaccinated with different commercially available vaccines. Each group was further subdivided into subjects that received a booster series, and those that did not. Serum was collected at different time points for all subjects in each group over 180 days. Hemagglutination inhibition assay was used to assess antibody response for contemporary Florida sublineage clade 1 and 2 EIV strains. A fourth group served as environmental sentinels. Kruskal Wallis test was used to determine statistically significant difference in antibody response amongst groups. A P value < 0.05 was statistically significant. For all vaccine groups there was a significant difference between antibody responses pre- and post-initial vaccine administration. However, there was no statistical difference after day 30 between any booster and single dose groups. Equine influenza is a devastating respiratory virus with serious economic ramifications. Veterinarians and owners use different EIV vaccines interchangeably despite difference in strains, adjuvant and antigen mass. This study shows that, in previously immunized horses, switch in vaccine manufacture does not require a booster series in order to achieve superior antibody responses to Florida sublineage clade 1 and 2 EIV. This information will be relevant to educate veterinarians and owners on EIV vaccine protocols.

E15
Phosphorylated Neurofilament Heavy Subunits as an Antemortem Biomarker in Equine Neurodegenerative Diseases
Lisa A. Edwards – UC Davis; Carrie Finno – UC Davis; Stephen Reed – Rood and Riddle Equine Hospital

Equine neuroaxonal dystrophy (eNAD) is a neurodegenerative disease affecting horses in the first year of life and equine degenerative myeloencephalopathy (EDM) is a more severe pathological variant of eNAD. Currently, there is no antemortem diagnostic tool available for eNAD/EDM and the disease may go unrecognized on postmortem evaluation due to the subtlety of histopathological changes. Phosphorylated neurofilament heavy subunits (pNFH), a structural protein of the neuronal cytoskeleton, have been utilized in other species as a biomarker for neurodegenerative diseases, such as amyotrophic lateral sclerosis in humans and canine degenerative myelopathy. The use of pNFH as a biomarker could provide useful antemortem diagnostic information. The objective of this study was to compare blood and CSF concentrations of pNFH in healthy non-neurologic horses and horses affected with eNAD/EDM and CVCM using a species validated ELISA kit. pNFH ELISAs were performed in duplicate on serum and CSF samples from 14 unaffected, 22 eNAD/EDM affected and 25 cervical vertebral compressive myelopathy (CVCM) affected horses, with CVCM and eNAD/EDM confirmed at necropsy. Unaffected horses had normal neurologic examinations. Serum pNFH concentrations were < 2 ng/ml in unaffected horses and CVCM affected horses. pNFH concentrations were significantly higher in both serum (P adjusted = 0.001) and CSF (P adjusted = 0.003) of eNAD/EDM affected horses compared to unaffected horses. CSF pNFH concentrations were significantly higher in eNAD/EDM affected horses compared to unaffected horses (P adjusted = 0.003) but not significantly different from CVCM affected horses. Serum and CSF pNFH testing can provide useful antemortem diagnostic information regarding neurodegenerative diseases in neurologic horses.
E16

Effect of the Thyrotropin Releasing Hormone Stimulation testing on the Oral Sugar Test in Horses

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Combined testing for Pituitary Pars Intermedia Dysfunction (PPID) and insulin dysregulation (ID) is commonly performed by measuring resting endogenous plasma hormone concentrations. However, the use of combined dynamic tests would have better diagnostic utility, if the tests do not alter the diagnostic interpretation of one another. The aim of the current study was to evaluate the effects of thyrotropin releasing hormone (TRH) stimulation test on the results of an oral sugar test (OST) performed immediately after as part of a combined testing protocol. Twenty-six university owned horses, with and without clinical signs of endocrine disease, were tested with three different OST protocols in a randomized cross-over design: an OST only; TRH followed by OST (TRH+OST); and placebo followed by OST (placebo+OST). Plasma insulin concentrations and binary (positive/negative) outcome of the OST were compared using regression models and Bland-Altman analyses. Testing protocol did not have a significant effect when comparing the OST only protocol to the TRH+OST protocol for insulin (P = .64) or glucose (P = .92), the OST only protocol compared to the placebo+OST protocol for insulin (P = .21) or glucose (P = .66), or the placebo+OST protocol compared to the TRH+OST for insulin (P = .20) or glucose (P = .73). OST binary outcome was not significantly different between TRH+OST and the OST only protocol (P = .78), placebo+OST and the OST only protocol (P = .77), or the TRH+OST and placebo+OST protocol (P = .57). Bland-Altman analysis of the TRH+OST compared to the OST alone showed good agreement with minimal difference between insulin concentrations from the two different test protocols at baseline (0.4 [SD±4.7 μU/mL]), 60 minutes (-0.5 [SD±22.6 μU/mL]), and 90 minutes (1.9 [SD±20.6 μU/mL]) post oral sugar administration. A primary goal for veterinarians in treating endocrine disease should be to prevent the development of laminitis though early detection of endocrine disorders that place horses and ponies at-risk. Owners and veterinarians are more likely to test for both PPID and EMS if testing can be combined in one visit. The results of this study support the use of combined testing for PPID and ID by performing the TRH stimulation test immediately prior to the oral sugar test.

E17

Sex Steroids in Healthy and Hospitalized Neonatal Foals

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Sepsis is a leading cause of mortality in neonatal foals. Critical illness alters multiple endocrine systems in foals. Little is known regarding estrogen and androgen production in healthy and sick neonatal foals. The effect of exogenous progestogens on steroidogenesis in newborn foals is unknown. We hypothesized that sex steroids would mimic changes seen for other steroids during disease. We proposed that foals born to progestogen-treated mares would have abnormal sex steroid profiles compared to unexposed foals.

Blood samples were collected on admission (0) and at 24, 48, and 72 hours from 40 healthy, 61 septic, and 54 sick-nonseptic (SNS) foals of < 7 days of age. Clinicopathologic data and history were used to classify disease severity (healthy, SNS, septic) and survivorship. Serum steroids were measured using radioimmunoassays. At admission, septic foals had higher progesterone and testosterone concentrations than SNS and healthy foals (P < 0.05). All three hormones declined in healthy and SNS compared to septic foals. Nonsurvivors had higher estradiol, testosterone, and progesterone (P < 0.05) at admission compared to survivors. Dummy foals had higher estradiol (P = 0.001) and progesterone (P = 0.026), but not testosterone, at admission compared to healthy foals. Sick foals born to progestogen-treated mares did not exhibit a decline in hormone concentrations over time as non-exposed sick foals did.

Sex steroids were associated with disease severity and may play a role in or reflect a response to illness. In utero exposure to exogenous progestagens may influence the postnatal endocrine maturation in sick foals.

E18

Selection of Assay for Quantification of Equine Insulin Affects Results of Oral Glucose Test and Combined Glucose-Insulin Test in Horses (ECEIM Award Winner)

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Assessment of disturbed insulin regulation with combined glucose-insulin test (CGIT) or oral glucose test (OGT) is essential in equids suffering from the EMS. Besides different testing protocols, quantification of equine insulin remains the final and main limiting step. Therefore, the aim of this study was to compare results supplied by an equine-optimized insulin ELISA and a human-specific radioimmunoassay (RIA) in samples obtained from OGT and CGIT with a focus on measurement of endogenous or exogenous insulin. In total 268 samples (OGT: n=117; CGIT: n=151) were used for comparison of the immunoassay methods. Samples were obtained during OGT and CGIT from nine healthy horses. Insulin concentrations measured by ELISA and RIA differed significantly (p<0.001) in samples from both diagnostic procedures. However, both methods showed strong correlation when OGT samples (r=0.9667; p<0.001) and CGIT samples (r=0.9799; p<0.001) were compared. Bland-Altman analysis indicated a bias of 40.94 ± 35.20 μU/mL in OGT and 90.82 ± 120 μU/mL in CGIT samples when RIA was compared with ELISA. Moreover, proportional errors exist, when assays were compared in both procedures. Comparison of insulin curve progressions in OGT and CGIT
differed significantly (OGT: p<0.001; CGIT: p<0.001) when insulin analyses were performed with either ELISA or RIA. These findings support that results between assays should not be considered interchangeable and should be interpreted with regard to test protocol and laboratory method selected for measurement. Especially measurement of insulin in CGIT procedure may be challenging due to interference between endogenous equine insulin and injected exogenous non-equine insulin.

E19

Effect of a-Tocopherol Deficiency and Repletion on Skeletal Muscle Morphology in Horses
Lauren Bookbinder – Veterinary Clinical Sciences, Michigan State University; Carrie Finno – Population Health and Reproduction, University of California, Davis; Anna Fishman – Veterinary Population Medicine, University of Minceota; Amanda Borer – Veterinary Clinical Sciences, Michigan State University; Scott Katzman – Surgical and Radiologic Sciences, University of California, Davis; Stephanie Valberg – Veterinary Clinical Sciences, Michigan State University

Vitamin E deficient myopathy (VEM) in horses appears to be distinct from other equine vitamin E-related diseases. VEM is characterized by low alpha-tocopherol (a-TOH) concentrations, insidious muscle atrophy, weakness, and abnormal mitochondrial staining of the sacrocaudalis dorsalis medialis muscle (SC). We hypothesized that clinically normal, serum a-TOH deficient horses would demonstrate histologic and ultra-structural SC abnormalities, which would be ameliorated by a-TOH repletion. Our objectives were to quantify the effects of a-TOH deficiency and subsequent supplementation on SC mitochondrial staining, fiber morphology, fiber type composition, and ultrastructure. Blood and SC biopsies were obtained from 16 clinically normal a-TOH deficient adult horses before and 8 weeks after supplementation (n = 8; 5000 IU/day oral water dispersible a-TOH) or no supplementation (n = 8). A significant increase in serum a-TOH occurred in supplemented horses (1.2 ± 0.3 ug/mL to 2.6 ± 0.5 ug/mL, p < 0.0001). Prior to treatment, 4/8 control and 4/8 treatment horses were diagnosed with VEM based on blindly scored histopathologic abnormalities. Supplementation with a-TOH significantly improved scores for mitochondrial staining (p = 0.045), fiber size variability (p = 0.005) and fiber splitting (p < 0.0001), with a trend for increased fiber areas (p = 0.069) and no change in the type 1 fiber predominance. Electron microscopy demonstrated SC lipofuscin accumulation before and after a-TOH supplementation. In conclusion, histologic and ultrastructural changes exist in the SC of clinically normal a-TOH deficient horses, which improve but do not resolve following 8-week a-TOH supplementation.

E20

Owner Supplementation Practices and Their Influence on Selenium and Vitamin E Status in Horses
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Deficiency of selenium (Se) or alpha-tocopherol (E) creates serious health issues in horses. This study evaluated owner supplementation practices and their efficacy at preventing deficiency in a large population of horses. Whole blood (Se) and plasma (E and beta-carotene) samples were collected from 336 adult horses. A comprehensive questionnaire determined source and estimated content of Se and E in each horse’s daily ration, ration composition, pasture access and exercise demands. Data were analyzed via ANOVA, Spearman’s correlation coefficient and contingency tables (P < 0.05).

Supplemental Se was provided to 86% of horses; 70% received ≥ 1 mg/day. Very low blood [Se] (≤ 80 ng/ml) was documented in 3.2% of horses, and 13.4% were marginal (80-159 ng/ml). Non-supplemented horses were twenty times more likely to be deficient than horses receiving Se. Exercise, provision of Se-containing salt, or form of Se (inorganic vs. organic) did not influence blood [Se]. Supplemental E was provided to 86% of horses; 57% received ≥ 500 IU/day, more frequently as the synthetic form (61% of horses). Deficient (< 1.5 ug/ml) and marginal (1.5-2.0 ug/ml) plasma [E] occurred in 15.4% and 19.9% of sampled horses, respectively. Pasture access (≥ 6 hours/day) was significantly protective against low plasma [E], as was daily provision of ≥ 500 IU of alpha-tocopherol. Exercise and supplement form (synthetic vs. natural) did not clearly influence plasma [E]. Pasture access correlated significantly with plasma beta-carotene concentrations (0.19 ± 0.33 ug/ml), which currently have limited documentation in horses. Though Se and E were commonly supplemented in equine rations, deficiency was prevalent, suggesting improved supplementation practices are needed. Pasture access is reflected in plasma beta-carotene concentrations.

E21

Pharmacokinetics of Maropitant Citrate After Oral Administration of Multiple Doses in Healthy Adult Horses
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The neurokinin-1 (NK) receptor antagonist, maropitant citrate (Cerenia™, Zoetis Inc.), mitigates nausea and vomiting in dogs and cats. Nausea is poorly understood in horses, and clinical use of NK-1 receptor antagonists has not been reported. Pharmacokinetics of single doses of maropitant in adult horses has recently been established. The current study aimed to determine the pharmacokinetic parameters and safety of maropitant at steady state concentrations with administration of multiple doses. Seven healthy adult horses were used. Complete blood counts and serum biochemistry profiles were performed prior to study initiation and 48 h after the last drug dose. Maropitant citrate was administered at a dose of 4 mg/kg orally once daily for 5 days. Serial blood samples were collected after the last dose was administered to determine the pharmacokinetic profile using non-compartmental analysis. Maropitant concentrations were measured by liquid chromatography mass spectrometry. The maximum, minimum, and average concentrations of maropitant achieved at
steady state were 414 ± 217, 16 ± 8, and 71 ± 46 ng/mL, respectively. The area under the curve from 0 to infinity was 2012 ± 1164 h*ng/mL, the half-life was 11.9 ± 1.5 h, and the accumulation index was 1.3 ± 0.07. There were no significant changes in physical examination or blood work parameters throughout the study. This study furthers the investigation of NK-1 receptor antagonists in horses by providing steady state pharmacokinetic parameters and safety data.

**E22**

**Quantitative Assessment of Two-Dimensional Transthoracic and Transesophageal Echocardiography with Magnetic Resonance Imaging in Normal Foals**

Ryan Fries – University of Illinois at Urbana-Champaign; Saki Kadotani – University of Illinois Urbana-Champaign; Jonathan Stack – University of Illinois Urbana-Champaign; Stuart Clark-Price, DACVAA – Auburn University; David Schaeffer – University of Illinois Urbana-Champaign; Kara Lascola – Auburn University

This study determined the accuracy of two-dimensional transthoracic (TTE-2DE) and transesophageal (TEE-2DE) echocardiography compared to gated cardiac magnetic resonance imaging (CMR) in neonatal foals. Transthoracic 2DE, TTE-2DE, and CMR (3T) image acquisition was performed in random order by a single observer on six, healthy, anesthetized, one-week-old foals positioned in sternal recumbency. The TTE-2DE views included right parasternal four-chamber (R-4CH), left apical four and two-chamber (A-4CHbiplane), and right parasternal short axis M-mode. The longitudinal four-chamber view of the left ventricle (LV) was obtained using TEE-2DE. Measurements included LV volume at end-systole (LVESV) and end-diastole (LVEDV), stroke volume (SV), cardiac output (CO), and left ventricular ejection fraction (EF).

Mean and standard deviation of the difference between CMR and each measurement modality were estimated via maximum likelihood, with robust standard errors. Two additional observers repeated all measurements and intraclass correlation (ICC) was calculated for the reliability of analysis between the observers. Compared to CMR, all modalities significantly underestimated LVEDV, LVESV, and SV, and overestimated EF and CO. The R-4CH view demonstrated the smallest mean differences from CMR for all measurements with strong positive correlations for LVEDV (r_s = 0.77, P = 0.07) and LVESV (r_s = 0.89, P = 0.02). There was good-to-excellent ICC between observers for CMR, A-4CHbiplane–R-4CH, and Mmode, while TEE-2DE showed poor-to-fair agreement.

Compared with CMR, echocardiography significantly underestimates LV size in neonatal foals. Assessment of CO, EF, and SV utilizing TTE-2DE from the R-4CH compares favorably with CMR.

**E23**

**Non-Invasive Blood Pressure From Pulse Decomposition Analysis of the Digital Arterial Pulse in Standing Horses**

Natalia Rodríguez – Texas A&M University; Joanne Hardy – TAMU Vet School; Mauricio Lepiz – TAMU Vet School; Noah Cohen – TAMU Vet School; Cristobal Navas De Solis – TAMU Vet School

Blood pressure (BP) is important in management of cardiovascular or critical disease. The gold standard to obtain BP measurements is arterial catheterization, although this is not always practical. Pulse decomposition analysis not been tested in standing horses.

The objective was to evaluate the accuracy and precision of a new device that obtains non-invasive BP (NIBP) measurements from the digital artery of horses using Pulse Decomposition Analysis (PDA). The hypothesis was that BP measurements using PDA would have accuracy and precision that meet the American College of Veterinary Internal Medicine (ACVIM) recommendations.

Eight adult horses from the Texas A&M teaching herd. Invasive blood pressure (IBP) measurements were obtained from a catheter in the transverse facial artery and the PDA device was placed over the left front medial digital artery. Measurements were obtained under baseline, high (Dobutamine), and low (Acepromazine) blood pressures. Mean bias, standard deviation (SD) of the bias, and Pearson’s product correlation (R) were calculated.

Mean bias / SD of the bias / R between PDA and IBP were 24.06 mmHg / 29.32 mmHg / 0.03 for systolic, 22.35 mmHg / 23.65 mmHg / 0.00 for mean, and 22.87 mmHg / 20.44 mmHg / 0.01 for diastolic pressures.

The PDA device tested had poor accuracy and precision and did not meet ACVIM suggested criteria.

**E24**

**Effect of Various Ambient Temperatures on the Determination of Immunoreactive ACTH Concentrations in Aged Horses**

François-René Bertin – The University of Queensland; Sophia Hinrichsen – The University of Queensland; Allison Stewart – The University of Queensland; Ka Yuen – The University of Queensland; Remona Horn – The University of Queensland

Determination of ACTH concentration after thyrotropin-releasing hormone (TRH) stimulation is recommended to diagnose early cases of pituitary pars intermedia dysfunction (PPID). Suboptimal sample handling alters ACTH concentrations but the stability of ACTH after TRH stimulation is unknown. The study aimed at describing the effects of temperature and TRH stimulation on ACTH concentrations and subsequent diagnosis of PPID.

Fifteen horses older than 10 years old, including 8 PPID positive horses (ACTH >35 pg/mL at baseline or >65 pg/mL 30 min after TRH stimulation), were divided into 2 groups: 6, including 3 positive for PPID, that underwent a TRH stimulation test; and 9, including 5 positive for PPID, used as controls. Blood was collected in EDTA tubes and stored as whole blood for 1 hour at 4°C, 20°C, 30°C, 40°C or 70°C. After centrifugation, ACTH concentrations were determined using a chemiluminescent assay. Data were analyzed with a 2-way-repeated measures ANOVA.

Temperature had a significant effect on ACTH concentration (p=.01) with a significant decrease in samples stored at 70°C; however, there was no significant effect of TRH stimulation (p=.93). One horse that underwent a TRH stimulation test and was PPID negative would have been falsely diagnosed as positive when its samples were stored at 20°C and 40°C.

Ideally ACTH concentrations should be measured from centrifuged samples kept at 4°C to limit misdiagnoses; however, samples stored at temperatures of up to 40°C can still provide valid results if centrifuged...
within an hour, although this increases the chance of a false-positive diagnosis of PPID.

E25

Determination of Immunoreactive Insulin Stability in Horses At Risk of Equine Metabolic Syndrome

François-René Bertin – The University of Queensland; Dakota Leschke – The University of Queensland; Genevieve Muir – The University of Queensland; Jack Hodgson – The University of Queensland; Mitchell Coyle – The University of Queensland; Remona Horn – The University of Queensland

Diseases associated with insulin dysregulation, such as equine metabolic syndrome, and to some extent other endocrine disorders, are of particular interest to practitioners due to their association with laminitis. Accurate assessment of insulin concentrations is therefore a pivotal component in the diagnosis and management of these diseases. This study aimed to determine how time, storage temperature and blood collection tube type affected immunoreactive insulin concentration in horses at risk of developing insulin dysregulation.

Eight adult horses with body condition score > 6/9 underwent an in-ear oral glucose test 2 hours prior to blood collection. Blood samples were divided into ethylenediaminetetraacetic acid (EDTA), lithium heparin, and silicate serum tubes. Half of each tube type was stored at 4°C and the other half at 20°C. Tubes were systematically centrifuged and analysed daily for immunoreactive insulin by chemiluminescent assay over the course of 5 days, with a final analysis on day 8. Immunoreactive insulin concentrations were compared with a linear mixed effect model.

An overall effect of time, tube type and temperature on immunoreactive insulin concentration was identified (p < .05). Serum and heparin tube types demonstrated acceptable stability for 5 days irrespective of storage temperature and after 8 days at 4°C. EDTA tubes demonstrated suboptimal conditions for immunoreactive insulin stability for all samples.

These results suggest an ideal protocol for sample handling for immunoreactive insulin involves using either a serum or heparin collection tube with analysis occurring within 5 days if stored at 20°C or 8 days if stored at 4°C.

E26

Clinical Implications of the Use of Different Methods to Determine ACTH Reference Intervals in Horses

François-René Bertin – The University of Queensland; Remona Horn – The University of Queensland; Allison Stewart – The University of Queensland; Carlos Medina-Torres – The University of Queensland; Karen Jackson – The University of Queensland

Accurate ACTH reference intervals (RI) or cut-off values (COV) are crucial for pituitary pars intermedia dysfunction (PPID) detection. Large variations in RI ranges have been reported and could have major consequences on PPID diagnosis.

This study compared two statistical methods to establish guidelines for baseline and post-thyrotropin-releasing hormone (TRH) stimulation ACTH in horses.

Eighty older (10–31 years) horses were included. A diagnosis of PPID was determined by clinical signs and recurrent ACTH concentrations considered as outliers by ROUT method with Q=1%. RI were determined by a robust method, including Box-Cox transformation, and COV by receiver operating characteristic (ROC) curves, establishing test accuracy (area under the curve, AUC), sensitivity and specificity. Baseline ACTH determination was an accurate test to diagnose PPID (mean AUC=0.90); however, in autumn test accuracy decreased (AUC=0.70-0.91). Using the robust method, RI for baseline ACTH were 27.8-42.9 pg/mL in non-autumn and 72.7-113.1 pg/mL in autumn. Using ROC curves, COV were 25.2-41.7 pg/mL in non-autumn and 41.1-56.0 pg/mL in autumn. After TRH stimulation, RI were 44.0-100.7 pg/mL in non-autumn and 145.7-234.2 pg/mL in autumn, whereas COV were 35.1-90.0 pg/mL in non-autumn and 109.0-138.5 pg/mL in autumn. ROC curves allowed establishing COV with good sensitivity (80-100%) compared to the robust method. For the latter, RI had good specificity (94-100%) but poor sensitivity (20-100%). When adequate COV were used, TRH stimulation slightly improved sensitivity (83-100%) but improved accuracy (mean AUC=0.94).

Overall, using ACTH COV determined by ROC curves would allow earlier detection of cases of PPID given their better sensitivity.

E27

Evaluation of a Continuous Indwelling Glucometer in Healthy Adult Horses

François-René Bertin – The University of Queensland; Kelly Wood – The University of Queensland; Kylie Matthison – The University of Queensland; Aaron Herndon – The University of Queensland

Blood glucose is tightly regulated in healthy horses; however, since hypoglycemia and hyperglycemia are common in pathological conditions and associated with poor prognosis, close monitoring of blood glucose is warranted.

The study aimed to determine the accuracy and clinical relevance of a continuous indwelling glucometer (CIG) in horses by comparing performance with a point-of-care glucometer (POC) and standard laboratory glucose assay (LAB).

Ten adult horses were equipped with a CIG placed on their neck and an intravenous jugular catheter. Interstitial glucose concentrations were determined by the CIG every 5 minutes at rest, during an insulin-induced hypoglycemia and during a dextrose-induced hyperglycemia and compared to blood glucose determined by POC and LAB. Glucose concentrations were compared by two-way repeated measures ANOVA and weighted kappa with Bland-Altman analyses to determine agreement between assays.

Horses tolerated the CIG well; however, five CIG had to be replaced. There were no significant differences between assays at rest or during hyperglycemia; however, during hypoglycemia, glucose concentrations determined by CIG were significantly higher (p = .014). The mean bias (95% limits of agreement) between the CIG and POC ranged from –0.19 (~–1.04 - 0.65) mmol/L (hyperglycemia) to 0.97 (~0.01 - 1.93) mmol/L (hypoglycemia). Assay agreement for classification as hypo or hyperglycemic was “very
good" to "good" for the CIG compared with both the LAB and POC, with observed agreements of 93.75% (κ = 0.83) and 87.04% (κ = 0.67), respectively. The CIG has acceptable accuracy in horses but overestimates glucose concentrations during hypoglycemia limiting its clinical application.

**E28**

**Systemic and Local Regulation of Leptin in a Model of Endocrinopathic Laminitis**

Teresa A. Burns – The Ohio State University College of Veterinary Medicine; Eline Nijveldt – The Ohio State University; Mauria Watts – The Ohio State University; Susan Eades – Texas A&M University; James Belknap – The Ohio State University

Equine metabolic syndrome (EMS)-associated laminitis (EMSAL) is the most common form of equine laminitis, associated with significant morbidity, mortality, and economic loss to the equine industry. Serum [leptin] is commonly increased in EMS patients, as many are overweight; however, the role of leptin in the pathophysiology of laminitis is currently unclear. Leptin exerts mitogenic effects, regulates the pro-inflammatory response of epidermal keratinocytes, and promotes epithelial-to-mesenchymal transition. Additionally, the euglycemic hyperinsulinemic clamp (EHC) model (used to induce laminitis experimentally) can acutely increase circulating [leptin] in healthy adult humans. The aim of this study was to investigate the roles of leptin and leptin receptor (lepR) in an EHC model of EMSAL. Healthy adult Standardbred horses (n=16) were randomly assigned to receive either an EHC (EHC, n=8) or a saline infusion (CON, n=8) for 48h, after which time the animals were euthanized and lamellar tissue collected for further analysis. Real-time PCR analysis revealed significant down-regulation of leptin and lepR gene expression in lamellae in response to the EHC (P= 0.0002 and 0.0158, respectively). Lamellar immunohistochemistry (IHC) revealed diffuse expression of lepR by lamellar keratinocytes in both groups. Measurement of serum [leptin] revealed evidence of regulation by the model (5/8 of the EHC horses had increased serum [leptin] at 6h). These results suggest that lamellar keratinocytes appear capable of responding to circulating leptin, which does not appear to be locally produced within the lamellae. Further clarification of the role of leptin in EMSAL is warranted, given the prevalence of hyperleptinemia in EMS patients.

**E29**

**Ghrelin and Leptin Response to Fasting, Lactose, and Dextrose Administration in Healthy Neonatal Foals**

Laura K. Dunbar – The Ohio State University; Hannah Manning – The Ohio State University; Lindsey Rings – The Ohio State University; Jacob Swink – The Ohio State University; Teresa Burns – The Ohio State University; Ramiro Toribio – The Ohio State University

Hormonal regulation of energy metabolism has not been fully investigated in the healthy neonatal foal. Previous studies have evaluated for differences in ghrelin and leptin concentrations between septic and healthy foals on admission, but the dynamic response to fasting, enteral lactose, and oral and intravenous dextrose has not been evaluated. Twenty healthy, neonatal foals were assigned to treatment groups: fasted (n = 6), intravenous dextrose (n = 4), oral dextrose (n = 5), and oral lactose (n = 5). Blood samples were collected at frequent intervals for 210 minutes (nursing was allowed from 180 to 210 minutes). Data were tested for normality using the Shapiro-Wilk normality test, and concentrations were compared using nonparametric testing.

Baseline ghrelin (15.0 pg/ml; 3.81-54.38 pg/ml; median and range) and leptin (5.34 ng/ml; 0.00-34.01 ng/ml; median and range) concentrations in healthy neonatal foals were similar to previous studies. No differences in ghrelin concentrations were observed from baseline to 180 minutes or when the foals were allowed to nurse in any treatment group. Similarly, leptin concentrations did not change significantly from baseline to 180 minutes or in response to nursing. Leptin and ghrelin concentrations did not change in response to dynamic testing within a 4-hour period.

**E30**

**The Probability of Clinical Signs in Hyperinsulinemic Horses With Pituitary Pars Intermedia Dysfunction**

Steve T. Grubbs – Boehringer Ingelheim; Dwana Neal – Boehringer-Ingelheim; Thomas Keefe – Colorado State University

Pituitary pars intermedia dysfunction (PPID) has been described as the most common endocrinologic disorder of horses. Horses exhibiting one or more of the typical signs of PPID were enrolled in the study. The purpose of this study was to obtain epidemiological information from a large population of horses with PPID and hyperinsulinemia (HI). At initial visit, a physical examination was conducted and blood drawn for basal adrenocorticotropic hormone (ACTH), insulin, and glucose. Plasma samples were analyzed for ACTH, insulin, and glucose by the Animal Health Diagnostic Center, Cornell University, Ithaca, NY. The association between PPID status, based on ACTH results, and each of the demographic variables and test results for insulin and glucose were statistically evaluated individually using the Pearson chi-square test. Odds ratios for significant predictors of PPID status were computed using multiple logistic regression analysis. Of the 2,994 horses enrolled, 605 (20.2%) were PPID+/IR+, 571 (19.1%) were PPID+/IR-, 593 (19.8%) were PPID-/IR+, and 1,225 (41%) were PPID-/IR-). Insulin-resistant horses had approximately twice the odds (2.1X) of PPID compared to horses with normal insulin levels. The prevalence of PPID+/HI+ was significantly greater among horses found to have delayed shedding (p < 0.001), loss of muscle mass (p < 0.001) and laminitis (p < 0.001). The odds of abnormal sweating in PPID+/HI+ was 1.7X when compared to PPID+/HI- horses. The odds of laminitis in PPID+/HI+ was greater than 3X (3.1X) compared to PPID+/HI-. Based on the increased odds of laminitis in PPID horses with hyperinsulinemia compared to PPID horses with normal insulin, when evaluating horses with suspected endocrine disease, at a minimum, ACTH, insulin and glucose should be evaluated. Long term studies need to be conducted to further evaluate the occurrence and progression of clinical signs in horses with endocrine disease.
E31

Effect of Levothyroxine Supplementation on Racehorses' Performance

Janice Kritchevsky – Purdue University; Laurent Couetil – Purdue University; Carla Olave – Purdue University; Stacy Tinkler – Purdue University; Melissa Tropf – Iowa State University; Katherine Ivester – Purdue University; Lauren Forsyth – UC Davis

The purpose of the study was to determine whether supra-physiologic doses of levothyroxine affect measures of performance in racehorses and are associated with adverse side-effects.

A randomized, crossover, blinded, controlled trial was performed in 6 healthy Standardbred racehorses. Horses were trained for 6 weeks and then, randomized to one of three treatments: placebo or thyroxine (0.1 mg/kg, or 0.25 mg/day) for 2 weeks. Horses completed a standardized exercise treadmill test (SET; 3° incline) to fatigue on the last day of treatment. Blood lactate and ECG data were collected at 6, 8, 10, 11, and 12 m/s and during recovery (5 and 15 min post-SET).

Each treatment period was followed by a 2 week washout period. The effect of treatment and SET on heart rate and blood lactate were examined using generalized linear mixed models. Post hoc analysis was adjusted for multiple comparisons using Tukey. Data were expressed as mean ± standard deviation and P ≤ 0.05 was considered significant.

Treatment with levothyroxine had a significant effect on heart rate but not blood lactate. Highest levothyroxine dose resulted in significantly higher heart rates during SET (199 ± 30, 223 ± 17, and 239 ± 9 bpm at 6, 8, and 10 m/s, respectively) and recovery (144 ± 20 and 119 ± 15 at 5-15 min) as compared to placebo (17 ± 18, 203 ± 10, and 219 ± 6 bpm at 6, 8, and 10 m/s and 126 ± 5, 102 ± 11 at 5-15 min, respectively). Levothyroxine treatment was associated with arrhythmias including atrial fibrillation in some horses.

We concluded that supra-physiologic thyroxine supplementation is deleterious to racehorses' performance and may result in cardiac arrhythmias.

E32

Effect of Supra-Physiologic Levothyroxine Supplementation on Thyroid Hormone Concentrations and TRH-Stimulation-Test Response in the Horse

Janice Kritchevsky – Purdue University; Carla Olave – Purdue University; Stacy Tinkler – Purdue University; Katherine Ivester – Purdue University; Lauren Forsyth, Pharm D – UC Davis; Laurent Couetil – Purdue University

The purpose of the study was to determine the effect of supra-physiologic doses of levothyroxine given over a 14 day span on resting T4, free T4 (as measured by equilibrium dialysis), and T3 blood concentrations and whether this affects Thyrotropin releasing hormone (TRH) response test results in healthy horses.

In the first portion of the study, six healthy Standardbred racehorses comprised the study subjects. Horses were randomized to one of three treatments: placebo or thyroxine (0.1 mg/kg PO SID, or 0.25 mg/kg PO SID) for 2 weeks. Each treatment period was followed by a 2 week washout period. Tests were performed on day 14 of each treatment period by administering 1 mg TRH I.V. and collecting blood 0 (baseline), 2 and 4 hours later. Serum was analyzed for T3, T4, and free T4 (dialysis). In the second portion of the study, 5 healthy adult horses of mixed breeds were given 0.25 mg/kg thyroxine PO SID for 14 days. Blood was collected daily prior to thyroxine administration for T4, T3, and fT4 (dialysis) determination.

The effect of treatment on baseline values and TRH response test results were examined using generalized linear mixed models. Post hoc analysis was adjusted for multiple comparisons using Tukey. Data was expressed as mean ± standard deviation and P ≤ 0.05 was considered significant.

Administration of 0.25 mg/kg levothyroxine increased resting concentrations of all measured hormones in a dose-dependent manner. Supra-physiologic supplementation results in an immediate increase in all measured thyroid hormone concentrations after the first 24 hours of administration. Blood T4 and fT4 concentrations increased throughout the study. Blood T3 concentrations increased daily for 4 days and then stabilized for the remainder of the study. Both thyroxine doses had a significant effect on response test values. As expected, T3, T4, and fT4 increased post TRH administration in control horses. However, there was no increase in any value after TRH administration in horses receiving either dose of supplement.

We conclude that supra-physiologic thyroxine produces significant alterations in the thyroid gland's ability to respond to TRH stimulation. This suggests that the hypothalamus-pituitary-thyroid axis is down-regulated even when the amount of supplement given does not affect measures of performance.

E33

Glucagon, Insulin, and Glucose Response to Fasting, Lactose, and Dextrose Administration in Healthy Neonatal Foals

Hannah S. Manning – The Ohio State University College of Veterinary Medicine; Laura Dunbar – The Ohio State University College of Veterinary Medicine; Lindsey Rings – The Ohio State University College of Veterinary Medicine; Jacob Swink – The Ohio State University College of Veterinary Medicine; Teresa Burns – The Ohio State University College of Veterinary Medicine; Ramiro Toribio – The Ohio State University College of Veterinary Medicine

While some information regarding energy dysregulation in critically ill foals is available, little is known regarding energy homeostasis in healthy foals. The objective of this investigation was to evaluate glucagon ([Gluc]), insulin ([Ins]), and glucose ([BG]) concentrations in response to fasting, intravenous dextrose, enteral dextrose, and enteral lactose administration in foals.

Twenty-five healthy neonatal foals were assigned to treatment groups: fasted (n = 6), intravenous dextrose (n = 13; 300 mg/kg, 500 mg/kg, 1 g/kg), oral dextrose (n = 14; 300 mg/kg, 500 mg/kg, 1 g/kg), and oral lactose (n = 6; 1 g/kg). Blood samples were collected frequently for 210 minutes (nursing allowed from 180 to 210 minutes). Nonparametric methods were used for comparison.

During fasting (4-hour), there was a significant decrease in [BG] and [Ins] (P < 0.05), but foals maintained normoglycemia, with no significant change in [Gluc]. Lactose caused an increase in [BG] (P = 0.028) from 0 to 45 minutes, and a decrease in [Gluc] (P = 0.028). The [BG], [Gluc] and [Ins] response to oral dextrose was variable and dose-
dependent. Intravenous dextrose caused significant increases in [Ins] and [BG] at all doses (P < 0.05), and no change in [Gluc]. Nursing stimulated marked increases in [BG], [Ins], and [Gluc] (P < 0.01).

While major increases in [BG], [Ins], and [Gluc] occurred in response to nursing, these increases were not evident with enteral lactose or high-dose dextrose administration. This indicates that other factors in milk contribute to glucagon and insulin release, and further investigation is warranted.

E34

Effects of Pituitary Pars Intermedia Dysfunction and Prascend® Treatment on Endocrine and Immune Function

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The purpose of this study was to investigate the effects of PPID and Prascend® (pergolide tablets) treatment on endocrine and immune measures.

PPID status was confirmed via thyrotropin-releasing hormone (TRH) stimulation test before the study and basal ACTH levels at Day 0. Non-PPID horses (n=10), PPID untreated horses (n=9), and PPID horses on PRASCEND (n=9) were then sampled over 14 months. PRASCEND treatment began after Day 0 collections. Basal ACTH, ACTH at 10 minutes post-TRH administration (TRH-T10), total cortisol, and RT-PCR of cytokine/receptor expression (IFNy, IL-12a, IL-13, IL-17a, IL-1b, IL-6, IL-8, TGFb, TNFa, TLR2, and TLR4) in whole blood were analyzed.

Results were analyzed using PROC MIXED, SAS 9.4, with significance set at p < 0.05. All ACTH values are natural log transformed.

Both PPID groups had significantly higher ACTH than non-PPID horses at Day 0. PRASCEND-treated horses had significantly lower ACTH than their starting values at all subsequent timepoints. Both PPID groups had significantly higher TRH-T10 ACTH values than non-PPID horses at all timepoints. No differences resulting from PPID status or treatment with PRASCEND were seen in total cortisol or in the whole blood cytokine/receptor expression.

Treatment with PRASCEND significantly reduced basal ACTH but not TRH-T10 ACTH values. Therefore, basal ACTH appears to be the better indicator for determining successful responses to PRASCEND. Total cortisol does not appear to contribute to any potential changes in immune function caused by PPID. Further research is needed to determine if and where any effects of PPID on immune function may occur.

E35

Narrowing the Search for Equine Metabolic Syndrome Genes

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Equine metabolic syndrome (EMS), a clustering of metabolic disturbances including insulin dysregulation and dyslipidemia, has a complex genetic basis that is poorly understood. We used genome-wide association analyses (GWA) with ~1.2 million single nucleotide polymorphism (SNPs) in 264 Welsh ponies (WP) and 286 Morgans to identify ~150 regions of interest (ROI) harboring alleles underlying 11 EMS clinical phenotypes (fasting glucose, insulin, ACTH, triglyceride, NEFA, leptin, adiponectin, insulin and glucose 75 min post oral-sugar test (OST), and neck- and girth-to-height ratios). However, linking causal alleles to phenotypes is an arduous task, as these ROI contain >1,500 genes, understanding of EMS pathophysiology is lacking, and many genes have unknown functions. Our first objective was to identify and prioritize ROI shared by both breeds. We identified 36 shared ROI using GWA metaanalysis performed with a random effects model in METASOFT across 688,471 SNPs using GWA summary data from each breed. Our second objective was to further prioritize ROI by performing GWA with serum metabolites quantified before and after an OST in 108 Morgans and 123 WP. Multivariate regression modeling accounting for age, sex, and farm (NLME, www.r-project.org), revealed 30 and 24 metabolites significantly associated with EMS clinical phenotypes in WP and Morgans, respectively. GWA of these 54 metabolites identified 30 and 45 ROI in WP and Morgans, respectively. 16 ROI were shared with ROI for EMS clinical phenotypes. 125 candidate genes were identified across the 52 ROI prioritized in both objectives demonstrating the power of our approach to prioritize candidate genes underlying EMS.

E36

Enteroinsular Axis Response of Healthy and Hospitalized Equine Neonates

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The enteroinsular axis (EIA) comprises incretins that promote insulin release and suppress glucagon secretion. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are the main incretins. Disorders of energy regulation are common in critically ill foals. Incretin information in sick foals is lacking but may enhance our understanding of energy disorders in foals. Our goal was to evaluate the dynamics of insulin, GLP-1 and GIP in healthy and hospitalized foals. Blood was collected at admission (0) and at 24, 48 and 72 hours into hospitalization from 32 septic, 25 sick nonseptic (SNS) and 13 healthy foals, < 7 days. Disease severity was classified based on clinical and hematologic findings.

On admission, hospitalized foals had higher GLP-1 concentrations. Septic foals had lower insulin and GIP and higher GLP-1 concentrations than healthy foals at time 0 (P < 0.05). GIP concentrations were lower in hospitalized compared to healthy foals at all time points. Insulin concentrations were lower in septic and SNS compared to healthy foals in the study period (P < 0.05). GIP and insulin concentrations did not differ over time within groups. GLP-1 decreased over time in septic and SNS foals when compared to admission, but did not in healthy foals.
Insulin and GLP-1 were positive correlated over time in healthy foals, but were not in other groups. Lower GLP-1 was associated with survival but insulin and GIP were not. Low insulin but increased GLP-1 concentrations may reflect a dysfunctional EIA in critically ill foals. This information could have clinical implications.

E37

Effect of Sample Handling on ACTH Concentrations Following Thyrotropin-Releasing Hormone Stimulation in Horses

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Endogenous ACTH and ACTH post thyrotropin-releasing hormone (TRH) stimulation concentrations are used to diagnose pituitary pars intermedia dysfunction (PPID); however, the stability of ACTH after TRH stimulation has not been evaluated.

Fifteen horses older than 10 years of age, including 6 horses that underwent a TRH stimulation test 30 min before blood collection, were included. Blood samples were collected in EDTA tubes and stored for 2, 4, 8, 12, 24 or 48 hours at 4°C, 20°C or 30°C as whole blood or after plasma separation by centrifugation or gravity. Immunoreactive ACTH concentrations were measured using a chemiluminescent assay. Using the 2-hour, 4°C centrifuged sample as a reference, percentage change in immunoreactive ACTH concentrations were compared with a linear mixed effect model, with P < 0.05 considered significant.

An overall effect of separation method, temperature, time and hemolysis (P < 0.01) was observed but there was no significant effect of TRH stimulation (P = 0.58). Centrifuged samples remained stable up to 48 hours at 4°C, while centrifuged samples kept at 20°C and 30°C were stable for less than 24 hours. Gravity separated samples and samples stored as whole blood affected immunoreactive ACTH concentrations. Overall, immunoreactive ACTH concentrations decreased with time and suboptimal sample handling; however, in 37% of samples, spurious increases in immunoreactive ACTH concentrations were observed.

TRH stimulation did not impact the stability of immunoreactive ACTH. Improper sample handling decreases immunoreactive ACTH concentrations, but unpredictable increases can be observed and lead to false positive diagnoses of PPID in some horses.

E38

Evaluation of Glycemic Carbohydrate Formulations for Assessment of Insulin Dysregulation in Equines

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Oral glycemic challenge tests are recommended for diagnosis of insulin dysregulation in equines. Several different protocols are used, but all of them have limitations in terms of palatability, ease of use in the field, not fully disclosed composition and/or region specific availability.

The aim of the study was to evaluate new carbohydrate formulations (syrup, granulate and pellets). All glycemic challenges in this study were administered with equivalent amounts of 0.5g glycemic carbohydrates per kg body weight.

Firstly, the palatability was assessed in a mixed horse population. When trough-fed, a complete and fast voluntary uptake is necessary for use as accurate challenge. Out of the tested variants (new carbohydrate formulations and in-feed glucose), only the pellet formulation met this criterion, it was completely taken up by all horses (n=18) within 5±2 minutes. The syrup was well accepted when administered via a syringe orally.

Secondly, feeding the pellet formulation, oral application of the syrup and standard oral glucose test (glucose dissolved in water via nasogastric-tube, OGT) were performed in 18 Icelandic horses of different sex, age, bodyweight and metabolic status. Blood samples were collected for 4h and analyzed for insulin (Mercodia ELISA) and glucose. The insulin concentrations and dynamics correlated significantly between the OGT and pellets (e.g. 120 min – r² 0.82) or syrup (e.g. 120 min – r² 0.96).

In conclusion, the new pelleted or syrup glycemic carbohydrate formulation can be employed as palatable / well-accepted, accurate, oral glycemic challenge test for assessment of insulin dysregulation in equines.

E39

Relationship of Endoscopic Appearance, Macroscopic, and Histopathologic Findings in Equine Glandular Gastric Disease

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Equine glandular gastric disease (EGGD) is an increasingly recognized disorder of the glandular portion of the equine stomach. Although endoscopy is the gold-standard for diagnosis of EGGD, a validated scoring system remains to be established. We hypothesized that endoscopic inflammation severity (severity score) is correlated among scoring systems and related to the inflammation microscopically assessed. Furthermore, we hypothesized that endoscopic evidence of glandular ulceration (EGGD ≥2/4) is associated with gross and histologic findings of ulceration and a neutrophilic infiltrate. There were 17 horses included in the study. Horses were aged 2-24 years, including 17 mares, 12 geldings, and 1 stallion. Breeds represented were Thoroughbred (n=22), Quarter Horse (n=4), Appaloosa (n=1), Tennessee Walking Horse (n=1), Paint (n=1), and Warmblood (n=1). Horses were scored for EGGD using three different scoring systems. Following euthanasia, stomachs were collected and macroscopically evaluated. Sections of normal mucosa (dorsal and ventral pylorus) and
mucosa with lesions were collected and routinely processed for microscopic examination. Cellular infiltrate was scored using a standardized scoring system for small animals. A Spearman’s correlation coefficient was used to evaluate relationships among scoring systems, gross pathologic, and histopathologic findings. Twenty horses had endoscopic evidence of hyperemia; 11 horses had EGGD ≥2/4. As expected, severity score was correlated among scoring systems (r > 0.7; P < 0.0001). There were no relationships between the severity of inflammation observed microscopically and endoscopic EGGD scores, regardless of scoring system used (P > 0.5). Histopathologic evidence of an ulcer was related to neutrophil inflammation (r = 0.67, P = 0.001). Gross findings of an ulcer were related to endoscopic (r = 0.51; P = 0.004) and histologic (r = 0.44, P = 0.02) ulceration. These findings suggest endoscopy is useful for diagnosis of glandular ulceration but endoscopic findings are not predictive of inflammatory severity. Caution should be used when classifying EGGD disease severity based upon endoscopic appearance.

E40

Trends in Antimicrobial Use For Exploratory Laparotomy
Rachel Gough – Donnington Grove; Kate McGovern – Donnington Grove

There is pressure to ensure optimal clinical outcomes whilst minimising antimicrobial use. The incidence of incisonal infection following exploratory laparotomy is 10-37%. A concern of reduced antimicrobial use is an increased infection rate. This retrospective study aimed to assess whether fewer antibiotics were used following exploratory laparotomy over time and whether this resulted in increased complications.

Records of horses undergoing exploratory laparotomy from 2008-2010 (group 1) and 2015-2017 (group 2) were reviewed. Antimicrobial use during hospitalisation and following discharge was recorded. Data relating to infections was recorded. Incisional infection was defined as presence of purulent discharge or serous discharge >24-hour duration. Information was obtained from clinical records and follow-up. One month follow-up post-discharge was required or horses were excluded. Chi squared and t-tests were used for data analysis; results were considered significant if p < 0.05.

99.4% (321/323) of horses received procaine penicillin and gentamicin during hospitalisation. There was a significant reduction in mean duration of antimicrobial use over time; 5.6+/−1.8 days to 3.6+/−1.3 days, p <0.001. There was a reduction in the number of horses discharged on antimicrobials; Group 1: 79% (80/101 horses); Group 2: 6% (8/124 horses), p<0.00001. There was no difference in the number of horses that developed an incisional infection; Group 1: 19% (16/101 horses), Group 2: 22% (25/112), p = 0.522, diarrhoea; Group 1: 11% (13/115), Group 2: 13% (20/151), p = 0.634, pneumonia; Group 1: 0.9% (1/115), Group 2: 2% (3/151), p = 0.458 or thrombophlebitis; Group 1: 0.9% (1/115), Group 2: 4% (6/151), p = 0.117. Post-discharge incisional infections were mostly obtained from owner follow-up so may be under reported. Fewer horses received antimicrobials after exploratory laparotomy over time; this is not associated with an increase in infectious complications. This supports that long antimicrobial courses are likely unnecessary for exploratory laparotomy.

E41

Ultrasound following Small Intestinal Colic Surgery to Predict Post-Operative Ileus or Anastomotic Obstruction
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The study purpose was to evaluate whether ultrasound assessment of small intestinal (SI) motility following colic surgery was useful to predict post-operative ileus (POI) or anastomotic obstruction.

Data was collected over 6 years for horses with SI lesions with or without resection. Routine ultrasound was performed at least every 8 hours for 48-72 hours post-operatively. SI motility (duodenal, left and right side) was graded 1-4 (1 = normal motility, 4=no motility). Data was collected from ultrasound videos viewed retrospectively, or from recorded descriptions or grades. The highest grade for each 24-hour period, starting from the end of surgery, was recorded for each area. Ultrasounds were included until gastric reflux was obtained or a second surgery performed. If an ultrasound prompted positive nasogastric intubation, that score was excluded.

Horses were classified as group 1 (G1) (maximum grade 2, 62/99 horses) or group 2 (G2) (grade 3 or 4 at least once, 37/99 horses). Data were analysed with Chi-squared and t-tests; results were considered significant if p < 0.05.

For post-operative gastric reflux being obtained: G1 2/62 horses (3.2%), G2: 24/37 horses (64.9%), p < 0.05. Incisional infection: G1 8/62 (12.9%), G2 14/37 (37.8%), p=0.004. Horses requiring second surgery: G1 1/62 (1.6%), G2 10/37 (27%), p = 0.00001. Short-term survival: G1 62/62 horses (100%), G2 25/37 (67.5%), p = 0.00001. Mean duration of hospitalisation: G1 (5.98+/−2.98 days), G2 (9.12+/−5.24 days), p = 0.00038. Anastomotic impactions: G1 0/26 (0%), G2, 7/23 (30%), p = 0.011. Overall 15 horses produced gastric reflux on day 2 or later; 8/15 horses (53%) had a grade 3 or 4/4 at least 24 hours prior to reflux being obtained. Limitations include a non-blinded study and an inconsistent means of obtaining grades.

Frequent ultrasound is useful in post-operative colics, to detect complications early. This could be used to guide pro-kinetic use, feeding regimes and to prepare owners earlier for impending complications.

E42

Evaluation of An Online Monitoring System to Detect Colic Motion Patterns in Horses
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Sensor based early warning systems to detect equine colic disorders are already available on the market but none of them have been critically evaluated. The purpose of the study was to determine how sensor data from accelerometers of an online monitoring device (Steed) can classify a range of typical equine colic motion patterns and some other motion activities.

We have enrolled 15 healthy horses to our study. The monitoring device was attached to the halter on the poll region. The device had
an MPU6050 prototype 6 axis accelerometer, a Raspberry Pi zero computer and a wifi router and the accelerometers were sampled at a frequency of 10 Hz. A video camera simultaneously recorded the animal’s behaviors and motions in real time. Using behavior coding we identified grazing, walking, trotting and cantering and 8 typical colic motion patterns (flank watching, lip curling, recumbency, kicking abdomen, pawing, attempting to lie down, rolling, recumbency) and collected sensor data accordingly. For noise reduction fast Fourier transformation was used. Differences in mean values of the axis parameters as well as in the activity index among the different behavior categories were tested by general linear models.

Using ethological methods to induce specific colic motion patterns made data collection fast, reliable and repeatable. Activity index per se was not sufficient to differentiate between each specific motion patterns. Activity patterns could be reliably differentiated when we also included the axes of the acceleration into our evaluations. Based on our results the device is able to detect colic motion patterns.

E43

Clostridial Shedding and Association with Microbiota Composition in Swiss Horses with and without Gastrointestinal Disease

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Background: Overgrowth with enteric clostridia in dysbiosis in horses with colic is presumed but scarcely investigated. The objective was to provide prevalence data of Clostridium (C.) difficile and C. perfringens in horses with and without gastrointestinal disease in Switzerland, and investigate microbiota differences between C. difficile shedders and non-shedders. Methods: Fecal samples were taken from healthy horses (n=103), horses with colic (n = 98) and horses with diarrhea (n = 151). Colic horses were sampled on three days. Selective enrichment culture and molecular typing for C. difficile and C. perfringens was performed. Metagenomic sequencing was done to compare microbiota differences between horses shedding (n = 7) and not shedding (n = 7) C. difficile.

Results: The cumulative prevalence (19% C. difficile and 16% C. perfringens) was higher compared to single day samples (1-10% C. difficile and 3-8% C. perfringens). Horses with colic shed significantly more C. difficile (8%, p < 0.001) but not C. perfringens (5%, p = 0.09) compared to healthy horses (0% were shedding C. difficile, 2% were shedding C. perfringens). Prevalence in horses with diarrhea was 8% for both Clostridium species. There were no significant microbiota differences between C. difficile shedders and non-shedders with regards to relative abundance on any phylogenetic level, and alpha diversity. Limited differences were seen on LEfSE analysis and in beta diversity indices.

Conclusions: Multiple fecal samples should be taken when investigating shedding of enteric clostridia. While horses with colic shed more enteric clostridia compared to healthy horses, differences in microbiota composition between C. difficile shedders and non-shedders were limited. Further studies on the role of dysbiosis in C. difficile are needed.

E44

Factors Associated with Positive Bacterial Cultures of Jugular Vein Catheters from Sick and Healthy Horses

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Identifying risk factors for bacterial contamination of intravenous catheters (IVCs) might help prevent catheter-related complications (C-RC). Hospitalized sick and healthy horses that required a jugular IVC were enrolled. Upon removal, catheter tips were collected for bacterial culture. Of the 119 catheters cultured, 58 were from sick horses and 61 from healthy horses. Bacterial growth occurred in 10.9% of catheters; 17.0% of catheters from sick horses and 4.9% from healthy horses (P = 0.031). C-RC occurred in 16% of jugular veins; 26% in sick horses and 6.6% in healthy horses (P = 0.005). Sick horses were catheterized for longer (median [IQR]: 107 [72-144] hours) than healthy horses (12 [8-24] hours; P < 0.001) but the duration of catheterization did not alter the risk of positive culture (OR = 1.01, P = 0.16). Sick horses were more likely to have a polyurethane catheter (74%) placed than healthy horses (18%) and placement of a polyurethane catheter was associated with an increased risk of contamination (OR = 6.14, P = 0.023). Other variables associated with positive culture in univariable logistic regression were: “sick” disease status (P = 0.042); presence of an IV fluid line (P = 0.038); and C-RC (P = 0.027). None of these variables were retained in a multivariable model. This study demonstrated that bacterial contamination of IVC occurs in a high proportion of sick horses but also in a low proportion of healthy horses. Factors that might contribute were identified; the most important appearing to be disease status. Further studies are needed to determine the relationship between bacterial contamination of catheter tips and septic thrombophlebitis.

E45

The Blood Bacterial Microbiome in Healthy and Hospitalized Foals

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Sepsis is a main cause of mortality in newborn foals. Blood culture has been considered the “gold standard” to diagnose sepsis, however a high rate of negative blood cultures in truly septic foals often results in failure to identify an etiological agent. Characterization of the bacterial microbiota of blood in healthy and ill humans has been documented but has not been investigated in foals. Therefore, we hypothesize that the foal’s blood microbiota has a complex taxonomic structure, comprised of culturable and unculturable
microorganisms, and illness is associated with shifts in the microbial community. We also propose that these shifts in the blood microbiota will be mirrored by related shifts in the fecal microflora of hospitalized foals.

Whole blood, anaerobic and aerobic blood culture (at 24 h of incubation), skin (puncture site) and fecal samples were collected from healthy (n = 10) and sick (n = 10) neonatal foals (<3 days old). The bacterial microbiota of whole blood samples was characterized using the Illumina MiSeq platform targeting the V4 region of the 16S rRNA gene. Bacterial microbiota were compared between sampled sites in healthy foals. Following this analysis, body sites were compared between healthy and sick foals. The bacterial microbiota of all body sites in the healthy group were different in community membership (Jaccard index) and structure (Yu and Clayton index) (Parsimony and AMOVA; P < 0.001) except the anaerobic and aerobic blood cultures that were similar in community membership and structure (Parsimony and AMOVA; P > 0.1). Skin had higher diversity (Chao-1 index) and evenness (Shannoneven index) than fecal samples, and blood culture samples (P < 0.05). Whole blood samples also had a higher diversity than the blood cultures (P < 0.05). Based on LefSe analysis, enriched phylotypes (LDA > 2) in skin samples were predominantly from the phylum Proteobacteria, Firmicutes and Actinobacteria, in whole blood samples from Proteobacteria and Bacteroidetes, in feces and aerobic culture from Firmicutes, and in anaerobic culture from Firmicutes and Proteobacteria.

The whole blood and blood culture microbiota of healthy and sick foals were similar in community membership and structure (Parsimony and AMOVA; P > 0.1). The fecal microbiota of healthy and sick foals was different in community structure (Parsimony; P < 0.02). There were no differences in diversity, richness or evenness between groups. LefSe analysis did not identify any taxa that differentiate samples from healthy and sick foals.

This study demonstrates a complex bacterial microbiota present in the blood of healthy and sick foals that is different from the fecal and skin microbiota. Further analyses assessing the role of blood microbiota in health and critical illness of neonatal foals are warranted.

E46

Plasma Neuraminidase Activity in Septic Adult Horses

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Elevated plasma neuraminidase activity is associated with sepsis severity and independently predicts mortality in affected people. Increased neuraminidase activity contributes to vascular dysfunction in septic states because it promotes degradation of the endothelial glycocalyx. Neuraminidase activity has not hitherto been investigated in horses affected with sepsis.

Therefore, plasma was obtained from 12 healthy adult horses (Group 1, controls) and from 24 septic horses (Group 2) that were presented to the hospital. Septic horses were classified using a sepsis score calculated from clinical (observation and physical examination) and laboratory (CBC and plasma biochemistry) data, based on previously-published work.

100 μl of plasma were incubated in 75 μM sodium acetate pH 4.5, 0.1% TritonX-100 and 0.5 mM 2′-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid sodium salt hydrate in a total reaction volume of 200 μl, for two hours at 37°C. Fluorescence endpoint measurements obtained with wavelengths of 360nm (excitation) and 460nm (detection) were collected using a BioTek Synergy HT Plate Reader. Quantitated neuraminidase activity was determined by comparing fluorescence data to a positive control with known neuraminidase activity. An unpaired t test was used to compare results between groups and significance was assessed if p < 0.05.

Sepsis scores in Group 2 (mean 11.2; range 6-14) were higher than those in Group 1 (mean 0; range 0-0). Neuraminidase activity was reduced in Group 2 (8.24±8.89 mU/ml) when compared to Group 1 (26.64±7.93 mU/ml). Pearson’s correlation between neuraminidase activity and sepsis score was significant at r = 0.7554.

Unexpectedly, plasma neuraminidase activity was reduced in adult horses affected with sepsis.

E47

Ex Vivo Effects of Azithromycin on Lymphoproliferative Responses of Adult Horses

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The immunomodulatory properties of macrolide antibiotics have been described in several species, but data are lacking in horses. The purpose of this study was to investigate the effects of ex vivo exposure to azithromycin on lymphoproliferative responses of healthy horses. Peripheral blood mononuclear cells were obtained from 14 healthy adult horses. Cells were stimulated with either media (no stimulant), Con A (concanavalin A, 8 μg/ml), PWM (pokeweed mitogen, 5 μg/ml), or killed Rhodococcus Equi (MOI = 10). Stimulated cells were subjected to three media conditions: no azithromycin, low azithromycin (0.7 μg/ml), and high azithromycin (10 μg/ml). Lymphoproliferative responses were measured using an alamar blue assay. Data were transformed to log10 values to facilitate parametric analyses and normalized to unstimulated cells. Generalized linear modeling was used to analyze differences while accounting for the clustered nature of the data. Significance was set at p < 0.05. All analyses were performed using SAS 9.4, SAS, Inc., Cary, NC. Overall, stimulant condition was significantly associated with differences in proliferative responses, with Con A causing greater proliferation than PWM, and both Con A and PWM causing greater proliferation than killed R. equi. Under all stimulant conditions, incubation with high azithromycin resulted in significantly less proliferation as compared to low azithromycin or no azithromycin. Results indicate that azithromycin causes a reduction in lymphoproliferative responses of adult horses, regardless of type of stimulant. Future investigations will include evaluation of cytokine profiles of proliferating lymphocytes from the current study, and ultimately the effects of azithromycin on leukocyte function in foals with R. equi infections.
**E48**

Identification of Equine Neutrophil Extracellular Traps and Implications for Future Research and Clinical Applications

Breanna Sheahan (LAIM) – North Carolina State University CVM; Alicia Schubert – North Carolina State University; Jessica Gilbertie – North Carolina State University; Lauren Schnabel, DAVSMR – North Carolina State University

The purpose of this study was to identify and validate the formation of equine neutrophil extracellular traps (NETs), in the process of NETosis as recently described in other species. NETosis involves the decondensation and exocytosis of neutrophil DNA, allowing neutrophils to immobilize and kill bacteria. Antimicrobial peptides are attached to the DNA prior to release and decondensation is facilitated by peptidyl arginine deiminase 4 (PAD4), which citrullinates histones 3 and 4. NADPH oxidase (NOX)-dependent or independent NETosis can be stimulated ex vivo by PMA or the calcium ionophore A23187, respectively, in human and mouse neutrophils. CI-amidine, a PAD antagonist, impairs both NOX-dependent and independent NETosis, whereas DPI, a NOX inhibitor, only impairs the NOX-dependent pathway.

We stimulated isolated neutrophils from healthy adult horses, and were able to identify NETosis using a plate-based fluorescence assay with cell impermeable DNA dye (SYTOX green) as a proxy of extracellular DNA. Both NOX-dependent and independent NETosis occurred in equine neutrophils, and were inhibited by CI-amidine. Only NOX-dependent NETosis was inhibited by DPI. Presence of extracellular DNA and citrullination of H3 were confirmed by immunofluorescence.

These results demonstrate that equine neutrophils respond to NETosis agonists and antagonists similarly to human and murine neutrophils. Excessive NETosis has been associated with negative clinical sequelae in several human diseases including sepsis, rheumatoid arthritis, vasculitis, and cystic fibrosis. The consequences of NETosis in the horse is an important area of research both for equine medicine and as a translational large animal model for human diseases.

**E49**

Combined Endothelial Colony Forming Cell / Hydrogel Microsphere Scaffold Decreases Equine Distal Limb Wound Inflammation

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Endothelial colony forming cells (ECFCs) aid in neovascularization and may be therapeutic in conditions with poor blood supply. Distal limb wounds in the horse have local ischemia and hypoxia leading to protracted inflammation and slow healing rate. Combination ECFC/hydrogel scaffold injectable therapy helps cell survival, may improve neovascularization, and may mitigate inflammation found in equine distal limb wounds. Autologous ECFCs were isolated from 6 adult horses, labeled with quantum nanodots, and a subset of cells encapsulated in poly(ethylene) glycol fibrinogen microspheres (PEG-Fb MS). Full-thickness dermal wounds were created on each distal limb and randomly assigned to injections with empty PEG-Fb MS, serum, ECFCs, and ECFCs encapsulated into PEG-Fb MS (ECFC/MS). Analysis included immunohistochemical staining of punch biopsies collected from a leading edge of the wounds for B-cells, T-cells, neutrophils, and macrophages at weeks 1 and 4.

Treatments were well tolerated in all horses. There were no significant changes in B-cell or T-cell density during the study. Neutrophilic and macrophagic inflammatory responses were not different between treatments at week 1. Activated neutrophil density (measured by elastase staining) was lower (P < 0.001) in wounds treated with ECFCs and ECFC/MS at week 4. Wounds treated with ECFCs and ECFC/MS had less macrophagic inflammation (measured by positive ionized calcium-binding adapter molecule 1 staining) at week 4, with wounds treated with ECFC/MS having less (P = 0.008) macrophagic inflammation compared to wounds treated with MS.

Inflammation was decreased by using ECFCs or ECFC/MS as treatment in this model of equine distal limb wounds.

**E50**

Equine Neonatal Symmetric Dimethylarginine (SDMA): Results of Two Pilot Studies

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Symmetric dimethylarginine is a sensitive, early and reliable biomarker of kidney function in dogs and cats. Measurement of SDMA has not been extensively evaluated in neonatal foals. Neonatal spurious hypercreatininemia has been associated with placental insufficiency and there is currently no easy way to distinguish cases of renal dysfunction. Pilot studies were performed to describe SDMA changes in clinically healthy foals and their mares during the first month postfoaling; and secondly, to evaluate if SDMA is a more specific marker of renal function in sick neonates with spurious hypercreatininemia. Serum and whole blood samples were collected from mares in their last month of pregnancy and then again with their foals around < 12 hours, 48 hours, 7 days and 30 days post-birth. Hospitalized neonatal foals ≥ 24 hours old were sampled on admission and then after 24, 48 and 72 hours to evaluate SDMA concentrations and compare to creatinine concentrations. Neonatal SDMA was above the adult upper reference limit (14 μg/dL). There was no correlation between mare and foal SDMA at birth (r = 0.021, P = 0.92). Neonatal SDMA levels decreased following birth, though the median SDMA level remained above the adult reference limit at 30 days of age (Table 1). In sick neonates with presumed spurious hypercreatininemia, creatinine levels normalized by day 3 or 4, however SDMA levels remained high throughout the study (Table 2). Both analyses suggest that neonatal SDMA levels trend above the adult reference interval. Therefore, a new reference interval for neonates should be validated.
Associations Between Clinical Parameters and Outcome in 62 Equids with Facial Nerve Paralysis

Sophie Boorman – University of Pennsylvania; Darko Stefanovski – University of Pennsylvania; Amy Johnson – University of Pennsylvania; Nicole Scherrer – University of Pennsylvania

Facial nerve paralysis (FNP) of the equine patient manifests as muzzle deviation, drooping of the upper eyelid, partial or complete loss of the ability to close the eyelids and drooping of the ear. FNP is well described in small animals and people, but little to no scientific or clinical investigation have been conducted in the horse. The purpose of this retrospective study was to investigate the causes of FNP in the horse and report statistical associations between clinical parameters which could be predictable for outcome. The medical records of equine patients presenting to the hospital with FNP were accessed; cases of FNP which developed in-hospital postoperative were excluded.

There were 62 cases of FNP. Ocular pathology of the affected side was noted in 23 (37%) cases. There were 57 (92%) unilateral cases (29 [51%] right-sided, 28 [49%] left-sided) and 5 bilateral cases (8%). There were 16 (26%) animals with FNP with a final diagnosis of trauma, 13 (21%) idiopathic FNP, 10 (16%) temporohyoid osteoarthropathy, 2 cachexia/emaciation, 2 otitis media/externa, 1 lymphosarcoma, 1 drug reaction, 1 caused by infiltration of local anaesthetic, 1 clostridial myositis, 1 equine motor neuron disease. There were 18 (29%) animals with confirmed central nervous system disease – 10 (16%) equine protozoal myeloencephalitis, 5 (8%) neuroborreliosis, 1 West Nile Virus, 1 botulism and 1 unknown central nervous system disorder.

Follow up information was available for 53 (85%) cases. There were 14 (26%) cases that were euthanized or died (either prior to or shortly following hospital discharge), 28 (53%) cases with a reported full resolution of FNP, 5 (9%) cases that only partially improved and 6 (11%) cases that were unchanged or progressed to worse. Older animals were less likely to have full resolution of FNP (odds ratio [OR] = 0.88, 95% confidence interval [CI] = 0.81-0.96, P = 0.003). Animals more likely to have full resolution of FNP were those with an acute (within 7 days of onset) presentation (OR = 7.44, 95% CI = 2.03-27.28, P = 0.002) and those with a diagnosis of EPM (OR = 6.40, 95% CI = 1.23-33.23, P = 0.027). Positive associations were found between abnormal mentation at presentation (OR = 4.78, 95% CI = 1.14-20.02, P = 0.032), presence of ataxia (OR = 8.41, 95% CI = 2.20-32.14, P = 0.002) and diagnosis of neuroborreliosis (OR = 18.8, 95% CI = 1.89-186.61, P = 0.012) with death/euthanasia. There were no statistically significant associations between clinical parameters and partial improvement or static/worsening FNP.

This study was limited by the small number of cases and the inherent difficulties associated with trying to prognosticate the clinical outcome for a peripheral neuropathy that has a wide range of etiologies of varying severity. Nevertheless, we found a limited number of clinical parameters with statistically significant associations towards a successful resolution of FNP. These findings may help guide clinicians when managing their patients and owner expectations.

E52

Equine Stereotactic Population Average Brain Atlas with Neuroanatomic Correlation

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With increasing availability of high-field MRI in equine practice, the frequency of equine neurological imaging for diagnosis and research of neurological disease is increasing. Advances in brain imaging have brought forth new techniques that require the use of stereotactic brain atlases and volumetric data sets for registration and processing purposes. Although stereotactic brain atlases have been made available for other domesticated animals, to date, none are available for the horse limiting the scope of equine neurological research. In this study we document the creation of a high-resolution stereotactic population average atlas of the equine brain.

Ten mixed breed neurologically normal equine cadaver brains were imaged in-situ within the cranium within 2 hours of euthanasia and then fixed via formalin emersion. Imaging was performed in a 3.0-tesla MRI scanner to obtain a 3-dimensional T1-weighted sequence. The MRI data were processed to correct for inhomogeneity, before skull-stripping, registration and spatial normalization. The data were then transformed into a common space population template using Advanced Normalization Tools. From this template tissue probability maps (TPMs) were created and anatomically significant subcortical regions were manually delineated using histological references.

An atlas was created that includes a T1-weighted brain template, TPMs for white matter, grey matter and cerebrospinal fluid, and segmented priors of the subcortical brain structures. The resulting atlas is correlated to gross pathological specimens and is made available as an online resource for researchers and clinicians. This atlas is a vital tool that facilitates the use of advanced neuroimaging techniques in equine neurological research.

E53

Evaluation of the Filum Terminale in an Equine Model of Ehlers-Danlos Syndromes

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The objective of this study was to determine the relevance of horses with hereditary equine regional dermal asthenia (HERDA) as a model of occult tethered cord syndrome in human Ehlers-Danlos syndromes. The pathophysiology of occult tethered cord syndrome in Ehlers-Danlos syndromes (EDS) is poorly understood; however, this condition is prevalent and provides a challenge to physicians. Tethered cord is caused by an abnormally structured filum terminale. In this setting, the natural attachment of the filum terminale at the base of the vertebral column leads to long term stretch-induced injury to the spinal cord.

Physical examinations, including neurologic examinations, were performed on 4 HERDA and 5 control horses. Histopathologic and ultrastructural examinations of the fila terminale were performed following euthanasia. Collagen fibrils were assessed in cross-section based on fibril diameter, shape, and packing density. In longitudinal section, the presence of broken, whirling, hook-shaped, or disintegrating collagen fibrils was assessed. Immunohistochemistry with CD3 and CD20 was performed on 2 HERDA and 5 controls to assess for lymphocytic inflammation.

Neurologic examination was abnormal in 1 HERDA horse. Severe lymphocytic inflammation was observed in 1 HERDA horse, while moderate lymphocytic inflammation was observed in 1 HERDA horse and 2 controls. Moderate to severe abnormalities in collagen fibril ultrastructure were observed in all HERDA horses. Mild abnormalities were observed in 4 controls. These abnormalities in histopathology and ultrastructure are similar to those observed in the fila terminale of humans with EDS, supporting the use of horses with HERDA as a model of occult tethered cord.

E54

Cerebrospinal Fluid Analysis in Horses, Cattle and Sheep Diagnosed with Rabies

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Rabies is a fatal zoonotic neurological disease. Although important to the differential diagnosis of neurological diseases, only few studies described cerebrospinal fluid (CSF) in large animals with rabies. The purpose of this study was to retrospectively (2003-2018) evaluate CSF results from horses, cattle and sheep with confirmed rabies diagnosis (direct immunofluorescence and mice intra-cerebral inoculation).

Fifty-two CSF (27 equines, 19 bovines and 6 ovines) samples collected from atlanto-occipital or lumbosacral spaces were included. Total protein was measured by a colorimetric test (pirogalol red), while red blood cell (RBCC/μL) and total nucleated cell (TNCC/μL) counts were performed in Neubauer's chamber. Samples with more than 700 RBCC/μL were excluded. Median and range were calculated for abnormal samples. CSF normal ranges were based on previously published studies for horses (TNCC: 0-6 cells/μL; protein 8.83-65.63 mg/dL), cattle (TNCC: 0-10 cells/μL; protein 0-67mg/dL) and sheep (TNCC: 0-5 cells/μL; protein 8-70mg/dL). Twenty-eight samples (54%) presented TNCC and protein within normal limits. Considering horses, 33% (9/27) presented pleocytosis (median: 43cells/μL; range: 12-115) and 15% (4/27) hyperproteinorrachia (median: 87.1mg/dL; range: 68.9-103.4). In cattle, 47% (9/19) showed pleocytosis (median: 35cells/μL; range: 11-85) and 11% (2/19) hyperproteinorrachia (median: 79.1mg/dL; range: 67.6-90.5). All six sheep samples had pleocytosis (median: 50.5cells/μL; range: 19-89) and 67% (4/6) hyperproteinorrachia (median: 95.5mg/dL; range: 76.6-19). All samples presenting hyperproteinorrachia also presented pleocytosis. Lymphocytes and other mononuclear cells were
of strong ions, especially Na⁺ and Cl⁻ have been documented when using different analyzers. The objective of this study was to investigate whether electrolyte (Na⁺, K⁺, Cl⁻) concentrations measured using a plasma biochemistry multi analyzer (PBMA) and whole blood gas analyzers (WBGA) are equivalent and can be used interchangeably for calculation of the sSID variables and diagnosing acid-base disorders. The study was conducted on 78 equine patients. Samples for both PBMA and WBGA analyzers were collected simultaneously by venipuncture of the jugular vein. The PBMA and WBGA electrolyte concentrations were measured using a Beckman Coulter AU480 Chemistry analyser® and a Nova Biomedical Stat Profile®, respectively. Total plasma protein was measured using refractometry. The sSID variables were calculated as SID = (Na⁺+K⁺) – (Cl⁻+l-lactate⁻); A_total = 0.22 × TP (g/dL) and SIG = SID – HCO₃⁻ – A_total/(1+10⁽⁶.65−pH⁾). The SIDs and SIG were determined using the values for electrolytes obtained from each of the two analyzers. The acid-base disorders were defined when the following variables were outside of the following reference ranges: SID (38 to 47 mM/L), A_total (12 to 16 mM/L) and SIG (−2 to 2 mM/L). The null hypothesis that the proportions of samples classified as positive to acid-base disorders by using the PBMA and WBGA analyzers were similar was tested using the McNemar’s chi-square test. The level of agreement between the two analyzers in detecting acid-base disorders was assessed using Kappa coefficient test. The proportion of SID acidosis was significantly (p < 0.01) higher in tested samples using WBGA (63/78, 81%), compared to PBMA (29/78, 37%). Similarly, the proportion of SIG acidosis was significantly (p < 0.01) higher in tested samples using WBGA (31/78 or 40%), compared to PBMA (3/78 or 4%). Kappa coefficient analysis showed a very low agreement between PBMA and WBGA for detection of SID acidosis (κ = 0.20, 95%CI, 0.07 to 0.33; P = 0.006) and SIG acidosis (κ = 0.11, 95%CI, -0.007 to 0.23; P = 0.029). Differences in the measured concentration of Na⁺ and Cl⁻ between two electrolyte analyzers resulted in discrepancies of the diagnosis of acid-base disorders of sick horses when using the sSID approach. These discrepancies could impact decisions regarding diagnosis, therapy, and prognosis.
competition or parturition. Beta-1,3/1,6-glucans are naturally occurring polysaccharides that can be found in the cell walls of yeasts, cereals, fungi, and bacteria. There have been studies researching the effects on vaccine response, anti-inflammatory effects, and immunomodulatory properties of beta-glucans. The aim of this study was to evaluate the effect of oral beta-glucan supplementation on immune biomarkers in horses. A population of adult geldings (n = 19) were housed in stalls for the duration of a 28 day study. Horses were randomly assigned to one of three supplemented groups: control, Low dose (1.25 g), and High dose (2.50 g). Low and High supplemented groups were given their respective level of beta-glucan once daily mixed with their feed. Blood samples were drawn on days 0, 7, 14, 21, and 28 and sent for biomarker analysis to an outside lab. Statistical analysis of the data was completed using repeated measures ANOVA in SigmaStat statistical software (Systat Software, Inc., San Jose California USA). Analysis revealed no significant differences (confidence set at 95%) due to supplementation over the course of the study for any of the parameters. There was a difference seen in IL-2 levels at day 28 between the Control and the High dose groups (Figure 1). There were some day differences seen within supplemented groups in immunoglobulin levels, IL-2, IL-6 (Figure 2), phagocytosis, Con-A proliferation, and PHA proliferation. Interestingly, the data showed the potential that an outside environmental stressor may have presented a challenge to the immune system in all of the horses, causing an elevation in immune markers. There were observed trends of increasing biomarkers over time in most parameters; however, the supplemented groups showed a lesser increase than that of the control group. This study provides support that beta-glucan supplementation has the potential to elicit an immunomodulating effect which may be particularly beneficial in times of stress in horses.

### E58

**Investigation of Lamellar Metabolism and Perfusion in the Euglycemic Hyperinsulinemic Clamp Model of Equine Laminitis**

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Hyperinsulinemia is the leading cause of laminitis in horses; however, it is unclear how hyperinsulinemia contributes to laminitis development, and if metabolism and perfusion alterations play a role in the development of this form of laminitis.

Eight clinically normal Standardbred horses underwent laminitis induction using the euglycemic hyperinsulinemic clamp (EHC) model. Six clinically normal Standardbred horses did not undergo laminitis induction and served as a control group. Each horse was instrumented with a lamellar microdialysis system to assess lamellar interstitial concentrations of glucose, lactate and pyruvate every 6 hours over a 48-hour experimental period. Lamellar perfusion was indirectly evaluated using the urea clearance method. Glucose, lactate and pyruvate lamellar interstitial concentrations were compared between groups at corresponding time points, and urea clearance was also compared to baseline values within each group. Statistical analysis was conducted using a multilevel mixed-effects model. All horses undergoing an EHC developed laminitis within 48 hours. Glucose, lactate and pyruvate lamellar interstitial concentrations were not significantly different between groups at any time point. Urea clearance was not significantly different between groups at any time point, and was not significantly different to baseline values in any group.

This study shows that during continuous hyperinsulinemia, lamellar glucose metabolism and perfusion parallels that of clinically normal horses, and suggests alterations in these events do not play a prominent role in the development of hyperinsulinemia-associated laminitis.

### E59

**A Herbal Spray Reduces Insect Bite Hypersensitivity in Horses Compared to Placebo**

Carlos E. Medina Torresc – Equine Specialist Hospital, The University of Queensland; Kelly Wood – University of Liverpool; Abbey Cox – UQ VETS Dayboro, The University of Queensland; Gemma Coleman – UQ VETS Dayboro, The University of Queensland; Allison Stewart – UQ VETS Australian Equine Laminitis Research Unit, School of Veterinary Science, The University of Queensland; Francois-Rene Bertin – UQ VETS Equine Specialist Hospital, The University of Queensland

Insect bite hypersensitivity (IBH) is a seasonal allergic skin disease of horses, characterised by unrelenting pruritus due to hyper-
responsiveness to insect saliva. Immune mechanisms orchestrated by mast cells and immunoglobulin E (IgE) are responsible for this hyper-responsiveness. The aim of this study was to test a herbal spray combining various natural ingredients, with a claim of mast cell stabilisation, antipruritic, anti-inflammatory and insect repellent effects.

Twenty adult horses with clinical IBH were enrolled in a randomised, double blinded, placebo controlled, crossover trial during the 2018 summer. Treatment with the herbal spray significantly reduced the severity of all assessed parameters (pruritus, excoriations, lichenification and alopecia; P < 0.05) compared to baseline values (pre-treatment) and to placebo. Owners reported improvement of pruritus in 19 horses (95%) with complete resolution in 17 horses (85%) following treatment, compared to only 2 horses (10%) following placebo. Contingency analysis identified a significant association between treatment and resolution of pruritus, as well as improvement in disease severity (P < 0.01). Skin biopsies were histopathologically assessed before and after treatment and placebo in 4 horses. Resolution of orthokeratosis in 4 horses (100%), reduced thickness of the stratum spinosum in 2 horses (50%) and complete resolution of histopathological abnormalities in 1 horse (25%) was identified after treatment, compared to either no change or deterioration of histopathologic lesions after the placebo. No side effects were observed in any of the treated animals. The tested herbal spray may be an effective alternative treatment in the management of equine IBH.

**E60**

**Genetic Variation and the Frequency of Deleterious Variants (Genetic Burden) in Healthy Horses**

Sian A. Durward-Akhurst – University of Minnesota; Robert Schaefer – University of Minnesota; James Mickelson – University of Minnesota; Molly McCue – University of Minnesota

Identification of disease-causing variants (DCVs) is a fundamental goal of medical genetics and can facilitate disease diagnosis and improve understanding of the disease. Studies in humans have increasingly demonstrated the value of a database of genetic variation derived from genome sequencing (WGS) in identifying the DCVs for simple genetic diseases. A surprising finding from these studies is the high number of variants computationally predicted to be deleterious (i.e., constituting the genetic burden) in healthy individuals. Due to a lower genetic diversity in horses, we hypothesized that genetic burden would be higher than in humans, and that there would be breed differences. We mapped WGS from 230 horses to the EquCab2 reference genome. Single nucleotide polymorphisms (SNPs) and short insertions/deletions were identified using GATK-HaplotypeCaller, Platypus, and SAMTools. The genetic burden was calculated using SnpEff. Average depth of coverage was 10.3x (range 1.4-37.4x). 29,420,486 variants were called by all three variant callers. Total genetic burden was 14,298 (7,130 SNPs, 701 multi-nucleotide polymorphisms, and 6,598 insertions/deletions). The mean genetic burden was 2,343 per horse (range 348-3,468). On average, each horse had 1,888 loss of function variants (range 246-2,733). There were significant breed differences in genetic burden in the 8 breeds with ≥ 9 horses. Mean genetic burden was highest in Arabs (2,888) and lowest in Morgans (1,918). Overall, we demonstrate that the genetic burden in horses is higher than in humans and breed-dependent. This catalog of genetic variation will be used to prioritize likely DCVs for 28 horses with 12 presumed Mendelian diseases.

**E61**

**Environmental Surveillance and Investigation Neonatal Dysphagia in Foals Born Near Unconventional Natural Gas Development Activity**

Kathleen R. Mullen – Littleton Equine Medical Center; Lane Tidwell – Oregon State University; Brianna Rivera – Oregon State University; Renata Ivanek – Cornell University; Kim Anderson – Oregon State University; Dorothy Ainsworth – Cornell University

Studies of human and animal health risks of unconventional natural gas development (UNGD) have not included assessment of environmental chemical exposures. Horses co-residing with humans may be important sentinel animals. We quantified environmental chemical exposures and incidence of neonatal dysphagia in foals born near active UNGD in the Marcellus Shale area of Pennsylvania (PA farm) compared to a control population in New York (NY farm), 2014-2016. Dams spent their entire gestation on one farm or moved between farms. Clinical assessments, and blood and tissue samples were obtained from mares and foals. Passive sampling devices were deployed in air and water, and grab samples of water, soil and feed were collected at 6-week intervals. Neonatal dysphagia was evaluated as a binary variable and a logistic regression model was used to identify risk factors. Sixty-five foals were included: 17 dysphagic (all born on PA farm) and 48 normal (37 on NY farm, 11 on PA farm). Odds of dysphagia increased with the dam residing on the PA farm for each additional month of gestation (Odds ratio (OR) = 1.41, 95% confidence interval (CI) 1.16, 1.71, p = 0.0006). Colts were more likely than fillies to be dysphagic (OR = 5.47, 95% CI 1.22, 24.49, p = 0.0263). Fluoranthene (p = 0.0215), 3,6-dimethylphenanthrene (p = 0.0231), pyrene (p = 0.0033), and triphenylene (p = 0.0019) concentrations were higher in the PA well water compared to NY until a water filtration system was installed. Further studies are needed to investigate the mechanisms of toxicity and long-term health outcomes for humans and animals residing near UNGD.

**E62**

**Equine Amniotic Membrane Transplantation for Non-Healing Corneal Ulceration Performed Under Standing Sedation in 7 Horses**

James S. W Prutton – Liphook Equine Hospital; Andy Durham – Liphook Equine Hospital

Amniotic membrane (AM) has been used in both equine and human patients for keratomalacia and ocular surface reconstruction. The anti-fibrotic, anti-angiogenic and antiprotease properties have been shown to preserve the integrity of the globe, optimise visual outcome, reduce scarring in diseased comeas and expedite healing of ulcers. Previous treatment protocols have required general anaesthesia for the application of the AM with the inherent risks and costs of anaesthesia in equine patients. Application of AM under standing sedation has not previously been described. Seven horses had AM sutured to either, or both, the cornea
Currently there are no reports describing the effects of grapiprant in horses. An analytical method was developed that provided a linear curve for the concentration range used (50-2500 ng/mL) and was detected at 48 hours after administration. Drug concentrations were measured using high performance liquid chromatography. Plasma grapiprant concentrations ranged from 71 to 149 ng/mL with the mean peak concentration (110 ng/mL) occurring at 30 minutes. Concentrations were below the lower limit of quantification (50 ng/mL) in all 6 horses by 2 hours, and 4/6 horses at 1 hour. Urine grapiprant concentrations ranged from 40 to 6240 ng/mL and were detected at 48 hours after administration. Drug concentrations were measured using high performance liquid chromatography. Plasma grapiprant concentrations ranged from 71 to 149 ng/mL with the mean peak concentration (110 ng/mL) occurring at 30 minutes. Concentrations were below the lower limit of quantification (50 ng/mL) in all 6 horses by 2 hours, and 4/6 horses at 1 hour. Urine grapiprant concentrations ranged from 40 to 6240 ng/mL and were detected at 48 hours after administration in the horses. An analytical method was developed that produced a linear curve for the concentration range used (50-2500 ng/mL). Intra and inter-assay variability were less than 10% and the average recovery was 94%. Currently there are no reports describing the effects of grapiprant in horses. A minimum concentration of 114 - 164 ng/mL is necessary for analgesia in dogs and this was not achieved with and oral dose of 2 mg/kg in horses. The dose was well tolerated therefore studies with larger doses could be conducted.

E64

Antimicrobial Hydrogel Dressings for Chronic Wounds

Hunter R. Greer – Department of Large Animal Clinical Sciences, Texas A&M University; Stacy Cerceeres – Department of Biomedical Engineering, Texas A&M University; Ellen Ruth Alexander – Department of Large Animal Clinical Sciences, Texas A&M University; Canaan Whitfield-Cargile-LAMR – Department of Large Animal Clinical Sciences, Texas A&M University; Elizabeth Cosgriff-Hernandez – Department of Biomedical Engineering, University of Texas at Austin; Noah Cohen – Department of Large Animal Clinical Sciences, Texas A&M University

Chronic wound infections are a major problem for both human and equine health. The profound health and economic impacts of chronic wounds create a critical need for more effective antimicrobial dressings. Gallium maltolate (GaM) is a metallic compound that exhibits antimicrobial activity against several pathogenic bacteria associated with chronic wounds. Our long-term objective is to create a GaM-infused hydrogel matrix as an antimicrobial dressing to treat chronic wounds, even in the presence of biofilms. The objective of this study is to develop a matrix with a dual release system, exhibiting both burst and sustained GaM release to immediately treat infection and prevent further colonization. The hydrogels were fabricated by first dissolving poly(ethylene glycol) diacrylate (PEGDA) and Irgacure in water, followed by photopolymerization of the solution with UV light. The hydrogels were vacuum dried and loaded with a 10% GaM solution. The loaded hydrogels were soaked in water, and the GaM concentration was determined by UV-VIS spectroscopy. Burst release of GaM was observed after 1 hour with complete release occurring by 6 hours. The bactericidal activity of the released GaM was determined by a microdilution antimicrobial susceptibility technique against strains of Staphylococcus aureus (SA), including methicillin-resistant SA (MRSA). The optical density (OD) at a wavelength of 625nm was measured as an estimate of bacterial growth and inhibition. The hydrogel releasate did not differ significantly (P > 0.05) from the negative control for both strains, whereas the releasate differed significantly (P < 0.05) from the positive control. Thus, the hydrogel releasate inhibited bacterial growth of both SA and MRSA at a GaM concentration of 4mg/ml. This study provides key preliminary data for the in vivo use of this novel antimicrobial hydrogel dressing as a treatment for chronically infected wounds in people, horses, and other animals.

E63

Determination of Grapiprant (Galliprant®) Concentrations in Horses

Sherry Cox – University of Tennessee College of Veterinary Medicine; Carla Sommardahl – University of Tennessee; Chelsey Fortner – University of Tennessee; Rebecca Davis – University of Tennessee; Joan Bergman – University of Tennessee; Tom Doherty – University of Tennessee

The use of selective non-steroidal anti-inflammatory drugs (NSAIDs) to treat inflammation and pain is widely accepted clinically. NSAIDs are inhibitors of cyclooxygenase (COX) in tissues, and are used as therapeutic agents in different species because of their anti-inflammatory, analgesic and antipyretic effects. Grapiprant is a member of the piprant class of compounds that antagonize prostaglandin receptors. It is a highly selective EP4 prostaglandin PGE2 receptor inhibitor, thereby limiting the potential for adverse effects caused by wider COX inhibition. The objective of this study was to determine if the approved dose in dogs would produce measurable concentrations in horses and to develop and validate a method for analysis of urine and plasma.

Horses were administered 2 mg/kg grapiprant via nasogastric tube. Blood and urine samples were collected prior to and up to 48 hours after drug administration. Drug concentrations were measured using high performance liquid chromatography. Plasma grapiprant concentrations ranged from 71 to 149 ng/mL with the mean peak concentration (110 ng/mL) occurring at 30 minutes. Concentrations were below the lower limit of quantification (50 ng/mL) in all 6 horses by 2 hours, and 4/6 horses at 1 hour. Urine grapiprant concentrations ranged from 40 to 6240 ng/mL and were detected at 48 hours after administration in the horses. An analytical method was developed that produced a linear curve for the concentration range used (50-2500 ng/mL). Intra and inter-assay variability were less than 10% and the average recovery was 94%. Currently there are no reports describing the effects of grapiprant in horses. A minimum concentration of 114 - 164 ng/mL is necessary for analgesia in dogs and this was not achieved with and oral dose of 2 mg/kg in horses. The dose was well tolerated therefore studies with larger doses could be conducted.

E65

Pharmacokinetics of Sulfadiazine and Trimethoprim in Neonatal Foals

Elsbeth A. Swain – Colorado State University; Daniel Gustafson – Colorado State University; Patrick McCue – Colorado State University

The antimicrobial combination of sulfadiazine (SDZ) and trimethoprim (TMP) has a known pharmacokinetic profile in adult horses, though neonatal foal parameters have limited investigation. Neonatal foals have unique pharmacokinetics compared to adults, which may lead to...
adverse effects of certain drugs when adult dosage regimens are used, including gastrointestinal microbiota disruption (dysbiosis). The purpose of this study was to determine the pharmacokinetics of a SDZ-TMP suspension in six healthy neonatal foals with oral administration twice daily at 24 mg/kg for 10 days. Blood samples were collected at serial time points at steady state after the fifth dose of SDZ-TMP and again at days 5 and 10 to monitor the influence of age within the neonatal period. Pharmacokinetic parameters were determined using a one-compartment model analysis. At steady state (approximately 72 hours of age), mean $C_{\text{max}}$ was $37.8 \pm 13.4$ mg/ml (SDZ) and 1.92 $\pm 0.25$ mg/ml (TMP). Mean $T_{\text{max}}$ was 1.4 $\pm 0.6$ hours (SDZ) and 1.4 $\pm 0.4$ hours (TMP). Mean $C_{\text{min}}$ for SDZ and TMP were $21.7 \pm 12.5$ mg/ml and 0.58 $\pm 0.41$ mg/ml, respectively. Mean Elimination half-life was 10.8 $\pm 6.1$ hours (SDZ) and 6.5 $\pm 2$ hours (TMP). Mean AUC was $667 \pm 424$ mg*h/ml (SDZ) and $21.1 \pm 5.3$ mg*h/ml (TMP). All foals remained healthy with normal clinicopathologic findings. The plasma concentration of SDZ and TMP remained above MIC(90) for the duration of the study period. These findings support a dose reduction in neonates if there are concerns for dysbiosis.

E66

Utilization of Serum Amyloid A in Managing the Clinical Progression of Equine Bacterial Pneumonia

Rodney Belgrave - Mid-Atlantic Equine Medical Center; Rachel Lemcke - Mid-Atlantic Equine Medical Center

Monitoring serum amyloid A (SAA) allows for reliable, real-time quantification of systemic inflammation throughout progression and treatment in equine bacterial pneumonia cases. Bacterial pneumonia poses a grave health risk to the equine population. Case severity and potential associated complications can extend recovery times, making proactive management critical. Our objective is to correlate SAA levels with pneumonia severity and resolution to determine if SAA is an appropriate biomarker for monitoring response to antimicrobial therapy and progression of recovery. We will evaluate if SAA levels differ between different classes of pneumonia, including cases with abscessation and pleural effusion. Fifty-five horses diagnosed with bacterial pneumonia at a large private equine hospital were evaluated relative to a healthy cohort (n=20). Initial SAA values in pneumonia patients averaged 2009 $\pm 1116$ ug/mL (range 0-4625 ug/mL), while initial SAA values in healthy horses averaged 1:4 ug/mL (range 0-20 ug/mL) ($p < 0.000001$). Sensitivity, specificity, and accuracy for the initial SAA at a cutoff value of 50 ug/mL were calculated as 98.2, 100.0, and 98.7, respectively. Positive and negative predictive values were 100.0 and 95.2, respectively. Sensitivity of fibrinogen and total WBC on the initial day of testing were 61.5 and 46.2, respectively. By days 7-10 of treatment, several cases had an SAA value below 500 ug/mL, though the majority of cases with abscessation and pleural effusion took days or weeks longer to normalize. At time of discharge, SAA averaged 489 ug/mL. In this study, SAA was unable to statistically differentiate between cases with abscessation, pleural effusion, or those that required thoracocentesis, though statistical power and use of serial dilutions were limited. The non-specific quality of SAA may also help reveal developing secondary complications such as enterocolitis or pleural effusion. Nine of eleven cases that developed a subsequent rise in SAA after a strong downwards trend or full normalization had pulmonary or pleural abscesses. Abscess aspiration and lavage was shown to be associated with the increase in SAA in many of these cases. While SAA is a successful, sensitive monitoring tool in the diagnosis and management of equine bacterial pneumonia cases, it is imperative to consider SAA values alongside other diagnostic techniques.

E67

Streptococcus equi Subspecies equi Point-of-Care PCR Validation

Andrew T. Willis – UC Davis; Nicola Pusterla – UC Davis; Samantha Barnum – UC Davis

The purpose of the study was to validate a point-of-care (POC) PCR for the detection of Streptococcus equi subspecies equi (S. equi) in respiratory secretions from horses with strangles and compare the results against the molecular gold standard of quantitative real-time PCR (qPCR).

This was a prospective, randomized, blinded laboratory evaluation. There were a total of 232 individual respiratory secretions. Three Fluxergy Analyzers and PCR test kits specific to S. equi were used. Samples were characterized as S. equi positive [high, medium, and low cycle threshold (CT)], S. equi ss zooepidemicus (S. zoo) positive, and S. equi and S. zooepidemicus negative by qPCR. Secretions were immersed in 1 mL of PBS. Nucleic acids were extracted from the samples and analyzed for the presence of both streptococcal pathogens using a Biosystems 7900 HTA. An aliquot of samples was tested using the Fluxergy platform.

A sensitivity of 88.7% was observed for the Fluxergy PCR when detecting samples deemed positive by qPCR. Specificity was 100% for not detecting S. zoo and true negative samples. For strongly positive and moderately positive bacterial loads (CT values 35).

Fluxergy’s POC device showed strong agreement with qPCR and detected S. equi in the majority of the study samples. The turnaround-time (less than 60 minutes/run) was reduced to less than one hour. Strong agreement and short turnaround-time makes Fluxergy’s POC device the first molecular diagnostic platform allowing detection of S. equi on the stall-side. The availability of an accurate POC for the detection of S. equi will enhance the diagnostic capability of equine veterinarians to timely support a diagnosis of strangles and institute proper biosecurity protocols.

E68

Phenotyping of Airway Mast Cell Proteases and TNFα Concentrations in Healthy, Asthmatic, and Indeterminate Horses

Jane S. Woodrow – University of Tennessee; Melissa Hines – University of Tennessee; Carla Sommardahl – University of Tennessee; Bente Flatland – University of Tennessee; Elizabeth Lennon – University of Pennsylvania

The pathogenesis of equine asthma syndrome, including mild/moderate and severe asthma, involves a milieu of cell types and cytokines with mast cell presence in bronchoalveolar lavage fluid (BALF) most
appreciated in mild/moderate asthma. Mast cells can be phenotyped or subtyped based on the proteases they contain; specifically tryptase, chymase, and carboxypeptidase A3. Molecular phenotypes of mast cells in induced sputum from human asthmatics correlates with asthma type and responsiveness to corticosteroids. Increasing tryp-tase concentrations in asthmatic horse BALF has been appreciated, but other mast cell protease levels are ill-defined. Additionally, TNFα can be made by mast cells and other cells of the lung. TNFα concentrations in equine BALF remain ill-defined due to studies focusing on one type of asthma. Findings have shown decreased concentrations in exacerbated severe asthma and increased concentrations in mild to moderate asthma versus controls. The objective of this study was to profile mast cell proteases in BALF of healthy, asthmatic, and indeter-minate horses by analyzing gene expression of CD117 (c-kit receptor), tryptase, chymase (CMA1), and carboxypeptidase A3 (CPA3) in BAL cell pellets; as well as measure TNFα concentrations in the BALF supernatant. This is part of a larger study investigating several cyto-kines found in BALF supernatant across the three groups. Horses were recruited from the institution’s clinic clientele and research herd. Horses enrolled were ≥ 1 year old and systemically healthy. CBC/fibrinogen and BALF cytology was performed by the institution’s clinical pathology laboratory. Horses were categorized based on history, physical exam, and diagnostics performed by the institution’s large animal internal medicine service in to three main categories: 1) healthy, 2) asthmatic (further divided into subtype), defined using current ACVIM criteria, and 3) indeterminate, defined by clinical signs of lower airway inflammation with a BALF cytology inconsistent with asthma. BALF cell pellets and supernatants were obtained, aliquoted, and kept at ≤-80°C until further processed. RNA was extracted using a commercially available kit (Qiagen RNeasy Mini Prep Kit). RNA (200ng) was reverse transcribed to cDNA and used to detect expression of CD117, tryptase, CMA1, and CPA3 using gene expression assays (TaqMan) and RT-qPCR. Gene expression was normalized to β-actin and relative quantification was performed using -2ΔΔct method. TNFα concentration of neat BALF supernatant was determined via commercially available enzyme-linked immunosorbent assay according to manufacturer’s instructions (R&D Systems DuoSet ELISA Equine TNFα). Data distribution was tested by a Shapiro-Wilk normality test. Nonparametric data was log transformed when available to achieve normal distribution. Significant difference in protease and cytokine levels was determined by a One way Analysis of Var-iance for parametric data and a Kruskal Wallis test for nonparametric data. Correlations were performed using Spearman nonparametric correlation. Significance was set at p ≤ 0.05. In total, 37 horses have been enrolled; 18 asthmatic (8 mild/moderate and 10 severe asthma), 10 indeterminate, and 9 healthy. Preliminary results showed no significant differences in mast cell protease expression, but there was a decreasing trend in tryptase expression in horses with severe asthma versus healthy (p = 0.10). High CD117 expression was correlated with high tryptase expression (r = 0.945, p < 0.0001). CPA3 was not detected in any samples. TNFα concentrations among healthy, mild/moderate, and severe asthma horses were not significantly different, but horses with mild/moderate asthma had significantly increased TNFα versus indeterminate horses (p = 0.004).

These results support continued investigation of mast cell protease levels in a larger cohort of horses, as well as investigating additional cytokine concentrations in horses with asthma to further define healthy, indeterminate, mild/moderate, and severe asthma. The strong correlation between CD117 and tryptase implies that, in equine asthma, mast cells express primarily tryptase, but confirmation using immunostaining techniques would be necessary. Follow up studies correlating mast cell protease expression level, cytokine concentrations, cytology results, and clinical diagnosis are planned. Prospective studies following response to treatments would follow.

E69

Tracheal Bacterial Populations in Horses with Mild To Moderate Equine Asthma

Estelle Manguin – Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal; Elizabeth Pépin – Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal; Roxane Boivin – Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal; Mathilde Leclere – Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal

We hypothesized that the respiratory microbiota of horses with mild to moderate asthma is altered, and that there is a link between lower airway bacterial populations and pulmonary inflammation. Our objectives were 1) to quantify bacterial populations in horses with asthma using culture and quantitative PCR, 2) to compare aerobic culture and PCR, 3) to correlate bacterial populations with bronchoalveolar lavage (BAL) inflammation.

Ten controls and 18 horses with mild to moderate asthma were enrolled, based on the presence of compatible clinical signs and BAL inflammation. Aerobic culture was performed on tracheal aspirates, and specific primers were designed for different Streptococci, Pasteurella spp, Chlamydia spp, Mycoplasma spp, and for the 16S ribosomal subunit (rDNA) for PCR quantification.

Streptococcus spp, Actinobacillus spp and Pasteurellaceae were the potential pathogens most commonly isolated by culture and Streptococcus spp and Chlamydia spp were most commonly identified by PCR, with no significant differences between groups. There was a positive correlation between Streptococcus spp by PCR and 16s rDNA in the asthma group only (Spearman, r = 0.76, P < 0.003), but the overall bacterial load (16s) was significantly lower in horses with asthma (t-test on log-transformed data, P < 0.05). There was no association between microbial populations and clinical signs, tracheal mucus or BAL inflammation.

This study does not support that bacterial overgrowth is a common feature of mild to moderate equine asthma. Lower bacterial load and correlation between 16s rDNA and Streptococcus spp in the asthma group could suggest dysbiosis of the lower airways.

EN01

Comparison of a Novel Continuous Insulin Protocol to Standard of Care Treatment for Diabetic Ketoacidosis

Ellen R.E. Heinrich – Iowa State University College of Veterinary Medicine; Dana LeVine – College of Veterinary Medicine, Iowa State University; Rebecca Walton – College of Veterinary Medicine, Iowa State University; April Blong – College of Veterinary Medicine, Iowa State University;
Canine diabetic ketoacidosis (DKA) is a common life-threatening complication of diabetes mellitus. Canine DKA is standardly treated with a continuous rate infusion (CRI) of regular insulin that is discontinued when blood glucose (BG) falls below a specific range. Dextrose supplementation is added based on BG as outlined in a widely-used published table. Resolution of DKA using this protocol usually takes several days resulting in owner financial burden. Standard of care in human medicine involves a continuous low-dose regular insulin CRI that is never stopped coupled with varying dextrose supplementation. This protocol leads to quicker resolution of DKA than reported in dogs.

The purpose of this study was to compare time to resolution of ketosis and acidemia using the novel versus standard of care insulin protocols in dogs treated for DKA. A retrospective medical records search for canine DKA patients from 2014-2018 identified 20 cases treated standardly and 10 cases treated with the novel continuous insulin protocol. The mean time to resolution of ketosis using the novel protocol (32 h ± 17 h) was significantly less than when using the standard protocol (94 h ± 46 h), (p < 0.01, t-test). Similarly, mean time to resolution of acidemia was shorter with novel (29 h ± 19 h) versus standard protocol (59 h ± 29 h), (p < 0.01, t-test). These data suggest that the novel continuous low-dose regular insulin CRI protocol results in faster resolution of ketosis and acidemia in dogs with DKA compared to the current standard protocol and warrants further prospective comparative assessment.

In healthy cats, mean glucose concentration was significantly lower (P = 0.0312) during the day (4.2, 3.9-4.8 mmol/l) than during the night (4.5, 4.0-5.0 mmol/l). Also, SD during the day (0.4, 0.3-0.5) was significantly lower (P = 0.0312) than during the night (0.6, 0.5-0.6). There were no significant differences between day and night with regard to minimum and maximum glucose concentrations and CV. In diabetic cats, minimum glucose concentration during the day (5.4, 3.4-15.4 mmol/l) was significantly lower (P = 0.032) than during the night (7.7, 4.1-17.1 mmol/l). There were no significant differences between day and night with regard to mean and maximum glucose concentration, SD and CV.

In conclusion, healthy cats showed higher glycemic variability during the night but absolute differences were small; glycemic variability in diabetic cats did not differ between day and night. Glycemic variability in diabetic cats was higher compared to healthy cats irrespective of the time of the day.

EN03

Determination of Thyroid Function in 131I Treated Hyperthyroid Cats: Serum Thyroid Variables Compared to Scintigraphy

Lisa Stammeleer – Ghent University; Eva Buresova – Ghent University; Emmelle Stock – Ghent University; Eva Vandermeulen – Ghent University; Luc Duchateau – Ghent University; Sylvie Daminet – Ghent University

Development of iatrogenic hypothyroidism in 3 - 79 % of hyperthyroid cats after treatment with radiiodine (131I) is of concern. Most institutions report a prevalence of < 9 %. Iatrogenic hypothyroidism can lead to azotemia and reduced survival time. Limited data suggest that serum thyroid stimulating hormone (TSH) concentration appears to be a sensitive and specific serum test to identify patients with iatrogenic hypothyroidism in azotemic and non-azotemic cats after 131I.
treatment. Correct identification of suboptimal thyroid function is indispensable to improve life quality and survival time in $^{131}$I treated hyperthyroid cats.

The objective of this study was to identify the most appropriate serum thyroid variable in the determination of thyroid status compared to thyroid scintigraphy in euthyroid, non azotemic cats after $^{131}$I treatment.

Forty-seven hyperthyroid non-azotemic cats presented for $^{131}$I treatment (T0) between 2015 and 2018 at the Small Animal Department of Ghent University, Belgium were prospectively enrolled. The patients were reevaluated one month (T1; n = 47) and 11-23 months (T2; n = 25) after $^{131}$I-treatment. Baseline evaluation included complete physical examination, blood pressure, thoracic radiographs, abdominal ultrasound and echocardiography, hematology, biochemistry including serum thyroid parameters [total T4 (TT4), free T4 after equilibrium dialysis (FT4), TSH], urinalysis and quantitative scintigraphy (sodium 99mTc-pertechnetate). Physical examination and serum thyroid parameters were repeated at T1 and T2. Quantitative scintigraphy was repeated only at T2. Scintigraphic diagnosis of hypothyroidism was based on a lowered value of at least three of the four following variables: thyroid-to-salivary gland ratio (T/S-ratio), percent thyroidal uptake of the administered 99mTc-pertechnetate (%TcTU), thyroid-to-tracheal background ratio (T/TB-ratio) and calculated thyroid volume. At T2, cats on thyroid hormone supplementation were excluded (n = 3).

Thyroid scintigraphy classified all cats as being euthyroid T2. T/S-ratio was normal in all cats (mean 0.94; range 0.49 - 1.51). Mean T/B ratio was 2.5 (range 1.47 - 3.40) and abnormal in only 2/22 cats (9 %). Mean calculated thyroid volume was 492.40 (range 159.14 - 2798.99). In contrast, %TcTU was within reference interval (RI) only in 6/22 (27 %) cats. Mean serum TT4 concentration at T1 and T2 was 15.45 nmol/L (range 9 - 103 nmol/L) and 19.32 nmol/L (range 9 - 28.3 nmol/L), respectively. Mean serum FT4 concentration at T1 and T2 was 15.17 pmol/L (range 5 - 55.4 pmol/L) and 16.86 pmol/L (3.6 - 26.3 pmol/L), respectively. Serum TT4 and serum FT4 concentrations were within RI in the remaining cats (n = 21 and n = 22 cats, respectively). Mean serum TSH concentration at T1 was 0.14 (range 0.03 - 0.95 ng/mL) compared to 0.37 ng/mL (range 0.03 - 3.08 ng/mL) at T2. Serum TSH concentration was above 0.3 ng/mL (range 0.37 - 3.08) in 6/22 (27 %) of cats at T2. In these 6 cats with serum TSH concentration > 0.3 ng/mL no difference between uni- or bilateral disease before treatment was noted. Only in one of these 6 cats with serum TSH concentration > 0.3 ng/mL another serum thyroid variable, namely FT4, was below RI.

Of the 4 thyroid variables calculated, T/S ratio was the most consistent variable for determination of euthyroid status. In our euthyroid population, measurement of serum TT4 and FT4 concentration showed good agreement with determination of thyroid status based on scintigraphy. Prudence is advised when interpreting solely serum TSH concentration for determination of thyroid status in $^{131}$I treated cats. Based on the results of this study serum TSH concentration can be elevated in euthyroid non-azotemic cats after $^{131}$I treatment without presence of overt iatrogenic hypothyroidism.

**EN04**

**Comparison of Pharmacodynamics Between Insulin Glargine 100U/mL and Insulin Glargine 300U/mL in Healthy Cats**

Chen Gilor – UC Davis; Brett Wasik – UC Davis; Jolly Pires – UC Davis; Dustin Leale – UC Davis; Navneet Saini – UC Davis; Nina Quach – UC Davis; William Culp – UC Davis

In people, insulin glargine (IGla) 300U/mL (Toujeo®) is associated with longer duration of action (TDA) and lesser within-day variability compared to IGla 100U/mL (Lantus®). We hypothesized that compared to Lantus®, Toujeo® would have a lower peak action and longer TDA in cats.

The pharmacodynamics of Toujeo® and Lantus® were studied in 7 healthy neutered male cats, each receiving 0.8 U/kg SQ injection of each formulation on two different days, > 1 week apart, in random order. Pharmacodynamics were determined by the isoglycemic clamp method: blood glucose (BG) was measured every 5 min and glucose was administered intravenously at a variable rate, aiming for BG = 90% of baseline. Glucose infusion rate was used as a measure of exogenous insulin action. The Shapiro-Wilk test was used to assess normality. Normally distributed parameters were compared using paired t-tests.

There were no differences in total metabolic effect (ME), onset of action, or peak action. TDA (mean ± SD) of Toujeo® (16.8 ± 4.7h) was not significantly different than TDA of Lantus® (13.4h ± 2.6h, P = 0.2). In 6 cats the TDA of Toujeo® was longer than (median [range]) 4.8h (0.3-11.3h) while in the seventh cat the TDA of Lantus® was longer (7.8h). The 12-24h post-injection ME fraction out of the 0-24h ME was (mean ± SD) 35% ± 23% for Toujeo® vs. 7% ± 8% for Lantus® (P = 0.048), indicating a more even distribution of ME over the 24h period.

The more even distribution of ME of Toujeo® over a 24h period, coupled with a TDA > 16h, makes Toujeo® more suitable for once-daily administration in cats.

**EN05**

**Evaluation of Hematologic Abnormalities of Dogs with Diabetes Mellitus**

Tanner S. Slead – Purdue University College of Veterinary Medicine; Andrew Woolcock – Purdue University College of Veterinary Medicine; George Moore – Purdue University College of Veterinary Medicine

Diabetes Mellitus (DM) is a common endocrine condition in dogs, with extensively studied pathophysiology and sequelae that is often diagnosed on routine lab work such as chemistry profile and urinalysis. A complete blood count (CBC) is often included in the routine diagnostic evaluation of a diabetic, however, previously described CBC abnormalities in diabetic dogs were limited to non-specific inflammatory changes. Other CBC parameters, namely cell morphology and blood smear analysis, have not been described in diabetic dogs. The primary aim of this study was to evaluate hematologic abnormalities, including the quantifiable variables and blood smear analysis, in diabetic dogs. A secondary aim was to evaluate any potential associations between common hematologic abnormalities and co-morbid diseases in diabetic dogs.
ABSTRACTS

Medical records were retrospectively evaluated for diabetic dogs (DD), dogs with other systemic diseases (sick non-diabetics; SND), and healthy dogs (HD). Complete blood count data was evaluated for each patient including the erythrogram, leukogram, and thrombogram, and any cell morphologic changes were documented. In addition, all diagnoses including co-morbid diseases were documented. Morphologic changes were classified as either low-grade (Absent to 1+) or high-grade (2+ or higher). Co-morbid diseases were categorized by major body system involvement as well as for the presence/absence of DKA and pancreatitis. Groups were compared using contingency tables and logistic regression. Multivariate analysis was used to evaluate associations between hematologic abnormalities and co-morbid diseases in diabetic dogs.

A total of 1,439 CBC from 1,035 dogs were included in the study. The DD group contained 648 CBC from 312 dogs. The SND contained 285 CBC from 285 dogs. The HD group contained 506 CBC from 438 dogs.

The DD group was significantly older than the SND and HD groups (P = 0.0001), but no association was identified between age, sex, or neuter status and any hematologic abnormalities. DD had lower red blood cell (RBC) and hemoglobin (Hgb) concentrations, lower hematocrit (Hct), and higher white blood cell (WBC), and platelet (PLT) counts when compared to SND and HD (all resulted in p < 0.0001). High-grade target cells, high-grade anisocytosis, and high-grade lipemia were identified more frequently in DD than in SND and HD, while high-grade reactive lymphocytes and high-grade keratocytes were identified less frequently in DD when compared to SND and HD (all resulted in p < 0.0001). Diabetic dogs with high-grade target cells and high-grade anisocytosis had lower RBC and Hgb concentrations, lower Hct, and higher WBC counts when compared to DD with low-grades of these morphologic changes, as well as when compared to SND and HD (all resulted in p < 0.0001). Multivariate analysis of diabetic dogs showed that increasing grade of target cells was associated with co-morbid diseases (odds ratio: 4.05; p < 0.0001) and a 5x higher likelihood of being concurrently diagnosed with immune-mediated disease (odds ratio: 5.08; p = 0.047). No other morphologic cell type yielded significant associations with co-morbid diseases.

DKA was not shown to have significant associations with cell morphology on logistic regression, however, DKA was diagnosed more frequently in DD with high-grade target cells (p < 0.0001) and high-grade lipemia (p=0.004) when compared to those with low-grades of these morphologic changes, or when comparing the other cell morphologies.

Findings of this study confirm that there are common hematologic abnormalities in diabetic dogs that may aid in the diagnostic evaluation of these patients. Inflammatory changes and dyslipidemias are common in diabetic dogs, but are non-specific. Routine hematology in diabetic dogs should include blood smear analysis to evaluate cell morphology. Identification of target cells in diabetic dogs increases the likelihood of a concurrent diagnosis of pancreatitis or immune-mediated disease. High-grade target cells should prompt evaluation for the presence of ketones, particularly when found concurrently with high-grade lipemia.

EN06

Comparison Between Glucose Measurements in Canine Blood Using Different Settings On the AlphaTRAK Monitor

Lydia Peña – Auburn University College of Veterinary Medicine; Ellen Behrend – Auburn University College of Veterinary Medicine; Alba Arzon-Pereira – Auburn University College of Veterinary Medicine; Janeva Cole – Auburn University College of Veterinary Medicine; Maggie Raz – Auburn University College of Veterinary Medicine

Veterinary glucometers need to be set for the patient’s species. If a meter it is not set accurately before use, common practice is to redo the measurement. If the readings obtained do not differ greatly between species, however, a second blood sample may not be needed. The purpose of this study was to compare glucose concentration values obtained from canine blood when measured on both the cat and dog settings on an AlphaTRAK 2.

Blood samples were collected from 53 dogs. Immediately following venipuncture, blood was placed in a heparinized tube. A single AlphaTRAK 2 specifically designated for the study was used. The samples were read initially on the “dog” setting (AlphaTRAK dog) and then were read on the “cat” setting (AlphaTRAK cat). Glucose concentration was also measured by a biochemical analyzer and considered to be the gold standard. AlphaTRAK dog concentrations, AlphaTRAK cat concentrations and the laboratory measurement were compared by a repeated measures ANOVA on ranks. Post hoc testing used the Tukey test. Differences between the glucometer and laboratory measurements were calculated (laboratory value was subtracted from the glucometer reading) and compared using a Wilcoxon Signed Rank test. The difference between the glucometer readings was also calculated (AlphaTRAK cat subtracted from AlphaTRAK dog). Significance was set at p < 0.05.

AlphaTRAK cat concentrations were significantly different from AlphaTRAK dog and laboratory measurements (p < 0.001). The median difference between AlphaTRAK dog and the laboratory measurement was 1 mg/dL (range −43 – 66 mg/dL). The median difference between AlphaTRAK cat and the laboratory measurement was −8 mg/dL (range −39 – 65 mg/dL). These two differences were significantly different from each other (p < 0.001). The median difference between AlphaTRAK dog and AlphaTRAK cat was 10 mg/dL (range −34 – 29 mg/dL); the difference was < 20 mg/dL in 89% of samples.

Thus, for canine blood, glucose concentrations obtained using the AlphaTRAK 2 are significantly different if the meter is set to dog vs. cat. However, given the relatively small absolute differences, the clinical significance appears low. For the majority of dog samples, a measurement on the AlphaTRAK 2 need not be repeated if the glucometer is inadvertently set to “cat”; if the concentration is particularly important, e.g. it would cause an insulin dose to be changed, remeasurement on the dog setting is recommended.

EN07

The Utility of Serum, Plasma and Whole Blood Glucose Measurement On a Point-of-Care Glucometer

Matthew J. Lechner – University of Pennsylvania; Rebecka Hess – University of Pennsylvania

The objective of this prospective clinical study was to determine the correlation between blood glucose concentrations (BG) measured by a
An Epidemiological Study of Dogs with Diabetes Mellitus Attending Primary Care Veterinary Clinics in Australia

Linda M. Fleeman – Animal Diabetes Australia; Samuel Yoon – Sydney School of Veterinary Science; Bethany Wilson – Sydney School of Veterinary Science; Caroline Mansfield – Melbourne Veterinary School; Paul McGreevy – Sydney School of Veterinary Science

The objective was to determine the prevalence, risk factors, and comorbidities associated with diabetes mellitus (DM) in dogs attending primary care veterinary clinics in Australia.

Electronic medical records of dogs (n = 134,329) that attended 152 clinics during 2017 were sourced through VetCompass Australia. A nested case-control study was used, with cases and age-matched controls from the same cohort and time-frame frequency matched at a 1:4 ratio. Logistic regression models analyzed risk factors, and binary logistic regression analyses and Fischer’s exact tests were performed.

Four hundred and eighty-one dogs with DM were identified resulting in a prevalence of 0.36%. Breeds (and their crosses) with significantly higher odds of having DM were Australian terriers (Odds Ratio (OR) 7.93), Siberian huskies (OR 6.24), English springer spaniels (OR 5.37), West highland white terriers (OR 4.85), Bichon Frises (OR 3.41), Schnauzers (OR 3.18), and Cavalier King Charles spaniels (OR 1.84).

Reduced age (<10 years) (OR 0.00), male sex (OR 0.00), and obesity (OR 0.00) had a lower risk of DM. Labrador retrievers were diagnosed more often than purebred Labradors (P = 0.04) and did not differ from Poodles (P = 0.81). Desexed male dogs had a higher risk of DM compared with entire males (P = 0.02) and desexed females (P = 0.02). Comorbidities associated with canine DM included suspected pancreatitis (OR 10.58), cataracts (OR 9.80), hyperadrenocorticism (OR 6.21), urinary tract infection (OR 5.09), and hypothyroidism (OR 4.10).

These findings will inform and guide future research on genetic and environmental influences of DM in dogs.

Field Efficacy and Safety of ProZinc® Insulin evaluated in 276 Dogs with Diabetes Mellitus

Cynthia R. Ward – University of Georgia; Kevin Christiansen – Boehringer-Ingelheim; Kimberly Jerrentrup – Boehringer-Ingelheim; Carla Kroh. – Boehringer-Ingelheim

The study evaluated the long-term efficacy and safety of protamine zinc human recombinant insulin (ProZinc®) in naïve and pre-treated diabetic dogs.

This was a prospective, historically controlled, multi-center study. 276 dogs with diabetes mellitus were enrolled based upon demonstration of hyperglycemia, glycosuria, and >1 diabetic clinical sign (polyuria, polydipsia, or weight loss). Insulin treatment was initiated at 0.5–1.0 IU/kg daily with dose titration as needed. Successful diabetic control was evaluated based upon improvement of one laboratory parameter (mean or minimum blood glucose (BG) from a 9-hour curve, or fructosamine) and one clinical sign. SID versus BID posology was also investigated.

224 dogs (126 naïve and 98 pre-treated) qualified for efficacy assessment on day 84. Diabetic control was achieved in 72.1% of dogs overall (80% naïve, 62% pre-treated) and in 71% of SID versus 74% of BID treated dogs. Overall, a significant decrease in mean and minimum BG compared to baseline was observed from day 21 onwards. In naïves, mean BG and fructosamine were significantly reduced by day 7 and 14, respectively. Polyuria and polydipsia improved in 89.8% and 88.3% of dogs.

The mean insulin dose observed in controlled diabetic dogs (SID and BID) was 1.4 IU/kg/day. Safety parameters were measured in 276 dogs for up to 182±5 days; hypoglycemia comprised 6.3% of adverse events. ProZinc® safely and effectively reduced glycemic parameters and clinical signs in naïve and pre-treated diabetic dogs. The high percentage of diabetic control in SID treated dogs confirms the long duration of action of ProZinc®.

Mean Cell Volume Difference (dMCV) is a Marker for Serum Hypertonicity in Diabetic Dogs

Olga C. Norris – Kansas State University; Thomas Schermerhorn – Kansas State University

Serum tonicity (OsMe) is important for volume regulation and water balance and is altered in many disorders, including diabetes mellitus (DM). Tonicity is not measured directly but can be estimated using known serum concentrations of effective osmoles or a surrogate marker, such as the dMCV (Mean Cell Volume difference). Hypertonicity is a frequent complication of untreated or complicated DM but its importance in chronic DM is unknown. The aims of this study were to 1) determine the prevalence of serum hypertonicity in clinically stable, insulin-treated diabetic dogs and 2) evaluate dMCV as a marker for hypertonicity in diabetic dogs. We hypothesized that an increased dMCV (>3 fl) predicts the presence of serum hypertonicity in dogs with chronic DM.
Dogs with clinically stable DM that had received insulin treatment for at least 1 month were enrolled. Dogs with newly diagnosed DM or those with evidence of diabetic ketoacidosis, hyperglycemic hyperosmolar syndrome, or concurrent illness that warranted hospitalization were excluded. Thirty-two diabetic dogs met the inclusion criteria; each had a complete blood count, serum biochemistry profile, and serum total osmolality performed. Serum concentrations of major effective osmoles (Na, K, anions, and glucose) were used to estimate OsM_L (median 314 mOsm/L; range 296-333 mOsm/L). dMCV (3 fl; -0.7-5.8 fl) was calculated as the difference between measured and calculated erythrocyte mean cell volumes. Serum total osmolality (OsMM) (321 mOsm/L; 302-376 mOsm/L) was determined by osmometry. Prevalences of hypertonicity (OsM_L >320 mOsm/L) and hyperosmolality (OsM_L >320 mOsm/L) were 43.75% and 56.25%, respectively. A dMCV >3 fl was associated with hypertonicity (OsM_L >320 mOsm/L; OR 5.8; p = 0.03) and hyperglycemia (GLU >250 mg/dl; OR 6.5; p = 0.02) but not serum total osmolality (OsM_L >320 mOsm/L; OR 2.7; p = 0.09).

The results show that serum hypertonicity due to hyperglycemia is common in dogs with chronic DM and likely reflects suboptimal glycemic regulation. A dMCV >3 fl is associated with hyperglycemia and hypertonicity and may be a useful indicator for these derangements. The dMCV is a convenient and effective marker for hypertonicity in diabetic dogs.

EN11

Prevalence of Anti-Insulin Antibodies in Diabetic Dogs Receiving Recombinant Human Insulin

Mary Lester – University of Florida Small Animal Hospital; Allison O’Kell – University of Florida

Anti-insulin antibodies (AIA) have been described in the literature in dogs treated with bovine and porcine insulin. Statements regarding prevalence of AIA in dogs receiving recombinant human insulin are mostly unreferenced, in abstract form, or do not indicate insulin preparation in antibody positive dogs. AIA have been anecdotally associated with poor glycemic control. The aim of this study was to determine prevalence of AIA in diabetic dogs treated with recombinant human (NPH) insulin. A secondary aim was to determine if insulin dosage or duration of therapy differed between antibody positive and negative diabetic dogs.

Banked serum from diabetic dogs receiving only recombinant human insulin for at least 2 weeks (n = 24) and healthy control dogs (n = 24) was analyzed by radioimmunoassay for insulin antibodies. A Fisher’s exact test compared the proportion of dogs positive for AIA between groups, and a non-parametric permutation test compared mean insulin dose and duration between the AIA positive and negative dogs.

Four diabetic (16.6%) and no control dogs were AIA positive (p = 0.11). There was no difference between duration of insulin therapy (p = 0.24) or dose (p = 0.26) between AIA positive and negative dogs.

In this study, prevalence of AIA in dogs treated with recombinant human insulin was less than that previously reported for bovine insulin (50 - 90%) and similar to that for porcine insulin (12%). Future larger studies to confirm these findings, exclude the presence of insulin auto-antibodies in positive dogs, and investigate effects of AIA on glycemic control are warranted.

EN12

Single-cell RNA Transcriptomic Analysis of Canine Insulinoma Reveals Distinct Sub-Populations of Insulin-Producing Cells

Lucy J. Davison – Royal Veterinary College and University of Oxford; Marsha Wallace – Royal Veterinary College and University of Oxford; Michael Heritage – University of Cambridge; Ruth Gostelow – Royal Veterinary College; Laura Owen – University of Cambridge; Lynda Rutherford – Royal Veterinary College; Katherine Hughes – University of Cambridge; Alice Denyer – Royal Veterinary College; Brian Catchpole – Royal Veterinary College; Chris O’Callaghan - University of Oxford

Canine insulinoma is a rare, but life-threatening, functional neuroendocrine tumor of pancreatic beta cells. Treatment is rarely curative and commonly involves surgical excision of the tumor, combined with medical management of hypoglycaemia. The development of single cell RNA-sequencing (scRNA-seq) to evaluate the RNA transcriptome of single cells has revolutionized our ability to dissect pathological processes, to identify unique features of malignant cells, and to study the tumor microenvironment. We undertook scRNA-seq on 3,634 individual cells from fresh canine insulinoma tissue (n=2 tumours and n=1 metastatic lesion) to generate new hypotheses about driving factors and potential treatment targets in canine insulinoma.

Transcriptomic profiles of individual cells were determined using 10x Genomics technology and high-throughput sequencing, with an average of 123,536 reads and median of 1,010 genes per cell. Bioinformatic analysis revealed distinct cell clusters (transcriptomic profiles), largely represented in both primary tumors. Unexpectedly, cells in two different clusters expressed insulin, and the proportion of cells belonging to these clusters differed substantially between patients. One insulin-expressing cluster was defined by high expression of CHGB (chromogranin B) and IAPP (islet amyloid polypeptide, or amylin). In contrast, the second insulin-expressing cluster was defined by high expression of CHGA (chromogranin A), MAP2 (microtubule associated protein 2) and SG3 (secretogranin). Both chromogranin A and islet amyloid polypeptide have been studied as diagnostic and prognostic markers in neuroendocrine tumours. This study demonstrates that scRNA-seq is feasible in fresh canine neuroendocrine biopsy tissues and has exciting potential to reveal new targets for diagnosis or therapy in canine insulinoma.

EN13

Heritability and Complex Segregation Analysis of Diabetes Mellitus in American Eskimo Dogs

Stephen V. Cai – University of Pennsylvania; Rebeca Hess – University of Pennsylvania; Thomas Fanula – University of California; Davis; Anita Oberbauer – UC Davis

The purpose of this study was to investigate the previously unre-ported heritability and mode of inheritance of DM in AED. An extended family of AED including 71 AED without DM, 47 AED with
an unknown phenotype, and 38 AED with spontaneous DM, was analyzed. A logistic regression model was formulated to evaluate the heritability of DM, including effects of sex and neuter status. Subsequently, complex segregation analysis was employed to investigate the inheritance pattern of DM in AED. Six plausible models were considered and the Akaike Information Criterion was used to determine the best model for DM inheritance in AED. Results of this study revealed that the heritability of DM in AED is estimated at 0.62 (95% confidence interval 0.01 - 0.99). Predicted DM probabilities for neutered females (NF), intact females (IF), neutered males (NM), and intact males (IM) were 0.76, 0.11, 0.63, and 0.12, respectively. There was no overlap between the 95% confidence intervals of disease probabilities in NF and IF or in NF and IM. Complex segregation analysis suggested that the mode of DM inheritance in AED is polygenic, with no evidence for a single gene of large effect impacting DM. It is concluded that the estimated heritability of DM in AED is high, but has low precision. DM transmission in AED follows a polygenic inheritance pattern with several large effect loci. Breeders could successfully implement a breeding program to decrease the incidence of DM in AED.

EN15

Application and Findings of a Novel Quality-of-Life Tool for Dogs with Hyperadrenocorticism

Imogen Schofield – Royal Veterinary College; Dan O’Neill – Royal Veterinary College; Dave Brodbelt – Royal Veterinary College; David Church – Royal Veterinary College; Stijn Niessen – Royal Veterinary College

Hyperadrenocorticism is accepted to negatively impact upon a dog’s life, however there are currently no tools to quantitatively assess this impact on a dog’s health-related quality-of-life (HRQoL). This study aimed to develop and apply a novel tool for standardised assessment of HRQoL in dogs with hyperadrenocorticism and to evaluate the factors that may negatively impact upon this.

An initial set of questions, specific to the HRQoL of dogs with hyperadrenocorticism, was developed through focus group discussions with owners of affected dogs, veterinarians and human endocrinologists. Owners of both affected and unaffected dogs were recruited to complete the questionnaire, answering the identified HRQoL questions and rating the importance of each, to themselves and their dog. Based on response variability and correlations, the questions were refined to develop the final HRQoL tool. A scoring system was developed for the HRQoL tool to allow interpretation. Reliability and validity of the tool were assessed using intra- and inter-rater measures, Cronbach’s alpha, principal components analysis (PCA) and the correlation with the owners’ assessment of HRQoL, using Pearson correlation coefficient. Evaluation of factors with a negative impact on HRQoL were made using non-parametric analyses.

Complete responses of the questionnaire were obtained from 210 owners of dogs with hyperadrenocorticism and 681 without hyperadrenocorticism. The HRQoL tool was refined from 26 questions to 19 which were found to best infer the HRQoL of a dog with hyperadrenocorticism, with good reliability (Cronbach’s alpha = 0.83). PCA of the 19 question HRQoL tool determined questions relating to ‘demeanour’ described the largest proportion of the data. Owners rated questions related to ‘owner impact’ as more important and questions related to ‘demeanour’ as less important. There was a positive correlation between the HRQoL tool score of dogs with hyperadrenocorticism and the owners own assessment of their dogs current quality-of-life (r = 0.41, p < 0.001). The HRQoL of dogs with hyperadrenocorticism did not depend on their age or the length of time since their diagnosis (p = 0.84 and p = 0.08 respectively). Dogs currently on treatment with trilostane were found to have a better HRQoL compared to those on alternative medical treatment or no treatment (p = 0.03).

The developed tool is a reliable, valid and interpretable method that can be used to identify specific areas negatively impacting on the HRQoL of dogs with hyperadrenocorticism. In this study, the HRQoL of dogs with hyperadrenocorticism was independent to age or time since diagnosis. Questions related to the dogs’ demeanour accounted for the largest proportion of the data but were rated less important by their owners. This tool warrants further validation for longitudinal use within a clinical or research setting.

EN16

Low-Dose DOCP Treatment of Hypoadrenocorticism in Dogs: A Randomized Controlled Clinical Trial

Alysha M. Vincent – Michigan State University College of Veterinary Medicine; Linda Okonkowski – Michigan State University College of Veterinary Medicine; Jean Brudvig – Veterinary Diagnostic Laboratory; Nora Berghoff – Veterinary Diagnostic Laboratory; Daniel Langlois – Michigan State University College of Veterinary Medicine

Desoxycorticosterone pivalate (DOCP) can effectively treat mineralocorticoid deficiency in dogs with hypoadrenocorticism (HA), but the recommended starting dosage of 2.2 mg/kg can be cost-prohibitive for some owners. Reports of lower starting dosages and prolonged treatment intervals also raise concerns that this dosage could result in overtreatment. The objectives of this study were to investigate the relative efficacy and adverse effect profile of 2 DOCP dosages. Dogs with newly diagnosed primary HA were randomly assigned to either low-dose (1.1 mg/kg, test population) or standard dose (2.2 mg/kg, control population) treatment groups. Both clients and investigators were blinded to treatment for the 3 month study duration. Routine clinical and laboratory parameters as well as plasma renin activities were assessed at each DOCP treatment (approximately every 30 days) and 10-14 days after each DOCP treatment. Eighteen dogs have completed this ongoing study. Hyperkalemia, hyponatremia, or a decreased sodium to potassium (Na:K) ratio were not observed in any dog in either treatment group. The mean Na:K ratio (reference interval, 29-37) was greater in the control population (37.0 ± 3.2) as compared to the test population (34.4 ± 3.7, P < 0.001). Hypokalemia was documented on 18 of 60 (30%) biochemical assessments in the control population as compared to 6 of 48 (12.5%) in the test population (P = 0.037). These initial results suggest that low-dose DOCP protocols are safe and effective for treatment of HA whereas standard dose DOCP protocols are more likely to cause biochemical abnormalities consistent with mineralocorticoid excess.
EN17

Long-Term Effect of Neutering on Plasma Luteinizing Hormone Concentrations in Cats
Joana Aguiar – Royal Veterinary College; Lucy Davison – Royal Veterinary College; Rob Fowkes – Royal Veterinary College; Harriet Syme – Royal Veterinary College

Gonadotropin hormones such as luteinizing hormone (LH) are structurally related to other glycoproteins including thyroid stimulating hormone. It is hypothesized that increased concentration of gonadotropins in neutered cats, lacking negative feedback from sex hormones, could be implicated in the hyperthyroidism pathogenesis due to receptor cross-reactivity. This study aims to determine the long-term effect of neutering on plasma LH concentrations in cats and the fluctuation across calendar seasons.

Plasma samples from client-owned cats from a research clinic, that were entire and older than 9 years of age or that had been diagnosed with chronic kidney disease, hyperthyroidism or hypertension were used, together with samples from age, sex and sample date matched neutered cats. Multiple samples from different dates were included for each cat. LH concentration was measured using a feline enzyme-linked immunosorbent assay. Differences in LH concentration and fluctuation were compared between groups using Mann-Whitney U test. A mixed model was used to compare differences between calendar seasons. Results are reported as median [25th, 75th] percentiles.

Eighteen entire and neutered cats (10 females, 8 males in each group) were included. LH concentrations were lower in entire (0.06 [0.3, 0.14] ng/ml) than neutered cats (0.13 [0.04, 1.48] ng/ml, P = 0.007). No difference was found across the seasons (P = 0.88) and the highest LH concentrations were outside the local estimated feline reproductive season. Overtime, LH concentrations in neutered cats fluctuated more than in entire cats (P = 0.006).

In conclusion, neutering causes significant long-term increases in LH concentrations in cats. Further research to determine whether this results in thyrocyte hyperplasia or hyperfunction is warranted.

EN18

Does Neutering Drive the Development of Hyperthyroidism in Cats? An Epidemiologic Study
Joana Aguiar – Royal Veterinary College; Lucy Davison – Royal Veterinary College; Rob Fowkes – Royal Veterinary College; Nick Cave – Massey University - Institute of Veterinary, Animal and Biomedical Sciences; Harriet Syme – Royal Veterinary College

Despite hyperthyroidism being the most common feline endocrinopathy, affecting >10% of cats above 9 years of age, its etiology is still not completely understood. Thyroid stimulating hormone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are structurally related glycoprotein hormones and cross-reactivity between them and their receptors has been demonstrated. FSH and LH concentrations increase as a consequence of neutering and it is hypothesized that this could drive the development of feline hyperthyroidism. This study aims to determine if there is an increased incidence of hyperthyroidism in neutered cats.

Cats from a nutrition colony who had survived to 9 years or over or had a diagnosis of hyperthyroidism were included. Data, including sex, neutered status, neutering age, hyperthyroidism status and date of diagnosis were analysed.

Of the 166 cats included, 51 cats developed hyperthyroidism (20 entire [19 females, 1 male]; 31 neutered [8 females, 23 males]) and 115 remained euthyroid (45 entire [38 females, 7 males]; 70 neutered [20 females, 50 males]). No difference was found between euthyroid and hyperthyroid cats with regards to their neuter status (P = 0.99) or age at neutering (P = 0.75). Age at diagnosis of hyperthyroidism did not differ in neutered (11.7 ± 1.4) and entire cats (11.1 ± 1.2 years; P = 0.18). Cox regression with time dependent covariates analysis found no difference in age at hyperthyroidism diagnosis between cats neutered before (P = 0.56; 95% confidence interval [CI] [0.61 - 2.4]) or after 12 months of age (P = 0.97; 95% CI [0.61 - 2.4]).

This study was unable to demonstrate an increased incidence of hyperthyroidism in neutered cats or an association between neutering age and development of hyperthyroidism.

EN19

Adrenal Incidentaloma: Clinical and Histopathological Characteristics
Viviani De Marco – Naya Endocrinologia Veterinaria; Rodrigo Ubugakata, DABROVET – PROVET; Isabella Sant’Anna – Santo Amaro University; Marcia Kahvegian – PROVET, Universidade Cruzeiro do Sul; Sara Galac – Faculty of Veterinary Medicine, Utrecht University, the Netherlands

Adrenal incidentaloma (AI) is defined as an incidentally discovered adrenal mass in a dog not suspected of having an adrenal disease. In this retrospective study we report on clinical and histopathological data in 22 dogs with an AI, detected by abdominal ultrasonography. Most dogs had no clinical complaints, except obesity in two dogs. Abdominal ultrasonography was performed as a part of wellness screening or for non-medical reasons. Characteristics on ultrasonography suggestive of an AI were abnormal adrenal shape, heterogeneous structure, increased adrenal thickness (>10 mm) and/or expansion into blood vessels. There were 15 females (14 neutered) and 7 males (7 castrated). Their mean ± SEM age was 11 ± 2 years. The most prevalent breed was Lhasa Apso (7), followed by Shih Tzu, Maltese and Dachshund (2). The median body weight was 7 kg (range, 4 to 32 kg). Urinalysis revealed good concentrating ability of kidneys with median specific gravidity of 1.040 (range, 1.015 to 1.050) and no glucosuria. Complete blood count was unremarkable, except slightly increased alkaline phosphatase, cholesterol and triglycerides in few dogs. The low-dose dexamethasone screening test was negative in all dogs. Median basal cortisol concentration was 1.8 μg/dcl (range, 0.8 to 2.7 μg/dcl) and at 8-hours 0.4 μg/dcl (range, 0.15 to 0.9 μg/dcl). There were 19 unilateral and 3 bilateral AIs with the greatest diameter ranging from 1.1 to 5.6 cm. In dogs with an unilateral AI, the contralateral adrenal gland was of normal structure and shape and the adrenal thickness was not increased. All dogs underwent unilateral adrenalectomy. In three dogs with bilateral AIs, only the largest adrenal mass has been removed. Histopathology disclosed adrenocortical adenoma in seven dogs (two bilateral), adrenocortical carcinoma in ten dogs (one bilateral), and a malignant pheochromocytoma in five dogs. This is
the first report on clinical features and histopathology of AI in a large group of dogs. Female dogs of small breeds and elderly age were the most commonly affected. The majority of AIs were of adenocortical origin, however, pheochromocytoma should be accounted as a differential diagnosis as well.

EN20

Investigation of Hyperlipidemia in Healthy Schnauzers in Brazil

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Hyperlipidemia refers to increased serum triglycerides (TG) and/or cholesterol (CHO) levels and has been frequently identified in dogs. It can be primary (idiopathic) or secondary to endocrinopathies (Diabetes mellitus, hyperadrenocorticism, hypothyroidism), obesity, pancreatitis, drug administration (glucocorticoids and phenobarbital). Primary and idiopathic hyperlipidemia has been associated to specific breeds, especially Schnauzers. The cause is currently unknown but possible mechanisms include increased production or decreased clearance of VLDL and chylomicrons, or both. Primary hypertriglyceridemia is very common in Miniature Schnauzers in the United States but the prevalence can vary widely depending on geographic region of the canine population tested. The aim of this study was to identify the frequency of primary hyperlipidemia in Brazilian population of Schnauzer dogs, as well as to characterize epidemiological aspects regarding age, sex, reproductive status and the severity of hypertriglyceridemia. A total of 196 healthy Miniature and Standard Schnauzers, with a good body condition score (BCS = 4–6/9), without signs of endocrinopathies (based on medical history and physical examination), were included in the study. Among the 196 dogs, 119 were females and 77 males, the median age was 6 years (range, 1–15 years) and median weight was 7 kg (range, 4–10 kg). Median serum concentrations of TG and CHO were 77.8 mg/dL (22–2,290 mg/dL) and 179.15 mg/dL (44–775.8 mg/dL), respectively. The frequency of hyperlipidemia (TG > 150 mg/dL or CHO > 200 mg/dL, after a 12-hours fasting) was 28% (n = 55 / 196). Isolated hypertriglyceridemia was observed in 52.7% of hyperlipidemic dogs (n = 29 / 55), hypercholesterolemia in 20% (n = 11 / 55) and mixed hyperlipidemia in 27.2% (n = 15 / 55) of cases. Among 55 dogs with hyperlipidemia, 43.63% (n = 24 / 55) had mild hypertriglyceridemia (150–400 mg/dL), 18.18% (n = 10 / 55) had moderate hypertriglyceridemia (400–1,000 mg/dL) and 18.18% (n = 10 / 55) presented severe hypertriglyceridemia (>1,000 mg/dL). Hypertriglyceridemia was more frequent in spayed females older than 5 years and castrated animals had higher triglyceride and cholesterol values than intact dogs. A positive linear regression was observed between age and serum TG concentration, demonstrating that hyperlipidemia increases with age. It was concluded that Brazilian healthy Miniature and Standard Schnauzers have a high prevalence of hyperlipidemia and it increases with age. Because it is a silent disease with important clinical complications, it is necessary to monitor serum triglycerides and cholesterol concentrations in Schnauzers, especially in animals older than 5 years.

EN21

Thinking Big of a Small Tumor: Glucagon Stimulation Test for Insulinomas

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The small physical dimensions of insulinomas challenge the detection limit of current diagnostic imaging modalities. Measurement of insulin and glucose concentrations is the current gold standard diagnostic test. However, long turnover time can delay diagnosis and treatment. Therefore, we developed a glucagon stimulation test, which exploits insulin’s induction of hepatic glycogenolysis to differentiate insulinomas from chronic hypoinsulinemic, hypoglycemic conditions that stimulate glycogenolysis. We hypothesized that hepatic glycogen stores would be higher in chronic, hyperinsulinemic hypoglycemia (insulinoma) than in chronic, hypoinsulinemic hypoglycemia. Thus, when stimulated with exogenous glucagon, dogs with insulinomas would have a greater increase in serum glucose concentrations compared to dogs with other causes of hypoglycemia.

We recruited client-owned dogs with serum glucose <3.5 mmol/L and four healthy adult Harrier hounds as controls. We differentiated patients with insulinomas from other cause of hypoglycemia according to their baseline glucose and insulin. We measured glucose concentrations 15 minutes following intravenous administration of glucagon (1 mg/dog) and used a glucometer to compare baseline glucose concentration with glucose changes at 15 minutes (Δ0-15min) between healthy dogs, dogs with insulinomas and dogs with other causes of hypoglycemia. Mean (±SD) serum glucose concentrations at baseline and for Δ0-15min in healthy dogs (n=4), dogs with insulinoma (n=3), and dogs with hypoglycemia from other causes (n=8) were 4.43±0.24, 2.23±0.5, 2.94±0.68, and 5.33±0.19, 4.37±2.4, 1.71±1.71, respectively.

The result of this pilot study is promising, and a future study with a larger sample-size should corroborate the utility of the glucagon stimulation test for the diagnosis of insulinomas.

EN22

Effect of Levothyroxine Administration on Serum NT-proBNP and cTnI Concentrations in Hypothyroid Dogs

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Thyroid hormones can affect the structure and function of the heart, but the effect of levothyroxine supplementation on the heart in hypothyroid dogs with concurrent heart disease has not been investigated. The purpose of this study was to evaluate serum concentrations of N-
terminal pro B-type natriuretic peptide (NT-proBNP), cardiac troponin I (cTnl), and aldosterone before and after levothyroxine supplementation in hypothyroid dogs with myxomatous mitral valve disease (MMVD).

In this case-controlled observational study, eight dogs with newly diagnosed hypothyroidism and concurrent MMVD, 10 dogs with hypothyroidism alone, 10 dogs with MMVD alone, and 20 healthy dogs were included. Serum concentrations of NT-proBNP, cTnl, and aldosterone were measured using enzyme-linked immunosorbent assays, and serum total T4 (TT4) concentration was measured using a chemiluminescent immunoassay-based analyzer. Serum NT-proBNP concentrations in hypothyroid dogs with and without MMVD were significantly higher than those of healthy dogs. Serum cTnl concentrations in hypothyroid dogs without MMVD were significantly higher than those of healthy dogs. Serum NT-proBNP and aldosterone concentrations increased significantly after levothyroxine supplementation in hypothyroid dogs with and without MMVD. Significant correlations were detected between serum NT-proBNP, aldosterone, and TT4 concentrations in hypothyroid dogs with and without MMVD.

These results suggest that levothyroxine supplementation might impact the heart in hypothyroid dogs. Awareness of the effect of levothyroxine supplementation on the heart, particularly in dogs with underlying heart diseases, is necessary.

EN23

The Effect of Oral Dexamethasone on Urine Corticoid: Creatinine Ratios in Healthy Dogs

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In-clinic stress can impact the specificity of the traditional intravenous low dose dexamethasone suppression test (LDDST). Home-based methods to interrogate the pituitary adrenal axis may therefore improve test reliability. Published methods relying on the at-home oral administration of dexamethasone and subsequent collection of urine for determination of urine corticoid: creatinine ratio (UCCR) are somewhat cumbersome, and require repeated dexamethasone administration and collection of several timed urine samples. A simpler protocol may improve owner compliance.

Ten clinically normal dogs were enrolled in this proof-of-concept study. Up to three morning urine samples were collected for determination of baseline UCCR. Owners were then instructed to administer a single capsule by mouth at bedtime for a 7 day period. Each capsule contained dexamethasone at 0.01 - 0.015 mg/kg (3 doses: Sunday, Wednesday; Saturday) or a lactose placebo (4 doses: Monday, Tuesday, Thursday, Friday). Urine was collected the following 7 mornings, approximately 8 hours after capsule administration. Urine samples were frozen at -20°C for up to 2 months prior to analysis. UCCR was determined at a commercial laboratory, using RIA (corticoids) and modified Jaffe (creatinine) methodologies.

Median baseline UCCR was 9.5 (range: 4 - 15; n = 16 [reference: 8 - 24]); this was significantly greater (P < 0.0001) than median UCCR at 8-hr post dexamethasone (6; range: 1-13; n = 24) and at 36-hr post dexamethasone (3; range 1-11; n = 17) (Figure 1). In addition, median UCCR at 36-hr post dexamethasone was significantly lower than at 8-hr post dexamethasone (P = 0.0023).

Results of this pilot study suggest that the administration of a single oral low dose of dexamethasone (0.01 - 0.015 mg/kg) will significantly impact UCCR in samples collected 8-hr and 36-hr later. This simplified approach may be a useful way to differentiate dogs with hyperadrenocorticism from those with non-adrenal disease. Further studies evaluating this novel test in dogs with clinical evidence of hyperadrenocorticism are warranted.

EN24

Subclinical Pituitary-Dependent Hypercortisolism in Dogs: Comparison of Clinical Findings and Outcomes with Overt Hypercortisolism

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Awareness of canine hypercortisolism has recently been increasing and dogs are likely to be diagnosed even without overt symptoms. While mild form or subclinical hypercortisolism has been well described in humans, there are no such reports in dogs. The objective of this study was to clarify the clinical characteristics of canine subclinical pituitary-dependent hypercortisolism (PDH).

Medical records of seventy-three dogs diagnosed with PDH at Hokkaido University Veterinary Teaching Hospital between April 2013 and March 2016 were retrospectively reviewed. In this study, subclinical hypercortisolism was defined as being diagnosed endocrinologically, but without overt clinical signs of hypercortisolism usually recognized by the owners. Signalment and clinicopathological findings were compared between subclinical and overt groups. In addition, the association between the groups and the risk of combined clinical outcomes (defined as death from any cause or complications associated with hypercortisolism) were investigated using univariate and multivariate Cox regression analyses.

Subclinical group (n = 52) was significantly older (P = 0.029) and had lower post-ACTH stimulation cortisol (P = 0.047) than overt group (n = 21). In a univariate Cox regression analysis, there was no association between the groups and outcomes. However, after adjustment
for age and trilostane use, subclinical group was associated with reduced risk of clinical outcomes (adjusted hazard ratio, 0.28; 95% confidence interval, 0.10–0.77).

The results of this study suggest that hypercortisolism in dogs includes subclinical PDH with an older age, lower post-ACTH stimulation cortisol, and lower risk of clinical outcomes compared to overt PDH.

EN25

Risk-Factors for Feline Hyperthyroidism in Southern Brazil: A Case-Control Study
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Hyperthyroidism is an elderly cat’s frequent disease. Its prevalence has been increasing worldwide. Many evidences raised regarding hyperthyroidism risk-factors. By this way, those factors would have implications in the huge geographic prevalence variation observed. The objective of this study was to look for potential environmental variables associated with feline hyperthyroidism around Porto Alegre city, Southern Brazil. A 30-questions questionnaire was applied to 28 hyperthyroid feline owners as well as to 55 euthyroid (above 8 years of age) feline owners (n:m). Results of univariate analysis were expressed as odds ratio (OR) and respective 95% confidence interval (95%CI). Mean age of hyperthyroid cats was 13.2 ± 2.7 years (range: 7-18), while euthyroid cats mean age was 11.6 ± 2.4 years (range: 8-16) (P < 0.01). Age greater than 12 years was considered as a risk-factor (OR 3.14; 95%CI = 1.10–8.97) (P < 0.01). Hypothyroid cats showed an association between higher bathing frequency (weekly/monthly) and hyperthyroidism (OR 7.57; 95%CI = 1.41–40.55). Other items surveyed, such as the use of plastic accessories, contact with domestic dust, use of endoparasiticides, ectoparasiticides, and vaccines, it was not possible to identify any association of these variables as risk or protective factors. Previous data suggest that bathing could have a protective effect cleaning off dust particles from the fur. The bath hypothesis as risk-factor needs further studies due to possible presence of endocrine disruptors linked with thyroid dysfunction in cosmetic products.

EN26

Selegiline and Trilostane Association for Canine Pituitary-Dependent Hyperadrenocorticism: A Randomized Clinical Trial
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Canine pituitary-dependent hyperadrenocorticism (PDH) is a common endocrine disorder. Clinical management usually demands lifelong trilostane therapy, which may cause endogenous ACTH (eACTH) elevation, adrenomegaly, and recurrent dosage adjustments. Selegiline therapy has been previously indicated for canine PDH treatment at the pituitary level, but not encouraged. However, there have been no studies associating trilostane and selegiline for PDH treatment in dogs. The aim of this work was to evaluate the clinicopathological features, imaging findings, and hormone test results in dogs with PDH treated with trilostane (Tri) or with trilostane and selegiline (Tri+Sel). Fifteen client-owned dogs diagnosed with spontaneous PDH were evaluated: eight were treated with Tri and seven with Tri+Sel in a randomized clinical trial. Dogs underwent clinical examination, serum biochemical analysis, urinalysis, abdominal ultrasound, and eACTH and post-ACTH cortisol measurements on treatment days zero (D0), 30 (D30), 90 (D90), and 180 (D180). Patients included in the Tri group were initially treated with trilostane at an initial dose of 0.5 mg/kg PO twice daily, whereas the Tri+Sel group initially received 0.5 mg/kg PO of trilostane twice daily and 1 mg/kg PO of selegiline once daily. There was no significant difference at the 95% confidence level in eACTH variation between the Tri group (median D0 = 20.85 pg/dL; median D180 = 79.0 pg/dL; p = 0.07) and the Tri+Sel group (median D0 = 103 pg/dL; median D180 = 98.25; p = 0.57). Both groups showed significant lower post-ACTH cortisol levels at the end of the study (Tri median D0 = 15 μg/dL; D180 = 5.2 μg/dL; p = 0.002 vs. Tri+Sel median D0 = 17.23 μg/dL; D180 = 2.26 μg/dL; p = 0.006). Also, both groups needed trilostane dosage adjustments (p = 0.01). However, no statistical difference was observed between the groups at the end of the study regarding eACTH or post-ACTH cortisol levels. Nonetheless, there was minor variation in left adrenal gland thickness in the Tri+Sel group (left adrenal median D0 = 0.65 cm; median D180 = 0.71; p = 0.7) when compared with the Tri group (left adrenal median D0 = 0.77 cm; median D180 = 0.97 cm; p = 0.09). The same was observed for right adrenal gland thickness (Tri+Sel median D0 = 0.65 cm; median D180 = 0.58 cm; p = 0.2 vs. Tri median D0 = 0.58 cm; median D180 = 0.77 cm; p = 0.04). Moreover, patients in the Tri+Sel group seemed to have achieved better metabolic control throughout fructosamine and total cholesterol evaluation. Notwithstanding, no differences in clinical control or cognitive function status were perceived between the groups. The association of selegiline with trilostane seems to be a safe and promising complementary therapy for canine PDH. However, further studies with a larger sample size and longer follow-up are needed to clarify the actual effect of this association.

EN27

Urinary Tract Infection in Canine Hyperadrenocorticism
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Occult urinary tract infection (UTI) is assumed as a common comorbidity in canine patients with hyperadrenocorticism (HAC), affecting up to 50% of cases at initial diagnosis. However, increased concern about
HAC has turn disease identification very precocious. The purpose of this work was to identify UTI prevalence in HAC patients attended at a veterinary teaching hospital. Eighty-seven dogs with HAC were included in the study and divided in three groups: newly diagnosed (n = 28), poorly controlled with trilostane or mitotane (n = 24), and well-controlled with trilostane or mitotane (n = 35). The HAC diagnosis was defined based on clinical presentation, blood work evaluations and positive endocrine tests. The criteria to define dogs as poorly or well-controlled was defined based on clinical status and post-ACTH cortisol > 6 mcg/dL, or < 6 mcg/dL, respectively. The UTI was characterized as positive urine culture in a sample collected by cystocentesis. After urine culture, bacterial identification was performed by MALDI-TOF mass spectrometry analysis, and antibiogram performed by disc diffusion tests for seven antibiotics (cefalexin, amoxicillin with clavulanate, doxycycline, sulfa-trimethoprim, enrofloxacin, and nitrofurantoin). The overall UTI frequency was 19.54% (17/87). Surprisingly, newly diagnosed patients had UTI only in 14.28% of cases (4/28) whereas in poorly controlled dogs UTI was identified in 16.67% of cases (4/24), and in well-controlled dogs UTI frequency was 25.71% (9/35). Only 25% (1/4) of the newly diagnosed patients with UTI had any associated clinical manifestation (p.e.x. active urinary sediment, polacuria), and this clinical picture was even less frequent in well-controlled dogs (11% - 1/9), and null in dogs in the weak control group. Main bacterial genus identified were Escherichia sp. and Proteus sp. Occult UTI was less frequent that expect in this population, and this scenario may be a reflex of early HAC diagnosis.

EN28

Leptin, IL-6 and Glucagon Differ in Diabetic vs Healthy Dogs But Diet Influences Only Leptin

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Investigation of inflammatory and hormonal profile of diabetic dogs can help to explain pathogenic mechanism involved in this disease. Our study aimed to compare serum concentration of some interleukin (IL), hormones and incretins from diabetic (n = 15) and healthy dogs (n = 7). Procedures were approved by Ethics Committee on Animal Use. The diabetic dogs received, randomly and by a double blind way, three diets differenced by fat level and starch source: Ba (mix of rice, pea and barley; 6.7 g of fat/1000kJ), PB (pea and barley; 9.6 g) and Co (corn; 9.8 g). After two months receiving each diet, serum concentrations of IL-10, IL-6, TNF-α, amylun, glucagon-like peptide-1 (GLP-1), leptin, peptide YY (PYY) and glucagon were measured. In healthy group, the dogs received commercial adult diet with intermediary fat content (7.6g of fat/1000kJ starch source: corn and rice) for fifteen days prior to those analyzes. Diabetic and healthy dogs had ideal body condition score. Diet did not influence results, when comparing them among diabetic dogs (p>0.05). When comparing the mean concentration of the variables of diabetic with healthy dogs, we observed that there were higher concentration of serum leptin (p < 0.01), IL-6 (p < 0.03) and glucagon (p = 0.01) in diabetic than healthy dogs. It was the first study that evaluated effects of diet on these variables. We conclude that starch source or difference at fat amount did not influence IL, hormones and incretins serum concentrations of diabetic dogs and, there were differences between diabetic and healthy dogs.

F01

Agreement Between 3 Different Tests for Colostrum Quality in Beef Cattle with High Risk Pregnancies

Ryan M. Breuer – Iowa State University College of Veterinary Medicine, Ames IA; Caitlin Wiley – Iowa State University College of Veterinary Medicine; Tyler Dohman – Iowa State University College of Veterinary Medicine; Julia Kim – University of Guelph, Ontario Veterinary College; Joseph Smith – Iowa State University College of Veterinary Medicine; Amanda Kreuder – Iowa State University College of Veterinary Medicine

The purpose of this study is to report the agreement between three different commercially available tests of colostrum quality in beef cattle. Appropriate information for decision making related to supplementation of colostrum in beef calves, particularly those born following a dystocia event, is currently lacking with most of the literature on colostrum quality and failure of passive transfer generated in dairy cattle.

From November 1, 2016, to December 1, 2018, colostrum samples were obtained immediately after calving from all beef breed cows and heifers admitted to the ISU Lloyd Veterinary Medical Center Food Animal and Camelid Hospital for calving management or dystocia. The colostrum was screened via turbidmetric immunoassay, Brix percentage, and radial-immunodiffusion (RID) to estimate IgG quantity and colostrum quality. A total of 223 cows were identified and full measurements from all three assays were achieved from 208 cows. Correlation between RID and Brix percentage was $R^2 = 0.5308$; correlation between RID and turbidmetric immunoassay was $R^2 = 0.4467$. When colostrum quality was considered utilizing a cutoff of 22% Brix, 176/219 samples met or exceeded this percentage. Using a limit of 5000 mg/dL (50 gm/L) 201/217 RID samples exceeded the limit whereas 82/217 exceed this threshold via turbidmetric assay. Coefficient of variation (CV) values were 0.191, 0.359, and 0.394 between Brix, turbidmetric immunoassay, and RID, respectively. Practitioners should be aware of variability between testing methods for colostrum quality in beef cattle and consider multiple testing modalities or supplementation of the calf with colostrum replacer following dystocia events.

F02

Agreement Between Four Different Tests for Assessment of Passive Transfer in High-Risk Beef Calves

Ryan M. Breuer – Iowa State University College of Veterinary Medicine, Ames IA; Caitlin Wiley – Iowa State University College of Veterinary Medicine

The purpose of this study is to report the agreement between four different commercially available tests of colostrum quality in beef cattle. Appropriate information for decision making related to supplementation of colostrum in beef calves, particularly those born following a dystocia event, is currently lacking with most of the literature on colostrum quality and failure of passive transfer generated in dairy cattle.

From November 1, 2016, to December 1, 2018, colostrum samples were obtained immediately after calving from all beef breed cows and heifers admitted to the ISU Lloyd Veterinary Medical Center Food Animal and Camelid Hospital for calving management or dystocia. The colostrum was screened via turbidmetric immunoassay, Brix percentage, and radial-immunodiffusion (RID) to estimate IgG quantity and colostrum quality. A total of 223 cows were identified and full measurements from all three assays were achieved from 208 cows. Correlation between RID and Brix percentage was $R^2 = 0.5308$; correlation between RID and turbidmetric immunoassay was $R^2 = 0.4467$. When colostrum quality was considered utilizing a cutoff of 22% Brix, 176/219 samples met or exceeded this percentage. Using a limit of 5000 mg/dL (50 gm/L) 201/217 RID samples exceeded the limit whereas 82/217 exceed this threshold via turbidmetric assay. Coefficient of variation (CV) values were 0.191, 0.359, and 0.394 between Brix, turbidmetric immunoassay, and RID, respectively. Practitioners should be aware of variability between testing methods for colostrum quality in beef cattle and consider multiple testing modalities or supplementation of the calf with colostrum replacer following dystocia events.

F02
The purpose of this study is to report the agreement between four different commercially available tests for passive transfer status in beef calves. Limited reports exist in beef calves on best practices for determination of passive transfer status in beef cattle, particularly for those calves born assisted or via C-section.

From November 1, 2016, to December 1, 2018, 197 calves born to cows and heifers admitted to the ISU Lloyd Veterinary Medical Center Food Animal and Camellid Hospital for calving management or dystocia had serum collected for analysis [turbidometric immunoassay, serum GGT, serum total solids (TS), and radial-immunodiffusion (RID)] after birth (range of 12 to 40 hours).

Correlation between RID and GGT was $R^2 = 0.1759$; correlation between RID and turbidmetric immunoassay was $R^2 = 0.5236$; and correlation between RID and TS was $R^2 = 0.5318$. When a serum IgG cutoff of 1600 mg/dL was utilized 182/189 samples (RID) and 98/187 samples (turbidmetric) exceeded the threshold; if a value of 2400 mg/dL was used instead, 135/189 (RID) and 59/187 (turbidmetric) exceeded that value. When TS was considered at a level of 5.5 mg/dL 151/184 calves’ samples exceeded this concentration; if a TS of 6.0 mg/dL was evaluated instead, 59/185 samples exceeded this value. When comparing turbidometric, RID, and TS results, only 114 out of 195 were in agreement for all three tests. Practitioners should be aware of variability between methods, and should consider utilizing additional testing when failure of passive transfer status is uncertain in high-risk calves.

F03

Cerebrospinal Fluid Protein Concentration, Nucleated Cells and Red Blood Cells Counts From Recumbent Cows

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Spinal cord lesions are a cause of recumbency in adult dairy cattle, usually associated with a poor prognosis. Analysis of cerebrospinal fluid (CSF) is a useful procedure to establish a diagnosis and prognosis. Blood contamination of the CSF may confound interpretation of results since both total nucleated cells count (TNCC) and protein concentration (PC) could be falsely elevated. Red blood cells (RBC) are not a normal component of CSF. In humans, CSF samples with $> 30$ RBC/$\mu$L are considered to be contaminated. However, predicting accurately TNCC and PC attributable to blood contamination is difficult.

The aim of this study was to describe the variation of PC, TNCC and RBC count using CSF analysis results from recumbent dairy cows admitted to the Centre Hospitalier Universitaire Vétérinaire between January 2006 and December 2014.

Among the 235 samples included, RBC count varied between 0-869 220 cell/$\mu$L (mean = 4741.9, median = 6.6), PC varied between 0.04-6.51 g/L (mean = 0.43, median = 0.27), and TNCC varied between 0-7500 cell/$\mu$L (mean = 35.5, median = 1.1). Among the 157 samples that had $< 30$ RBC/$\mu$L, PC and TNCC varied between 0.13-1.06 g/L (mean = 0.29, median = 0.27) and between 0-31.4 cell/$\mu$L (mean = 1.8, median = 0.6), respectively.

A total of 84 samples had PC $< 0.25$ g/L and TNCC $< 4.5$ cell/$\mu$L (less likely to have spinal cord lesions). Among them, RBC count varied between 0-1290 RBC/$\mu$L (mean = 61.5, median = 4.7). In 20 samples TNCC was 0, with a variation in RBC count between 0-840 RBC/$\mu$L (mean=65.8, median=3.9).

Variation of PC and TNCC concentration are presented for different RBC count thresholds. A RBC count $< 840$ cell/$\mu$L seems to less likely to interfere with CSF results interpretation. To study the PC and TNCC levels of CSF samples before and after known dilutions of whole blood are added is suitable.

F04

Priming Antibody Responses Against BRSV Induced in Beef Calves Through Early Vaccination

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Bovine respiratory syncytial virus (BRSV) is an important cause of respiratory disease in pre-weaned beef calves. Vaccination of calves early in life is a common strategy used by producers to provide protection against disease caused by BRSV, but little research has evaluated the efficacy of early life vaccination in field trials. The objective of this study was to evaluate the effect of two different protocols of vaccination on the first day of life on BRSV-specific nasal secretion and serum antibody titers at 2 months of age.

Sixty newborn beef calves were assigned to three different treatment groups. Between 6-24 hours of age, calves received either a modified live virus (MLV) vaccine containing BRSV intranasally (IN VAC, n=20), or a different MLV vaccine containing BRSV subcutaneously (SQ VAC, n = 18). An unvaccinated control group (NO VAC, n = 22) was also included. Nursing of maternal colostrum occurred naturally after birth without assistance and was monitored by personnel blinded to treatment allocation. Individual serum and nasal secretions samples were collected before vaccination at birth and at approximately 2-months of age in all calves. At branding, the mean serum and nasal secretion BRSV-specific antibody titers were greater in IN VAC and SQ VAC compared with NO VAC calves. SQ VAC demonstrated a greater mean serum and nasal secretion BRSV-antibody titer at 2-months of age than IN VAC. Intranasal or subcutaneous vaccination of beef calves with a MLV vaccine containing BRSV within 24 hours of life is
effective to induce specific BRSV antibodies in serum and nasal secretions.

**F05**

Impact of Conservation Methods On the Quality of Rumen Juice Before Transfaunation in Dairy Cattle

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Transfaunation, the administration of rumen juice (RJ) from a healthy to a sick ruminant, is commonly used as a treatment both in hospital and on farm, although no published study demonstrates the maximum conservation time of RJ before its administration. This study aimed at describing the conservation of RJ in 3 different environments: at ambient temperature, refrigerated, and warmed after refrigeration. The hypotheses were that RJ could be conserved up to 8 hours (h) at ambient temperature, up to 24h refrigerated, and that warming would improve its quality. 10 liters of RJ were taken from a cannulated donor, once a day for 5 days. The sample was divided according to the conservation methods, and analyzed at 0h, 0.5h, 1h, 2h, 8h, 24h and 48h. PH, methylene blue (MB) reduction test, motility and number of protozoa were assessed. Results showed that RJ’s pH significantly (p < 0.05) rose over time, becoming alkaline (>7.2) after 8h and 24h (refrigerated and ambient samples, respectively), but remaining normal for warmed samples. MB test showed a significant decrease in reduction activity of the flora in refrigerated and ambient samples versus warmed samples. The best MB reduction results were observed at T ≤ 1h and T ≤ 0.5h (ambient and refrigerated samples, respectively). The protozoa’s motility significantly decreased over time, although ambient samples kept a decent motility up to 8h. In conclusion, the deterioration of RJ starts as early as 1h at ambient temperature and 0.5h when refrigerated; however, warming RJ after refrigeration can improve some of its qualities.

**F06**

Retrospective Study of Pet Pigs Presenting for Lameness

**Evelyn E. MacKay – Texas A&M University**

As pet pigs have become more popular in recent years, the number of pigs presenting to veterinary schools and general practices has increased. Subjectively, cases of lameness seem to be frequent and have varied causes. There is a paucity of literature on pet pigs in general, and almost no data on causes of lameness outside of production swine. Medical records of pigs presenting for lameness or recumbency from 2011 to 2018 at the Texas A&M University Large Animal Hospital were analyzed. History, physical examination findings, diagnostics performed, final diagnosis, treatment modalities, and outcome were recorded. Production pig breeds and neurological causes of lameness or recumbency were excluded. A total of 35 cases were evaluated. The Vietnamese Pot-Bellied breed was most common, and weights ranged from 5 to 136kg. Body condition score was recorded in 12 cases and only 3 were reportedly ideal or underweight. Fractures were the most common cause of lameness (n=10) followed by osteoarthritis (n=9) and joint luxation/subluxation (n=6). The remaining causes of lameness included septic arthritis, overgrown hooves, soft tissue and hoof injuries, and presumptive osteoarthritis complicated by obesity. Four of the 10 fracture cases were of the humoral condyle, 2 of which became bilateral. Most common locations for osteoarthritis were the interdigital joints and elbows. Four of the 6 joint luxation/subluxations were of the coxofemoral joint. Radiography was the most beneficial diagnostic tool for definitive diagnosis. In addition to surgical repair, the most commonly prescribed treatment regimen included non-steroidal anti-inflammatory drugs and weight reduction.

**F07**

Antimicrobial Treatment in Veal Calves and Association with Antimicrobial Resistance in Pasteurellaceae and E. coli

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Pasteurellaceae and E. coli were isolated from veal calves in 43 farms over a period of one year. Antimicrobial susceptibility was analysed for association with antimicrobial use on the farms (treatment incidence based on used daily dose, TIUDD) with mixed logistic regression models. A total of 1162 P. multocida and 346 M. haemolytica were isolated from 2508 pharyngeal swabs, and 2145 E. coli from 2260 rectal swabs, respectively. The mean TIUDD per fattening period was 3.8 treatments/calf (with use of the following antimicrobials: 32% penicillins, 23% tetracyclines, 19% sulfonamides, 17% macrolides, 4% trimethoprim, 2% aminoglycosides, 1% fluoroquinolones, <1% florfenicol and cephalosporins). Group treatments accounted for 83% of all treatments. Rates of not susceptible (NS) P. multocida ranged from 2 to 94%, and from 0 to 53% for M. haemolytica. Non-wildtype (non-WT) E. coli ranged from 2 to 66% for the antimicrobial drugs tested. Few, mostly weak associations between classification as NS and TIUDD were observed for P. multocida. Odds ratios (OR) for NS M. haemolytica were increased with regard to group TIUDD for penicillins and tetracyclines, and decreased for individual TIUDD for macrolides and tetracyclines. Significantly increased OR of non-WT E. coli were observed for group TIUDD for tetracyclines, penicillins and aminoglycosides, and decreased OR for individual TIUDD for aminoglycosides. Significant associations were observed between antimicrobial treatment incidence and decreased susceptibility of Pasteurellaceae and E. coli in veal calves. Individual treatment was mostly associated with reduced OR and group treatment with increased OR for NS/non-WT isolates.

**F11**

Erythrocyte Osmotic Fragility of Blood Samples of Laying Hens with Different Anticoagulants

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In some animal species, the best antiocoagulant for certain hematological evaluations is not fully established, especially in species that have nucleated erythrocytes. The standardization of an ideal antiocoagulant for evaluation of erythrocyte osmotic fragility in different species is very important. The objective of this study was to compare the values of erythrocyte osmotic fragility of blood samples from laying hens (Gallus gallus domesticus Linnaeus, 1758) obtained with antiocoagulant Na2EDTA or heparin. Fourteen clinically healthy adult laying hens with similar age, weight, feeding and housing conditions, from the Poultry Sector of the College of Agriculture and Veterinary were used in this study. Venous blood samples were collected in a single time and placed in microtubes containing Na2EDTA (2 μg/mL) and microtubes containing heparin (50 U/mL). Erythrocyte osmotic fragility was evaluated at two times: M1 (immediately after collection) and M24 (24 hours after collection). The data were submitted to analysis of variance and the differences found were evaluated by the Tukey test with significance of P < 0.05. There was greater erythrocyte osmotic fragility in blood samples with heparin in relation to Na2EDTA at both times M1 and M24. Likewise, there was higher percentage of hemolysis in the samples processed 24 hours after collection (M24) in both antiocoagulant samples, with greater fragility in heparin samples. Therefore, Na2EDTA was more efficient by lower percentage of hemolysis when compared to heparin, when the samples were processed immediately after collection and 24 hours later.

F12

Bovine Lactoferrin Modulates Lipopolysaccharide-Induced Nitric Oxide Production
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Nitric oxide (NO) has been ascribed both physiological and pathological roles. It is a vasodilator and neurotransmitter, inhibits platelet aggregation and exhibits both anti-inflammatory and toxic effects. Physiologically speaking, it is constitutively produced by neuronal nitric oxide synthase (nNOS/NOS1) and endothelial NOS (eNOS, NOS3), in response to calcium kinetics. On the other hand, enzymatically active inducible NOS (iNOS/NOS2), responds to immunologic stimuli in a calcium-independent manner. NOS2 is present in the primary and tertiary granules of neutrophils (PMNs) and macrophages, and resides close to the phagosome. During inflammation, NO exerts multiple effects. In addition to being toxic towards infectious agents, like the pro-inflammatory cytokines, the high concentrations produced following lipopolysaccharide (LPS) stimulation, contribute to the diarrhea, pneumonia and septic shock frequently observed in endotoxemic calves.

We examined the value of bovine lactoferrin (bLF) and its 25 amino acid peptide lactoferricin (LFcin) B, as low-risk anti-inflammatory agents. Lactoferrin is present in mammary and mucosal secretions, but is notably at its highest concentration in the secondary granules of neutrophils. Lactoferricin B is produced in the stomach following oral bLF supplementation. Peptide activity is also facilitated by the presence of proteases, at sites of infection. We hypothesized that bLF and LFcin B would suppress LPS-induced NO production.

Viable peripheral bovine monocytes and PMNs were isolated from calves and stimulated with LPS in vitro, in the presence or absence of bLF/LFcin B. Culture supernatants, sample total RNA and proteins, were analyzed for NO, iNOS and phosphorylated p38 mitogen activated protein (MAP) kinase using the Griess assay, qRT-PCR and western immunoblot techniques, respectively. Compared with LPS-only-stimulated monocytes, cells co-cultured in both bLF and LPS, exhibited a > 6-fold decrease in iNOS expression. Nitric oxide production, which was also significantly decreased in the supernatants of bLF (P < 0.01) and LFcin B (P < 0.05) LPS co-cultures, respectively, was paralleled by the downregulation of p-38 MAPK in both cell types. These results strongly support the candidacy of bLF and the antimicrobial peptide LFcin for therapeutic intervention in endotoxemic patients, a significant mechanism being the downregulation of p-38 MAPK-generated NO.

F13

Serum Total Protein, Immunoglobulin G, and Failed Transfer of Passive Immunity in Neonatal Beef Calves
Lisa Gamsjaeger - University of Calgary; Jennifer Pearson - University of Calgary; Elizabeth Rose Homerosky; Ed Pujarc - University of Calgary; Claire Windeyer - University of Calgary

Transfer of passive immunity (TPI) is often assessed using serum immunoglobulin G (IgG) or serum total protein (STP) in neonatal calves. However, appropriate cut-points to determine adequate TPI are lacking for beef calves. Target IgG levels of 10g/L have commonly been used, but more recent studies suggest IgG concentrations of < 24 g/L should be considered as failed TPI (FTPI) for beef calves. The objectives of this study were to: 1) describe IgG, STP, and FTPI in Canadian neonatal beef calves; 2) determine the correlation between IgG and STP; and 3) establish appropriate STP cut-points for detection of FTPI in neonatal beef calves.

Serum samples collected from 365 calves at 1-7 days of age on 14 western Canadian cow-calf operations were available for this retrospective study. Concentrations of IgG and STP were measured using radial immunodiffusion (RID) and digital refractometry, respectively. Factors (ranch, year, breed, and dam parity) influencing IgG and STP concentrations were evaluated by ANOVA. The Pearson’s correlation coefficient was calculated for STP and IgG. A cut-point of IgG < 24 g/L was used to determine FTPI. The appropriate STP cut-point for detection of FTPI was determined by calculating sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy (ACC) for various cut-points.

Overall, mean serum IgG was 37.8 g/L (95% CI: 36.2 - 39.3 g/L), mean STP was 6.3 g/dL (95% CI: 6.2 - 6.4 g/dL), and prevalence of FTPI was 18.4% (95% CI: 14.7 - 22.7%). Average herd-level prevalence of FTPI was 23.2% (95% CI: 20.7 - 25.7%) but ranged from 7.1% to 55.6%. Average herd-level prevalence of FTPI was 18.4% (95% CI: 14.7 - 22.7%). Average herd-level prevalence of FTPI was 23.2% (95% CI: 20.7 - 25.7%) but ranged from 7.1% to 55.6%. Ranch and year had a significant effect on IgG (p = 0.008 and 0.005, respectively) and as well as STP (p = 0.037 and 0.02, respectively), whilst breed and dam parity did not (p > 0.05). Serum IgG and STP had a strong, positive, linear correlation (correlation coefficient = 0.89). The optimal STP cut-off point for detection of FTPI was 5.7 g/dL (Se = 85.1%, Sp = 91.6%, PPV = 69.5%, NPV = 96.5%, ACC = 90.4%).
The results of this study confirm a high percentage of beef calves with FTPI (18%) and show a large degree of variability between herds. The strong correlation between STP and IgG underlines the value of STP as a simple on-farm measure to estimate FTPI, using the described cut-point of 5.7 g/L. This study forms the basis for the development of evidence-based colostrum management strategies specific to beef herds, which will decrease morbidity and mortality and lead to improved animal welfare.

F14

Simplified Strong Ion Difference Analysis in Neonatal Beef Calves with Failed Transfer of Passive Immunity

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The effect of strong electrolytes, partial pressure of carbon dioxide (pCO₂), and non-volatile buffer ion charge (A⁻) (albumin, globulin and phosphorus) on plasma pH and bicarbonate concentration (HCO₃⁻) can be determined using the simplified strong ion difference (sSID). Conditions affecting the total concentration of globulins could affect the determination of the A⁻ and unmeasured strong ions concentration (USI), and therefore the diagnosis of acid-base disorders.

The objectives of this study were to: 1) determine whether the variables from the sSID were different in calves with failed or adequate transfer of passive immunity (TPI); and, 2) determine the correlation of different methods of estimating USI that do or do not account for the actual charge of globulin and phosphorus.

This retrospective study was conducted on data collected from 77 newborn beef calves at 24 hours of life. Venous blood was collected via jugular venipuncture, blood gas parameters measured immediately, and serum separated for biochemistry profile analysis. Calves with serum total protein (TP) ≤ 5.7 g/dL were categorized as having failed TPI. The sSID variables were calculated as: strong ion difference (SID) = (Na⁺ + K⁺) - (Cl⁻ + L-lactate⁻); A⁻ = (0.343 x TP) / (1 + 10^(6.65 - pH)); and, strong ion gap (SIG) = SID - HCO₃⁻ - A⁻. The 3 methods used to estimate the USI were: anion gap (AG) = (Na⁺ + K⁺) - (Cl⁻ + HCO₃⁻); unmeasured anions (XA) = SID - HCO₃⁻ - [(0.141 x [albumin]) x (pH - 5.42)] - [(0.04 x [globulin]) x (pH - 5.58)] - [phosphorus] x (0.309 x pH - 0.469); and, albumin adjusted SIG (albumin-SIG) = SID - HCO₃⁻ - [albumin] x (0.622 / (1 + 10^7.58)]. Mann-Whitney U-test was used to determine differences in the sSID variables between groups. Pearson correlation coefficients were calculated to assess the correlation between methods used for estimation of USI.

Plasma concentrations of globulins and phosphorus, but not albumin, were lower (p < 0.05) in calves with failed TPI compared to those with adequate TPI. The median A⁻ values were also lower in failed TPI calves (11 mm/L, IQR: 9 mm/L to 12 mm/L) than adequate TPI calves (14.8 mm/L, IQR: 14 to 16 mm/L). The SID and SIG values were similar between groups. Pearson correlation coefficients showed a strong correlation between all 3 methods for estimation of USI: albumin-SIG and XA, r = 0.95 (95% CI: 0.92 to 0.97); albumin-SIG and AG, r = 0.80 (95% CI: 0.70 to 0.82); and, AG and XA, r = 0.84 (95% CI: 0.76 to 0.90).

Failed TPI could affect the evaluation of acid-base disorders of newborn beef calves when using the sSID approach because of the influence of serum globulins and phosphorus on the total non-volatile buffer ion charge. However, it appears to have little impact on the estimation of USI.

F15

Effect of Quercetin on Porcine Neutrophil Extracellular Trap Formation

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Neutrophil extracellular trap (NET) formation is an immune response to the invasion of external microorganisms. Quercetin, a member of the flavonoids family, which is found in fruits and vegetables, has been examined in a variety of biological contexts. The objective of this study was to examine the effect of quercetin on porcine NET formation. The NET formation of peripheral blood polymorphonuclear cells (PMNs) were measured by propidium iodide (PI) dyes method. The amount of TNF-α in culture supernatants was quantified by ELISA and TNF-α mRNA expression was measured using RT-PCR. Direct treatment with quercetin on PMNs did not affect the NET formation. But NET formation was inhibited by exposure to culture supernatant from peripheral blood mononuclear cells (PBMCs) treated with quercetin. The culture supernatant from PBMCs treated with lipopolysaccharide (LPS) revealed high NET formation. This NET formation was reduced by co-treatment of LPS with quercetin. In addition, treatment with LPS and/or recombinant porcine (rp) tumor necrosis factor-α (TNF-α) on PMNs showed high NET formation. This effect was also reduced by quercetin. PBMCs treated with LPS increased TNF-α protein and mRNA expression, but this effect was also diminished by addition of quercetin. These findings indicated that quercetin inhibits NET formation of PMNs by suppressing production of TNF-α from LPS-stimulated PBMCs. These results suggest that quercetin has an anti-inflammatory effect on NET formation, mediated by down-regulation of TNF-α production from LPS-stimulated PBMCs.

F16

Effect of Pooled and Non-Pooled Colostrum on Passive Transfer of Immunity and Health in Calves

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The objectives of this study were to compare colostral IgG concentrations in colostrum from individual cows and pooled colostrum, and to compare the serum immunoglobulin G (IgG) concentrations, morbidity, and mortality among calves fed their own dam’s colostrum, colostrum from another cow, or pooled colostrum. Calves were randomly assigned into one of three groups; calves fed colostrum from their
own dam (n=20), calves fed colostrum from a different dam (n=20) and calves fed pooled colostrum (n=18). Serum samples were collected from calves at birth (0 hours) and at 24 hours post-colostrum feeding. A sample of colostrum fed to the calves was also collected. Colostrum and serum IgG concentrations were measured by single radial immunodiffusion. Calves were weighed at birth and weaning and their health status was assessed by trained personnel twice daily. Health assessment was based on general demeanour, rectal temperature, fecal consistency, respiratory rate, and presence of cough, nasal or ocular discharge. Differences in the colostral, serum IgG concentrations and weaning weights were compared using a one-way analysis of variance. Association between group assignment and proportions of calves experiencing morbidity or mortality were determined. Median (range) of colostral IgG concentrations were 99.4 (54.8 – 137.1), 95.2 (11.4 - 164.9) and 100.7 (73.0 – 128.0) g/L for own dam, other dam and pooled groups, respectively. Serum IgG levels at 0 hours prior to colostrum ingestion was 9 mg/dL across all calves indicating calves had not nursed prior to feeding colostrum. Median (range) serum IgG levels at 24 hours were 5204 (3,779 – 10,276), 5,566 (3,765 – 9,450) and 5,306 (3,131 – 8,950) mg/dL for calves that ingested colostrum from own dam, other dam, and pooled, respectively. This indicated that all calves had adequate passive transfer of immunity. We did not find any differences in the colostral IgG concentrations among the own dam, other dam and pooled colostrum sources (P > 0.05). Serum IgG concentrations at 24 hours were not different among the 3 calf groups (P > 0.05). Weaning weights, proportions of morbidity or mortality were not different (P > 0.05) among the calves fed colostrum from own dam, other dam or pooled colostrum. Pooling had no effect on passive immunity and calf health. Our study results suggests that on dairy farms with high median colostral IgG, pooling has minimal effect on passive immunity of colostral immunoglobulins.

F17

On-Arrival Blood Transcriptomes Identify Altered Immune Pathways in Stocker Cattle That Develop Bovine Respiratory Disease

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Bovine respiratory disease (BRD) is a multifactorial cause of morbidity and mortality in post-weaned beef cattle. Interactions between infectious agents, host immune response, and environmental risk factors are poorly understood. We hypothesize that altered transcriptional profiles in the blood of weaned, marketed, and transported calves impact regulatory pathways responsible for protection from and resistance to disease. Blood was collected from the jugular vein into Tempus tubes (ThermoFisher) from bull and steer calves (n=80, mean weight=206 kg) at stocker facility arrival. Animals were monitored for clinical signs of BRD based on the DART system. Cattle diagnosed with BRD within the first 14 days following arrival (n=6), and cattle without signs of BRD over the 84-day period of evaluation (n=5) were selected for blood RNA sequencing (Illumina HiSeq 3000). Sequencing reads (80M paired end/sample) were quality filtered and aligned to the bovine reference genome assembly ARS-UCD1.2. False discovery rate (FDR) adjusted p-values of 0.10 were applied to identify differentially expressed genes, utilizing the analysis tool edgeR. Sixty-three differentially expressed genes were identified between groups; 40 were downregulated and 23 were upregulated in diseased calves. The online resources DAVID v6.8 and String v10.5 were used to identify biological functions, pathways, and networks represented in calves that developed BRD based on differentially expressed genes. We identified pathways related to immune function, response to external stimuli, metabolic processes, and cell regulation, providing a profile of transcriptionally active pathways that differ between stocker cattle that develop BRD and those that do not.

F18

The Role of Hypophosphatemia in Downer Cow Syndrome: Retrospective Study of 995 Cases

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Hypophosphatemia is commonly observed in recumbent cows. Whether it truly has a causal role in the downer cow syndrome is still under debate. The objective of this retrospective study was to estimate the impact of low serum phosphate concentration on the prognosis for the downer cows, in particular during the periparturient period. The results of this study should contribute to investigate the possible role of hypophosphatemia in the etiology of the downer cow syndrome.

Medical records from all periparturient (3 weeks postpartum) recumbent dairy cattle presented between 1994 and 2016 at the Centre Hospitalier Universitaire Vétérinaire of the Université de Montréal were studied. Exclusion criteria included coxofemoral luxation and/or long bone/vertebra fractures. Several covariables were available. Only potentially important variables in the phosphorus homeostasis were retained for the multivariate model; history (age, duration of recumbency), hospitalization (diagnosis, final outcome) and blood analysis results (endotoxemia, calcium, potassium, BHB, AST, creatinine). The phosphorus concentration was considered normal when the value was between 1.05 mmol/L and 2.83 mmol/L. A multivariate logistic regression model was used to explore the association between predictors implied in the epidemiology of hypophosphatemia and survival. A total of 995 cows were included in the analysis. Among them, 545 (54.8%) survived after hospitalization. Hypophosphatemia was observed in 186 cows (18.7%). According to our results, hypophosphatemia was not significantly associated with survival of downer cows. However, AST, creatinine and duration of recumbency were significantly associated with survival. Our results suggest that the prognosis of periparturient downer cows is not affected by a low serum phosphate concentration and therefore, that the role of hypophosphatemia in the etiology of the downer cow syndrome is questionable.

### F19

**Comparison of Thoracic Ultrasonography and Thoracic Radiography to Detect Lung Lesions in Hospitalized Dairy Calves**

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The detection of lung lesions in dairy calves is commonly achieved using thoracic ultrasonography (TUS) and radiography (TR). However, it remains unclear from the literature if one diagnostic procedure is superior to another. The main objective of this study was therefore to evaluate the sensitivity (Se) and specificity (Sp) of TUS and TR to detect lung lesions in dairy calves with the hypothesis that TUS and TR have similar Se and Sp.

Fifty hospitalized dairy calves (≤7 days; ≤100kg; standing; with or without respiratory signs) were enrolled. After collection of clinical data, each calf was successively evaluated with TUS (positive if lung consolidation ≥1cm) and TR (positive if an alveolar pattern was visualised) by two blinded operators. Only calves with lesions detected by TUS or TR underwent a computed tomography (CT) (positive if lung consolidation was present) used as the gold standard. All imaging tests were performed within 24 hours. Se and Sp were estimated by a two-stage Bayesian method.

Among the 50 calves, 26 have lung lesions on CT (Table). SeTUS and SeTR were 0.68 (95% Bayesian credible Intervals (BCI):0.61-0.86) and 0.75 (95%BCI:0.70-0.91), respectively. SpTUS and SpTR were 0.88 (95%BCI:0.62-0.99) and 0.87 (95%BCI:0.62-0.99), respectively. There was only a mild difference between Se and Sp of both tests (SeTUS - SeTR: -1.45 (95%BCI:-1.72;-1.33) and SpTUS - SpTR: -1.75 (BCI:-1.92;-1.33)). In conclusion, in this study, TUS and TR have a good Se and Sp and TUS was mildly less performant than TR to detect lung lesions in dairy calves.

### Table: Descriptive data for the comparison between thoracic ultrasonography (TUS) and thoracic radiography (TR) in 47 dairy calves.

|       | TR+ | TR- | Total |
|-------|-----|-----|-------|
| TUS+  | 22  | 2   | 24    |
| TUS-  | 4   | 19  | 23    |
| Total | 26  | 21  | 47    |

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**GI01**

**Familial Protein Losing Enteropathy in Gordon Setters: A Genome Wide Association Study**

Elle Donnini - Iowa State University; Max Rothschild - Iowa State University; Muhammed Walugembe - Iowa State University; Albert Jergens - Iowa State University; Karin Allenspach - Iowa State University

Canine protein losing enteropathy (PLE) is a multi-factorial disease caused by interactions between the intestinal immune system, intestinal microbiota, and environmental factors in susceptible individuals. We have identified a line of Gordon Setters in the United Kingdom that were disproportionally affected by PLE at a young age. The aim of the present study was to identify genomic regions and single nucleotide polymorphisms (SNPs) which may predispose Gordon Setters to familial PLE. Genomic DNA from 106 Gordon Setters (6 affected dogs from an affected litter, 6 case controls from the same litter, 10 related affected dogs, and 84 non-affected dogs) was collected and extracted from buccal mucosal swabs. Genotyping of cases and controls was performed using the Canine Illumina HD SNP array and data generated were analyzed in PLINK using two analyses; the pairwise (case/control) fixation index (FST) and runs of homozygosity (ROH). Several regions and possible single SNPs on chromosomes 10, 12, 15, 17, 18, 21, and 23 were detected to be associated with familial PLE in Gordon Setters using the ROH method and FST windows approaches. Searching one Mb up- and down-stream of the most significant SNPs, as identified by single SNP ROH analyses as well as 200Kb before and...
Fecal samples were tested for the presence of previously reported risk factors. A secondary objective was to examine this dataset for the detection of T. foetus in 264 (16%) fecal samples. Fecal samples collected via fecal loop, or colon flush), and prior treatments administered for diarrhea including ronidazole. The only drug effective for clearing the infection, ronidazole, can be associated with treatment failure, but the incidence of this is unknown. Without assessment of the impact of collection conditions on PCR test results or knowledge of the effectiveness of ronidazole, it will remain difficult to optimize diagnosis and treatment failure, but the incidence of this is unknown. Without assessment of the impact of collection conditions on PCR test results or knowledge of the effectiveness of ronidazole, it will remain difficult to optimize diagnosis and treatment of T. foetus infection. In an effort to address these unknowns, the objective of this study was to determine any relationship between fecal PCR test results and treatment history. Association of Feline Tritrichomonas foetus Fecal PCR Results with Sample Collection Technique and Treatment History

Barry Hedgespeth – North Carolina State University; Stephen Stauffer – North Carolina State University; Jody Gookin – North Carolina State University

T. foetus is a common cause of chronic diarrhea in cats, yet diagnosis and treatment of the infection remain challenging. Fecal polymerase chain reaction (PCR) testing is considered to be the most sensitive means for diagnosis of the infection, but results are suspected to be influenced by fecal collection technique and by prior use of antimicrobial drugs. The only drug effective for clearing the infection, ronidazole, can be associated with treatment failure, but the incidence of this is unknown. Without assessment of the impact of collection conditions on PCR test results or knowledge of the effectiveness of ronidazole, it will remain difficult to optimize diagnosis and treatment of T. foetus infection. In an effort to address these unknowns, the objective of this study was to determine any relationship between fecal PCR test results for T. foetus and information gathered by convenience from the submission form accompanying samples submitted to a veterinary diagnostic laboratory. A secondary objective was to examine this dataset for the presence of previously reported risk factors. Fecal samples were tested for the presence of T. foetus ribosomal DNA using a validated single-tube nested PCR assay. When reported, information was collected regarding the cat’s age, breed, sex and neuter status, geographical location, whether the cat was from a single-versus multiple-cat household, method of fecal collection (voided, fecal loop, or colon flush), and prior treatments administered for diarrhea including ronidazole. A total of 1,808 fecal samples from 1,717 individual cats were submitted between January 2012 and December 2017. T. foetus was detected in 264 (16%) fecal samples. Fecal samples collected via fecal loop were more likely (odds ratio [OR] 1.8, 95% confidence interval [CI] 1.25-2.58, P=0.002) to be positive than samples collected by voiding or colonic flush. Neither the history of prior treatment nor the type of treatment was significantly associated with PCR result. After an initial positive PCR test, 15/20 (75%) cats treated with ronidazole had a negative follow-up PCR test. Junior cats (7 to 35 months of age) were more likely (OR 1.5, 95% CI 1.17-2.02, P=0.002) while senior cats (132 to 179 months of age) were less likely (OR 0.4, 95% CI 0.16-0.78, P=0.011) to have a positive PCR test result. Intact male cats were more likely (OR 2.2, 95% CI 1.58-2.95, P < 0.001) while spayed females were less likely (OR 0.7, 95% CI 0.53-0.98, P=0.043) to have a positive PCR test result. Purebreds, specifically Bengal, Abyssinian, Savannah, and Cornish Rex, were more likely than other breeds to have a positive PCR result.

In contrast to current recommendations, the results of this study identify that the use of a loop for fecal collection in cats increases the odds of obtaining a positive PCR test result for T. foetus. Administration of medications to cats prior to PCR testing did not impact PCR test results. Conversion from a positive to a negative PCR status was observed in 75% of cats treated with ronidazole. Identification of which cats have higher odds of infection, the optimal technique for sample collection, and percentage of cats responding to ronidazole will improve recognition, diagnosis, and treatment expectations in cats with T. foetus infection.

GI03

Pain Scores in Cats with Pancreatitis Compared to Control Cats

Susan Mehain – Washington State University Veterinary Teaching Hospital; Jennifer Slovak – Washington State University Veterinary Teaching Hospital; Nicolas Villarino – Washington State University Veterinary Teaching Hospital; Tamara Grubb – Washington State University Veterinary Teaching Hospital

Currently, there are only two validated pain assessment scales for cats: the UNESP-Botucatu multidimensional composite pain scale (UBMCPs) and Glasgow composite measure pain scale (GCMPS). These scales have been validated in cats with acute, post-operative pain. Pancreatitis is a common medical condition that can cause significant pain in affected individuals. Feline pancreatitis is typically chronic in nature and may be underdiagnosed because signs are vague and cats are notoriously known for masking their discomfort. The goal of this study was to determine if cats diagnosed with clinical pancreatitis had higher pain scores using the UBMCPs and GCMPS when compared to a control population of cats without clinical pancreatitis. This was a prospective study. A total of 18 cats presenting for suspected pancreatitis were screened, of which 13 qualified for enrollment based on clinical signs (ex. lethargy, dysrexia, anorexia, weight loss) and abnormal feline pancreatic lipase immunoreactivity (Texas A&M Spec fPLI) values. All 13 cats had initial pain scores on admission performed using both pain scales (UBMCPs and GCMPS). There were 7 cats enrolled as controls that had normal Spec fPLI values and had also received pain scoring. This study was approved by IACUC (ASAF #04802-008). The data were evaluated to determine if there was a significant difference between the initial pain scores of cats diagnosed with clinical...
Pain assessment in feline pancreatitis at diagnosis compared to recheck evaluation

Susan Mehain – Washington State University Veterinary Teaching Hospital; Jennifer Slovak – Washington State University Veterinary Teaching Hospital; Nicolas Villarino – Washington State University Veterinary Teaching Hospital; Tamara Grubb – Washington State University Veterinary Teaching Hospital

Cats can mask pain or have subtle signs that can be easily missed. Pain in cats is underappreciated, undertreated, and difficult to differentiate from behavioral manifestations of fear, stress, or anxiety. However, if pain goes undetected, an increase in disease morbidity and unnecessary suffering can result.

The objective of this study was to determine if cats with pancreatitis would have a detectable reduction in pain scores at a recheck evaluation based on use of validated pain scales. The pain scales utilized included the UNESP-Botucatu multidimensional composite pain scale (UBMCPS) and Glasgow composite measure pain scale (GCMPS), which rely on observations including posture, response to palpation, and facial expressions to assign a total pain score value. It was hypothesized that pain scores using the UBMCPS and GCMPs would be higher at the time of diagnosis compared to pain scores at a follow-up evaluation.

This was a prospective study. A total of 18 cats were screened for pancreatitis, of which 13 qualified for enrollment based on clinical signs and a feline pancreatic lipase immunoreactivity assay (Texas A&M; Spec fPLI) value of > 5.7 ug/L. Cats were recruited for the study based on a suspicion of pancreatitis. Exclusion criteria included a negative SNAP feline pancreatic lipase (fPLI) or severe concurrent disease. All 13 cats had initial pain scores performed using both pain scales previously described (UBMCPS and GCMPs). Pain was assessed by both a hands-on observer and a remote observer that assigned scores based on videos obtained. Of those 13 enrolled in the study, 10 cats had follow-up pain scores and Spec fPLI values measured. This study was approved by IACUC (ASAF #04802-008).

The data were evaluated with a paired t-test to determine significant differences between the observers’ mean scores, and between mean pain scores in cats at the time of diagnosis with pancreatitis and at follow-up. There was no difference between the observers’ mean scores for GCMPS and UBMCPS. There was sufficient evidence to suggest that cats had lower overall pain scores using both pain scales at follow-up compared to at the time of initial presentation (t = 2.6819, p = 0.0125 < 0.05 and t = 2.4688, p = 0.01782 < 0.05, respectively).

These results support that cats with pancreatitis have a detectable reduction in their pain scores at the time of recheck evaluation compared to their initial presentation at the time of diagnosis with pancreatitis.

GI05

Effect of Fecal Microbiota Transplantation on the Fecal Microbiome of Healthy Dogs Treated with Antibiotics

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Antibiotics cause profound fecal microbiome alterations. This study aimed to assess the efficacy of fecal microbiota transplantation (FMT) administered orally or per enema on fecal microbiome recovery after tylosin treatment of healthy dogs.

Sixteen healthy, parasite-free research dogs were enrolled. One dog with normal fecal dysbiosis index (DI) was selected and served as fecal donor prior to study start. All dogs were then treated with tylosin 20mg/kg daily (days 1-7), and randomly assigned to receive FMT either with one enema (day 8, n=6), or using daily oral capsules (days 8-21, n=6). Control dogs received daily placebo capsules (days 8-21, n=4). Fecal samples were collected and frozen at days 1, 8, 15, 22, 29 and 42. Quantitative PCR was performed on all samples for 7 bacterial groups, and DI was calculated. Two-way-ANOVA with pairwise comparison was used for statistical analysis, p

DI increased significantly in all groups at day 8 (mean difference 7.92, 95% CI 6.85 to 8.99). While DI decreased in all groups at day 15, dogs receiving oral FMT had a lower DI than controls at days 15 (−2.37, CI -0.35 to 4.39) and 22 (−2.36, 95% CI −0.34 to −4.38). For all groups, qPCR results for all bacteria were changed at day 8, and most normalized by day 15. However, normalization of qPCR for Clostridium hiranonis and Faecalibacterium was significantly delayed until day 22 in controls but not in FMT dogs.

These results suggest that FMT holds promise in correcting acute intestinal dysbiosis.

GI06

IL-13 and IL-33 mRNA are under-expressed in German shepherd dogs with inflammatory bowel disease

Aarti Kathrani – Royal Veterinary College; Victor Lezcano – Tuskegee University; Edward Hall – University of Bristol; Albert Jergens – Iowa State University; Jonathan Mochel – Iowa State University; Todd Atherly – Iowa State University; Karin Allenspach – Iowa State University

Ulcerative colitis (UC), a subtype of human inflammatory bowel disease (IBD), is primarily a result of Th2-mediated inflammation. Anti–cytokine biologic drugs targeting the Th2 pathway have been shown
to be effective for the treatment of UC. A recent genome-wide association study in German shepherd dogs (GSD) with IBD has implicated polymorphisms in the Th2 cytokine genes. Therefore, the aim of the study was to determine if the mRNA expression of the Th2 cytokines, interleukin (IL) -13 and IL-33 are altered in the duodenal mucosa of GSDs with IBD compared to non-GSDs with IBD and to healthy dogs. This research was a retrospective study using archived paraffin-embedded duodenal biopsies collected via endoscopy from 20 client-owned dogs diagnosed with IBD (10 GSDs and 10 non-GSDs) at Bristol Veterinary School and 8 healthy Beagle dogs from the Iowa State University Service Colony. A novel RNA in situ hybridization (ISH) technology, RNAscope was used to hybridize IL-13 and IL-33 mRNA probes onto at least 10 duodenal biopsy tissue sections from each dog. RNAscope signals were visualized using a microscope and semi-quantitative assessment, as well as all cell counts were performed by a single operator.

GSDs with IBD had significantly lower IL-13 and IL-33 mRNA expression compared to non-GSDs with IBD (IL-13 and IL-33: based on estimated total cell count and duodenal villous, subvillous, epithelium and lamina propria expression scores; P < 0.001) and healthy Beagle dogs (IL-13: based on duodenal villous, subvillous and lamina propria expression scores; IL-33: based on estimated total cell count, duodenal villous, subvillous, epithelium and lamina propria expression scores; P < 0.001).

GSDs with IBD exhibited under-expression of IL-13 and IL-33 mRNA in the duodenal mucosa compared to non-GSDs with IBD and healthy Beagle dogs. Similar to human patients with UC, these data indicate that Th2 cytokines could be implicated in the pathogenesis of IBD in GSDs.

GI07

Cytokine Production Following Stimulation of Ex-Vivo Whole Blood with Diet in Dogs with Chronic Enteropathy

Aarti Kathrani – Royal Veterinary College; Edward Hall – University of Bristol

Ex vivo whole blood stimulation assays have been used to characterize the cytokine response to intact and hydrolyzed protein diets in individual healthy immunotolerant cats. The present study aimed to use this assay to determine the cytokine response to diets being fed at the time of diagnosis to dogs with chronic enteropathy (CE) and to compare this to a control group of dogs presented for non-gastrointestinal (GI) causes.

Dogs with at least a 3 - week history of persistent or intermittent GI signs and dogs presented for non-GI related causes were prospectively recruited. For each case, residual blood from sampling for laboratory testing as part of the diagnostic investigation was placed into heparin. Ex vivo whole blood stimulation assays were performed using crude extracts of the diet currently being fed and provided by the owner. Saline was used as a negative control. Supernatants were collected and analyzed for tumor necrosis factor (TNF)-alpha, interleukin (IL)-10, and IL-4 using an enzyme-linked immunosorbent assay.

Thirty-five dogs with chronic GI signs were recruited; 4 were excluded due to confirmed or suspected GI neoplasia. The final case group consisted of 22 dogs with CE diagnosed on histopathology of GI biopsy and 9 with suspected CE. The non-GI group consisted of 18 dogs. Hydrolyzed protein diets elicited significantly lower IL-10 and TNF-alpha concentrations compared to commercial intact protein diets in dogs with confirmed or suspected CE (P-value 0.004 and < 0.001, respectively). Six out of 15 dogs with detectable IL-4 concentrations in the confirmed CE group had IL-4 to IL-10 ratios that exceeded the 95% confidence interval (CI) of the mean of the non-GI group (non-GI: 95% CI of IL-4:IL-10 = 0.64 - 2.71; confirmed CE: IL-4:IL-10 in 6 dogs = mean 22.40, range 2.77 - 89.11).

Hydrolyzed protein diets elicited a significantly reduced cytokine response when incubated with patient whole blood ex vivo compared to commercial intact protein diets in dogs with confirmed or suspected CE. The IL-4 to IL-10 ratio as a marker of dietary responsiveness warrants further investigation, together with assessment of the cytokine response to diet at the intestinal mucosal surface.

GI08

Fat-Soluble Vitamin Deficiencies in Canine Chronic Enteropathy

Kristen Maxwell – University of Tennessee; Elizabeth Lennon - University of Pennsylvania

Chronic enteropathies such as inflammatory bowel disease (IBD) can result in nutrient malabsorption. In human IBD patients, deficiencies in fat soluble vitamins have been frequently documented and treatment is recommended. In veterinary medicine, deficiency of vitamin D in IBD and intestinal lymphoma have been demonstrated in dogs and cats, and vitamin K deficiency has been suspected in cats with IBD. However, the incidence of deficiencies of vitamins A, E, and K are unknown. Vitamins A, D, E, and K all have immunomodulatory and/or anti-inflammatory properties, so deficiency of these vitamins could exacerbate intestinal inflammation. Therefore, recognition of deficiency and supplementation may be an important adjunct to successful treatment. The purpose of this study was to investigate whether dogs with chronic enteropathy have differences in serum fat-soluble vitamin concentrations.
concentrations of vitamins A, D and E compared to healthy dogs. We hypothesized that, like humans, dogs with chronic enteropathy would have lower concentrations of vitamins A, D and E than healthy controls. Blood samples were collected from healthy purpose-bred dogs, dogs with chronic enteropathy, and healthy client-owned dogs following IACUC approval and client informed consent. Dogs were included in the healthy group if they had no evidence of systemic disease based on history, physical examination, and had a complete blood count and serum or plasma chemistry profile performed within the past year. Dogs were included in the chronic enteropathy group if they had > 3 weeks of gastrointestinal symptoms and no underlying cause identified based on bloodwork and ultrasound findings, and had a serum vitamin B12 sample analyzed. Dogs were excluded if they had a concurrent condition known to affect serum vitamin concentrations, if they were eating a homemade diet, or receiving vitamin supplementation. Vitamins A as retinol and vitamin E as α-tocopherol were analyzed by isocratic ultra-performance liquid chromatography (UPLC) and vitamin D as 25-hydroxyvitamin D was analyzed by a commercially available radioimmunoassay kit at the Michigan State Veterinary Diagnostic Laboratory. Normality was assessed by a Shapiro-Wilk test. Differences between groups were assessed by a Kruskal-Wallis test (Vitamins A and E, which had 3 groups of dogs) or a Mann-Whitney U test (Vitamin D, which did not have a healthy client-owned group) and significance was set at p < 0.05. Thirty-three healthy purpose-bred dogs (Beagle and Hounds), 19 dogs with chronic enteropathy, and 6 healthy client-owned dogs (vitamins A and E only) were enrolled in the study. Vitamin D (25-hydroxyvitamin D) was significantly decreased in dogs with chronic enteropathy (median: 127 nmol/L; IQR: 63-318) compared to healthy purpose-bred dogs (median: 288 nmol/L; IQR 12.2-38.6), p = 0.02. Vitamin E as α-tocopherol was not significantly different between dogs with chronic enteropathy (median: 19.6 μg/ml; IQR 12.2-38.6), healthy purpose-bred dogs (median: 25.41 μg/ml; IQR 20.6-27.7), and healthy client-owned dogs (median: 26.2 μg/ml; IQR: 17.0-54.0), p = 0.44. However, there was an extremely wide variability in serum α-tocopherol concentrations in dogs with chronic enteropathy, and 5/19 dogs had serum α-tocopherol concentrations that were > 2 standard deviations below the mean of the healthy dogs, indicating that these individual dogs likely had a deficiency of vitamin E despite the fact that overall the population was not different from the healthy dogs. Vitamin A as retinol was not significantly different between groups (chronic enteropathy, median: 791.5 ng/ml; IQR: 504.3-1069.0; healthy purpose bred, median: 609.0 ng/ml; IQR: 537.0-715.5; healthy client owned: median: 657.0 ng/ml; IQR: 476.8-824.3), p = 0.25. Based on our preliminary data we can conclude that, consistent with previous reports, a subset of dogs with chronic enteropathy suffer from vitamin D deficiency. Additionally, a subset of dogs with chronic enteropathy have deficiency of vitamin E. Future studies assessing serum retinyl esters are planned, as most vitamin A is transported as retinyl esters in carnivores, so the lack of differences in retinol may not truly reflect Vitamin A status. Future studies will also assess the role of vitamin supplementation in dogs with vitamin deficiencies secondary to chronic enteropathy.

GI09

Duodenal Expression of Antimicrobial Peptides in Dogs with Idiopathic Inflammatory Bowel Disease and Intestinal Lymphoma

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Although antimicrobial peptides (AMPs) play an integral role in the regulation of intestinal microbiota and homeostasis, their expression in canine gastrointestinal diseases, including idiopathic inflammatory bowel disease (IBD) and intestinal lymphoma, remains unknown. The objective of this study was to investigate the intestinal expression of AMPs in dogs with IBD or intestinal lymphoma.

IBD was diagnosed in 44 dogs, small cell intestinal lymphoma in 25, and large cell intestinal lymphoma in 19 dogs; 20 healthy beagles (research colony dogs) were used as normal controls. Duodenal mRNA expression of six representative AMPs—lactoferrin, lysozyme, cathelicidin, secretory leukocyte peptidase inhibitor (SLPI), bacterial permeability increasing protein (BPI), and canine beta defensin (CBD103)—was quantified by real-time reverse transcription polymerase chain reaction. The relative expression of BPI, lactoferrin, and SLPI was significantly higher in dogs with IBD and intestinal lymphomas than in healthy controls. Interestingly, the expression patterns of AMPs differed between dogs with IBD and those with intestinal lymphomas. Increased expression of BPI differentiated IBD from dogs with intestinal lymphomas as well as healthy control dogs, with a sensitivity of 86.3%, a specificity of 100%, and an area under the curve of 0.948. These results suggest that the expression patterns of AMP aid in the diagnosis of canine IBD and intestinal lymphoma, although it remains uncertain whether the altered AMP expression is the cause or effect of mucosal inflammation.

GI10

Fecal Microrna 29a is Increased in Dogs with Chronic Enteropathy

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MicroRNAs are small, non-coding RNAs that regulate gene expression. MicroRNAs are associated with gut immunity and intestinal barrier function. A study in humans with irritable bowel syndrome showed that the glutamine synthetase gene (GLUL) regulates intestinal permeability. MicroRNA 29a (miR-29a) regulates the gene GLUL. The purpose of this study was to evaluate fecal miR-29a in dogs with chronic enteropathy (CE).

Forty-seven dogs were selected for this study: 25 healthy controls and 22 with CE. Among the 22 CE dogs, 7 were classified as having food-responsive enteropathy (FRE), and 15 as steroid-responsive enteropathy (SRE). Fecal samples were collected and RNA extraction was performed using the Stool Total RNA Purification Kit (Norgen Biotek). Samples were normalized using miR-26b as an endogenous control. The TaqMan system was used to perform reverse transcription PCR (RT-PCR) and subsequently real-time PCR (q-PCR). MIIR-29a
fold change expression was calculated. Significance was set as (P < 0.05).

The expression of miR-29a was significantly increased (P = 0.001) in the feces of dogs with CE compared to healthy control dogs. In addition, when comparing miR-29a expression among chronic enteropathy subgroups, dogs with SRE showed increased miR-29a expression (P = 0.0004) compared to healthy control dogs.

The observed increased expression of miR-29a suggests that a subset of dogs with CE may have increased intestinal permeability, as previously proposed. Future studies should correlate fecal miR-29a with histopathological findings and other measures of intestinal permeability.

GI11

Tylosin Causes Dysbiosis Associated with Altered Fecal Unconjugated Bile Acids in Healthy Dogs

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Tylosin is a commonly prescribed treatment for diarrhea in dogs. Oral antibiotics may alter the intestinal microbiota, which performs key bile acid biotransformation reactions. In human medicine, altered bile acid proportions are implicated in chronic intestinal disorders. The purpose of this study was to evaluate the impact of tylosin on the composition of the fecal microbiota and bile acid profile of healthy dogs.

Sixteen healthy client-owned dogs were randomized to receive oral tylosin at 20 mg/kg or a placebo capsule q12h for 7 days in a double-blinded fashion. Fecal samples were collected on days 0, 7, and 63. The fecal microbiota was analyzed by 16S rRNA sequencing, and proportions of primary vs. secondary fecal unconjugated bile acids (UBA) were determined using a gas chromatography-mass spectrometry assay. Parameters were compared using ANOVA or Friedman test, for parametric or non-parametric data, respectively, followed by corrections for multiple comparisons. Statistical significance was set at P < 0.05.

In the placebo group, there were no significant changes in the fecal microbiota or the proportions of fecal UBA over time. Bacterial species richness and evenness were decreased in dogs treated with tylosin at day 7. At day 63, the Shannon index remained lower (mean ± SD: 4.52 ± 0.83) than the day 0 index (mean ± SD: 5.23 ± 0.80; P = 0.047). Tylosin administration was associated with significant shifts in overall microbial composition as indicated by unweighted UniFrac distances between samples from the three time points (ANOSIM; R = 0.632, P = 0.001). In both groups at day 0, fecal UBA were predominantly secondary UBA (median, [range]: 94.2%, [8.1-100]). Tylosin administration was associated with an increase in the proportion of primary fecal UBA at day 7 (85.5%, [44.1 - 90.6]) compared to day 0 (61.1%, [0.2 - 22.7], P = 0.037). At day 63, there was a persistent significant decrease in the proportion of lithocholic acid compared to day 0 (P = 0.025).

Oral tylosin administration resulted in alterations of the fecal microbiota with concurrent changes in fecal UBA. These shifts did not uniformly resolve after the antibiotic was discontinued.

GI12

Evaluation of the Recovery of the Fecal Microbiome and Metabolome of Dogs Following Acute Diarrhea

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In dogs treated for chronic enteropathies, clinical remission may be achieved after weeks of treatment, but fecal dysbiosis may persist for months. In dogs with acute diarrhea, the recovery of the fecal microbiome and metabolome has not been studied. The aim of this study was to investigate the recovery of the fecal microbiome and metabolome of dogs with acute hemorrhagic diarrhea syndrome (AHDS) or acute uncomplicated diarrhea (AD).

Fecal samples were collected from 19 healthy control dogs (HC), 5 dogs with AHDS (0, 7, 14, and 90 days after diagnosis), and 5 dogs with AD (0, 6, and 30 days). Microbiota was characterized using Illumina sequencing of 16S rRNA genes, and analyzed with QIIME, and statistics were performed with ANOSIM. In addition, metabolites were extracted and untargeted liquid chromatography high resolution accurate mass spectrometry analysis was performed. Statistics were performed using MetaboAnalyst 4.0. Significance was set at p < 0.05.

Clinical scores for all dogs with AHDS were classified as severe at day 0, and had improved to clinically irrelevant scores by day 7 (p < 0.001). Dogs with AD had moderate to severe clinical scores at day 0, and mild to irrelevant scores at day 6 (p = 0.05). At the time of diagnosis, bacterial communities for both AHDS and AD groups were significantly different compared to healthy controls (p < 0.001). Differences persisted in all follow up samples for AHDS (p < 0.005) and AD (p < 0.001). Out of 1,112 metabolites identified, 261 were found to be significantly different in AHDS and/or AD compared to HC. Several amino acids, such as D-tryptophan (p < 0.001), were found to be increased in both AHDS and AD at day 0. Degradation products of tryptophan, such as indole and kynurenine, were also found to be increased in AHDS at day 0 (p < 0.001).

Dogs with AHDS and AD showed alterations in their microbiome and metabolome, compared to healthy dogs, which remained 30 and 90 days post-diagnosis, respectively. Our findings indicate that an acute episode of diarrhea may have a longer lasting impact on the fecal microbiome and metabolome than previously acknowledged.

GI13

Lymphatic Endothelial Cell Immunohistochemistry for Evaluation of Intestinal Lymphatics in Dogs with Chronic Inflammatory Enteropathy

Sara A. Wennogle – Colorado State University; Simon Preistnall – Royal Veterinary College; Alejandro Suárez-Bonnet – Royal Veterinary College; Siri Soontararak – Colorado State University; Craig Webb – Colorado State University

In dogs with chronic enteropathies, clinical remission may occur, but intestinal permeability is often found to be increased. The lymphatic system is integral to the immune response, and potential alterations in intestinal lymphatics have not been characterized in dogs with chronic enteropathies.

The purpose of this study was to investigate the recovery of the intestinal lymphatic system in dogs with chronic enteropathies, and evaluate the rate of recovery of the intestinal lymphatics post-diagnosis. The study included 5 dogs with chronic enteropathy (CE) and 5 healthy control dogs (HC). Lymphatic endothelial cell (LEC) immunohistochemistry was performed on small intestinal biopsy samples from the ileum. The lymphatic endothelial cell marker LYVE-1 was used to label LECs.

The observed changes in LEC immunohistochemistry were quantified using ImageJ. The results showed that the number of LECs was significantly reduced in dogs with CE compared to HC (p < 0.05). The recovery of LECs was slower in dogs with CE than in HC, with a persistent decrease in the number of LECs at day 90 post-diagnosis. The findings suggest that the intestinal lymphatic system may be impaired in dogs with chronic enteropathies, and that the recovery of the lymphatic system may be delayed compared to healthy dogs.
Lymphatic endothelial cell (LEC) immunohistochemical markers have identified intestinal lymphatic vasculature abnormalities in humans with inflammatory bowel disease (IBD), but have not been used to evaluate the intestinal lymphatic vasculature in a group of dogs with chronic inflammatory enteropathy (CIE). The objectives of this study were to utilize LEC markers to identify and quantify the intestinal lymphatic vasculature in endoscopic biopsies of CIE dogs and to evaluate whether measured parameters of the lymphatic vasculature correlate to serum albumin concentrations.

24 dogs with CIE were prospectively enrolled. Thirteen dogs had a serum albumin concentration < 2.5 g/dL (defined as CIE-protein-losing enteropathy [PLE]) and 11 dogs had a serum albumin concentration ≥ 2.5 g/dL.

LEC immunolabeling with Prox-1 and LYVE-1 was performed on endoscopic biopsies from 24 dogs with CIE. Duodenal and ileal villous lacteal width (VLW), proprial mucosal lacteal width (MLW), and number of proprial mucosal LEC were determined for each case and analyzed for correlation to serum albumin. Lacteal dilation scores via routine histopathology were assessed for correlation to VLW and MLW.

Serum albumin concentrations were correlated with mucosal LEC in the duodenum (p = 0.0332) and ileum (p = 0.0038) and with VLW (p = 0.0223) and MLW (p = 0.0006) in the ileum. LEC IHC identified presumptive proprial mucosal lymphangiectasia in some dogs. Lacteal dilation scores correlated to VLW in the duodenum (p = 0.02) and ileum (p = 0.008), but did not correlate to MLW in either section of intestine. LEC immunolabeling identified presumptive proprial mucosal lymphangiectasia in CIE dogs, particularly in the ileum of hypoalbuminemic dogs. Routine evaluation of villous lacteals alone is likely underestimating abnormalities of the lymphatic vasculature in dogs with CIE.

**GI14**

**Comparison of the Fecal Dysbiosis Index Between Growing Puppies and Adult Dogs**

Ammalis Cigarroa - GI Laboratory Texas A&M University College of Veterinary Medicine; Amanda Blake - Texas A&M University; Theresa Keating - Guide Dogs for the Blind; Patti Van De Coevering - Guide Dogs for the Blind; Jonathan Lidbury - Texas A&M University; Joerg Steiner - Texas A&M University; Jan Suchodolski - Texas A&M University

The fecal Dysbiosis Index (DI) is a recently developed, rapid quantitative PCR-based assay that measures the abundance of 8 bacterial taxa in a fecal sample to quantify the degree of fecal microbial dysbiosis in adult dogs. It is of interest to evaluate whether the DI differs in puppies during the postnatal period and at which age it resembles the distribution observed in adult dogs. Recent studies using 16S rRNA gene sequencing have shown that puppies after birth harbor predominantly aerobic bacteria such as E. coli, which are typically increased in adult dogs with dysbiosis. As puppies age, their microbiome shows a higher proportion of anaerobic bacteria, with a concurrent decrease in E. coli. We hypothesized that the DI is increased after birth and decreases with increasing age.

Fecal samples were collected from healthy puppies (n = 83) of different age groups: 1-2 weeks (n = 16), 3-4 weeks (n = 14), 5-6 weeks (n = 15), 7-9 weeks (n = 17), 10-16 weeks (n = 12), and 17-48 weeks (n = 9). Fecal samples were also collected from adult dogs (age range: 1-13 years; n = 13). Fecal DNA was extracted and analyzed by quantitative PCR for total bacteria, Faecalibacterium, Turicibacter, Stretotococcus, E. coli, Blautia, Fusobacterium, and Clostridium hiranonis. The data was also expressed as a single numerical value, the dysbiosis index (DI). Data were analyzed using a Kruskal-Wallis test followed by a Tukey’s multiple comparison test to identify differences between age groups.

Until 6 weeks of age, the DI was significantly increased compared to adult dogs (P < 0.001). This was due to a predominance of E. coli. Compared to 1-2 week old puppies, the DI was significantly decreased in the 7-9 week age group (P = 0.018), and the abundance of E. coli was significantly decreased in the 10-12 week age group (P = 0.014). The abundance of Turicibacter (P = 0.003), Faecalibacterium (P = 0.001), Blautia (P = 0.004), and C. hiranonis (P < 0.001) was increased in all other age groups compared to the 1-2 week age group. The DI for the 17-48 week age group was not significantly different compared to that of adult dogs (P = 0.998).

Newborn puppies have an increased DI driven by increased E. coli and a decreased abundance of anaerobic bacteria. The results of this study suggest that DI decreases during the postnatal period and does not differ significantly from adult dogs after 16 weeks of age.

**GI16**

**A Prospective Study of Effects of Fat Ingestion on the Intestinal Mucosa in Normal Dogs**

Su-Jin An – Gyeongsang National University Animal Medical Center; Dong-In Jung – College of Veterinary Medicine, Gyeongsang National University; Do-Hyeon Yu – College of Veterinary Medicine, Gyeongsang National University

A high-fat meal before the diagnostic procedure could help to effectively identify patients with intestinal lymphangiectasia increasing the conspicuity of the characteristic dilated lacteals. However, the accurate dose and timing of the high-fat meal are unknown. The purposes of this prospective study are to evaluate the effects of corn oil as fat on healthy beagle dogs by investigating physiological changes in the intestinal mucosa over time and we aim to establish the correct application of the high-fat diet to diagnose enteropathy.

The amount of corn oil was set at half the nutritionally recommended intake based on the AAFCO dog food nutrient profiles, to avoid exceeding the daily fat intake, considering the possibility that the excess may be judged similar to the abnormal change. The entire small intestine of five healthy dogs were scanned in real time by ultrasonography (Arietta 70, Hitachi Aloka®), and mucosal echogenicity evaluation was assigned at fasting, immediate (0 hour), 1 hour, 3 hours, 6 hours, and 12 hours after the ingestion of corn oil. Small intestinal wall thickness did not differ significantly over time. The conspicuity of hyperechoic foci, or horizontal lines parallel to the submucosa, or vertical striation lines to the submucosa was observed as early as immediately after ingestion and remained prominent up to 3 hours later (p < 0.05).

Based on the ultrasonography experiments, conventional endoscopy (GIF-160, Olympus®), capsule endoscopy (MC-1200-M, MiroCam®)
were performed at fasting, 3 hours, and 12 hours after the ingestion of corn oil. All dogs showed normal mucosal appearance and the color was pinkish white to yellowish. However, the mucosal roughness, pinpoint whitish mucosal foci and diffuse edematous aspect at 3 hours were somewhat confirmed, compared with the images at fasting ($p < 0.05$). In microscopic examination of biopsy samples obtained by conventional endoscopy, appropriate tissues were sectioned so that the long axis of the villi could be observed and variable degrees of lacteal dilatation, within normal variation, were present in these tissues. The percentage of the dilated villi to total villi was higher at 3 hours than at 12 hours ($p < 0.05$). Mucosal changes were considered physiological changes after fat ingestion; however, mild mucosal changes in the small intestine in healthy dogs from immediately after to within 3 hours from fat ingestion were observed. The intestinal mucosa gradually recovered after fat ingestion, it can be allowed for differentiation between normal and diseased intestines at 12 hours. Thus, post-12-hour examination in healthy subjects does not affect the diagnosis, and intestinal lymphangiectasia can be successfully diagnosed even at 12 hours after a low dose of high-fat intake.

Prevalence of Methanogens in Fecal Samples of Dogs with Chronic Enteropathy

Sandra Bermudez Sanchez – Gastrointestinal Laboratory, Dept. Small animal clinical sciences, Texas A&M University; Rachel Pilla – Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University; Joerg Steiner – Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University; Jonathan Lidbury – Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University; Jan Suchodolski – Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University

Methanogenic archaea are commensal microorganisms found in the mammalian intestinal tract that are responsible for methane production. Humans with irritable bowel syndrome have an increased abundance of methanogenic archaea. Limited data is available about the potential role of methanogenic archaea in the pathogenesis of intestinal disease of dogs. The aim of this study was to quantify methanogenic archaea in fecal samples from dogs with chronic enteropathy.

Fecal samples were collected from 14 healthy dogs and 20 dogs with chronic enteropathy (CE). Fecal DNA was extracted using a commercial kit (PowerSoil, QIAGEN). The abundance of methanogenic archaea was evaluated by quantitative PCR (qPCR), targeting the gene mcrA that encodes a specific enzyme involved in methanogenesis. A Mann-Whitney test was used for comparison of the abundance between groups, and Fisher’s exact test for comparison of dogs that are positive for methanogenic archaea. Significance was set at $p < 0.05$.

While dogs with CE did have a higher prevalence of methanogenic archaea (8/20 dogs or 40%) than healthy control dogs (3/14 dogs or 21%) this difference was not significantly different ($p > 0.295$). There was also no significant difference in the abundance of methanogenic archaea between groups ($p = 0.297$).

This study did not identify a significant difference in methanogens between dogs with CE and healthy control. Further studies in a larger cohort of dogs are warranted to determine whether an increase in sample size would lead to different findings or whether certain subgroups of dogs with CE are associated with a higher prevalence of methanogens.
GI18

Biomechanical Comparison of Two Percutaneous Gastropexy Techniques for Percutaneous Endoscopic Gastrostomy Tubes

Bradley Bishop – University of Florida; Alex Gallagher – University of Florida

Percutaneous endoscopic gastrostomy (PEG) tubes are placed to allow enteral nutrition support in a variety of disease conditions. Myriad complications can occur during placement of PEG tubes or in the post-operative period, including early tube dislodgement resulting in septic peritonitis. The objective of this study was to evaluate two percutaneous gastropexy techniques for securing PEG tubes using biomechanical assessment and evaluation of procedure time.

Eighteen canine cadavers were assigned to one of three groups: PEG tube only, PEG tube with T-fastener gastropexy, and PEG tube with U-stitch gastropexy. Time to completion of placement of PEG tube and gastropexy was recorded. After placement, the stomach and left abdominal body walls were removed and biomechanical testing performed.

T-fastener and U-stitch techniques required more force to induce failure than the PEG only technique (p = .016 and p = .006, respectively). Both techniques required more time than placing a PEG tube alone (p = .004 for both). There were no differences between groups for weight or sex.

Performing T-fastener or U-stitch gastropexy may decrease the risk of early PEG tube dislodgement in dogs. However, studies evaluating the techniques in live dogs are necessary to confirm this hypothesis. The extended procedure time to perform either of the percutaneous gastropexies was not clinically significant.

GI19

Quantification of Fecal Bile Acids and Clostridial Species in Puppies

Amanda B. Blake – Texas A&M University Gastrointestinal Laboratory; Annalis Cigarroa – Texas A&M University; Patricia Ishii – Texas A&M University; Jonathan Lidbury – Texas A&M University; Joerg Steiner – Texas A&M University; Jan Suchodolski – Texas A&M University

Bile acids are potent signaling molecules that affect numerous physiological functions through bile acid receptors in the gastrointestinal (GI) tract. Studies in humans have shown that secondary bile acids can inhibit the proliferation of Clostridium difficile, which is often found in healthy and diseased dogs. Clostridium hiranonis has been identified as an important converter of primary to secondary bile acids in the dog. Because alterations in bile acid metabolism are frequently seen in dogs with GI disease, it is important to assess the development of bile acid metabolism during the postnatal period. Therefore, the aim of this study was to quantify fecal bile acids, C. hiranonis, and C. difficile during the postnatal period of healthy puppies.

Fecal samples were also collected from healthy adult dogs (age range 1-13 years; n = 13). DNA was extracted for analysis of C. hiranonis and C. difficile by qPCR. A gas chromatography-mass spectrometry assay was used to quantify unconjugated fabe bile acids. Kruskal-Wallis tests followed by Dunn's multiple comparison tests were used to assess differences between age groups, and Spearman's Rho was used to assess correlations between variables.

The secondary bile acids lithocholic acid and deoxycholic acid, increased significantly in the 7-9 week age group when compared to the 1-2 week and 3-4 week age groups (p < 0.01). The abundance of C. hiranonis also increased significantly in the 7-9 week age group (p < 0.001), which coincided with a significant decrease of C. difficile abundance (p < 0.05). In the 7-9 week age group and older groups, secondary bile acid concentrations and C. hiranonis and C. difficile abundance did not significantly differ from adult dogs. Total secondary bile acid concentrations had a significant positive correlation with C. hiranonis abundance (ρ = 0.761; p < 0.001) and a significant negative correlation with C. difficile abundance (ρ = -0.591; p < 0.001).

Our results suggest that secondary fecal bile acid concentrations and C. hiranonis and C. difficile abundance were similar to those of adult dogs after 6 weeks of age, and that there is a relationship between secondary bile acid concentrations and C. hiranonis and C. difficile abundance in puppies.

GI20

Comparison of Plasma, Serum, and Whole Blood Amino Acid Concentrations in Healthy Dogs

Amanda B. Blake – Texas A&M University Gastrointestinal Laboratory; Patricia Ishii – Texas A&M University; Jonathan Lidbury – Texas A&M University; Joerg Steiner – Texas A&M University; Jan Suchodolski – Texas A&M University

Amino acids play an important role in the regulation of intestinal inflammation. Previous studies have shown serum concentrations of some amino acids, such as tryptophan and glutamic acid, to be altered in dogs with chronic enteropathies. Amino acids are usually measured in plasma or whole blood. However, there are no studies that have directly compared plasma, serum, and whole blood amino acid concentrations in dogs. This study aimed to identify differences in amino acid profiles of plasma, serum, and whole blood.

Plasma, serum, and whole blood samples were obtained from 46 healthy dogs. All samples were deproteinized within 48 hours of collection and stored at -80°C until analysis with a Biochrom 30+ Amino Acid Analyser (Biochrom Ltd, Cambridge, UK). Friedman tests followed by Dunn’s multiple comparisons tests were used to assess differences between the sample types.

Of the 38 amino acids evaluated, 32 had at least one significant difference between plasma, serum, and whole blood samples (p < 0.05). Concentrations of 23 amino acids were significantly higher in serum than in plasma (p < 0.05). Tryptophan had significantly lower concentrations in whole blood (median [min-max]: 32 μM [20-53 μM]) than plasma (59 μM [31-112 μM]; p < 0.001) and serum (60 μM [30-114 μM]; p < 0.001). Glutamic acid concentrations were highest in whole blood (61 μM [42-88 μM]) followed by serum (36 μM [25-58 μM]) and
plasma (27 μM [16-47 μM]), with all three sample types being significantly different from each other (p < 0.001).

In conclusion, this study suggests that while some amino acids are present in similar concentrations in plasma, serum, and whole blood, others are dependent on sample type and warrant strict adherence to sample type based reference intervals.

**GI21**

**Serum Cytokines before and after Treatment in a Cohort of Dogs with Chronic Enteropathy**

Julien Dandrieux - University of Melbourne; Leilani Santos - University of Melbourne; Lina Maria Martínez Lopez - University of Melbourne; Caroline Mansfield - University of Melbourne

Chronic enteropathy (CE) in dogs is characterised by gastrointestinal and systemic inflammation, but little information is available on systemic inflammatory cytokines. This prospective study had two objectives: to compare (1) the concentration of inflammatory cytokines (interleukin-2 [IL-2], IL-6, and tumour necrosis factor alpha [TNFα]) between dogs with CE at diagnosis and healthy dogs and (2) the concentration of IL-2, IL-6, and TNFα before and after treatment in dogs with CE.

Seven dogs with CE had serum taken at diagnosis and at remission with CE. The CE group included 3 dogs with food-responsive enteropathy, 3 dogs with antibiotic-responsive enteropathy, and 1 dog with steroid-responsive enteropathy. Overall, there was a reduction in all cytokines measured after treatment for CE. The reduction in median IL-2 pre-treatment compared to post-treatment was from 7.3 pg/mL [4.0 – 99.4] to 3.4 pg/mL [3.4 – 578.4], for IL-6 from 20.5 pg/mL [3.6 – 617.2] to 8.6 pg/mL [3.6 – 482.2], and for TNFα from 9.3 pg/mL [6.0 – 419.5] to 6.0 pg/mL [6.0 – 352.0]. Only the reduction in IL-6 was significant with a p-value of less than 0.05.

The CE group included 3 dogs with food-responsive enteropathy, 3 dogs with antibiotic-responsive enteropathy, and 1 dog with steroid-responsive enteropathy. Overall, there was a reduction in all cytokines measured after treatment for CE. The reduction in median IL-2 pre-treatment compared to post-treatment was from 7.3 pg/mL [4.0 – 99.4] to 3.4 pg/mL [3.4 – 578.4], for IL-6 from 20.5 pg/mL [3.6 – 617.2] to 8.6 pg/mL [3.6 – 482.2], and for TNFα from 9.3 pg/mL [6.0 – 419.5] to 6.0 pg/mL [6.0 – 352.0]. Only the reduction in IL-6 was significant with a p-value of less than 0.05. TNFα reduction trended to significance at p-value 0.06 while IL-2 reduction was found not significant. In addition, there was no significant difference between dogs with CE prior to treatment and healthy dog for IL-2, IL-6 and TNFα with p-values of respectively 0.25, 0.24, and 0.45. The results from this pilot study suggest that a sample size of 42 to 60 dogs (depending on the cytokine) are required to reach significance.

No significant difference was seen between healthy dogs and dogs diagnosed with CE. Dogs with CE had a reduction in serum IL-6 concentration with successful treatment, regardless of treatment category. Further studies are needed to determine the role of IL-6 in CE, the potential role of serum IL-6 as a disease biomarker and the use of cytokines to differentiate between subtypes of CE.

**GI22**

**Chronic Enteropathies and Pruritus in Dogs**

Ricardo Duarte Braz - Faculdades Metropolitanas de São Paulo; Nathalia Artacho - Faculdades Metropolitanas Unidas; Ana Carvalho - Gastrovet; Ana Balda - Faculdades Metropolitanas Unidas

A canine chronic enteropathy activity index (CCECAI) was published in 2007, and pruritus was included among the variables of interest in the evaluation of the severity of the disease. The objective of this study was to further assess the association between chronic enteropathies (CE) and pruritus in dogs.

Thirty dogs with CE in remission, defined as a CCECAI <5 (“mild” or “insignificant” disease) after treatment, and not receiving corticosteroids for at least one month were enrolled in the study. Owners were invited to answer a pruritus severity scale validated for dogs with atopic disease. Owners of 30 healthy dogs (control group) answered the same questionnaire.

The median pruritus severity score for dogs with CE was 3.4 (range: 0 – 8), which was significantly higher than dogs of the control group (median: 1.6; range: 0 – 6, P = 0.026, Z-Score = 2.22). The main limitation of this study is that dogs with CE were not evaluated with the pruritus severity scale at the time of diagnosis, only after remission. Therefore, the impact of treatment of CE on pruritus cannot be assessed. However, it is noteworthy that some dogs in remission were receiving drugs that are used to treat pruritic dermatitis (e.g., cyclosporine).

It may be concluded that dogs with CE, even those successfully treated, have a higher score of pruritus than healthy dogs. If CE and pruritus share the immune-mediated mechanisms or if pruritus is a consequence of CE is unclear.

**GI23**

**Survey of Endoscopic Techniques for Esophageal and Gastric Foreign Body Removal in Dogs and Cats**

Alexander Gallagher - University of Florida, CVM; Alexandra Wood - University of Florida, CVM

Esophageal and gastric foreign bodies (EFB and GFB) are a common occurrence in cats and dogs. The objective of this study was to evaluate the endoscopic techniques and instruments used by internists or criticalists based on type and location of the foreign body.

An online, anonymous survey was created using a commercial software and approved by the UF IRB. The survey was distributed to diplomates of the ACVIM and ACVECC through each college’s listserv. Data obtained included specialty, years in practice, practice type, available instruments, and preferred instrument for various foreign bodies by location and type. Data was analyzed using descriptive statistics.

268 surveys were completed with 74% from private referral practice and 21% from academic practice. For EFB, laparoscopic or endoscopic grasping forceps were used most commonly and with a flexible endoscope. An overtube was used ~50% of the time for EFB including sharp objects and bones. For GFB, grasping forceps or loop snares were used most commonly with baskets or loop snares used for round/smooth
Evaluation of the Effect of a Famotidine Continuous Rate Infusion on Intragastric pH in Dogs

Katherine Hedges – University of Tennessee College of Veterinary Medicine; Adesola Odunayo – University of Tennessee College of Veterinary Medicine; Josh Price – University of Tennessee; Silke Hecht – University of Tennessee College of Veterinary Medicine; M.K. Tolbert – Texas A&M College of Veterinary Medicine

Acid suppressant drugs play a critical role in the management of upper gastrointestinal ulcerative and erosive conditions. Famotidine, a histamine-2 receptor antagonist, remains a commonly used acid suppressant in veterinary practice, despite the superior efficacy of proton pump inhibitors in raising canine intragastric pH and a failure to reach the goals for the treatment of acid-related disorders ulceration in people (i.e. gastric pH ≥ 3 and 4 for 75% and 67% of the day, respectively). Some veterinary institutions administer famotidine IV as a continuous rate infusion (CRI), but the efficacy of this practice is unknown. Our study objective was to determine if famotidine administered as IV CRI in healthy dogs achieved the aforementioned goals. In a randomized, two-way crossover study, 9 dogs received famotidine IV at 1 mg/kg q12h daily or 1 mg/kg loading dose followed by 8 mg/kg/day for three days. Intragastric pH was compared at baseline (day 0) and on treatment days 1, 2, and 3 within and between treatments using repeated measures mixed model ANOVA. The famotidine CRI resulted in a significant increase in mean pH, mean percentage time (MPT) intragastric pH ≥ 3, and pH ≥ 4 (p ≤ 0.001) on all treatment days. Only the famotidine CRI achieved pH goals for treatment of acid-related disorders and did so on all treatment days. Administration of famotidine by continuous rate infusion is effective in increasing intragastric pH in healthy dogs to a degree that corresponds with healing of proximal GI tissue injury in humans. Thus, famotidine CRI may be an alternative to treatment with intravenous proton pump inhibitors in dogs.

Correlation between Mucosal and Fecal S100A12 Levels and Histologic Changes in Canine Chronic Inflammatory Enteropathy

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S100A12 is a protein released from activated mononuclear cells. The concentration of S100A12 in feces correlated with the severity of clinical signs, endoscopic lesions, and histologic inflammatory lesions in dogs with chronic inflammatory enteropathies (CIE). Also, fecal S100A12 concentrations might serve as a prognostic marker. These results are interesting as the mucosal cellular infiltrate in dogs with CIE is primarily lymphocytic-plasmacytic. Whether fecal S100A12 concentrations reflect the number and/or activity of intestinal mucosal mononuclear cells, or whether the protein is also produced by other cells has not been investigated. Thus, the aim of this study was to evaluate intestinal mucosal S100A12 positivity and its relationship with serum and fecal S100A12 concentrations in dogs diagnosed with CIE.

Intestinal lamina propria S100A12 positivity (cytoplasmic–membranous staining pattern) ranged from 1–223 positive cells/mm² in the stomach, 8–368 cells/mm² in the duodenum, 16–215 cells/mm² in the ileum, and 9–351 cells/mm² in the colon. S100A12 positivity in the duodenum correlated statistically significantly with that in the stomach and ileum (p = 0.66 and 0.69, both P < 0.01), but was inversely correlated with the severity of macrophage infiltration in the duodenum (p = -0.47, P = 0.042). Serum S100A12 concentrations ranged from 148–248 μg/L (median: 201 μg/L) in this study, with corresponding 3-day mean fecal S100A12 concentrations ranging from 31 to 2,448 ng/g (median: 381 ng/g). Fecal S100A12 concentrations strongly correlated with the number of S100A12+ cells along the entire gastrointestinal tract (r = 0.76, P = 0.028), whereas an inverse association was seen between serum S100A12 concentrations and colonic S100A12+ cell counts (r = -0.50, P = 0.043).

These results suggest that the intestinal mucosa in dogs with CIE contains an increased number of activated (pro-inflammatory) phagocytes expressing and secreting S100A12 protein, but the macrophage population seen on routine histopathology is predominantly mature (anti-inflammatory) with a reduced or absent expression of S100A12. Double-staining of intestinal tissues with CD64 and S100A12 will be needed to prove our hypothesis that the reverse relationship between the number of phagocytes detected on routine histopathology and S100A12+ cells is due to an increase of pro-inflammatory (CD64+) phagocytes in dogs with CIE.

Comparative Analysis of the Effect of Intravenously Administered Acid Suppressants on Gastric pH in Dogs

Amanda R. Kuhl – University of Tennessee College of Veterinary Medicine; Adesola Odunayo – University of Tennessee College of Veterinary Medicine; Josh Price – University of Tennessee College of
Gastrointestinal (GI) bleeding can result from multiple diseases in dogs such as GI neoplasia and NSAID toxicity. These conditions may result in severe hemorrhage and anemia, leading to an increase in treatment cost and patient mortality. An important adjunctive therapy in treating GI hemorrhage is maintaining an environment favorable to clot formation, which requires potent inhibition of gastric acid secretion. Thus, gastric acid suppression using intravenous acid suppressants like proton pump inhibitors (PPIs, e.g. pantoprazole, esomeprazole) and histamine-2 receptors blockers (e.g. famotidine) is commonly recommended in critically ill dogs with gastrointestinal hemorrhage. Pantoprazole is often used for the treatment of GI bleeding in hospitalized dogs. However, in our preliminary studies and published work by Seo et al., a constant rate of infusion (CRI) of famotidine and esomeprazole appeared to provide more potent acid suppression. A comparative evaluation was needed to confirm these findings.

Thus, our central objective was to evaluate the efficacy of intravenously administered esomeprazole, pantoprazole, and a CRI of famotidine in increasing the intragastric pH of dogs. We hypothesized that esomeprazole and famotidine CRI would provide superior acid suppression compared to pantoprazole in dogs and reach pH goals for the treatment of GI bleeding.

In a randomized, 3-way crossover study, nine healthy dogs received esomeprazole and pantoprazole at 1 mg/kg IV q12h and famotidine with a loading dose of 1 mg/kg followed by an 8 mg/kg IV CRI daily for three days. Intragastrian pH was recorded using continuous pH monitoring at baseline and the following three treatment days. Mean pH as well as the mean percentage time (MPT) the intragastric pH was ≥ 3 or ≥ 4 were compared among and within treatment groups using repeated measures mixed model ANOVA.

Significant increases in mean pH (p << 0.0001), MPT ≥ 3 (p = 0.0012), and MPT ≥ 4 (p = 0.006) were noted over time with all three treatments. Although no significant differences were observed among treatment groups for days 1 - 3 for mean pH, MPT ≥ 3, and MPT ≥ 4, only esomeprazole and famotidine CRI achieved the goals established for the treatment of gastroduodenal ulceration and gastroesophageal reflux disease in people on all treatment days. Although further study is warranted, famotidine CRI and esomeprazole may be superior acid suppressants compared to standard doses of pantoprazole.

Conjugated and Unconjugated Bile Acids in Feces From Dogs with Chronic Inflammatory Enteropathy

Alison C. Manchester – Texas A&M; Sara Wenngle – Colorado State University; Andrea Martinez Aguirre – Texas A&M; Amanda Blake – Texas A&M; Joseph Sorg – Texas A&M; Joerg Steiner – Texas A&M; Jonathan Lidbury – Texas A&M; Jan Suchodolski – Texas A&M

Altered fecal bile acid (BA) profiles have been identified in humans and dogs with chronic intestinal disorders. In healthy individuals, secondary BAs are more abundant in feces. This is due to a coordinated set of biotransformations whereby intestinal bacteria deconjugate and dehydroxylate conjugated primary BAs to form unconjugated secondary BAs. The contribution of conjugated BAs to the total fecal BA pool has not been investigated in dogs. The purpose of this study was to determine the contribution of conjugated BA to the total fecal BA pool in healthy dogs and dogs with chronic inflammatory enteropathy (CIE).

The study population was comprised of 11 client-owned dogs diagnosed with CIE based on histopathologic evaluation. Eight healthy client-owned dogs served as controls. A single fecal sample from each dog was lyophilized prior to analysis via high-performance liquid chromatography with an evaporative light scattering detector (HPLC-ELSD) to evaluate levels of cholic acid (CA), chenodeoxycholic acid (CDCA), taurocholic acid (TCA), glycocholic acid (GCA), taurochenodeoxycholic acid (TCDCA), glycochenodeoxycholic acid (GCDCCA), lithocholic acid (LCA) and deoxycholic acid (DCA). A Mann-Whitney test was used to fecal BA levels between groups. Statistical significance was set at P < 0.05.

Concentrations of total fecal BAs did not differ significantly between the groups. However, concentrations of primary BAs were higher in feces of dogs with CIE (median, [range]: 119.4 nmol/gm, [10.2 - 1057.0]; P = 0.026) than healthy dogs (22.6 nmol/gm, [14.0 - 33.7]). The percentage of primary BAs was also higher in feces of dogs with CIE (87.9%, [4.1 - 100.0]; P = 0.011) than healthy dogs (6.9%, [2.9-12.3]). Concentrations of secondary BAs were higher in feces of healthy dogs (250.6 nmol/gm, [125.2 - 1133.0]; P = 0.006) than dogs with CIE (29.5 nmol/gm, [0 - 589.0]). Conjugated BAs comprised (median 3.8%, [0.9 - 10.9]) of the total fecal BA pool in healthy dogs as compared to (12.3%, [0.8 - 59.2]; P = 0.051) in dogs with CIE. Dogs with CIE have altered fecal BA profiles. Further work is needed to determine the clinical implications of these findings.
Small breed dogs less than 10 kg were overrepresented (66 / 72) and there was no significant difference in body weight between ILCL, ISCL and CE. For the duodenum, minimal duodenal wall thickness was significantly thinner in ILCL (median 2.67 mm, P < 0.01) compared to ISCL (median 3.34 mm) and CE (median 3.59 mm). For the colon, on the other hand, minimal wall thickness was significantly thicker in ILCL (median 1.47 mm, P < 0.05) compared to ISCL (median 0.81 mm) and CE (median 0.92 mm). Indistinct or loss of duodenal wall layering was more frequently observed in ILCL than in ISCL and CE (57.1 % vs 4.2 % vs 8.3 %, P < 0.01) and indistinct or loss of jejunal wall layering was more frequently observed in ILCL than in CE (66.7 % vs 20.8 %, P < 0.05). Mucosal surface irregularity was more commonly observed in the duodenum of ILCL than in ISCL and CE (61.9 % vs 12.5 % vs 8.3 %, P < 0.01) and in the jejunum of ILCL than in CE (45.8 % vs 8.3 %, P < 0.05). Jejunal lymph nodes thickness were significantly thicker in ILCL (median 8.4 mm, P < 0.05) and ISCL (median 5.6 mm, P < 0.05) versus CE (median 3.9 mm). However, the current study revealed markedly enlarged lymph nodes (thickness > 10 mm) only in a small number of ILCL and ISCL (20 % and 19 %, respectively).

Although most ultrasonographic features of ILCL, ISCL and CE were overlapped in small breed dogs, thinning of duodenal wall, indistinct or loss of wall layering and irregular mucosal surface of the duodenum or jejunum and enlarged jejunal lymph nodes may be indicative of ILCL. Villous atrophy or ulceration may have contributed to small intestinal wall thinning and mucosal surface irregularity in ILCL, although further investigation is needed. The ultrasonographic features of ISCL and CE differ only in the presence of enlarged jejunal lymph nodes.

**GI29**

*Effect of Oral Superoxide Dismutase Supplementation on the Clinical Improvement of Canine Inflammatory Bowel Disease*

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Oxidative stress plays a crucial role in inflammatory bowel disease (IBD), and superoxide dismutase (SOD) helps ameliorate it in humans. However, the efficacy of SOD in canine IBD is unknown. Therefore, the objective of this study was to determine whether oral SOD supplementation restores clinical abnormalities in dogs with IBD. This study was conducted as a prospective, randomized, double-blind, clinical trial of 18 dogs with IBD for 90 days. The dogs were randomly assigned to three groups: the control group (standard IBD treatment, n = 6), group 1 (standard IBD treatment plus oral SOD 250 IU/day PO, n = 6), and group 2 (oral SOD 250 IU/day, n = 6). IBD clinical improvement was evaluated using the canine inflammatory bowel disease activity index (CIBDAI), an endoscopic assessment, a histopathologic evaluation, and serum C-reactive protein concentrations before (day 0) and after the treatments (days 7, 30, 70, and 90). CIBDAI scores decreased in all groups after the treatments. The CIBDAI score decreased on day 90 in the control group compared to days 0 and 7. The CIBDAI score decreased on days 70 and 90 in group 1 compared to day 0. The CIBDAI score decreased on days 70 and 90 in group 2, compared to day 0, and it also decreased on day 70 compared to day 7. The CIBDAI score of group 2 was significantly lower than that of the control group on days 30 and 70. Furthermore, the percentage change in the CIBDAI score of group 1 was significantly lower than that of the control group on days 7, 30, and 70. It was also lower in group 2 compared to the control group on days 30, 70, and 90. However, no significant differences were observed in the other variables. No side effects were detected after SOD supplementation. These results indicate that oral SOD supplementation is likely to ameliorate clinical signs without side effects in dogs with IBD, although the effect of SOD might not be associated with a histopathological improvement.
human. These abnormal DNA methylation could be involved in tumori-
genesis of the disease and could be also useful to provide diagnostic or prognostic markers that utilize specific DNA methylation changes.

GI31

Thromboelastographic Evaluation in Dogs with Acute Pancreatitis
Chul Park – College of Veterinary Medicine, Chonbuk National University; Junho Lee – Chonbuk National University; Juhee Rye Student – Chonbuk National University; Hyonjong Chung Student – Chonbuk National University

Pancreatitis has been known as the most frequent disease in the exo-
crine pancreas of dogs. Acute pancreatitis is a process of acute inflam-
ation of the pancreas that is involved in a variety of related tissues or remote organ systems that do not cause permanent changes. Acute pancreatitis has a variety of clinical symptoms including anorexia, vomiting, diarrhea, nervous disorders, melena, weight loss, hematem-
esis, leukocytosis, neutropenia, thrombocytopenia and coagulopathy. Thromboelastography(TEG) is able to identify all hemostatic steps including initiation, amplification, and proliferation of coagulation, including the dissolution of fibrin as well as the relationship between platelets and leukocytes. Therefore, the purpose of this study is to evaluate the degree of coagulopathy in dogs with acute pancreatitis using TEG analysis. This study consisted of ten healthy dogs and ten dogs with acute pancreatitis (patient group). A total of twenty dogs were tested for aPTT, PT, D-dimer, and platelets counts as a conventional coagulation test and also TEG analysis. Acute pancreatitis was diagnosed with history, clinical signs, physical examination, serum chemistry, cPLI ELISA kit test, and abdominal ultrasound. In the results, the value of Angle (table 1, p = 0.013) and the MA value (table 1, p = 0.035) showed a tendency to increase in patient group. Also, the value of R was significantly decreased in patient group (table 1, p = 0.010). In conclusion, this study showed the hypercoagulable states in dogs with acute pancreatitis compared to healthy dogs except the K value.

GI32

Evaluation of PTHrP as a Biomarker in Feline Pancreatitis
Jennifer E. Slovak – Washington State University College of Veterinary Medicine

Feline pancreatitis continues to be an elusive disease in our feline patients. Clinical signs are vague and non-specific, the etiology of the disease is unknown and the pathophysiology is poorly understood. Chronic pancreatitis in cats, as well as in humans, is characterized by a more fibrotic process versus acute inflammatory component; as a result of perpetuating inflammation from repeated injury or altered blood supply, leading to irreversible fibrosis. Parathyroid related protein (PTHrP) is expressed in many tissues and exerts multiple effects in disease states. In quiescent pancreatic acinar cells, PTHrP is present in low levels, however when an acinar cell is injured, there is upregulation of PTHrP expression, and subsequently PTHrP levels increase in human and rodent cells.

To date, there has been no investigation of PTHrP serving as a bio-
marker in cases of feline pancreatitis. Our hypothesis was that cats with chronic pancreatitis would have systemically elevated PTHrP levels. The study was descriptive and prospective. A population of cats diag-
nosed with feline pancreatitis (n=9) and a control population of cats without pancreatitis (n=9) were included. Cats were diagnosed with pancreatitis based on suspicion of clinical signs, supportive laboratory values (quantitative fPLi >5.4 μg/L), and ultrasonographic suspicion (irregular pancreatic margins and hyperechoic peri-pancreatic fat). All the enrolled cats also had serum PTHrP levels tested at the time of their diagnosis.

None of the cats in the pancreatitis group, and 0/9 cats in the control group had elevated systemic PTHrP levels. A Wilson score confidence interval on the proportion of cats having the PTHrP biomarker is 0.299, meaning we expect that 30% or less of the population will have the PTHrP biomarker. Although a small patient sample size was utilized, sys-
temic PTHrP is not likely a reasonable biomarker for feline pancreatitis.

GI33

Canned Gastrointestinal Diets and Feline Fecal Occult Blood Testing
Kate E. Spies – Washington State University College of Veterinary Medicine; Jennifer Slovak – Washington State University College of Veterinary Medicine

The goal of this study was to determine the number of positive FOB tests in a population of healthy cats fed canned veterinary prescribed gastrointestinal-formulated diets. A fecal occult blood (FOB) test rapidly and qualitatively screens patients’ stool for microscopic amounts of blood. Diets containing certain protein and vegetable sources as well as canned or moisture rich foods have been proposed as possible interferants of FOB tests. FOB tests are commonly utilized in human medicine, less frequently in canine medicine, and even more rarely in feline medicine to diagnose enteric pathology. In fact, compared to humans and dogs, there is limited literature available investigating FOB testing in cats.

| Table 1. Results of TEG value on healthy dogs (n=10) and dogs with acute pancreatitis (n=10). The reference range was based on the article (Bauer et al., 2009). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variables       | Healthy group   | Patient group   | Reference       | P-value         |
|                 | (n=10)          | (n=10)          |                 |                 |
| R(min)          | 2.7±0.2         | 1.6±0.2         | 1.8 – 8.6       | 0.010           |
| K(min)          | 2.0±0.2         | 1.3±0.2         | 1.3 – 5.7       | 0.710           |
| Angle(*)        | 65.4±3.3        | 77.9±1.7        | 36.9 – 74.6     | 0.013           |
| MA(mm)          | 61.4±2.8        | 71.9±2.3        | 42.9 – 67.9     | 0.035           |
The goal of this study was to determine the number of positive FOB tests in a population of healthy cats fed canned veterinary prescribed gastrointestinal-formulated diets. Ten clinically healthy, indoor-only cats aged 1 - 8 years old were initially fed their normal diets, then transitionally fed two gastrointestinal canned diets over a 7 - week period: canned hydrolyzed protein Hill’s Z/d diet® and canned gastrointestinal formulated Purina EN diet®. Two fecal samples were tested per cat each week using human point-of-care Hemocult® tests.

A logistic mixed effects regression analysis was performed to relate the number of positive FOB samples to the type of diet given. A likelihood ratio test was performed to test the effect of the diet and strong evidence was found to suggest that the diets differed in the odds of positive FOB tests p = 0.004. The odds of a positive FOB test from canned EN fed cats was significant p < 0.00001, and the odds of a positive FOB test from the canned Z/d fed cats was p < 0.935. Cats given a canned non hydrolyzed gastroenteric diet may have a greater likelihood of having a false positive FOB test than cats fed a canned hydrolyzed protein diet. FOB test results should be interpreted with caution in feline patients eating non-hydrolyzed canned foods.

**GI34**

**Clinical, Clinicopathologic, and Histologic Variables in Dogs with Chronic Enteropathy and Low or Normal 25(OH)D**

Sara A. Wennogle – Colorado State University; Simon Preistnall – Royal Veterinary College; Alejandro Suárez-Bonnet – Royal Veterinary College; Craig Webb – Colorado State University

The mechanism of low vitamin D status in dogs with chronic inflammatory enteropathy (CIE) is not well understood. The objective of this study was to improve understanding of the pathogenesis of low vitamin D in dogs with CIE by evaluating variables associated with intake of vitamin D, concentrations of other fat soluble vitamins and vitamin D serum binding proteins, and markers of systemic and intestinal inflammation in dogs with CIE and low or normal serum 25-hydroxyvitamin D (25(OH)D).

Fifteen dogs with CIE and low serum 25(OH)D concentrations and 15 dogs with CIE and normal serum 25(OH)D concentrations were prospectively enrolled. Clinical and clinicopathologic variables were compared between groups. Correlations between serum 25(OH)D and histopathologic variables were also assessed.

Higher canine chronic enteropathy clinical activity index (CCECAI) scores (p= 0.0032), lower serum α-tocopherol (p= 0.0007), cholesterol (p< 0.0001), and albumin (p< 0.0001) concentrations and higher serum C-reactive protein (CRP) (p= 0.0041) concentrations were more likely in CIE dogs with low serum 25(OH)D when compared to CIE dogs with normal 25(OH)D. Serum concentrations of vitamin D binding protein (VDBP) were not different between groups (p= 0.9105). Duodenal morphologic and inflammatory (p= 0.0016 and p= 0.0039, respectively) histopathologic scores and total histopathologic scores in duodenum and combined duodenum/ileum (p= 0.0003 and p= 0.0024, respectively) were negatively correlated with serum 25(OH)D.

The pathogenesis of low vitamin D status in dogs with CIE is likely multifactorial. Fat malabsorption deserves further study in dogs with low vitamin D and CIE. Loss of VDBP may not be significant mechanism of low vitamin D status in dogs with CIE.

**GI35**

**Amoxicillin-Clavulanate in Dogs with Acute Diarrhea: Minimal Alteration of the Intestinal Microbiome Composition**

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Short-term antibiotic treatment with amoxicillin-clavulanate is frequently used in dogs with acute diarrhea. Previous studies showed significant tylosin- and metronidazole-induced dysbiosis, however, the impact of amoxicillin-clavulanate on the intestinal microbiota has not been fully elucidated. This study aimed to evaluate the influence of amoxicillin-clavulanate on the intestinal microbiome of dogs with acute diarrhea.

Sixteen dogs with acute diarrhea were randomly assigned to either treatment- (AG: amoxicillin-clavulanate 12.5–25 mg/kg PO q12h for 7 days) or placebo-group (PG; lactose powder q12h for 7 days). Fecal scores were evaluated over 10 days. Fecal samples were collected on days 0, 6, and 30. Total bacteria and 8 bacterial groups (Faecalibacterium, Fusobacterium, Turicibacter, E. coli, Streptococcus, Blautia, C. hiranonis) were analysed by qPCR. A numeric value, the Dysbiosis Index (DI), was calculated. Results were compared among AG, PG and 8 healthy dogs (HG). On day 0, dogs with diarrhea showed lower abundances of Faecalibacterium compared to HG (p = 0.027). DI showed no significant difference between the dogs with diarrhea (median [range]: -1.44 [-5.31 to +5.45]) and the HG (-4.58 [-7.49 to +1.95]; p > 0.999). Comparing dogs with diarrhea, no differences were observed in the DI (p > 0.999), the assessed bacterial taxa (p > 0.949), or fecal scores (p > 0.999) between AG and PG.

Dogs with acute diarrhea treated with amoxicillin-clavulanate showed no faster improvement of stool-consistency compared to the placebo group. Dogs with diarrhea showed minimal alterations of intestinal microbiota but treatment with amoxicillin-clavulanate did not significantly influence the microbiome.

**HM01**

**Retrospective Evaluation of Splenectomy in the Treatment of Canine Primary Immune Thrombocytopenia**

Melissa R. Gettinger – Iowa State University College of Veterinary Medicine; Barbara Glanemann – Royal Veterinary College, University of London; Austin Viall – College of Veterinary Medicine, Iowa State University; Julia Veir – Veterinary Teaching Hospital, Colorado State University; Chad Johannes – College of Veterinary Medicine, Iowa State University; Laura Van Vertloo – College of Veterinary Medicine, Iowa State University
Splenectomy, an effective second-line therapy for people with steroid-refractory or dependent primary immune thrombocytopenia (pITP), has not been evaluated as a therapy for canine ITP. This study’s objective was to evaluate the efficacy and safety of splenectomy as a treatment for canine pITP.

In response to requests on specialty veterinary listservs, case information from 13 pITP dogs which underwent splenectomy was received from 6 institutions.

Reasons for splenectomy included refractory ITP (2/13, 15.4%), side effects of immunosuppressive therapy (3/13, 23.0%), splenic mass suspicious for neoplasia (6/13, 46.2%), or a combination (2/13, 15.4%). Splenic histopathology was benign in all cases.

Blood products were administered pre-operatively in 5/13 cases, and intraoperative complications were described in 1/13 cases (fluid-responsive hypotension). All patients survived surgery and were discharged. Post-operative complications included suspected thrombus (2/13) resulting in euthanasia in one dog, sudden death at home possibly due to hemorrhage (1/13), and incisional infection (1/13).

The clinician’s goal of splenectomy was met in all cases. Mean platelet count increased significantly from pre-surgery (124.3 K/µL +/- 119.2) to post-surgery (741.3 K/µL +/- 506.3) (p = 0.0064; paired t-test).

Relapse of ITP post-surgery was described in 2/13 cases, resulting in euthanasia in one. Of the dogs surviving >14 days post-operatively, 1 was lost to follow-up as of last documented communication (3 to 112 weeks post-splenectomy).

Splenectomy may be a useful therapy for canine pITP and warrants further prospective evaluation.

HM02

Cold Storage of Canine Platelet Concentrates in Additive Solutions: An in vitro Assessment

Sara Ravicini – Washington State University; Jillian Haines – Washington State University; Julianne Hwang – Washington State University; Kathrine Wardrop – Washington State University

Canine platelet transfusions are an essential tool to treat acute bleeding or prevent surgical complications in dogs with platelet disorders. Currently long-term storage is not advised, limiting the options for a potentially life-saving treatment, especially in emergency situations.

The goal of this study was to demonstrate that platelet additive solutions (PAS) can be used in place of plasma and that these solutions, when combined with cold storage at 4°C, reduce the occurrence and degree of platelet storage lesions, maintain platelet function, delay pathogen proliferation, and extend storage time of platelet concentrate for up to 7 days.

Platelet concentrates obtained from canine blood donors, were aliquoted into 4 separate bags containing 100% plasma (control) or 35% plasma and 65% of a PAS (Plasma-Lyte A, Isoplate, or InterSol). Samples were stored at 4°C without agitation. At days 0, 3, 5, and 7 samples were analyzed to determine the degree of platelet storage lesion as evaluated by measurement of metabolic markers, platelet activation markers, platelet aggregometry assessment; and bacterial growth. Platelet count and mean platelet volume, glucose, lactate, lactate dehydrogenase, pO2, pCO2 aggregation percentage via light aggregometry, activation percentages via flow cytometry detection of surface P-selectin, and bacterial contamination via culture were assessed to compare the 4 storage solutions.

Development of storage lesions was minimal, as demonstrated by maintenance of a mean pH >7.2 (p < 0.05) and mean lactate values < 4 mmol/L at day 7 (p < 0.05) in all solutions. InterSol did have a significantly lower pH than plasma and the other PAS (p < 0.05). Both lactate and pCO2 were significantly increased in plasma compared to all PAS (p < 0.05 and p < 0.01, respectively). Plasma, InterSol, Isoplate, and Plasma-Lyte A had mean (standard deviation) aggregation percentages of 27.8 (17.9), 5 (2.2), 28.9 (21), and 24.5 (10.4) respectively. InterSol had significantly lower aggregation than plasma, Isoplate, and Plasma-Lyte A at days 3, 5, and 7 (p < 0.01).

Glucose utilization did not vary significantly between any of the solutions. No significant difference was found between plasma and PAS for mean platelet volume, mean platelet component concentration, clumping, lactate dehydrogenase, platelet distribution width, and platelet count. No bacterial growth was found in any of the solutions.

Overall the PAS were comparable to plasma for the cold storage of platelets. Cold stored platelets showed minimal storage lesion development with no bacterial growth and, with the exception of InterSol, maintained function for up to 7 days.

HM03

Impact of Sampling Method on Thromboelastography Variables

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Thromboelastography (TEG) is currently regarded as state of the art method for the assessment of global coagulation. Standardisation of preanalytic conditions is pivotal to achieve accurate results. The aim of the study was to investigate the effect of two different blood sampling methods on TEG variables.

Blood from nine healthy beagle dogs was sampled simultaneously into citrated tubes. One tube was filled via the vacutainer port through a 20-gauge needle at the jugular vein and the second tube was filled by open tube catching through a 20-gauge cannula at the lateral saphenous vein. A kaolin activated trace was performed using a standardized protocol of 60 min stabilisation at room temperature with a TEG 5000 Analyser (Hemonetics) and the resulting variables were recorded.

Inferential statistics by Mann-Whitney test showed significant differences (P < 0.05) in the splitting point (SP) 2.98 min +/- 0.77 (median 2.9 min) vs. 3.97 min +/- 0.57 (median 4 min) and reaction time...
Shear stress by vacuum sampling seems to accelerate coagulation in jugular blood samples harvested by vacutainer. Thus there has to be a higher awareness of that already how a sample is drawn affects the results, and thereby sampling methods have to be standardized for thromboelastography to ensure accurate results. Data indicate that strict standardisation of the blood sampling method is required to ensure comparability of results.

**HM04**

**Gene Mutations Detected in Miniature Dachshunds Diagnosed with Myelodysplastic Syndrome in Japan**

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Miniature Dachshund is one of the most popular dog breeds in Japan. Occurrence of non-regenerative anemia in this breed is more common that in any other breeds, and most of them are diagnosed with myelodysplastic syndrome (MDS) from the peripheral blood and bone marrow characteristics. Therefore, we set the purpose of this study to explore gene mutations in Miniature Dachshunds diagnosed with MDS.

Whole exome sequencing (WES) analysis was conducted for comprehensive investigations of gene mutations in the cases. Genomic DNA was extracted from peripheral blood of 4 cases of Miniature Dachshunds diagnosed with MDS and 3 same breed dogs with normal CBC as control group. WES was performed using Illumina NextSeq 500. The alignment of processed reads to the canine reference genome (CanFam 3.1) was carried out using Genome Analysis Toolkit, and SnpEff was used for the annotations of the variants. Mutations that were detected in all four MDS cases but not in any control dogs were explored. Then, a new cohort composed of 20 Miniature Dachshunds diagnosed with MDS and 14 same breed dogs with normal CBC was examined for the presence of gene mutations.

The WES analysis revealed 59,495, 62,015, 61,851, and 61,844 variants in each dog with MDS. Of these variants, 13 variants in 10 genes were found in all four MDS cases but not in any control dogs. Validation for these 13 variants by Sanger sequencing confirmed the heterozygous point mutations of *Uromodulin Like 1 (UMODL1)* and X-Ray Repair Cross Complementing 5 (*XRCC5*) genes that resulted in single amino acid substitutions. In the new cohort, the mutations of *UMODL1* and *XRCC5* genes were found in 4 (20%) and 4 (20%) of the 20 MDS cases, respectively, but not in any of the 14 control dogs. The somatic mutations of *UMODL1* were observed in human MDS patients, and single nucleotide polymorphisms of *XRCC5* were shown to be associated with the susceptibility to MDS in humans. The present study indicated candidate gene mutations associated with MDS in Miniature Dachshunds, and further studies are needed to elucidate associations of the gene mutations with the development of MDS.

**HM05**

**Effects of Signalment and Phlebotomy Technique on Platelet Clumping in Cats**

Matthew Karnya – The Cat Clinic

Accurate measurement of platelet counts in cats can be difficult due to a high degree of platelet clumping that may confound automatic CBC analyzers. It has been suggested that phlebotomy technique may play a role in the occurrence and severity of platelet clumping. Several methods of phlebotomy are commonly used in feline medicine. The purpose of this study was to determine if the method of phlebotomy (jugular vein manual draw vs medial saphenous vein vacuum draw) influences the frequency or severity of platelet clumping, and to further investigate the effects of signalment (ie age and sex) on platelet clumping.

Samples were collected from cats presenting for blood collection at a group of primary care veterinary practices. Jugular samples were collected using a 22g 0.75” needle and a 3mL syringe by manual aspiration and medial saphenous samples were collected using a 22g 0.75” butterfly catheter by vacuum, both into a 2mL capacity K 2EDTA vacutainer-style collection tube filled to recommended capacity. Platelet counts, degree of platelet clumping, age, sex, and site of collection were recorded from each sample. Degree of clumping was scored on a 4 point system (none, mild, moderate, marked) as per standardized laboratory guidelines based on the percentage of platelets clumped. Cats with thrombocytopenia, thrombocytopenia, history of anticoagulant therapy, or history of thromboembolic events were excluded. Platelet count was determined by an automated analyzer, while degree of clumping was assessed by an experienced laboratory technician.

612 cats were included in the analysis. 37.6% of samples were collected from the jugular and 62.4% from the medial saphenous. 52% were from female cats and 48% from male, with a median age of 11 years (range 2m to 23y). Overall rates of platelet clumping were similar to those previously reported (10.46%). No significant difference was seen in clumping rates, clumping severity, or platelet counts between medial saphenous and jugular samples (Fig 1). There was no difference seen in any platelet variable based on age. Samples from neutered male cats had a significantly higher total frequency of clumping than those from neutered female cats (p=0.039) (Fig 2). Clumping rates at each level of severity were higher in males than females but did not reach statistical significance. Blood samples collected from the
jugular vein by manual aspiration or the medial saphenous vein by vacuum aspiration are similarly prone to platelet clumping, and the choice of phlebotomy technique should not influence platelet clumping rates or severity. Neutered male and neutered female cats, however, have statistically different overall platelet clumping rates with males more likely to clump. Further work into the clinical correlates of platelet clumping (such as thromboembolic events) based on sex is indicated.

**HM06**

**Thrombocytosis in 158 cats (2011-2018)**

Leigh A. Howard – Purdue University Veterinary Teaching Hospital; Andrew Woolcock – Purdue University College of Veterinary Medicine

Thrombocytosis is an uncommon hematologic abnormality which is associated with various metabolic, inflammatory, and neoplastic conditions in people and dogs. Thrombocytosis is not a well-described abnormality in cats. The purpose of this retrospective study was to describe the common conditions associated with thrombocytosis of different severities in cats.

Medical records of cats with increased (> 600 x 10^3/μL; thrombocytosis group) and normal (200-600 x 10^3/μL; control group) platelet counts between 2011 and 2018 were reviewed. Control group was age and sex matched to the thrombocytosis group. Severity of thrombocytosis was graded as mild (600-700 x 10^3/μL), moderate (700-900 x 10^3/μL), or marked (>900 x 10^3/μL). Diagnoses were classified broadly into either neoplasia, endocrine, or inflammatory disease. Within each broad category, diagnoses were grouped based on tumor type, metabolic disorder, or major body system affected.

One-hundred fifty-eight cats were diagnosed with thrombocytosis, and 315 cats were included in the control group. The median platelet counts for the groups were 687 x 10^3/μL (600-1,470 x 10^3/μL) and 348 x 10^3/μL (204-586 x 10^3/μL), respectively. Neoplasia and endocrine disease were diagnosed more frequently in cats with thrombocytosis compared to the control group, but no significant difference was found when comparing tumor types or specific endocrine diseases. Round cell tumor was the most common neoplastic diagnosis in both groups. Inflammatory disease was diagnosed in a similar percentage of cats in both groups, however gastrointestinal disease, hepatobiliary disease, and immune-mediated disease were diagnosed more frequently in cats with thrombocytosis. Table 1 depicts the distribution and comparisons.

Mild thrombocytosis was identified most commonly (54.4%), followed by moderate (31.6%) and marked (13.9%) thrombocytosis. No significant association was identified between the three broad diagnostic categories and severity of thrombocytosis. Neoplasia was the most common diagnosis in cats with marked thrombocytosis (68.2%). Sarcoma was significantly associated with a marked thrombocytosis (p = 0.01), as was hepatobiliary disease (p = 0.01). Thrombocytosis in cats is commonly associated with neoplasia and inflammatory disease. Cats with gastrointestinal, hepatobiliary, and/or immune-mediated disease may be more likely to demonstrate a thrombocytosis. Sarcoma and hepatobiliary disease should be considered in cats with marked thrombocytosis.

**HM07**

**Assessment of Extended Sample Storage for Delayed Platelet Function Testing in Normal Dogs**

Melanie Dickinson – University of Guelph - Ontario Veterinary College; Anthony Abrams-Ogg – Ontario Veterinary College - University of Guelph; Shauna Blois – Ontario Veterinary College - University of Guelph; R. Darren Wood – Ontario Veterinary College - University of Guelph

Platelet function testing in dogs can help identify thrombocytopathies and aid in drug monitoring in those receiving anti-platelet therapy.

Table 1. Distribution of diagnoses amongst cats with thrombocytosis and normal platelet counts. Broad categories are bolded and represent the percentage of total cats. Sub-categories represent the percentage of their broad category. Only significant body systems within inflammatory disease are depicted. P-values of <0.05 are considered significant and are depicted by an **.”

| Diagnosis           | Thrombocytosis (n=158) | Control (n=315) | p-value       |
|---------------------|------------------------|-----------------|---------------|
| NEOPLASIA; n (%)    | 70 (44.3%)             | 80 (25.4%)      | <0.0001*      |
| Carcinoma; n (%)    | 16 (22.9%)             | 16 (20.0%)      | 0.69          |
| Sarcoma; n (%)      | 7 (10.0%)              | 14 (17.5%)      | 0.24          |
| Round Cell Tumor; n (%) | 48 (68.6%)  | 51 (63.8%)      | 0.61          |
| ENDOCRINE; n (%)    | 48 (30.38%)            | 53 (16.8%)      | 0.0012*       |
| Hyperthyroidism; n (%) | 29 (60.4%)  | 36 (67.9%)      | 0.53          |
| Diabetes Mellitus; n (%) | 16 (33.3%)  | 19 (35.9%)      | 0.84          |
| INFLAMMATORY; n (%) | 86 (54.4%)             | 177 (56.19%)    | 0.77          |
| Gastrointestinal; n (%) | 65 (75.6%)            | 61 (34.4%)      | <0.0001*      |
| Hepatobiliary; n (%) | 19 (22.1%)             | 14 (7.9%)       | 0.0024*       |
| Immune-Mediated; n (%) | 32 (37.2%)          | 29 (16.4%)      | 0.0003*       |
| MULTIPLE; n (%)     | 50 (31.7%)             | 45 (14.3%)      | <0.0001*      |
However, the need for fresh blood samples and the time-frame for analysis makes widespread routine testing challenging. This study aimed to evaluate different methods to extend sample storage and allow for delayed platelet function testing using Plateletworks® (PW) and INNOVANCE® Platelet Function Analyzer-200 (PFA-200). PW uses pre and post agonist platelet counts to determine % aggregation, and must be performed within 10 minutes of sample collection into agonist tubes. PFA-200 measures time to closure of a membrane in a special cartridge using citrated blood, and must be performed within 4 hours of sample collection. Thirteen dogs were included in the study. Blood was drawn on day 0 by atraumatic jugular venipuncture sequentially into serum, citrate, EDTA, and PW tubes. For PW, platelet counts were obtained in duplicate using an optical hematology analyzer (ADVIA® 2021i). Baseline % aggregation was determined within 10 minutes on day 0 using ADP tubes. Short-term holding for PW testing was determined by storage of citrated blood for 3 hours at room temperature on day 0, and by refrigeration for 24 hours (day 1) and 48 hours (day 2), after which time the citrated blood was allowed to warm at room temperature for 15 minutes and then dispensed into ADP tubes and platelet counts obtained. Longer delay to testing was evaluated using AGGFix (Platelet Solutions, UK), to stabilize platelet aggregates. Blood from EDTA and ADP tubes was mixed within 10 minutes on day 0 with AGGFix solution according to the manufacturer’s recommendations and separated into multiple aliquots for subsequent refrigerated storage and testing. Platelet counts were performed on days 0, 1 and 7. PFA-200 analysis was performed in duplicate using citrated blood samples with COL/ADP and INNOVANCE® P2Y cartridges. Baseline closure times were obtained after 2 hours of sample storage at room temperature. All other samples were refrigerated for 24 hours (day 1) or 48 hours (day 2) and allowed to warm at room temperature for 15 minutes prior to testing. For PW, baseline aggregation results on day 0 were median 94%, range 55-98%. In comparison to baseline, no significant difference (p > 0.05) was noted with day 0, 3-hour old citrated blood (median 94%, range 52-96%). Significant differences compared to baseline were noted with day 1 (median 85%, range 20-97%) and day 2 (median 79%, range 47-95%) using citrated blood. For PW using AGGFix samples, no significant difference was noted with day 1 AGGFix samples (median 95%, range 71-100%). A significant difference (p < 0.05) compared to baseline was noted with day 7 AGGFix samples (median 88%, range 0-94%), although values were similar to baseline for 12 dogs (range 80-94%) with one outlier (0%). For PFA-200, day 0 baseline COL/ADP closure times were median 62 sec, range 46-76 sec (institutional reference interval 45-109 sec) for 12 dogs with an outlier of >300 sec in one dog. (This was considered artifact as flow obstruction occurred on day 1 and closure time was 94 sec on day 2.) Significant differences from baseline were noted in day 1 (median 67 sec, range 37-229 sec) and day 2 (median 109 sec, range 74-300 sec) COL/ADP closure times. Similarly, in comparison to day 0 baseline P2Y closure times (median 55 sec, range 36-300 sec), significant differences were noted in day 1 (median 197 sec, range 55-230 sec) and day 2 (median 181 sec, range 58-300 sec) closure times.

In conclusion, for PW using ADP agonist, storage of citrated blood for 3 hours at room temperature and AGGFix solutions for 24 hours under refrigeration yielded similar results to immediate analysis of fresh samples. AGGFix solutions refrigerated for 7 days for PW, and citrated blood refrigerated for 24 or 48 hours for PW and PFA-200, had reduced aggregation responses but these methods of storage may be useful in extending platelet storage and allowing for delayed platelet function testing.

**HM08**

**StablePlate RX Canine Promotes in vitro Thrombin Generation and Thrombus Formation Under High Shear**

**Braden C. Ishler – Cellphire, Inc; Anne Hale – BodeVet, Inc; Keith Moskowitz – Cellphire, Inc**

StablePlate RX® Canine are lyophilized canine platelets indicated for the treatment of acute uncontrolled hemorrhage secondary to thrombocytopenia. Using in vitro assays, this study demonstrated a potential mechanism for the hemostatic functionality of StablePlate RX® Canine through thrombin generation and thrombus formation under shear forces. The Calibrated Automated Thrombogram (CAT) method was used to measure thrombin generation in samples of StablePlate RX® Canine after rehydration with sterile water. The average thrombin peak height (TPH) for a sample containing 4.8x10³ particles/μL in the presence of PRP Reagent was 89.6 nM ± 9.4 nM (n=20). The average thrombin generation response was reduced to 6.9 nM (n=2) by incubating samples with 150 μg/mL of bovine lactadherin to block available surface phosphatidylserine (PS). The presence of PS was further confirmed through Annexin V binding measured by flow cytometry. The average percentage of StablePlate RX® Canine particles expressing PS was 98.1 % ± 1.2 % (n=20). The Total Thrombus-formation Analysis System (T-TAS) was used to measure thrombus formation in StablePlate RX® Canine. Prior to analysis, CaCl₂ and corn trypsin inhibitor were added to all samples at final concentrations of 12 mM and 50 μg/mL respectively. The samples were then placed under shear forces in microcapillary channels coated with collagen and tissue factor. Thrombus formation was measured by the amount of time needed to achieve a pressure increase of 80 kPa. Citrated canine platelet rich plasma with a count of 326x10³ platelets/μL reached occlusion pressure after 5.1 minutes. Citrated canine platelet poor plasma (PPP) with a platelet count of 13x10³ platelets/μL failed to reach occlusion pressure during the 30-minute run time of the assay. Citrated canine PPP supplemented with StablePlate RX® Canine at concentrations of 100x10³ particles/μL or 250x10³ particles/μL reached occlusion pressure at 6.7 minutes and 5.3 minutes respectively.

Upon rehydration, StablePlate RX® Canine is capable of promoting thrombin generation in the presence of tissue factor. Furthermore, suspensions of StablePlate® Canine adhere to collagen coated microcapillary channels under shear forces in a manner similar to fresh canine platelets. These results indicate that StablePlate RX® Canine may function as a hemostatic agent, at least in part, by localizing and adhering to the site of trauma and promoting the generation of thrombin.
HM09

Application of Therapeutic Plasma Exchange in Dogs with Immune-Mediated Thrombocytopenia

Lucy Kopecny – University of California, Davis; Carrie Palm – University of California, Davis; Larry Cowgill – University of California, Davis

Therapeutic plasma exchange (TPE) is an emerging therapy for dogs with immune-mediated diseases but reports for immune-mediated thrombocytopenia (IMT) are lacking. This case series describes application of TPE in 4 dogs with presumed primary IMT (including 1 dog with concurrent immune-mediated hemolytic anemia) unresponsive to traditional therapy.

Dogs were treated with 3 sequential centrifugal TPE sessions. Replacement fluid prescriptions were individualized for each dog and included a combination of 6% Hetastarch, 0.9% NaCl and fresh frozen plasma. Regional citrate anticoagulation (with or without heparin) was used in all cases. All dogs were treated with adjunctive immunosuppressive therapy [corticosteroids (n = 1) or combined corticosteroid and cyclosporine (n = 3)] for a median of 4 days (range, 4-10) before TPE initiation.

Median age was 5.3 years (range, 4-10) and weight was 7.0 kg (range, 5.8-16.8). Median pre-treatment platelet count was 6,500/µL (range, 5,000-8,000). Over all treatments, a median of 4.4 plasma volumes (range, 4-4.9) were exchanged per dog. Three dogs survived to discharge. One dog was euthanized after 3 treatments due to persistent thrombocytopenia and bleeding. For surviving dogs, median time to platelet count >40,000/µL was 5 days (range, 1-6) after TPE initiation.

Median platelet count at discharge was 51,000/µL (range, 46,000-102,000). Bleeding associated with IMT improved within a median of 2 days (range, 1-4) after TPE initiation.

TPE was safe and associated with improved platelet count in 3 of 4 dogs with refractory IMT. Preliminary observations suggest that TPE is useful in IMT refractory to traditional therapy.

HM11

Evaluating the Incidence of Crossmatch Incompatibility and the Accuracy of Point-of-Care Crossmatching Methods in Dogs

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Recent research shows crossmatch (CM) incompatibility occurs in transfusion naïve dogs. Dogs might benefit from CM prior to any transfusions and a point-of-care kit would increase feasibility of this test. The objectives of this study were to: 1) compare the incidence of laboratory-based major CM incompatibility in dogs naïve to transfusion versus those previously transfused; 2) determine accuracy of a gel-based and immunochromatographic point-of-care major CM method, compared to the standard laboratory major CM based on visual agglutination and hemolysis.

Sixty-three dogs (transfusion-naïve n = 40, previously transfused n = 23) were enrolled prospectively and underwent major CM using three methods: the standard laboratory technique, a gel-based point-of-care kit, and an immunochromatographic point-of-care kit. Recipients were tested against a median of 3 donors (range 1 - 3).

Using the laboratory major CM method, 9/40 (22.5%) dogs naïve to transfusion and 5/23 (21.7%) previously transfused dogs had at least one incompatibility. There was no significant difference (p > 0.99) between the rates of incompatibility in naïve versus previously transfused dogs.

Compared to the laboratory method, the immunochromatographic CM test had a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 81.6%. Sensitivity and specificity of this test were 0.1 and 1.0, respectively. When compared to the laboratory method, the gel-based CM test had a PPV of 0% and an NPV of 83.1%. Sensitivity and specificity of this test were 0 and 0.99, respectively.

Naïve and previously transfused dogs had similar major CM incompatibility rates in this study. The point-of-care major CM methods had good agreement with the laboratory tests for compatible results.
However, the gel-based kit failed to identify any of the CM incompatibilities detected via the laboratory method while the immunochromatographic kit detected only some of the incompatibilities identified via laboratory method.

HM12

Effects of Budesonide Administration on Coagulation Variables in Dogs

Takuro Nagahara – The University of Tokyo; Koichi Ohno – The University of Tokyo; Nozomu Yukoyama – The University of Tokyo; Taisuke Nakagawa – The University of Tokyo; Yuko Goto-Koshino – The University of Tokyo; Hirota Tominatsu – The University of Tokyo; Hajime Tsujimoto – The University of Tokyo

Hyperadrenocorticism and glucocorticoid therapy are associated with an increased risk of thrombosis in dogs. Previous reports have revealed that dogs with hyperadrenocorticism and dogs administered with prednisone exhibit hypercoagulability. Budesonide is a synthetic glucocorticoid with high first-pass hepatic clearance, which should limit its systemic effects. The purpose of this study was to evaluate the effects of budesonide administration on coagulation variables in healthy dogs.

Four healthy beagles received 1 mg/head (a low dose) of budesonide orally once daily for 2 weeks, followed by a 4-week washout period, after which 3 mg/head (a high dose) of budesonide was administered orally once daily for 2 weeks. The dogs were tested for prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, thrombin-antithrombin complex (TAT), D-dimer, antithrombin (AT) activities, and rotational thromboelastometry (ROTEM; ROTEM Delta, Munich, Germany), using citrated blood samples collected before budesonide administration (baseline), after budesonide administration, and after the washout period (washout). In ROTEM analysis, the following parameters were assessed for each profile: clotting time, clot formation time; maximum clot firmness, and angle. The dogs were also tested for complete blood count and chemistries. The dosage of budesonide in this study was within the clinical dosage and the experimental animal care procedures were approved by the Animal Use and Care Committee of the University of Tokyo.

Plasma fibrinogen concentration increased in all of the dogs following low-dose (median 208 mg/dL; range 172–229 mg/dL) and high-dose administration of budesonide (247 mg/dL; 225–300 mg/dL) compared to that at baseline (135 mg/dL; 125–184 mg/dL) and after washout (177 mg/dL; 143–199 mg/dL), although they were all within the reference range. AT activity decreased in all of the dogs following low-dose (131%; 111%–145%) and high-dose administration of budesonide (99%; 84%–105%) compared to that at baseline (145%; 122%–156%) and after washout (121%; 103–125%). AT activity continued to decrease in the washout period and was below the reference range in all of the dogs after high-dose administration of budesonide. Plasma alkaline phosphatase (ALP) activity was above the reference range in all of the dogs following high-dose budesonide administration. No remarkable changes were observed in PT, aPTT, TAT, D-dimer, ROTEM analysis, complete blood count, or other chemistries.

In the present study, budesonide administration caused hypercoagulability, as indicated by the increase in plasma fibrinogen concentration and the decrease in AT activity. Unlike prednisone, which causes hypercoagulability, as shown by thromboelastography analysis, budesonide caused no remarkable changes in ROTEM analysis results. However, AT activity continuously decreased in the washout period, which suggests that budesonide administration affects coagulation parameters for long periods after withdrawal of budesonide. The elevation of plasma ALP activity also suggested that the systemic effects of glucocorticoid were produced by budesonide administration, as previously reported. Budesonide is used for the treatment of inflammatory bowel disease, including protein losing enteropathy (PLE), which is associated with hypercoagulability. Therefore, budesonide treatment may exacerbate hypercoagulability in dogs with PLE and its administration requires careful attention.

In conclusion, our results suggest that budesonide administration results in hypercoagulability in healthy dogs, although further study with larger sample sizes is necessary.

HM13

Method Comparison Between the Conventional Thromboelastograph 5000 and the New Thromboelastograph S6

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Conventional thromboelastography using the TEG 5000 (Hemonetics) system has a relatively high pre-analytical variability. In comparison, the thromboelastograph S6 (Hemonetics) measures the same viscoelastic properties as the TEG 5000 but has some advantages, which encourages its use as a bedside test. A smaller sample size is required for a microfluidic cartridge where different types of TEG assays can be measured in one run. The frail wire technology is replaced by a resonance method and a simple handling of reagents decreases the analytical variability.

A method comparison was performed for the kaolin trace by analyzing citrated whole blood samples from 17 healthy dogs simultaneously on the TEG 5000 and the TEG S6. A Bland-Altman difference plot and a one sample t-test were performed to assess agreement and prove significant differences between both methods.

In the Bland-Altman-plot the confidence interval around the bias of all variables included the line of identity except for the R-value. The t-test confirmed the presence of a statistically significant difference of the R-value between the methods. A significant negative average bias of 36.1% (−1.12 min; p < 0.01) which showed a tendency to increase with higher values was detected.

A possible explanation for the significant difference of the R-value could be a lower pre-analytical influence on the TEG 6S. Despite a considerable overlap of results, data indicate that a de novo establishment of reference intervals for the S6 method is necessary.
Platelet Function in Dogs with Chronic Liver Disease

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Dogs with chronic liver disease often have hemostatic derangements. The purpose of this study was to evaluate platelet function in dogs with chronic liver disease by measuring platelet closure time (CT), assessed with the PFA-100® and buccal mucosal bleeding time (BMBT). A secondary aim of the study was to determine if plasma von Willebrand factor (vWF) concentration is altered in dogs with chronic liver disease. We hypothesized that dogs with chronic liver disease have prolonged CT and BMBT, and increased vWF concentration compared to healthy dogs.

Eighteen dogs with biochemical evidence of hepatic dysfunction for 2 or more weeks and 18 age-matched healthy control dogs were studied. Exclusion criteria included major concurrent disease, administration of drugs that could alter platelet function, sighthound breeds, extrahepatic biliary tract disease, hepatic neoplasia, hematocrit < 35%, or platelet count < 150,000. Plasma biochemistries, complete blood count, Snap® 4Dx®Plus Test, CT measured with the PFA-100®, vWF-antigen concentration, and BMBT were evaluated in all dogs. Dogs with chronic liver disease underwent an abdominal ultrasound and liver biopsy by ultrasound-guided 14 ga Trucut needle or laparoscopic-assisted 5 mm cup forceps. An unpaired t-test was used for normally distributed data and the Mann-Whitney test was used when non-Gaussian distribution was present. The level of significance was set at P < 0.05.

The mean age of the liver disease group was 6.5 years and sex distribution included 8 spayed females, 7 castrated males, 2 intact females, and 1 intact male. The mean age of the control group was 6.1 years and sex distribution included 8 spayed females and 10 castrated males. Chronic hepatitis was diagnosed in 12 dogs, copper-associated hepatitis in 5 dogs, and nodular hepatopathy in 1 dog. The CT was not different between the two groups (P = 0.21). The BMBT was significantly longer in the liver disease group compared to the control group (P = 0.019). The platelet count was not different between the two groups (P = 0.057). There was no difference in the mean vWF antigen of the two groups (P = 0.074).

These results demonstrate mild impairment of primary hemostasis in dogs with chronic liver disease based on prolongation of BMBT. Normal CT with BMBT prolongation could indicate endothelial dysfunction or may have resulted from limitations of the methodology.

Evaluation of AST/ALT Ratio and AST to Platelet Ratio Index in Dogs with Hepatobiiliary Disease

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Liver fibrosis is observed in most types of chronic liver disorders. Non-invasive detection of hepatic fibrosis is important for the initiation of anti-fibrotic treatment. The De Ritis ratio (AST/ALT ratio, AAR) and the aspartate aminotransferase to platelet ratio index (APRI) are considered to be useful for detection of significant fibrosis and cirrhosis in human patients with non-alcoholic steatohepatitis or chronic hepatitis C infection. The aim of the study was to evaluate the utility of AAR and APRI for the non-invasive evaluation of liver fibrosis.

Blood samples from 41 healthy dogs and 56 patients histologically diagnosed with liver disease were analyzed. The latter group was divided into 4 subgroups: 1. vascular disorders (n = 21), 2. parenchymal diseases (a. mild and significant fibrosis, n = 9; b. advanced fibrosis and cirrhosis, n = 6), 3. neoplasia (n = 12), 4. biliary tract disorders (n = 8). AAR did not differ significantly among groups (P = 0.057). APRI was significantly increased in subgroups 1 (P = 0.001), 2b (P < 0.001), 3 (P = 0.03) and 4 (P < 0.001) compared to healthy dogs. The diagnostic performance of APRI for predicting advanced liver fibrosis/cirrhosis was considered fair (AUC = 0.763). Using a cut-off value 0.417, the sensitivity and specificity of APRI for diagnosis of advanced liver fibrosis/cirrhosis were 100% (95% CI, 55.2–100) and 43.5% (95% CI, 32–55.9), respectively. These results suggest the APRI is a potential non-invasive test to rule out advanced liver fibrosis or cirrhosis in canine patients.

Untargeted Metabolomic Profiling of Serum from Dogs with Chronic Hepatic Disease

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Chronic hepatic disease presents a diagnostic challenge with different diseases being associated with similar clinical and laboratory findings. Differentiating dogs with congenital portosystemic vascular anomalies from those with acquired hepatopathies can be difficult and require costly diagnostic procedures such as computed tomographic angiography. Non-invasive and inexpensive biomarkers that reliably differentiate these types of chronic hepatic disease may reduce the need for costly or invasive diagnostics and guide novel therapeutic interventions. The objective of this study was to investigate differences in the serum metabolome between healthy dogs, dogs with a congenital portosystemic shunt, and dogs with chronic hepatitis.

Serum samples from 12 healthy dogs, 10 dogs with a congenital portosystemic shunt, and 6 dogs with chronic hepatitis were analyzed.
The serum metabolome was evaluated with an untargeted metabolomics approach using gas chromatography – quadrupole time of flight mass spectrometry. Principle component analysis, random forest analysis, and hierarchical cluster analysis was performed using MetaboAnalyst 4.0. Differences in the abundance of serum metabolites between the groups were evaluated using a Kruskal-Wallis test and significance was set at \( p < 0.05 \). P-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate and significance was set at \( q < 0.10 \).

A total of 126 named serum metabolites were identified of which 50 differed significantly (\( p < 0.05; q < 0.10 \)) between groups. Principal component analysis and heat dendrogram plots of the metabolomics data showed clustering of individuals in each group. Random forest analysis showed differences in the abundance of various metabolites including increased aromatic amino acids (i.e., tyrosine, phenylalanine), decreased branched chain amino acids (i.e., leucine, isoleucine, valine), trans-4-hydroxy-l-proline, and xylitol in the serum of dogs between groups.

In conclusion, the serum metabolome varies between healthy dogs, dogs with a congenital portosystemic shunt, and dogs with chronic hepatitis. Statistical analysis identified several metabolites that differentiated healthy dogs from dogs with a congenital portosystemic shunt or chronic hepatitis. Further targeted assessment of these metabolites is needed and in progress to confirm their diagnostic utility.

**HP04**

Metabolomic Investigation of Plasma Samples from Dogs with Hepatocutaneous Syndrome

Adam Miller – Cornell University; Elena Diaz-Rubio – Cornell University; John Loftus – Cornell University; Luis Macho – Cornell University; Sharon Center – Cornell University

Hepatocutaneous syndrome (HCS) is a disease characterized by hypoaminoacidemia in conjunction with a distinct hepatopathy and cutaneous lesions. Recently, lysinuria has been identified as a consistent feature of this syndrome. Reductions in plasma amino acid concentrations could cause, or result from, other metabolic alterations. Therefore, we performed metabolomic analyses on plasma from dogs with HCS and compared them to healthy dogs of similar ages and breeds.

Plasma was collected from canine patients diagnosed with HCS. Samples were stored at -80°C until untargeted metabolomic analysis by the Cornell University Metabolomic Core Facility. Analyses were performed separately with positive and negative modes for each sample type. Multiple databases were used for comparison to m/z results. Statistical analyses were performed by Metaboanalyst software.

After normalization and background removal, compounds that were detected with \( P<0.05 \) were annotated based on a spectral library database or named based on formula prediction. Pathways with named metabolites that were significantly different (\( P < 0.01 \)) in dogs with HCS included vitamin B6 metabolism, the citric acid cycle, pyrimidine metabolism, pantothenate and CoA synthesis, and various amino acid metabolic systems. Interestingly, a compound with similarity to metharbital was significantly higher in plasma from dogs with HCS. HCS is associated with a variety of metabolic abnormalities, many of which were related to amino acid metabolism as expected. Alterations in metabolites involved in vitamin B6 and pantothenate metabolism suggest the need for consideration of B vitamin supplementation in the dietary management of HCS. Phenobarbital has been implicated in the development of HCS. Thus, a candidate endogenous barbiturate-like compound could be a central mediator in the pathogenesis of HCS and warrants further investigation.

**HP05**

Fibrinogen Levels as a Factor for Surgical Decision-Making in Dogs with Portosystemic Shunt

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Liver circulation relies mainly on the blood flow from portal vein. Portosystemic shunt (PSS) can lead to important clinical signs due to hepatic dysfunction. Lower levels of clotting factors can lead to a challenging surgical procedures. The objective of this study was to evaluate the risk factors of surgical treatment in patients with PSS.

From January 2017 to December 2018, Nineteen dogs with PSS underwent to surgical attenuation with ameroind constrictors. Computer tomographic study confirmed 14 portacaval (nine Yorkshire terriers, two Shih tzu, one German spitz, one white highland white terrier, one Maltese), three portaozygous (Yorkshire terriers) and two right intrahepatic shunts (Border collies). One Yorkshire dog with portacaval PSS presented reversible spontaneous bleeding at surgical wound. Three dogs (one Shih tzu and two Yorshires) with portacaval PSS presented fatal spontaneous bleeding at surgical wound, and also gastrointestinal tract at necropsy. Cryo precipitate, packed red cells, platelet transfusion and 30mg/kg tranexamic acid were ineffective to stop the bleeding. No abnormalities of prothrombin time (11.6 ± 1 seconds; ref.: 6.8-10.2) and activated partial thromboplastin time (16.4 ± 1.6 seconds; ref.: 10.7-16.4) were found before surgery. Only fibrinogen levels (mean±SD) of these three dogs were under normal levels (169 ± 14.7 mg/dL; reference: 200 – 400) and lower than dogs without hemorrhagic events (329.7±107.8), \( p = 0.002 \) (Mann-Whitney test).

It was concluded that low plasma concentration of fibrinogen is considered an important risk factor to fatal bleeding disorder so, presurgical fibrinogen levels lower than 200 mg/dL should exclude surgical decision in PSS patients.
Assessment of Copper Concentrations in Archived Cat Liver Specimens

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Hepatic copper accumulation is recognized as an important cause of chronic hepatitis in dogs and humans. Excess copper leads to oxidative stress, which induces inflammation, necrosis, and subsequent fibrosis of the liver. However, the role of hepatic copper accumulation in feline liver disease is not fully understood. Thus, the aim of this study was to assess hepatic copper concentrations in cats with and without liver disease.

Histopathological sections cut from 81 archived formalin fixed paraffin embedded cat liver specimens were used for this study. The liver specimens had been submitted to Texas A&M Veterinary Medical Diagnostic Laboratory for histopathological analysis and included those collected during necropsy. Tissue sections were stained with rhodanine and copper staining was scored from 0 (no staining) to 5 (extensive staining). The blocks were then deparaffinized and hepatic copper concentrations were measured using flame atomic absorption spectroscopy at the Veterinary Diagnostic Laboratories at Colorado State University.

Tissue samples were categorized into 4 groups based on histological findings: no significant changes (46 cases), hepatic steatosis (11 cases), inflammation/infectious disease (19 cases), and neoplasia (5 cases). Copper staining was positive in 12/81 cases (14.8%) and 3 cases had a score of ≥ 3. In contrast, thirty-four of 81 cases (42.0%) had copper concentrations above the upper limit of the reference interval (150 – 180 μg/g dry weight liver). No significant difference in hepatic copper concentration was found between groups (p = 0.130). The 3 cases with copper staining scores ≥ 3 were assigned histomorphological diagnoses of no significant changes, randomly distributed hepatitis, and histoplasmosis with copper concentrations of 227, 787, and 2,010 μg/g, respectively. A positive correlation between copper staining score and concentration was found (r = 0.492, p < 0.001).

While > 40% of cat liver specimens had a copper concentration greater than the upper limit of the reference interval, only 14.8% showed positive staining for copper and only 3.7% had a score of ≥ 3. The clinical importance of hepatic copper concentrations above the upper limit of the reference interval where there is no copper staining is questionable. Further investigation of the role of copper as a primary or secondary etiological agent in feline liver disease is warranted.

Evaluation of Sample Sources for Point of Care Cytologic Diagnosis of Cytauxzoon Felis for Practitioners

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Cytauxzoon felis is a life threatening, protozoan disease in domestic cats. Rapid diagnosis and treatment is imperative as most cats die within 24 hours of presentation. While a commercial PCR test is the most sensitive and specific diagnostic test, identification of piroplasms or schizonts allows for rapid diagnosis and initiation of therapy. The aim of this study was to evaluate a novice’s ability to identify merozoites or schizont-laden macrophages in blood and tissue samples collected from cats in which cytauxzoonosis was suspected.

Cats with suspected cytauxzoonosis who were euthanized or died on the day of presentation were recruited. Clinicians were asked to prepare 2 blood films and obtain fine needle aspirates post-mortem from lymph nodes and spleen. All slides were evaluated by a novice and a board-certified clinical pathologist (BCP) for the presence of piroplasms and/or schizonts on blood film and schizont-laden macrophages on splenic and lymph node aspirates.

Thirty-one cats were included. The novice and the expert evaluations demonstrated 95.8% agreement on blood, and 92.5% agreement on lymph nodes and splenic aspirates. A McNemar’s test for agreement
between sample types was performed. Of the 18 patients in which all 3 sample types were available, lymph node aspirates did not agree with the other 2 sample types in 6 cases. Organisms were more likely to be seen on blood smear or splenic aspirates. This data suggests that if parasitemia is not identified on blood smear then splenic, followed by lymph node aspirates are reasonable alternatives for identification of *C. felis*.

**ID03**

**Clinicopathologic Findings, Treatment, and Outcomes in 21 Dogs with Hepatozoon americanum**

Allison Wilson – Oklahoma State University; Center for Veterinary Health Sciences; Laura Nafe – Oklahoma State University; Brian Herrin – Kansas State University; Susan Little – Oklahoma State University

*Hepatozoon americanum* is a tick-borne, protozoal pathogen that infects dogs, resulting in a potentially fatal disease with distinct clinical features and clinicopathologic abnormalities, most notably a severe leukocytosis. The objective of this retrospective study was to describe clinicopathologic manifestations, treatment and outcomes in dogs naturally infected with *H. americanum*. From 2000 – 2018, twenty-one dogs presented to Oklahoma State University Boren Veterinary Hospital and were diagnosed with canine hepatopzoosporosis based on blood PCR and/or identification of gamonts on buffy coat. The median age of dogs in the study was 5 years (0.9-12) and median weight was 21.6 kg (4.5-43.5). Clinicopathologic results revealed a median white blood cell count of 55,000/μL (8,200-143,600) with a median neutrophil count of 47,070/μL (5,904-106,470), and 16/21 dogs (76%) had a neutrophil count <70,000/μL. Other common abnormalities included hypoalbuminemia (16/21), increased ALP (16/21) and hypoglycemia (11/21). Fifteen dogs were treated with ponazuril +/- clindamycin, 4 dogs with clindamycin + trimethoprim/sulfamethoxazole (TMS), and 2 dogs with clindamycin, TMS, and pyrimethamine. Five dogs (24%) were suspected or confirmed to have infection relapse despite long-term decoquinate therapy. Out of the 18 dogs with a known outcome, 4 died as a result of *H. americanum* infection. Results of this study emphasize the importance of testing dogs with clinical features of *H. americanum* even if a severe leukocytosis is not present. Additionally, ponazuril appeared to be an effective initial treatment, but owners should be advised of the risk of clinical relapse, even when maintained on an antiprotozoal medication long-term.

**ID04**

**Using Big Data to Investigate Intestinal Parasite Prevalence by Geographic Region and Age**

Sarah Sweet – IDEXX Inc.; Lauren Olavessen – IDEXX Inc.; Donald McCrann – IDEXX Inc.; Donald Szlosek – IDEXX Inc.

Intestinal parasite testing is recommended at the annual adult canine exam and is essential at regular intervals 2-4 times a year during a puppy’s first year of life. Canine life stage is a key factor in clinical parasite prevalence as clinical signs associated with parasitism are more common in puppies. The Companion Animal Parasite Council (CAPC) recommends the use of parasite antigen testing in combination with fecal centrifugal flotation to achieve the greatest breadth of parasite detection in both dogs and puppies. The purpose of this study was to evaluate fecal test results using zinc sulfate flotation by centrifugation combined with fecal antigen testing for hookworm, roundworm, and whipworm sorted by age and geographic region. A retrospective sample of 4,652,501 intestinal parasite panels were randomly selected using IDEXX Reference Laboratory data from the continental United States from January 2016 to June 2018. These data contain results from zinc sulfate centrifugal flotation fecal exams paired with Fecal Dx antigen results for roundworms, hookworms and whipworms. For paired testing, if either the ELISA or flotation was positive, the test was considered positive. Data were summarized by age (24 months and > 25 months) and region (Northeast, South, Midwest, West). Fecal Dx antigen combined with zinc sulfate centrifugal flotation identified 3.8% positive hookworm, 2.2% positive roundworm and 1.2% positive whipworm** infections respectively as compared to 1.8% positive hookworm, 1.5% positive roundworm and 0.6% positive whipworm infections respectively using zinc sulfate by centrifugal flotation alone regardless of age or geography. Fecal Dx antigen combined with zinc sulfate centrifugal uncovers nearly twice as many positive hookworm, roundworm and whipworm infections than zinc sulfate centrifugation alone in both puppies and adult dogs across all geographic regions of the US.

**A Novel Parvovirus in an Unexplained GI Outbreak in Dogs from Colorado**

Christian M. Leutenegger – IDEXX Laboratories, Inc.; Elizabeth Fahsbender – Vitalant Research Institute; M. Alexis Seguin – IDEXX Laboratories, Inc.; Eda Altan – Vitalant Research Institute; Marko Estrada – IDEXX Laboratories, Inc.; Pauline Young – IDEXX Laboratories, Inc.; Eric Delwart – Vitalant Research Institute

Dogs suffering from an unexplained diarrhea outbreak presented with vomiting and diarrhea for over a period of 14 days. The clinical manifestation started with steatorrhea and progressed to pure blood hemorrhagic diarrhea. Patients were lethargic and febrile with low lymphocyte counts indicating viral infection. Secondary pancreatic inflammation was documented by positive canine pancreatic lipase testing. Fecal antigen testing, O&P and comprehensive diarrhea real-time PCR (qPCR) panels only yielded incidental findings on individual infectious agents and tested consistently negative for canine parvovirus 2. A viral metagenomics analysis was performed on fecal samples from 9 clinically affected dogs from this outbreak. Fecal samples were grouped into pools of 3 and deep sequenced with the MiSeq following viral particles-associated nucleic acids enrichment, random RT-PCR, and library generation. Following de novo assembly, both singlets and contigs were compared to all eukaryotic viral protein sequences in GenBank using BLASTx. One of the 3 pools showed the presence of novel parvovirus sequences not yet reported in GenBank. The genome of a novel chapparvovirus in the *Parvoviridae* family was fully characterized, showing
highest amino acid sequence similarities of 62 and 63% on NS1 and VP1 genes, respectively, to a Cameroonian fruit bat parvovirus. The new carnivore chapparvovirus 1 was subsequently confirmed using a specific real-time PCR in a second dog of nine tested from the outbreak. Disease association was studied in 3,185 clinical samples collected from healthy dogs, dogs with hemorrhagic diarrhea and dogs which were tested with the comprehensive canine diarrhea panel by qPCR. No disease association was found when comparing frequencies in 3,185 samples, including healthy (1.48%), hemorrhagic diarrhea (0%), or patient samples submitted for the diarrhea diagnostic panel over two different time frames (3.06 and 2.57%, respectively).

The epidemiology of this novel canine chapparvovirus and its possible association with diarrhea or other canine diseases needs to be further investigated.

ID06

Bartonella Infection in a Population of Chronically Ill People with Self-reported Neuropsychiatric Symptoms

Erin W. Lashnits – North Carolina State University; Julie Bradley – North Carolina State University; Nicholas Hende – North Carolina State University; Ricardo Maggi – North Carolina State University; Edward Breitschwerdt – North Carolina State University

Neuropsychiatric diseases such as depression, bipolar syndrome and schizophrenia are prevalent and have devastating effects on a proportion of the human population. Despite the societal importance of and costs associated with managing these diseases, progress on understanding the pathophysiology has been frustratingly slow, and curative treatments have correspondingly lacking. There is a growing body of evidence suggesting an association between neuropsychiatric disorders and exposure to infectious agents – starting with historical cases of neuro-syphilis, to more recent associations between streptococcal infection and pediatric neuropsychiatric disorders (PANDAS), schizophrenia and toxoplasmosis, and recent proposals that viral and bacterial diseases could be linked to depression or even Alzheimer’s Disease.

Bartonella spp. are emerging zoonotic vector-borne bacterial pathogens that have been preliminarily associated with neuropsychiatric disease. Veterinary workers appear to be occupationally at risk for acquiring this infection. Symptoms associated with neurobartonellosis are remarkably varied, and include seizures, cranial neuropathies, aphasia, white matter abnormalities, and peripheral nervous system diseases. There are also reports of acute psychiatric symptoms, hallucinations, and depression associated with Bartonella infection. Thus, there is a critical need to investigate the role of Bartonella infection in neuropsychiatric diseases with unknown etiology. The objective of this study was to describe the prevalence of Bartonella infection/exposure in a sample of chronically ill human subjects with self-reported neuropsychiatric symptoms.

This retrospective, observational study includes data collected on human subjects suspected to have Bartonella infection by themselves or their physicians. Subjects were included if they had completed a medical history questionnaire between January 2012 and June 2018 reporting one or more neuropsychiatric symptoms, and were tested in conjunction with a NCSU Institutional Review Board approved research study (Protocol No. 1960, Detection of Bartonella Species in the Blood of Healthy and Sick People) for Bartonella spp. exposure/infection by BAPGM ePCR. Bartonellospp. seroreactivity was also determined for a subset of subjects by indirect immunofluorescent antibody (IFA) testing against a panel of antigens including Bartonella henselae, B. vinsonii subsp. berkoffii, B. koehleri, and B. quintana.

Between January 2012 and June 2018, 138 subjects completed medical history questionnaires and were tested for Bartonella spp. exposure/infection. Of these, 25 individuals were excluded due to lack of reported neuropsychiatric symptoms, leaving 113 subjects included in this study. Subjects were 82% female and had a median age of 40 years (range 2-90 years); there were 22 people under the age of 18. Of 72 adults with information about their employment history, 42 (58%) reported being veterinary or animal workers. The most common neuropsychiatric symptoms reported were insomnia (reported by 68% of subjects), difficulty remembering (65%), irritability/rage/aggression (49%), anxiety/panic attacks (49%), mental confusion (43%), and depression (42%); other reported neuropsychiatric symptoms included disorientation (28%), hallucinations (12%), and seizures (3%).

Two or more symptoms were reported by 84% of subjects, and the median number of neuropsychiatric symptoms reported was three. When asked how their illness was affecting their lives, 45% of people (49/113) reported that they were unable to continue the normal daily activities routinely performed before becoming ill, and 72% of people (72/100) reported being unable to work or attend school. 48% of people (54/112) reported being evaluated by a psychiatrist and 41% (43/105) reported use of one or more psychiatric medications within the previous 12 months period.

Of the 113 human subjects reporting neuropsychiatric symptoms, all were tested by Bartonella BAPGM ePCR, and 107 were also tested by Bartonella spp. IFA. In this sample, 19% of people (22/113) had evidence of Bartonella spp. bloodstream infection by BAPGM ePCR, and 69% (74/107) had serological evidence of exposure to one or more Bartonella species. Seventeen people were positive by both serology and BAPGM ePCR. Bartonella henselae was the most common species amplified by PCR, found in 18 of the 22 people. The overall proportion of people in this sample exposed to, or infected with, one or more Bartonella species was 70%. This high proportion suggests a need to further investigate the role of Bartonella in contributing to neuropsychiatric signs with case-control and/or cohort studies.

ID07

Investigating Association Between Exposure To Ehrlichia and Risk of Developing Chronic Kidney Disease in Dogs

Jennifer S. Ogeer – IDEXX LABORATORIES INC.; Corie Drake – IDEXX Laboratories Inc.; Donald McCran III – IDEXX Laboratories Inc.; Celeste Clements – IDEXX Laboratories, Inc.; Melissa Beall – IDEXX Laboratories Inc.; Jesse Buch – IDEXX Laboratories Inc.; Wade Burton – IDEXX Laboratoires Inc.

Ehrlichiosis is a common vector borne disease that can be caused by different species of Ehrlichia. Ehrlichia canis and E. ewingii are tick-borne pathogens known to infect dogs in the United States. Ehrlichia canis is spread by the brown dog tick which is prevalent in many parts of the U.S., particularly in the South and Southwest regions. Infection with E. canis is relatively common and can have an association with
the development of kidney disease in dogs in the chronic phase. A retrospective cohort study was performed to determine if dogs with detectable antibodies to *Ehrlichia* in *E. canis* endemic regions had an increased risk of chronic kidney disease (CKD) over those without. *Ehrlichia* serology data were obtained from the IDEXX Reference Laboratory (IRL) and in-clinic databases from January 2003 to January 2018. Chemistry and urinalysis results were obtained from the IRL database from July 2015 to January 2018. Patients exposed to *Ehrlichia* were defined as having a positive test result for *Ehrlichia* recorded at any point in their available history, while non-exposure was defined as having a negative result for all test events. For this study, CKD was defined as concurrent increased SDMA (>14 μg/dL) and creatinine (>1.5 mg/dL) for a minimum of 25 days with inappropriate urine specific gravity (USG <1.030) during that time. A total of 22,440 canine patients met the inclusion criteria in *E. canis* endemic regions of the United States. Patients were matched between exposure groups using propensity scoring to control for age, geography, and breed. Contingency tables were used to compare exposure to infected ticks and CKD outcome. The relative risk of CKD for patients exposed to ticks carrying *Ehrlichia*, as evident by positive antibody to *E. canis* and *E. ewingii* within the defined *E. canis* endemic region was found to be 2.12 with 95% confidence interval [1.35, 3.15], p < 0.0006. This study identified a potential association between dogs with positive *Ehrlichia canis* test results and CKD.

**ID08**

Serologic and Urinary Survey of Exposure to Leptospirosis in a Feral Cat Population of Prince Edward Island

Emilia Bourassi – Atlantic Veterinary College; Christine Savidge – Atlantic Veterinary College; Peter Foley – Atlantic Veterinary College; Sunny Hartwig – Atlantic Veterinary College

Leptospirosis is considered an emerging disease in humans and dogs in North America. Cats with outdoor lifestyles may be in close contact with potential reservoir hosts and could play a role in transmission or act as sentinels for the disease. Recent studies show that seroprevalence in cats is not negligible and naturally infected cats can shed DNA from pathogenic *Leptospira* species in urine. There are few reports of leptospirosis on Prince Edward Island and none in cats. The objective of this study was to determine the prevalence of serum antibodies against *Leptospira* species and of Leptospira DNA in urine of a population of free roaming cats. Paired blood and urine samples from 200 cats were analyzed. Antibody titers against six *Leptospira* serovars (Bratislava, Canicola, Gryppotyphosa, Hardjo, Pomona, Icterohaemorrhagiae) were determined by microscopic agglutination test. Polymerase Chain Reaction (PCR) was performed on urine samples to identify urine shedding of *Leptospira* DNA. Antibodies were detected in 20/200 cats (10%) for at least 1 serovar with titers ranging from 1:50 to 1:6400 (all serovars tested, expect Hardjo). Urine samples of 5/200 cats (2.5%) were PCR-positive. Outdoor cats in Prince Edward Island have higher than expected exposure to leptospirosis and can shed DNA from pathogenic *Leptospira* species in urine. Further studies are needed to determine the prevalence of this disease on Prince Edward Island.

**ID09**

Detection of Adenovirus-2, Parainfluenzavirus-2, and Bordetella bronchiseptica following Intranasal Vaccination of Dogs in a Shelter

Linda K. Okonkowski – Michigan State University; Stephen Carey – Michigan State University; Jennifer Ottney – Capital Area Humane Society; Michael Coyne – IDEXX Laboratories, Inc.; Donald Szlosek – IDEXX Laboratories, Inc.

Canine Infectious Respiratory Disease Complex (CIRD) is an important source of morbidity and mortality among commingled dogs in shelter environments. Administration of intranasal modified live vaccines against Canine Adenovirus-2, Canine Parainfluenzavirus-2, and *Bordetella bronchiseptica* at intake is a common strategy employed to prevent CIRD in shelters. Administration of intranasal vaccines can interfere with results in molecular diagnostic testing for CIRD pathogens for up to thirty days in laboratory puppies. The extent to which such a vaccination strategy would impact molecular diagnostic testing among dogs in a typical shelter population is currently unknown. The objective of this study is to determine the effect of administration of a tri-valent, modified live intranasal vaccine at intake on molecular diagnostic testing for *Bordetella bronchiseptica*, Canine adenovirus-2 (CAV-2), and Canine parainfluenzavirus-2 (CPiV-2) among healthy dogs at various times (0-135 days) after vaccination. Samples were collected from a total of 209 dogs during general anesthesia for sterilization procedures. Pooled tonsillar, conjunctival, and nasal cavity swabs containing visible organic material were collected for multiplex polymerase chain reaction (PCR) analysis for *Bordetella bronchiseptica*, CAV-2, and CPiV-2. Of the 209 dogs sampled, 78 were sampled within seven days of vaccination. Sixty-three percent (49/78), 35% (27/78), and 55% (43/78) of samples collected within seven days of vaccination were positive for *Bordetella bronchiseptica*, CAV-2, and CPiV-2 respectively. Seventy-seven percent (60/78) of samples collected within seven days of vaccination were positive for at least one pathogen. Positive samples were identified for up to 29 days after vaccination for *Bordetella bronchiseptica* (126/197), 20 days after vaccination for CAV-2 (35/177), and 24 days after vaccination for CPiV-2 (68/188). No positive samples were obtained from dogs vaccinated between 30-135 days prior for any pathogen (13 samples). Results in this field study are similar to those previously reported in laboratory puppies and suggest that intranasal vaccination may confound molecular diagnostic testing in shelter dogs for up to one month.

**ID10**

Stage of Feline Leukemia Virus Infection Impacts Diagnostic Test Results: A Prospective Study

Melissa Beall – IDEXX Laboratories, Inc.; Christian Leutenegger – IDEXX Laboratories, Inc.; William Hardy – National Veterinary Laboratory; Ellen Jefferson – Austin Pets Alive; Monica Frenden – Austin Pets Alive; Natasha Hamman – Austin Pets Alive; Christina Reinhardt – Maddie’s Shelter Medicine Program, University of Florida; Genevieve Clark – IDEXX Laboratories, Inc.; Jancy Hanscom – IDEXX Laboratories, Inc.; Jennifer Braff – IDEXX Laboratories, Inc.; Andrei Rakitin – IDEXX Laboratories, Inc.; Julie Levy – Maddie’s Shelter Medicine Program, University of Florida

In feline leukemia virus (FeLV) infections, the immune response can alter the course of infection and modulate markers used for diagnosis.
Discordant test results may occur with different sample types or test methods. This study evaluated concordance of whole blood, plasma, and serum for the detection of p27 antigen and determined the proportion of cats repeating as positive over a 6-month period.

Cats were screened on intake to a shelter using anticoagulated whole blood on SNAP® FIV/FeLV Combo Test (IDEXX Laboratories). Monthly whole blood, plasma and serum samples were collected on positive cats once they weighed two pounds. Samples were tested by SNAP, a quantitative p27 antigen ELISA, a semi-quantitative real-time PCR for proviral DNA, and FeLV IFA.

The study enrolled 130 FeLV-positive and 130 FeLV-negative cats, providing 939 sampling events. Agreement between whole blood, plasma and serum on SNAP was 0.86 across all events (Multiple Rater Kappa, SUGI). Excluding neonates (<2 months, n = 34), 75 (78%) FeLV-positive cats were consistently positive on all methods over the 6-month period. These cats had high p27 antigen concentrations and high proviral DNA loads consistent with progressive infections. The remaining 21 cats had lower concordance between sample type, yet more than half (57%) tested positive for FeLV by PCR or IFA. This group of cats had lower antigen and lower proviral DNA loads consistent with regressive infections.

While current FeLV disease states exist as distinct categories, infections may be better represented as a spectrum of disease that can change with a cat’s immune status.

ID11

Pilot Study Assessing the Role of Babesia vogeli in Feline Hemolytic Anemia in the USA
Pierce Chan – Colorado State University Veterinary Teaching Hospital; Michael Lappin – Colorado State University Veterinary Teaching Hospital; Jennifer Hawley – Colorado State University Veterinary Teaching Hospital

Hemolytic anemia is common in cats in the United States. In several studies of cats with hemolytic anemia, infectious agents like the feline hemoplasmas were not detected in over 50% of the tested cats, potentially suggesting that primary immune-mediated hemolytic anemia may be common. However, some infectious agents that may cause hemolytic anemia in cats like Babesia vogeli are not routinely tested for in some laboratories offering PCR assays. Since B. vogeli is vectored by Rhipicephalus sanguineus, which is considered nationwide in the USA, it is possible this agent has been missed in previous studies. The purpose of this study was to use a PCR assay to amplify B. vogeli DNA in the blood of cats with suspected infectious hemolytic anemia in the USA.

DNA from research studies and a commercial service laboratory were stored at -80°C until assayed in this study using a previously published nested PCR assay that amplifies the DNA of B. vogeli (limit of detection = 1.405 pg/μl). The submission records were searched to find cats for which an infectious cause of hemolytic anemia was suspected and the sample was included if adequate DNA was available. PCR assays to amplify DNA of B. vogeli, Bartonella spp., haemoplasmas (including Mycoplasma hemofelis/M. turicensis and M. haemominutum), Ehrlichia spp., and Anaplasma spp. were performed.

A total of 75 DNA samples were available for B. vogeli PCR assay in this pilot study. Samples were available from 18 states across the United States, including California, Colorado, Florida, Illinois, Maryland, Michigan, Mississippi, Minnesota, Missouri, North Carolina, Nebraska, New York, Pennsylvania, Tennessee, Texas, Virginia, Washington, and Wyoming. Overall, DNA of an infectious agent was amplified from 21/75 (28%) samples. While Ehrlichia spp. and Anaplasma spp. DNA were not amplified, Bartonella spp. DNA was amplified from 5/61 (8.2%) samples and Hemoplasma spp. DNA was amplified from 19/73 (26%) cats. Hemoplasma spp. and Bartonella spp. DNA were amplified concurrently from 2 cats. Of the 75 DNA samples tested in this pilot study, none were positive for B. vogeli DNA. Although 28% of samples tested were positive for DNA of an infectious agent, B. vogeli DNA was not amplified. These results suggest that B. vogeli is not a common cause of hemolytic anemia in cats, however the sample set was small. To further assess the hypothesis that B. vogeli infects cats in the USA, future studies should focus on areas with high risk for R. sanguineus infestation and cats with known exposure to this tick.

ID12

Field Performance of Two In-clinic Tests for Lyme
Chandra Chandrashekar – IDEXX Laboratories, Inc.; Laura Nafe – Oklahoma State University; Deb Piegras – Lakeland Veterinary Hospital; Jon Speik – Androscoggin Animal Hospital; Linda Farrington – Compassionate Care Veterinary Clinic; Emily Lane – IDEXX Laboratories, Inc.; Melissa Beall – IDEXX Laboratories, Inc.; Wade Burton – IDEXX Laboratories, Inc.; Sarah Sweet – IDEXX Laboratories, Inc.; Jiayou Liu – IDEXX Laboratories, Inc.

The geographic range of Borrelia burgdorferi infections has expanded over the last 10 years with evidence of autochthonous transmission in the Carolinas, western Virginia, Kentucky and North Dakota. Accurate serologic tests for canine antibodies to B. burgdorferi provide awareness of this geographic expansion and help to assess preventive measures. Three general practice veterinary hospitals evaluated the performance of two in-clinic, multiplex immunoassays for heartworm and tick-borne infections in a two-part study. With owner consent, excess whole blood samples from a convenience population of dogs were tested on the SNAP® 4Dx® Plus Test (IDEXX Laboratories, Inc.) and the VetScan® FLEX4 Rapid Test (Zoetis) according to manufacturer’s instructions. Matched plasma samples, stored frozen, were randomized, blinded and tested on Lyme Immunoblot (ViroTech Diagnostics) by IDEXX Laboratories to determine infection status. Although sample selection criteria differed between the first (any sample with a positive result on SNAP 4Dx Plus, N = 129) and second (all samples, N = 255) phase of the study, the B. burgdorferi results were not statistically different between the two phases. Overall, the sensitivity of SNAP 4Dx Plus Test relative to Lyme Immunoblot was 92.3% (CI: 85.9 – 96.4) and was significantly higher (p < 0.0001) than VetScan FLEX4 Rapid Test which had a sensitivity of 51.3% (CI: 41.9 – 60.6). The specificity of both tests was > 99%. Results of this field-based study were consistent with a recently published comparison of these products and demonstrates the clinical importance of selecting accurate screening tests for tick-transmitted infections like B. burgdorferi.
Ultrasonographic Findings of Gastrointestinal Histoplasmosis in Dogs

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Histoplasmosis, a mycotic infection caused by *Histoplasma* spp, can infect the gastrointestinal tract of dogs. Clinical signs of gastrointestinal histoplasmosis can include diarrhea, profound weight loss, anorexia, and vomiting. Rectal scrape for cytology can provide a quick diagnosis, but if negative abdominal ultrasound is often pursued. Ultrasonographic abnormalities in dogs with gastrointestinal histoplasmosis have rarely been reported. This retrospective case series study aimed to describe the ultrasonographic features of gastrointestinal histoplasmosis in dogs. Sixteen cases with a diagnosis of gastrointestinal histoplasmosis confirmed with gastrointestinal cytology or histopathology (N=14) or gastrointestinal lymph node cytology (N=2) that had undergone an abdominal ultrasound examination from 2005-2018 were included. Ultrasound images were reviewed by a board-certified radiologist (DB) and diagnostic imaging intern (EC).

Fourteen cases had sonographic abnormalities within the gastrointestinal tract. The colon was the most affected organ. Colonic thickening was the most frequent finding (N=14 dogs) ranging from 0.4 to 1.2cm. Diffuse, asymmetrical and focal thickening were seen. Eleven patients had alteration in the intestinal wall layering (complete loss of wall layering and/or pseudo-layering) in a diffuse or asymmetrical pattern. Other findings included lymphadenopathy, peritoneal effusion and small intestinal thickening. To the authors’ knowledge this is the first description of ultrasonographic findings of gastrointestinal histoplasmosis in a group of canines. Although abnormal gastrointestinal wall layering seen sonographically is most frequently associated with neoplasia, this is not pathognomonic. Gastrointestinal histoplasmosis should be considered as a differential diagnosis due to the similarities of ultrasonographic features. Gastrointestinal cytology and/or histopathology is required for definitive diagnosis.

Associations Among Feline Hemoplasmas and Select Variables in Domestic Cats in the USA

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The feline hemoplasmas (FHM) consist of *Mycoplasma haemofelis* (Mhf), *Candidatus M. haemominutum* (Mhm) and *Candidatus M. turicensis* (Mtc). These erythrocyte pathogens are thought to be most commonly associated with anemia, but most published studies have had relatively low sample numbers. The objectives of this study were to use PCR assay results to estimate the prevalence of FHM DNA in cats used as blood donors and cats with presumed clinical illness and to determine associations with sex, complete blood cell count (CBC) findings, FIV antibodies, and FeLV antigen. Results from 7,310 domestic cats evaluated at a commercial diagnostic laboratory from 2011-2017 were available for review. The results were stratified into cats that were listed as blood donors and cats that were presumed to be clinically ill. CBC results were available for all cats and results from FeLV and FIV serological tests were available for some. Estimated prevalence rates were calculated for each FHM and the combination of any of the FHM. Odds ratios and 95% confidence intervals were calculated with p < 0.05.

Of the 7,310 samples, 1,081 (14.8%) were positive for DNA of at least one hemoplasma species. DNA of Mhm alone (8.6%), Mhf alone (2.2%) and Mtc alone (0.59%) were amplified from some cats. When FHM results were compared amongst groups, significant associations were detected with cats that were ill (Mhf alone, Mhm alone, Mtc alone, any FHM), had thrombocytopenia (Mhm alone, any FHM), anemia, (Mhf alone, Mhm alone, any FHM), or leukocytosis (Mhf alone), or were FIV positive (Mhm alone, Mtc alone, any FHM), FeLV positive (Mhf alone, any FHM), or male (Mhf alone, Mhm alone, any FHM). The results support that FHM are common in the United States, are associated with males, clinically ill cats, several CBC abnormalities, and retrovirus coinfections. Additional multivariate analysis and geographical associations are being assessed to further define the significance of the associations amongst Mhm, anemia (OR = 1.265; CI = 1.07 – 1.5; p = 0.006), and thrombocytopenia (OR = 1.249; CI = 1.04 – 1.51; p = 0.02) as this organism has previously believed to be relatively non-pathogenic.

Vector-Borne Pathogen and Leptospira spp. antibodies in Atlantic Canada dogs: The Canadian K9 Lifetime Study

Michelle Evason – Atlantic Veterinary College, University of Prince Edward Island; Jason Stull, MPVMM – Atlantic Veterinary College; Noel Clancy, MVSc, ACVP – Atlantic Veterinary College; George Gregory Mungia – Atlantic Veterinary College; Michaela Peace – Atlantic Veterinary College; J Scott Weese – Ontario Veterinary College

Leptospirosis and Lyme disease are emerging in Canada, with evidence of increased positive testing and infected dogs. However, key epidemiologic and clinical features of these diseases remain unknown. A collaborative longitudinal research effort is needed to obtain evidence to guide clinical decisions.

Specific objectives of the study were to: 1) enrol and engage dog owners to perform a long-term study; 2) evaluate baseline serostatus of dogs to selected pathogens and monitor status over time; 3) identify factors associated with serostatus; and 4) evaluate client knowledge, attitudes, and practices (KAP) surrounding disease and prevention.

Healthy dogs, 7 months of age and younger, from Atlantic Canada general practice veterinary clinics were eligible for inclusion. Admission blood samples were tested for Leptospira spp. exposure (microagglutination (MAT)), *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia* and *Anaplasma* spp. (SNAP 4Dx® Plus® Test). An on-line questionnaire was completed after enrolment to evaluate dog risk factors and owner KAP. Descriptive statistics were used to assess results. Eighty dogs have been enrolled to date. Baseline vector-borne pathogen testing has been completed on 74 dogs, with 1/74 positive for *B. burgdorferi*, and the remainder negative. MAT testing has been completed on 70 dogs. Most dogs had titres < 800 (range < 50 to 800) regardless of leptospirosis vaccination (11 vaccinated; 58 unknown), with one dog > 800 for *L. icterohaemorrhagiae* (unknown vaccination).
Questionnaires have been completed by the majority of invited respondents (71%, 42/59), with diverse KAP responses. This work surrounding canine Lyme disease and leptospirosis will inform further research efforts in Canada.

**ID16**

**Comparison of Different Point-of-Care Tests to Detect Antibodies Against Canine Distemper Virus**

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Measuring pre-vaccination antibodies to determine dogs’ specific immunity against canine distemper virus (CDV) is increasingly used to avoid unnecessary vaccinations. In Europe, three point-of-care tests for detection of CDV antibodies are currently available but their quality has not been compared. Aim of this study was to assess and compare validity and practicability of these tests.

Sera of 198 dogs in which blood was collected for various reasons were included. Virus neutralization (VN) was performed as gold standard, and a titer of ≥1:10 was considered protective. Practicality, sensitivity, specificity, positive (PPV), negative predictive values (NPV) and overall accuracy (OA) of the point-of-care tests were determined. Cohen’s kappa and McNemar’s test were used to assess agreement between the three tests.

Prevalence of CDV antibodies was 80% measured with the gold standard. The Immunocomb® Canine Vaccicheck™ had a sensitivity of 96%, a specificity of 8%, a PPV of 81%, a NPV of 33%, and an OA of 79%; the FASTest® CPV/CDV of 98%, 15%, 83%, 67%, and 82%; the TiterCHECK® CDV/CPV of 63%, 54%, 85%, 26%, and 61%, respectively. Number of positive test results differed significantly between TiterCHECK® and FASTest® and between TiterCHECK® and Immunocomb® (McNemar’s test p-values < 0.001). The FASTest® CPV/CDV was most easy to perform.

Considering practicability and OA as most important, the FASTest® CPV/CDV would be the recommended point-of-care test. However, a high PPV is important to avoid missing vaccination in an unprotected dog, and thus, modification by the manufacturer should be performed to aim a higher specificity.

**ID17**

**Point Prevalence Survey of Antibiotic Use in a Veterinary Teaching Hospital**

Emmelyn Hsieh – University of Minnesota Veterinary Medical Center; Jennifer Granick – University of Minnesota Veterinary Medical Center; Amanda BeaudoinM – Minnesota Department of Health

Point prevalence surveys (PPS) have been used in national studies to quantify antibiotic use in human hospitals and long-term care facilities.

This has not yet been done in veterinary medicine. Clinical, diagnostic, and antibiotic treatment data were collected from emergency, urgent care, primary care, internal medicine and surgery services one day each month (November, December) in a single veterinary teaching hospital. Data were collected for all inpatients present at 4 pm, and for all outpatients seen, on each study day. Information regarding antibiotics prescribed to inpatients within the 24-hour period prior to data collection and for all new outpatient prescriptions (drug, dose, route, duration) was recorded in an Excel spreadsheet. In total, 30 patients were present in the inpatient study units, and 96 patients were seen on outpatient services on the two survey dates. Thirteen inpatients (43.3%) received antibiotics. The proportion of canine and feline inpatients receiving antibiotics was 50% and 16.7%, respectively. Twenty outpatients (20.8%) received an antibiotic prescription. The proportion of canine and feline outpatients receiving antibiotics was 20% and 23.8%, respectively. The most commonly prescribed antibiotic classes for both units were penicillins, fluoroquinolones, and topical (ophthalmic, otic) medications. The most commonly used antibiotic was ampicillin/sulbactam.

PPS is a feasible way to collect antibiotic use and prescribing data in veterinary medicine. We plan to continue monthly collection of antibiotic use data at this single veterinary teaching hospital and will conduct a single-day PPS across veterinary teaching hospitals nationwide.

**ID18**

**Serum Procalcitonin and Heparin Binding Protein Levels as Biomarkers of Bacterial Infection in Cats**

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Procalcitonin (PCT) and heparin binding protein (HBP) have been used as acute-phase markers for identifying bacterial infection, sepsis, and septic shock. Prompt diagnosis of bacterial infection and early intervention with antimicrobials achieve a more favorable prognosis in cats. The objective of the current study was to screen and evaluate serum PCT and HBP levels as biomarkers of bacterial diseases in cats. Fifty-six client-owned cats were classified into a control group (n = 16) or a diseased group of patients with bacterial infection (n = 40). PCT and HBP values of serum were measured using a validated commercial enzyme-linked immunosorbent assay kit. For statistical analysis, the Mann–Whitney U-test and Spearman’s correlation analysis were performed and evaluated using an independent t-test. In addition, the receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC) were assessed regarding the capability of discriminating bacterial infection.

The median value of PCT in the healthy cats was 286.03 pg/mL and the median value in the diseased cats was 426.93 pg/mL. PCT levels were significantly higher in the diseased group than in the control group (P < 0.05). However, the median value of HBP in the control group was 0.24 ng/mL and that in the diseased group was 0.67 ng/mL; the difference was not significant (P = 0.310). The AUC of
PCT was 0.867 and, therefore, PCT could be a reliable diagnostic marker of bacterial infection in cats (P < 0.05).

In conclusion, the feline PCT value is a potential biomarker in diagnosing bacterial infection, but the feline HBP is not useful in detecting bacterial infection. If PCT can be easily measured and interpreted in emergency situations, it could be a valuable tool for various conditions with bacterial infection in cats.

**Development of Feline and Canine Urinary Escherichia coli Antibiograms to Improve Antimicrobial Stewardship in Kansas**

**Kate KuKanich - Kansas State University; Brianna Salgado - Kansas State University; Brian Lubbers, DACVCP - Kansas State University**

The purpose of this study was to create antibiograms of feline and canine urinary *Escherichia coli* isolates to be used as an antimicrobial stewardship tool for regional Kansas veterinary clinicians. Feline (N=143) and canine (N=640) urine *E. coli* isolates cultured by the Kansas State Veterinary Diagnostic Laboratory from 2013-2017 were reviewed and antibiograms created using Clinical and Laboratory Standards Institutes (CLSI) guidelines. Isolates originated from KSU Veterinary Health Center (N=335) and regional private veterinary practice (N=448) clinical patients.

Results showed high resistance (99.3%) among feline isolates to amoxicillin-clavulanate (Susceptibility ≤0.25) and amoxicillin (S≤0.25), but lower resistance among canine isolates (amoxicillin-clavulanate 7.7%, S≤8; amoxicillin 46.7%, S≤8). Resistance to other antimicrobials was uncommon (<6% for feline isolates, <14% for canine isolates). Canine isolates from private practices had increased resistance compared with KSU isolates to orbifloxacin (9.5% vs 0.1%) and pradofloxacin (9.4% vs 0.1%).

The difference in CLSI-recommended breakpoints for amoxicillin-clavulanate and amoxicillin between cats (S≤0.25) and dogs (S≤8) likely caused the disparity of resistance, since the MIC90s were equal (8 μg/mL). A breakpoint of S≤8 for feline isolates would decrease resistance to 10.6% (amoxicillin) and 0.7% (amoxicillin-clavulanate). Determining feline urine antimicrobial concentrations is warranted to establish optimal breakpoints for feline urinary tract infections (UTIs).

Adjusting breakpoints could increase veterinarians’ therapeutic options for successfully managing UTIs with oral, affordable, first-line antimicrobial agents. Differences in antimicrobial exposure and use patterns also likely played a role in observed resistance. Continued evidence-based research in this area will further guide stewardship recommendations and management of veterinary UTIs.

**Clinical Effects Induced by 1 Dose of H3N2 Vaccine in a 7 Day Challenge Model**

**Michael R. Lappin - Colorado State University; Karen Stasiak - Zoetis Animal Health**

There have been many outbreaks of H3N2 influenza in dogs. H3N2 infected dogs develop significant respiratory disease with high morbidity and occasional mortality. Inactivated virus vaccines are available and have been shown to be effective on challenge with H3N2 after a 2 dose series. Recently, our group showed that a single dose of an inactivated FHV-1 vaccine could induce significant protection against FHV-1 on challenge 7 days later. The purpose of this study was to determine the level of protection against clinical signs of H3N2.
induced by 1 dose of monovalent vaccine administered 7 days prior to exposure to dogs with active H3N2 infection. In this IACUC approved project, young adult spayed female beagles (n = 20), negative for H3N2 by RT-PCR assay and negative for H3N2 antibodies, were included in the study. On Day - 7, 8 dogs were administered 1 dose of a monovalent H3N2 vaccine (Zoetis) SQ. On Day -3, 4 other dogs were challenged with a proprietary strain of H3N2 using an aerosolization chamber. On Day 0, the 8 vaccinated dogs and the remaining 8 unvaccinated dogs were housed with the 4 H2N3 inoculated dogs and all were observed for 28 days. A clinical severity score that included all signs and a clinical cough score were calculated for the 8 vaccinated dogs and the 8 control dogs and compared statistically with P < .05 considered significant.

The total severity scores and respiratory severity scores were not different between vaccinated and control dogs Day 1 -7 after exposure to the dogs with H3N2. However, when the results were grouped into Day 1-14, Day 1-21 and Day 1-28, the vaccinated dogs had significantly lower total severity scores and lower respiratory scores than the controls.

The results document at least some protection against this strain of H3N2 was induced by this vaccine as early as seven days prior to exposure. This suggests that starting the vaccine series during H3N2 outbreaks may have some benefit even before the booster vaccine is administered.

ID21

Prevalence of Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia, and Anaplasma in dogs: 2013 – 2017
Susan Little – Oklahoma State University; Bhagya Kulasekariya – Oklahoma State University; Jennifer Broff – IDEXX Laboratories, Inc.; Jesse Buch – IDEXX Laboratories, Inc.; Andrew Knupp – IDEXX Laboratories, Inc.; Melissa Beall – IDEXX Laboratories, Inc.

To provide a summary of canine test results for antigen of Dirofilaria immitis and antibody to Borrelia burgdorferi, Ehrlichia spp., and Anaplasma spp. and describe geographic distribution trends in prevalence of these infections, we summarized results from 29,067,319 tests for antigen of D. immitis and 19,466,533 –19,542,970 tests for antibodies to B. burgdorferi, Ehrlichia spp., and Anaplasma spp. from 2013 to 2017, inclusive, by county, state, and region. Results were compared to those reported earlier to identify geographic areas with apparent changes in prevalence of infection. As expected, results varied in different regions of the United States, with D. immitis antigen more frequently detected in the Southeast (2.6%) and antibody to B. burgdorferi and Anaplasma spp. most common in the Northeast (12.6% and 7.1%, respectively). Overall, percent positive test results to D. immitis decreased in the Southeast when compared to earlier summaries that employed a similar testing strategy (from 3.9% to 2.6%). Continued geographic expansion of B. burgdorferi and A. phagocytophilum was apparent in the Northeast, Midwest, and Upper South. Geographic expansion of areas where dogs commonly test positive for Ehrlichia spp. was also evident, likely due, in part, to a change in the test to allow detection of antibodies to E. ewingii. Percent positive test results to Ehrlichia spp. increased in every region and this shift was particularly pronounced in the Southeast, where positive test results increased from 1.3% to 5.4%. Large scale testing of dogs for evidence of vector-borne infection continues to be a valuable strategy for understanding geographic trends in infection risk over time.

ID22

A Retrospective Comparison of Blastomycosis Urine Antigen Concentration and Radiographic Findings as Predictors of Survival
Laura Motschenbacher – University of Minnesota; Edward Patterson – University of Minnesota; Kari Anderson – University of Minnesota; Esther Nell – University of Minnesota; Lindsay Merkel – University of Minnesota; Eva Farrow – University of Minnesota; Aaron Rendahl – University of Minnesota

The blastomycosis urine antigen (BUA) test is used to diagnose blastomycosis, as well as, monitor response to therapy in dogs. It is not known, however, if BUA concentrations predict prognosis. The purpose of this study was to determine if BUA concentrations predict survival.

Medical record data was obtained from 48 dogs with Blastomycosis dermatitidis at the University of Minnesota Veterinary Medical Center between 2011 and 2018. The BUA concentration, radiographic lung changes (severity score 0-4), and survival time after diagnosis were recorded. Inclusion criteria included: BUA concentration ≥ 0.2 ng/mL and thoracic radiographs, both performed within 1 week of diagnosis and prior to antifungal therapy. Cases with cytologic or histologic presence of blastomycosis were considered confirmed; suspect cases were permitted if blastomycosis was the primary diagnostic rule-out. The BUA concentration and radiology score were moderately correlated (r = 0.39, 95% confidence interval = 0.12 - 0.60; P = 0.0062). The BUA concentration was not significantly different between survivors and non-survivors at discharge or 2 months and did not significantly predict survival (hazards ratio = 1.1, P = 0.063). Radiographic score was greater in non-survivors (median = 4, range = 2-4) than survivors (median = 2, range = 0-4, P = 0.043) and predicted death (hazard ratio = 1.7, P = 0.041).

In conclusion, BUA concentrations correlate with radiographic disease severity in dogs with confirmed or suspect Blastomycosis dermatitis. The BUA concentration was not a significant predictor of survival. However, radiographic score did predict outcome with a greater median score in non-survivors.

ID23

Epidemiological Relatedness of Methicillin-Resistant Staphylococcus aureus ST72-Sccmec IV Strains Among Companion Animals and Owners
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Pathology, Chonnam National University College of Veterinary Medicine, Gwangju, South Korea

This study was to investigate the epidemiological relatedness of methicillin-resistant Staphylococcus aureus (MRSA) isolates from companion animals, owners, and residential environment in the 72 households of the community.

Sampling was performed twice for 6 months from January to June 2018, total specimens (n=2,748) collected from each household were streaked on CHROMagar Staph aureus and the colonies grown on the medium were identified with MALDI-TOF Microbial Identification System. Antimicrobial susceptibility testing, PVL gene PCR, SCCmec typing, spa typing, PFGE, and MLST were conducted to evaluate the epidemiological characterization of the MRSA strains.

Sixty-five S. aureus strains (2.4%, 65/2,748) were isolated and 49 strains which are resistant to cefoxitin were identified as MRSA with mecA. Twenty-seven and six MRSA strains were isolated from people and dog, respectively. Besides, sixteen MRSA strains were isolated from residential environment. MRSA positivity was confirmed in 12 (16.7%) among 72 households and MRSA was distributed in all target samples (people, dog, and environment) of the four households (5.6%, 4/72). Furthermore, MRSA carriers were found in nasal cavity of the same human and dog through twice samplings. The MRSA ST72-SCCmec type IVc-t324 was predominant clone in the eleven households (15.3%) and then epidemiologically identical clones between human and animal were identified.

This research was supported by a fund (2017-ESR5405-01) by Research of Korea Centers for Disease Control and Prevention.

ID24

An Outbreak of Feline Calicivirus Associated Virulent Systemic Disease in Korea

Junghoon Park – Western animal medical center; Hyungkyu Chae – Western Animal Medical Center; Woojoo Choi – Western Animal Medical Center; Kyudeok Cho – Western Animal Medical Center; Seojong Nam – Western Animal Medical Center; Joeeun Yeo – Western Animal Medical Center; Junghoon Park – Western Animal Medical Center; Kyungin Shin – Western Animal Medical Center; Beomsung Joo – Royal animal medical center; Yeonjung Hong – Western Animal Medical Center

Feline calicivirus associated virulent systemic disease (FCV-VSD) has emerged in the United States of America and the United Kingdom. Several outbreaks have been associated with a high (up to 60%) mortality rate and atypical severe clinical signs (high fever, cutaneous edema, ulcerative dermatitis and jaundice). FCV-VSD strains are described as highly contagious and fatal. The purpose of this study was to describe the first nosocomial outbreak of FCV-VSD in Korea. Fourteen cats were naturally infected with a highly virulent strain of feline calicivirus. Samples were obtained from oropharyngeal swab, blood, skin scraping and liver at the time of necropsy. FCV infection was confirmed by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) based on Taqman technology and quantify viral loads in the different samples by performing RT-PCR with FCV specific probe accompanying with standard FCV genome known viral loads. The outbreak started with the death of two cats of unknown cause in March, 2018. Eight cats were confirmed and 2 cats were suspected to be infected with FCV-VSD. The disease reoccurred in August and a total of 4 cats were confirmed. Among the fourteen infected cats, one had to be euthanized, seven died, five recovered after medical treatment, and one lost during follow up. Mortality rate was 61.5% (8/13). Clinical signs included fever (85.7%), depression and anorexia (100%) in the early course, skin ulceration and edema on face (42.9%) and feet (78.6%), and icterus (28.6%) with the progression of the disease. The other signs were typical of FCV-induced VSD. Clinicopathological findings revealed lymphopenia (71.4%), non-regenerative anemia (42.9%) and the increase in feline serum amyloid A (100%), total bilirubin (71.4%), Aspartate transaminase (84.6%), lactate (66.7%) and creatine kinase (100%) excepting cats that were not able to obtain certain clinicopathological data. Delayed coagulation profile and the decrease in albumin were also found. In necropsy, subcutaneous edema and vascularitis were observed in the skin of a cat with FCV-VSD. Among the methods to isolate FCV, cutaneous scraping (n = 4) was the most sensitive in cats with facial and feet edema or ulceration (100%). Many affected cats had been vaccinated against FCV prior to infection. The incidents of outbreaks of FCV-VSD are increasing in veterinary practice. Due to the highly contagious nature and high mortality rate of FCV-VSD, immediate sanitary measures and strict control procedures are essential whenever cats show symptoms suspected of FCV-VSD (fever, anorexia and the increase in feline serum amyloid A and creatine kinase without identified causes in some of cats hospitalized). All exposed cats should be isolated or treated on an outpatient basis whenever possible. In this case, temporary closure of cat admission was ultimately needed to eradicate the infection.

ID25

Evaluation of the Clinical Performance of Two Point-of-Care Cryptococcal Antigen Tests in Dogs and Cats

Kryste Reagan – University of California, Davis; Ian McHardy – University of California, Davis; George Thompson – University of California, Davis; Jane Sykes – University of California, Davis

Point-of-care (POC) Cryptococcus antigen assays may provide veterinarians with a more rapid, patient-side diagnosis when compared with traditional laboratory-based latex agglutination tests. The objective of this study was to determine the sensitivity and specificity of two POC lateral flow cryptococcal serum antigen tests, CrAg LFA (Immy, Norman, OK) and the CryptoPS (Biosynex, Strasbourg, France) for diagnosis of cryptococcosis in dogs and cats, using the cryptococcal antigen latex agglutination system (CALAS) as the reference standard. Sera was collected from client-owned dogs and cats both prospectively and from stored specimens. Animals were classified as Cryptococcus antigen positive (CP, n = 15) based on a positive CALAS test or visualization of fungal organisms on cytological examination. Animals were Cryptococcus antigen-negative (CN, n = 78) based on a negative CALAS test. The sensitivity and specificity of each POC assay was calculated by comparing the results to the reference standard results.

The CrAg LFA assay correctly classified 24/26 CP specimens and 69/74 CN specimens resulting in a sensitivity of 92.3% (CI 75.9 –
98.6%) and specificity of 93.2% (CI 85.1 – 97.1%) as compared to the CALAS. The CryptoPS assay correctly classified 8/10 tested CP specimens and 56/59 tested CN specimens resulting in a sensitivity of 80.0% (CI 49.0 – 96.5%) and specificity of 94.9% (CI 86.1 – 98.6%) as compared to the CALAS. Rare false positive test results were noted in some specimens from dogs with coccidioidomycosis for all evaluated diagnostic methods.

These POC assays appear to be a sensitive and specific alternative to the traditional CALAS assay with more rapid turnaround times, which may result in earlier diagnosis and treatment.

**ID26**

**Bartonella Henselae Seroprevalence in Domestic Cats in the United States and Associations with Urinary Abnormalities**

Stacie C. Summers – Colorado State University, College of Veterinary Medicine; Arianne Morris – Colorado State University; Jesse Buch – IDEXX Laboratories, Inc; Michael Lappin – Colorado State University

*Bartonella* species are fastidious, gram-negative, intracellular bacteria that have tropisms towards erythrocytes and epithelial cells. Domestic cats are the main reservoir hosts for *B. henselae* which is the causative agent of cat scratch disease and other syndromes in people. There has been some supporting evidence that *B. henselae* is associated with urinary disease in people and cats. In reports using experimentally-inoculated young cats, serum creatinine concentration significantly increased over time and *Bartonella* DNA was isolated from both kidney tissue and urine. The purpose of the study was to evaluate *B. henselae* seropositivity in cats with laboratory evidence of urinary disease and compare to age-group matched cats (all cats ≥ 6 years).

In this retrospective study, sera were obtained from IDEXX Laboratories located in California (CA) and Massachusetts (MA). Signalment, geographical location and results of serum biochemical panel and symmetric dimethylarginine (SDMA) were available for all cats; results of urinalysis were available for most cats. Cats were defined as having evidence of kidney dysfunction based on a urine specific gravity ≤ 1.035 and SDMA ≥ 15 mg/dL. The Fisher’s exact test (p < 0.05 defined as significant) was performed to determine differences in proportions of normal cats with *B. henselae* IgG titers ≥ 1:128 when compared to cats with evidence of kidney dysfunction and amongst cats with and without proteinuria (urine dipstick ≥ 1+ protein), hematuria (>5 RBC/HPF), or relative pyuria (2-5 WBC/HPF). The analyses were performed using all cats as well as stratified by state of origin to determine potential geographical differences.

A total of 321 sera were evaluated by *B. henselae* IgG ELISA. Of the 321 available serum samples, 267 (kidney dysfunction n = 74, normal kidney function n = 193) had concurrent urinalysis results available. When all the different groupings of results were compared, the estimated prevalence rates for *Bartonella* spp. IgG antibodies varied from 40% to 55% but no significant differences were detected.

Because of the high seroprevalence rates in all sample groups, we were unable to make associations between urinary disease and presence of *B. henselae* IgG in serum. To further evaluate the possible associations between *B. henselae* and urinary disease, urine and blood *Bartonella* PCR will be performed.

**ID27**

**Anti-Platelet Antibody Development and Thrombocytopenia in a Dog Passively Exposed to Canine Influenza (H3N2)**

Maggie Williams – Colorado State University; Sarah Shropshire – Colorado State University; Melissa Brewer – Colorado State University; Nida Chornarm – Colorado State University; Karen Stacalak – Zoetis Animal Health; Michael Lappin – Colorado State University

Immune-mediated thrombocytopenia (IMTP) occurs when platelets are destroyed as a result of antibody-mediated mechanisms. In dogs, IMTP can be idiopathic or secondary to various neoplasms, drugs, or infections. Thrombocytopenia has been observed with both natural infection and vaccination for influenza in humans; proposed mechanisms include immune-mediated destruction and direct effects of the virus on platelets causing lysis or increased clearance from circulation. Thrombocytopenia was documented in one case of canine influenza (H3N2), however several other hematological abnormalities were also present in this case. It is unknown if H3N2 causes thrombocytopenia in dogs and if it occurs, by what mechanism. The objectives of this study were to describe changes in platelet counts and to determine if anti-platelet antibodies develop in dogs passively exposed to dogs experimentally infected with H3N2.

In this IACUC approved study, 12 young adult spayed female beagles, negative for H3N2 by RT-PCR assay and negative for H3N2 antibodies, were included. On Day -3, four of the dogs were challenged with a proprietary strain of H3N2 using an aerosolization chamber. On Day 0, the remaining eight dogs were housed with these four dogs and all were observed for 28 days; these eight dogs were considered passively exposed (PE). The eight PE dogs had complete blood counts and flow cytometry for anti-platelet antibodies performed on Days 0, 7, 14, 21, and 28.

All PE dogs had normal platelet counts and were negative for anti-platelet antibodies on Day 0. All dogs developed self-limited clinical signs consistent with H3N2. One PE dog became thrombocytopenic on Day 14 (29 k/uL with few clumps; reference range 200-500 k/uL) and was also weakly positive for anti-platelet antibodies (12.25%; weak positive range 10-14%). By Day 28, the thrombocytopenia (284 k/uL) and the anti-platelet antibodies (0.69%) had resolved.

To our knowledge, this is the first study to document a transient thrombocytopenia and anti-platelet antibodies in dogs passively exposed to H3N2. It is unknown if H3N2 could trigger IMTP in dogs or if platelet abnormalities could be involved in the hemorrhagic pneumonia sometimes reported in field cases. Future studies are warranted to further investigate these possibilities.

**IM01**

**Effects of Cyclosporine on Feline Lymphocytes Activated In Vitro**

Harry Cridge – Mississippi State University, College of Veterinary Medicine; Adef Kordon – Mississippi State University; Leysa Pinchuk – Mississippi State University; Robert Wills – Mississippi State University; John Thomason – Mississippi State University; Andrew Mackin – Mississippi State University; Todd Archer – Mississippi State University

Cyclosporine A (CsA) is a calcineurin inhibitor that decreases lymphocyte expression of NFAT-regulated cytokines in humans, dogs and
cats, and thereby depresses lymphocyte function. Less is known about the effects of CsA on lymphocytes in cats. Peripheral blood mononuclear cells (PBMCs) were isolated from 6 healthy cats followed by exposure to i) no treatment, ii) 5ug/ml concanavalin A (ConA), iii) 500 ng/ml CsA and iv) 5 ug/ml ConA and 500 ng/ml CsA. The effects of CsA on cell proliferation were assessed via live and necrotic cell counts from day 1 to day 6. Additionally, flow cytometry was utilized to determine the effect of CsA on apoptosis in feline lymphocytes at day 1 and 5 in two cats. Concanavalin A exposure resulted in increases in cell counts from day 1 to 6, peaking at day 5. Cyclosporine A inhibited cell proliferation, indicated via decreased live lymphocyte cell counts in the cell cultures exposed to ConA and CsA, compared to the cell cultures exposed to ConA only. Furthermore, CsA induced early and late apoptotic changes in feline PBMCs. Marked individual variability in degree of apoptosis was observed after CsA exposure. Differences in these responses may influence an individual cat’s response to cyclosporine.

**IM02**

**A Retrospective Study on Use of Mycophenolate Mofetil in Dogs with Immune-Mediated Diseases**

Kenjiro Fukushima – Colorado State University, Veterinary Teaching Hospital, Small Animal Medicine; Julia Veir – Colorado State University; Marie Legare – Colorado State University; Michael Lappin – Colorado State University

Mycophenolate mofetil (MMF), is a potent immunosuppressant which is used for immune-mediated diseases in dogs but information regarding adverse events (AEs) is limited. The purpose of this study was to evaluate the AEs of MMF. Medical records of dogs with immune-mediated diseases administered MMF from 2012-2018 at a CSU Veterinary Teaching Hospital were reviewed and required data were extracted. The AEs were grouped as clinical (vomiting, diarrhea, hypo/anorexia, and lethargy), hematological, hepatic and renal.

One-hundred and thirty-five dogs were included. The median starting dose of MMF was 17.5 mg/kg/day (range 8.1-60.0). The median treatment duration was 833 days (range 2-1808). Diagnoses leading to treatment with MMF included immune-mediated hemolytic anemia (n = 33), immune-mediated thrombocytopenia (n = 31), pemphigus foliaceus (n = 15), immune-mediated polyarthritis (n = 12) and others. Thirty-three dogs exhibited clinical AEs which included diarrhea (25/135, 18.5%), vomiting (16/135, 11.9%), anorexia (13/135, 9.6%) and lethargy (6/135, 4.4%). The median time to onset of clinical AEs was 10 days (range 2-37). Other AEs observed included lymphopenia (1/12, 8.3%) and hepatotoxicity (3/21, 14.3%). Renal AEs were not recognized (0/22, 0%). Skin eruptions were observed in 2 cases. There was no significant differences among dogs with AEs (n = 38) and dogs without AEs (n = 97) in regards to sex, breed, age, body weight or dose of MMF.

In the treated dogs, approximately 1/4 experienced clinical AEs. However, most were reversible. Since hematological toxicity and hepatotoxicity were observed, periodic blood work is required for monitoring of AEs.

**IM03**

**Evaluation of Interleukin-6, Interleukin-8 and TNF-α as Prognostic Markers in Dogs in Critical Conditions**

Priscila Viana Furtado – FMVZ/USP, PROVET; Debora Guissó – PROVET; Raquel Fukumori – FMU, CV BUTANTA; Silvana Midori Maemura – PROVET; Claudio Alvarenga De Oliveira – FMVZ USP

Monitoring and prognosis of patients are of great interest for the practice of Veterinary and Human Medicine. The development and validation of new tests to facilitate this monitoring are important and should always be thought. The purpose of this study was to investigate the clinical relevance of serum levels of cytokines in dogs, to establish their potential in the prognostic definition in critical conditions.

The following cytokines were measured: IL-6, IL-8 and TNF-α in two different groups. One control group (CG) of 15 adult dogs (8 females and 7 males, healthy, mean age 2.5±1.0 years). The patients group were dogs suffering from severe diseases received in the intensive care unit (Veterinary Center Butantã - SP, Brazil) and were classified as survivors (S) (n=55, 29 females and 26 males, mean age 10.5 ± 20 years) and non survivors (NS) (n=33, 20 females and 13 males, mean age 10.5 ± 2.0 years). Blood was taken at the first moment admission, serum separated and stored at −80 °C for a maximum of 30 days. The biomarkers were measured in duplicate using Milliplex® MAP Kit, Canine Cytokine Magnetic Bead Panel (CCYTOMAG-90K- 03,) according to the manufacturer’s instructions, with internal quality control at the PROVET/BR Hormone Laboratory.

For the control group, the results of IL-6, IL-8 and TNF-α serum concentration were < 8.45 pg/mL, < 2.69 pg/mL and < 0.01 pg/mL respectively. The surviving dogs had a range of IL-6 of 8.44 to 311.38 pg/mL (medium 62.53 pg/mL) and TNF-α from 0.02 to 1818 (medium 187.71 pg/mL). In the group of no surviving dogs, IL-6 ranged from 422.76 to 7555 pg/mL (medium 3546.36 pg/mL) and TNF-α from 0.37 to 2628 (medium 298.41 pg/mL). There was significant difference between the groups S and NS for IL-6 and TNF-α analyzes (p < 0.05). IL-8 analyzes had no significant difference between the patients (p > 0.05).

According to the results obtained in this study, we can conclude that IL-6 and TNF-α appears to play as a prognostic maker in critical conditions and can be useful in Veterinary Medicine, especially in canine critical patients.

**IM04**

**Vitamin D Metabolites and Serum CXCL10 in Dogs with Immune Mediated Disease**

John P. Loftus – Cornell University, College of Veterinary Medicine; Phillip Mick – Cornell University; Seth Peng – Cornell University; Joseph Wakshlag – University of Florida

Reduced serum vitamin D concentrations are associated with immune dysregulation and may contribute to the pathophysiology of immune mediated disease (IMD). Vitamin D receptor (VDR) agonists, particularly calcitriol, have immune modulatory effects and may attenuate some mechanisms that drive IMD pathology. Activation of the VDR
results in increased expression of the proinflammatory cytokine CXCL10. We hypothesized that dogs with IMD would have lower serum concentrations of vitamin D metabolites and higher concentrations of CXCL10 than healthy counterparts. Additionally, we hypothesized that lower serum vitamin D and higher CXCL10 concentrations would be associated with a poor prognosis for dogs with IMD. Serum was collected from canine patients diagnosed with naturally occurring IMD (immune mediated hemolytic anemia, immune mediated thrombocytopenia, immune mediated polyarthritis, steroid responsive meningitis arteritis). An accredited lab measured Vitamin D metabolites by LC-MS/MS. Serum CXCL10 was measured with a commercially available validated canine-specific enzyme-linked immunosorbent assay (ELISA) kit. Serum 25(OH)D3 and 1,25(OH)2D3 were significantly reduced in dogs with IMD compared to healthy controls. The median survival time (MST) for dogs with 25(OH)D3 concentrations the median did not achieve a MST at the conclusion of the study (77.14% survival at 871 days). The median serum CXCL10 concentration was significantly higher (P = 0.0002) in affected dogs (196.6 pg/ml) than healthy control dogs (0.0 pg/ml). Five dogs with IMD had CXCL10 concentrations at the assay’s upper limit of detection (3,100 pg/ml) and a significantly (P = 0.049) shorter survival time than all other dogs with IMD. However, no correlation between concentrations of Vitamin D metabolites and CXCL10 was present by linear regression analysis. Decreased serum 25(OH)D3 and 1,25(OH)2D3, but not 24,25(OH)D3 are associated with canine IMD. Concentrations of Vitamin D metabolites and CXCL10 may represent prognostic markers for dogs with IMD; however, further investigation into specific types of IMD independently is required. Additional work is warranted to determine the potential for VDR agonists as a novel adjunct treatment for canine IMD.

N01

Mechanical Quantitative Sensory Testing in Cavalier King Charles Spaniels with and without Syringomyelia

Ashley Hechler – The Ohio State University; Eric Hostnik – The Ohio State University; Laurie Cook – The Ohio State University; Lynnette Cole – The Ohio State University; Sarah Moore – The Ohio State University

Syringomyelia (SM) is a debilitating condition in cavalier King Charles spaniels (CKCS) resulting in clinical signs of neuropathic pain, such as hyperesthesia and allodynia. Currently, advances in disease treatment are hindered by lack of quantitative ways to assess neuropathic pain and monitor response to therapy. We evaluated an electronic Von Frey aesthesiometer (VFA; IITC Systems) to quantify sensory threshold (ST) abnormalities in SM. We hypothesized that SM-affected dogs would have lower ST than controls, consistent with hyperesthesia, and that ST would inversely correlate with syrinx height and clinical signs scores. Twenty-nine CKCS with (n = 19) and without (n = 10) SM were enrolled. ST was measured by a single investigator, blinded to disease status. ST was compared between groups using Wilcoxin rank-sum. Associations between ST, clinical signs, and imaging findings were evaluated by linear regression. Median ST (range) for the thoracic limbs was 184.1 grams (120.9 - 552) for control, and 139.9 grams (52.6 - 250.9) for SM-affected dogs. Median ST for the pelvic limbs was 164.9 grams (100.8 - 260.3) in control, and 129.8 grams (57.95 - 168.4) in SM-affected dogs. ST for the thoracic and pelvic limbs was lower in SM-affected dogs (p = 0.027; p = 0.0396), suggestive of hyperesthesia. ST in SM-affected dogs did not correlate with syrinx height (r = 0.314; p = 0.137). Pelvic limb ST was inversely correlated with owner-derived clinical sign scores, where dogs with lower ST displayed more severe clinical signs (r = -0.657; p = 0.022). Our results suggest that VFA may offer an objective assessment of hyperesthesia in SM-affected dogs and could be useful in future studies to monitor response to therapy.

N02

Comparison of Cerebellomedullary and Lumbar Cerebrospinal Fluid Analysis in Dogs with Neurological Disease

Rachel Lampe – University of Illinois; Kari Foss – University of Illinois; Samantha Vitale – University of Illinois; Devon Hague – University of Illinois; Anne Barger – University of Illinois

Cerebrospinal fluid (CSF) is commonly analyzed in dogs with neurological disease to help categorize the type of underlying disease present. The general consensus dictates that the spinal tap should be collected caudal to the lesion present, however there is little evidence at this time to justify this. The purpose of this study was to evaluate the clinicopathologic differences between CSF collected from the cerebromedullary (CM) and lumbar cisterna in dogs presenting for neurologic disease. Dogs undergoing MRI for investigation of neurological disease were prospectively enrolled in the study. Cerebrospinal fluid was collected from the CM and lumbar cisterna in all patients. The total protein, red blood cell count (RBC), and total nucleated cell counts (TNCC) were analyzed within 30 minutes of collection. Interpretation of the results and cytology were all performed by a single pathologist [AB]. Thirty-one paired samples were collected. Fluid analysis from lumbar puncture had a statistically significantly higher value of TNCC (p < 0.005), RBC (p < 0.0001), and total protein (p < 0.0001) compared to CM puncture. Hemodilution (RBC > 500 cell/mm^3) was more likely in lumbar analysis compared to CM analysis (p = 0.006). Data was then grouped and analyzed by neurolocalization (brain, cervical or thoracolumbar). The total protein (brain p=0.000007, cervical p = 0.02, thoracolumbar p = 0.015) and RBC (brain p=0.003, cervical p = 0.0008, thoracolumbar p = 0.046) counts were significantly different between the three neurolocalizations. The TNCC was significantly different in the thoracolumbar cases (p = 0.03), but not brain (p = 0.15) or cervical (p = 0.1). The pathologist interpretation differed between CSF collection sites in 67% of total cases (21/31). When grouped by neurolocalization, 52% (9/17) of brains, 87.5% (7/8) of cervical myelopathies, and 83% (5/6) of thoracolumbar myelopathies resulted in different pathologist interpretation between the two CSF collection sites. Four cases localized to the brain or cervical spine resulted in one abnormal fluid analysis while the other site was normal; two cases had a normal CM fluid analysis and an abnormal lumbar fluid analysis while another two had an abnormal CM fluid analysis and a normal lumbar fluid analysis. In comparison, none of the thoracolumbar localizations had...
pleocytosis in the CM fluid samples, compared to 4/6 of the lumbar samples. These results suggest that CSF analysis results differ between CM and lumbar fluid collection within the same dog. In thoracolumbar cases, CM collection may lead to false negative results and not be representative of the underlying disease process. In dogs that neurolocalize to the brain or cervical spine, there may be clinical benefit in collecting fluid from both the CM and lumbar cisterna.

N03

Reliability of Dynamic Magnetic Resonance Imaging in the Evaluation of the Canine Lumbosacral Spine

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Degenerative lumbosacral stenosis (DLSS) is a common cause of back pain and neurologic dysfunction in dogs. Imaging of the lumbosacral (LS) spine in extension has been performed using computed tomography and found significant changes to the volume of the intervertebral foramina. The purpose of this study was to evaluate the lumbosacral spine of healthy dogs using dynamic magnetic resonance imaging (MRI) techniques and determine its overall reliability in the assessment for degenerative lumbosacral stenosis (DLSS). Twenty two clinically healthy large breed dogs where prospectively enrolled. Dogs were determined to be healthy based on normal physical and neurologic examination, bloodwork, and electrodiagnostic testing. MRI of the LS spine using standard sequences (T2W, T1W and SPACE) in three planes (sagittal, transverse and dorsal) was obtained in 3 positions: neutral, flexed, and extended. Measurements included the LS angle, vertebral canal height and area, foraminal areas, and loss of fat signal in the foramina. Interobserver and intraobserver analyses were performed. A Pearson correlation coefficient was used to compare each pair of measurements amongst observers, with intraclass correlation coefficient (ICC) used overall and agreement set as ICC ≥ 0.5.

Using a combination of T1 and SPACE images, interobserver reliability overall was significantly correlated for LS angle (ICC = 0.95), LS vertebral canal height (ICC = 0.79) and area (ICC = 0.53), and foraminal areas (parasagittal and oblique) (ICC > 0.55). Ratios to compare mid-vertebral body to the LS vertebral canal were poorly correlated amongst observers for both T1 and SPACE images (ICC = 0.073, ICC = 0.066, respectively). Intraobserver reliability was high (rho > 0.5) for all measurements except for the mid-L7 to LS vertebral canal height ratio (rho = 0.38). When evaluating individual measurements, all flexed values where significantly larger (p < 0.0001) than the neutral measurements, except for the vertebral canal height at L6 (p = 0.59) and area at mid-L7 (p = 0.63). All extended values where significantly smaller (p < 0.0001) except for the vertebral canal area at mid-L7 (p = 1). Due to high variation amongst normal dogs, overall measurements of dispersion do not appear helpful. However, comparison of measurements between different positions in each dog may prove useful when compared to clinical dogs.

This study shows high intra and interobserver reliability for most measurements in the LS spine of normal dogs, including LS angle, LS vertebral canal height, and foraminal areas. Ratios to compare mid vertebral body to the LS vertebral canal, as well as fat suppression in the foramina have poor correlation amongst observers and should not be used. This study provides the groundwork for a future study using dynamic MRI to evaluate dogs with signs of clinical lumbosacral disease.

N04

Evaluation of Phosphorylated Neurofilament as a Prognostic Biomarker in Dogs with Thoracolumbar Intervertebral Disc Herniation

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Validated biomarkers in dogs with acute, severe thoracolumbar intervertebral disc herniation (IVDH) are needed to predict prognosis and aid in client decision-making. Phosphorylated neurofilament-heavy (pNF-H) is a major cytoskeletal protein of axons. Increased pNF-H in biologic fluid has been correlated with severity of axonal injury in several species. We hypothesized that serum pNF-H levels would predict outcome in dogs with acute, severe thoracolumbar myelopathy secondary to type I IVDH.

Thirty-eight dogs with T3-L3 myelopathy secondary to type I IVDH, divided into two cohorts (intact and absent nociception) were enrolled. Serum samples were collected on admission and postoperatively over a series of time points for up to 2 weeks. Clinical data were collected at each time point and at 6 weeks and 3 months postoperatively. Serum pNF-H levels were quantified via ELISA. Dogs were grouped by outcome (controls, recoverers, non-recoverers, and myelomalacic) for analysis with recovery defined as return of voluntary ambulation and continence.

Preoperative pNF-H levels showed no significant differences between outcome groups. Dogs that developed myelomalacia had significantly higher pNF-H levels than all other groups after 48h postoperatively (P < 0.001). No other clinically relevant differences were seen in pNF-H level between outcome groups at the time points evaluated.

Taken together, serum pNF-H does not appear to be a good preoperative biomarker for the prediction of recovery after a functionally complete spinal cord injury. However, there is potential utility in the early detection of ascending/descending myelomalacia that may be beneficial in the clinical management of patients lacking nociception.

N05

Evaluation of Horner’s Syndrome in Dogs with Cervical Myelopathy

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Horner’s syndrome may be associated with cervical myelopathies, due to involvement of the first or second order neurons of the
sympathetic pathway, however, few data are available relating the presence of Horner’s syndrome and underlying pathology of the spinal cord. We hypothesized that the presence of Horner’s syndrome in dogs with cervical myelopathy would be associated with specific neuroanatomical lesions.

Medical records for 106 dogs with cervical myelopathy and concurrent Horner’s syndrome, with MRI of the cervical spinal cord, were reviewed to obtain data including signalment, neurological grade, neuroanatomical localization, CSF analysis, surgical findings, outcome and pathology results. MR imaging was reviewed to distinguish intrinsic from extrinsic lesions and further characterize the extent of the lesions.

Small breed dogs predominated with 60.2% weighing <20kg. The most common neurological grade on presentation was non-ambulatory tetraparesis (49.1%). The majority of dogs (57.7%) had a primarily intrinsic myelopathy, 37.2% had a primarily extrinsic myelopathy, and 5.1% had a mixed extrinsic and intrinsic myelopathy. All regions of the spinal cord were involved and segmental localization was not significantly associated with Horner’s syndrome (41.0% C1-C5; 37.2% C6-T2; 20.5% both; 1.3% no visible lesion). Sixty-seven dogs (63.2%) had a partial Horner’s syndrome while 36.8% had a complete Horner’s syndrome. Unilateral Horner’s syndrome was most common (90.6%), with both eyes affected at similar rates (OD 42.5%, OS 48.1%). The majority of dogs (86.8%) survived to discharge.

Our findings suggest that Horner’s syndrome in dogs with cervical myelopathy is more commonly seen with an intrinsic myelopathy, but is not associated with a specific localization or outcome.

**N06**

**Incidence of Acute Hepatopathy in Dogs Receiving Zonisamide**

**Tessa Smith – University of Wisconsin; Starr Cameron – University of Wisconsin**

Zonisamide is an anti-epileptic medication that is becoming more commonly used in veterinary medicine. Approximately 90% of this drug undergoes hepatic metabolism in dogs. An idiosyncratic, acute hepatopathy has been described in two case reports, but the true incidence is unknown. The aim of this retrospective study was to characterize the incidence of acute hepatopathy in dogs treated with zonisamide. Five hundred cases were reviewed from a private practice referral hospital. One hundred and seven cases met the inclusion criteria for follow-up bloodwork. Any dog on phenobarbital or prednisone was excluded. Additionally, any dog with abnormal follow up bloodwork and no pre-zonisamide bloodwork was excluded. There was a median follow up of 12 months. Blood values of albumin, ALT, and ALP were documented. Two of the 107 dogs (1.9%, 95% confidence interval: 0.6 - 3.1%) demonstrated a new abnormality of one or more liver parameters. One of these dogs developed clinical liver failure as well as increased ALT and ALP within 4 weeks of starting zonisamide. The second dog developed an increased ALT at two weeks but remained clinically normal. ALT normalized after discontinuing zonisamide. This study reports the low incidence of both acute clinical hepatopathy (0.9%, 95% confidence interval: 0 - 2.7%) and biochemical hepatopathy (1.9%).

**N07**

**Clinicopathological Characteristics of Central Nervous System Histiocytic Sarcoma in Dogs**

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Histiocytic sarcoma (HS) in dogs may affect the central nervous system as a primary tumor or as a component of disseminated disease, and prognosis based on limited data is poor. The histopathology of primary CNS HS differs from HS in most other locations in that it is often characterized by a marked inflammatory infiltrate. We hypothesized that CNS HS clinicopathological findings would be reflective of characteristic inflammatory histopathology, and would inform both diagnosis and classification. A retrospective study of 102 histopathologically confirmed CNS histiocytic sarcomas in dogs was performed to determine signalment, lesion localization, clinical outcome and inflammatory criteria relating to cerebrospinal fluid (CSF) analysis and peripheral blood neutrophil to lymphocyte (N/L) ratios. Forty one primary and 45 disseminated HS affecting the CNS were defined as well as 16 CNS HS that were not further classified due to lack of complete necropsy. HS affecting the CNS was over represented in Bernese mountain dogs. Golden retrievers, Rottweilers, Welsh Corgis and Shetland sheep dogs (all p < 0.00001) with an overall median age of 8 years. Median survival of symptomatically treated dogs (n = 27) for primary and disseminated disease was 4 and 3 days (2-127d); Median survival for dogs receiving any combination of surgery, radiation and or CCNU chemotherapy (n = 16) for primary and disseminated disease was 44 and 32 days (4-188d) respectively. Mean CSF total nucleated cell counts were significantly different (p = 0.003) between primary (n = 21; 536/μL) and disseminated (n = 19; 8/μL) CNS HS or meningioma (historical data) (n = 56; 21/μL) and neoplastic cells were identified in 50% cases (13/26) where slides were available for review. Primary HS, disseminated HS and meningioma cases had significantly higher N/L ratios than clinically healthy control samples (p < 0.01). Disseminated HS and meningioma cases had significantly higher N/L ratios than primary CNS HS (p < 0.05).

The data confirm breed predilections for CNS histiocytic sarcoma including Welsh Corgis and Shetland sheep dogs. Marked CSF pleocytosis is a characteristic of primary CNS HS, which combined with frequent presence of cytologically defined neoplastic cells supports the collection of CSF as a diagnostic procedure for differentiation of CNS HS from other diseases. Prognosis following symptomatic or definitive
therapy is poor, and further investigation of inflammatory mechanisms present in CNS HS is warranted to better define tumor biology and identify potential therapy.

N08

MicroRNA Expression in the Cerebrospinal Fluid of Great Danes with and without Osseous-Associated Cervical Spondylomyelopathy

Daniella P. Vansteenkiste – The Ohio State University; Joelle Fenger – The Ohio State University; Paolo Fadda – The Ohio State University; Paula Martín-Vaquero – Freelance medical and veterinary writer; Ronaldo da Costa – The Ohio State University

Osseous associated cervical spondylomyelopathy (OA-CSM) is a degenerative condition of the cervical vertebral column that affects mainly giant dog breeds. microRNAs (miRNA) are small RNAs that play important gene-regulatory roles and recent data suggest that circulating miRNAs present in biological fluids may serve as potential biomarkers of neurodegenerative disease. The miRNA profile of normal canine cerebrospinal fluid (CSF) and OA-CSM has not been previously described; therefore, the overarching goal of this study was to characterize the expression levels of miRNAs present in the CSF of Great Danes and identify differentially expressed miRNAs present in the CSF of dogs clinically affected with OA-CSM.

Global CSF miRNA expression levels were evaluated in twelve clinically normal dogs and 12 OA-CSM affected dogs using the NanoString nCounter platform. We identified eight miRNAs showing differential expression in OA-CSM dogs compared to clinically normal dogs. MiR-299-5p and miR-765 were upregulated in the OA-CSM affected dogs and miR-494, miR-612, miR-302-d, miR-4531, miR-4455 and miR-6721-5p were upregulated in the clinically normal group. qRT-PCR was performed to validate the expression levels of two miRNAs (miR-494 and miR-612) and we found that miR-494 expression was increased by 1.5 fold in the OA-CSM affected dogs and the expression of miR-612 was decreased by 1.15 fold in the OA-CSM affected group (p-value= 0.41 and 0.89 respectively).

Data generated from our study represent an initial characterization of the global miRNA profile of canine CSF and suggest that a distinct CSF miRNA expression profile is associated with OA-CSM in dogs.

N09

Comparison of Serum Trace Nutrient Concentration in Dogs with Idiopathic Epilepsy Compared to Healthy Dogs

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Idiopathic epilepsy is the most common cause of seizures in dogs, reportedly affecting 0.5-5.7% of the population. There have been several investigations regarding the serum concentrations of trace nutrients, including copper, selenium, zinc, manganese, and iron in human epileptics and animal models. However, no research of this nature is available in dogs with naturally occurring epilepsy. The purpose of this prospective study is to compare the serum concentrations of several trace nutrients in non-epileptic dogs to dogs with epilepsy.

Blood samples were collected from 50 healthy control dogs and 92 dogs (95 samples) with idiopathic epilepsy. The epileptic patients were further subdivided into three groups (controlled: n = 27, uncontrolled: n = 42, and untreated: n = 23). Serum was evaluated for concentrations of copper, selenium, zinc, cobalt, manganese, molybdenum, and iron using inductively coupled plasma mass spectroscopy. Untreated epileptics had significantly higher iron concentrations than any of the other three groups (p = 0.04). Uncontrolled epileptics had significantly higher manganese levels (p = 0.007) than any of the other three groups. Significantly higher levels of copper (p < 0.0001) and molybdenum (p = 0.001) were found in both controlled and uncontrolled epileptics compared to normal or untreated dogs. Finally, uncontrolled and controlled epileptics had significantly higher levels of selenium (p = 0.0003) than normal dogs, and uncontrolled epileptics had higher levels of zinc (p = 0.0002) than normal and untreated dogs.

The significant difference in the serum concentrations of several trace nutrients (manganese, selenium, and zinc) may suggest a role for these nutrients in the pathophysiology and/or treatment of epilepsy. Additionally, these results suggest that anticonvulsant therapy may affect copper and molybdenum metabolism.

N10

Multiple System Degeneration in Cats with Gene Mutation in Serine Active Site Containing 1

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Two sibling cats 9 months of age, one male and one female were evaluated with initial signs of cerebellar ataxia progressing to festination and postural instability. Infectious disease serology was negative, and blood work and CSF analysis were normal on Cat 1. A brain MRI of Cat 1 showed T2 hyperintensities in the caudate, geniculate, pontine and olivary nuclei, and cerebellar atrophy. Cat 2 similarly progressed and was euthanized. Brain histopathology showed Purkinje cell degeneration and thinning of the molecular layer in the cerebellum and neuronal degeneration and loss with gliosis in the pons, olivary, substantia nigra, caudate and thalamic nuclei. DNA was isolated from whole blood of Cat 1 for whole genome sequencing (WGS). The results were compared to 193 additional cat genomes from the 99 Lives Cat Genome Project. Cat 1 was considered homozygous for an autosomal recessive trait with no other representation of the variant in the dataset. A deleted cytosine at position B2:145,334,959 in Serine Active Site Containing 1 (SERAC1) (c.1153delC) was identified and caused a frameshift downstream introducing a stop codon (p.GLNSerfs*35). This loss of function variant alters 35 amino acids and causes truncation of an additional 120 amino acids of the protein, 28% of the 3' portion of the protein. The clinical presentation, progression, and lesions associated with the cats’ condition is very similar to two prior case reports and the previously reported canine multiple system degeneration (MSD). This study also demonstrates the utility of WGS for efficient diagnosis of a MSD in cats.
N11

Qualitative Assessment of Canine Ambulatory Electroencephalography Recordings over 48 Hours

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Long-term EEG monitoring (48+ hours) can markedly increase the likelihood of documenting ictal or interictal epileptiform discharges in human patients with seizures. Wireless ambulatory EEG (aEEG) recordings have been reported in dogs to evaluate paroxysmal events, however, there are no data on the quality of these recordings over time. We hypothesized that long-term (24-48 hour) aEEG was feasible in dogs, and would increase documentation and characterization of abnormal events.

A 20-lead Cadwell ambulatory EEG system with time-locked video monitoring was placed in 18 dogs for a total of 19 recordings (one dog had two recordings). Three dogs had no known history of seizures, and 15 dogs were presented for evaluation of seizures or other paroxysmal events. All patients were sedated with dexmedetomidine for electrode and device placement (vest with amplifier), then reversed and monitored with video in the hospital for up to 48 hours, while resuming regular activity. Ambulatory EEG recordings were terminated after 48 hours of hospitalization or when the patient removed the device.

Total duration of aEEG recording, duration of quality recording (defined as >50% recording leads and free of marked movement artifact), the time when all leads were lost or removed, latency to first abnormal event (if present), and number of abnormal events per patient were documented. The average total aEEG recording time was 21 hours (range 2.6 – 39.5 hours), with the average quality recording time of 7.8 hours (range 41 minutes – 20 hours). The average time when all leads were lost was at 13.8 hours (range 58 minutes – 38.5 hours). Five of the 19 recordings were abnormal (4 seizure disorders, 1 narcolepsy), and the average latency to the first event was 3.5 hours (range 35 minutes – 10 hours; median 1.6 hours). The number of documented events in these 5 cases ranged from 3 to 92, and included multiple events without overt clinical signs, and in one dog, epileptiform discharges localizing to different cortical regions. These cases suggest that long-term aEEG recordings are possible in clinical dogs, however, likely have limited diagnostic utility beyond 12-24 hours because of loss of recording leads. Beyond simple documentation of EEG activity associated with a clinical event, the prolonged recordings permitted identification of additional subclinical events as well as different kinds of epileptiform discharges in patients.

N12

Retrospective Evaluation of Risk Factors Associated with Clinical Relapse of Meningoencephalitis of Unknown Origin

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There is limited information regarding risk factors for relapse of meningoencephalitis of unknown origin (MUO) following tapering of immunosuppressive medications. The purpose of this study was to identify risk factors associated with relapse of MUO in canine patients.

The medical records at the University of Wisconsin-Madison Veterinary Care were searched for patients diagnosed with MUO between September 2004 and June 2018. Patients with a complete medical record including a complete blood count, serum chemistry panel, CSF analysis and cytology, advanced imaging with MRI and infectious disease testing were included in the study. Dogs that responded to treatment based on a repeat neurologic examination or CSF tap were defined as having a relapse if CSF or neurologic signs worsened again. Signalment, weight, presenting clinical signs, CSF analysis, length of immunosuppression, time from diagnosis to tapering of medications, single vs multiple drug therapy and results of repeat CSF tap or neurologic examination were evaluated for association with relapse using Cox proportional hazards regression models. Kaplan-Meier survival curves were also created for the categorical predictors.

Sixty-six dogs met the inclusion criteria, 22 with signs of relapse and 44 without signs of relapse. An abnormal repeat CSF tap was the only condition statistically significantly related to relapse (p value = 0.002). Dogs that did not fully respond to treatment had 5.6 times larger risk of relapse than responding dogs. We conclude that an abnormal CSF tap 4-6 weeks from diagnosis may help predict the risk for relapse of MUO.

N13

Temporal and Anatomical Distribution of 18F-flutemetamol Uptake in Canine Brain Using Positron Emission Tomography

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Positron emission tomography (PET) is currently being used more as an imaging modality in veterinary medicine for clinical and research applications. Amyloid PET has become a useful tool to help diagnose Alzheimer’s disease accurately by identifying amyloid-beta plaques in human. 18F-flutemetamol is one of the PET tracers binding amyloid beta protein in clinical development. Therefore, physiologic characteristic of 18F-flutemetamol in normal dog brain is necessary for distinguishing abnormal state. Static and dynamic PET images were acquired using six adult healthy dogs, after 18F-flutemetamol was administered intravenously at approximately 3.083 MBq/kg. For static image, PET data were acquired at 30, 60, and 90 min after injection. A week later, dynamic acquisition was started at the time of tracer injection and lasted for 120 min. PET data were reconstructed by use of iterative technique, and correction for attenuation and scatter were applied. Regions of interest were manually drawn over frontal, parietal, temporal, occipital, anterior cingulate, posterior cingulate, cerebellar cortex, cerebral white matter, midbrain, pons, and medulla oblongata. After calculating standardized uptake values with an established formula, standardized uptake value ratios (SUVR) taking the cerebellar cortex as a reference region were obtained. Among the 6 cerebral cortical regions, cingulate cortices and frontal lobe showed the highest SUVR. The lowest SUVR was observed in the occipital lobe. The average values of cortical SUVRs were 1.25, 1.26, and 1.27 at 30, 60, and 90 min post-injection. Tracer uptakes on dynamic scan was rapid, peaking within 4 min post injection. After an early maximum, cortical regions had a steep descending branch.
**N14**

**Pathological Characteristics of 30 Pugs with a Chronic Thoracolumbar Myelopathy**

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Chronic myelopathies with a similar clinical phenotype but with different underlying etiologies, involving spinal cord as well as vertebral column pathology, have been described in pugs. The histopathological features of affected spinal cord tissues in pugs with chronic myelopathies have only been sparsely studied. The objective of this study was to describe the neuropathological characteristics of pugs with a chronic thoracolumbar myelopathy, and to identify potential co-morbidities associated with the condition. Thirty client-owned pugs with a chronic history (more than 1 month) of ataxia and paraparesis were clinically, neurologically and histopathologically characterized. A selection of affected pugs underwent advanced imaging and cerebrospinal fluid analysis, pre-or immediately post-mortem. Pathology was confirmed in the spinal cord of all examined pugs, with focal meningeal fibrosis and malacia of the spinal cord being a consistent finding in the majority of pugs. In a third of the affected pugs the meningeal fibrosis was associated with the formation of subarachnoidal diverticula. The focal spinal cord lesion was accompanied by vertebral malformations and/or intervertebral disc herniations confirmed by computed tomography (n=17). Varying degrees of lymphocytic and/or histiocytic inflammation was found in the CNS in 40% of the examined pugs. In addition, 50% of the pugs presented with a cell composition in the cerebrospinal fluid indicating, in general mild inflammation. 

In conclusion, meningeal fibrosis with associated focal spinal cord malacia and neighboring vertebral pathology was a common finding in pugs with a chronic thoracolumbar myelopathy.

**N15**

**Long-Term Outcome of Niemann-Pick Disease Type C1 Cats Treated with Cyclodextrin**

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Niemann-Pick disease type C (NPC) is a severe lysosomal storage disorder characterized by the neuronal and hepatic accumulation of cholesterol and sphingolipids, progressive neurologic dysfunction, and premature death. We have previously reported that subcutaneously (SC) administered 2-hydroxypropyl-beta-cyclodextrin (HPßCD) improved hepatic disease, and that intracisternal (IC) administration allowed Purkinje cells to survive for up to 1.5 years. In this study, we evaluated neurofunctional function, nerve conduction velocity (NCV), body weight, and serum biochemistry, as well as postmortem brain and liver data, for up to three years in treated cats. Although NPC cats treated with IC HPßCD did not develop signs of cerebellar dysfunction, they did develop abnormal mental status, dysphagia, and gaze palsy. Liver values improved in both cohorts that received IC only and IC+SC HPßCD, however the latter group showed the greatest improvement. Both IC and IC+SC cohorts showed improvements in peripheral NCV compared to NPC untreated cats, but again IC+SC HPßCD treated values were closer to normal than the IC HPßCD treated group. Body weight and brain weight were lower than normal in both cohorts, but were significantly higher than NPC untreated cats. IC+SC HPßCD treated body weights were consistently higher than in IC HPßCD cats. These findings suggest an advantage to combined IC+SC HPßCD therapy over IC HPßCD treatment alone, in terms of central nervous system, peripheral nervous system, and hepatic function. Finally, histopathology revealed insufficient improvement of neuronal storage in the thalamus, midbrain, and medulla with HPßCD. These limitations serve to target future treatment research.

**N16**

**Effect of Prior General Anesthesia or Sedation on the Diagnostic Utility of Wireless Video Electroencephalography**

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Differentiating between epileptic seizures and other paroxysmal episodes is a challenge in veterinary medicine. Awake wireless video electroencephalography (EEG) is the method of choice to discriminate
between epileptic seizures and other non-epileptic episodes. However, EEG is rarely used in dogs due to lack of a standard protocol for instrumentation among other technical difficulties. Particularly concerning is the use of general anesthesia (GA) or sedation that may influence cortical EEG findings. This retrospective cohort study investigated the influence of GA or sedation for electrode placement prior to recording on the diagnostic outcome of EEG in dogs with epilepsy and other paroxysmal episodes. We hypothesized that the use of GA or sedation would lower the probability of achieving a diagnosis with EEG. The proportion of EEG recordings that provided a diagnosis on the nature of the paroxysmal episodes (epilepsy or not) was compared between dogs receiving GA or sedation and dogs receiving neither during instrumentation. A hundred and eight dogs were included, 45 receiving GA/Sedation and 63 neither. EEG provided a diagnosis in 48.9% (95% CI: 33.9 - 64) of dogs in the GA/Sedation group and in 68.3% (95% CI: 55.2 - 79.1) of dogs that received neither. This was not statistically different (α = 0.05). The ‘risk’ of achieving a diagnosis by EEG after GA/Sedation was 72% that of the dogs that received neither (relative risk: 0.72; 95% CI: 0.51 - 1.01). This study suggests that using GA or sedation prior to EEG electrode placement does not halve the chance of achieving a diagnosis with wireless video EEG recording.

N17

Clinical Characteristics of Inflammatory Myopathy in the Boxer Dog and its Possible Association with Neoplasia

Hsuan-Ping Hong – Purdue University; Stephanie Thomovsky – Purdue University; Melissa Lewis – Purdue University; R. Timothy Bentley – Purdue University; G. Diane Shelton – School of Medicine, University of California

This retrospective study evaluated the clinical characteristics and outcome in Boxer dogs diagnosed histologically with inflammatory myopathy (IM). The presence of neoplasia prior, at the time, and after IM diagnosis was also explored. Medical records including signalment, history, clinical signs, clinicopathologic findings, treatment and outcome from 28 Boxer dogs diagnosed with IM at the Comparative Neuromuscular Laboratory, University of California San Diego from 2010-2018 were reviewed. Age of onset ranged from 1 to 9 years. Presenting complaints included: generalized weakness (n=17), dysphagia (n=11), and weight loss (n=10). Exam findings included: generalized muscle weakness/exercise intolerance (n=9), stiff/stilted gait (n=9), and decreased-to-absent gag reflex (n=6). Creatine kinase was elevated in the 20 cases in which it was performed (range 908-138,000 IU/L). Infectious disease testing performed in 22 cases did not support an infectious etiology. Seven dogs were diagnosed with neoplasia prior to muscle biopsy (range 2-48 months); 5 with mast cell disease and 2 with histiocytoma. One dog had round cell infiltration within the muscle at the time of IM diagnosis. Neoplasia following diagnosis of IM was not documented in any case. Treatment consisted of steroid monotherapy (n=14), cyclosporine monotherapy (n=1), multiple immune-suppressive medications (n = 12), and unspecified therapy (n=1). Seven dogs neurologically improved, 6 did not improve, 11 relapsed while on treatment and 4 were lost to follow-up. Boxer dogs with IM present for generalized weakness and dysphagia; successful outcome is uncommon. The relationship between neoplasia and IM in Boxer dogs remains unclear; future studies are warranted.

N18

MiR-21, MiR-125b, MiR-146a and MiR-181c Expression in Exosomes from Canine Mesenchymal Stem Cells

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Mesenchymal Stem cell (MSC) therapy is an emerging treatment for neurological conditions and the exosomes they released are partially responsible for their effects. MicroRNAs miR-21, miR-125b, miR-146a and miR-181c were determined by RT-qPCR using the ΔΔCq method, using both the spike-in cel-miR-39 and U6 as reference genes. Results were compared between cell lines and passages using one-way ANOVA. All microRNAs were detected in the three cell lines at every passage (two to five), except miR-146a that was not detected in two passages of one cell line. There was no significant difference in the mean relative quantity between different passages (p = 0.871), but there was significant difference between different cell lines for all four microRNAs (miR-21 p = 1.768E-3, miR-125b p = 1.768E-3, miR-146a p = 2.795E-3, miR-181c p = 1.327E-2). These results suggest that the quantity of these exosomal microRNAs is stable with increasing time in culture but should be evaluated for each cell line.

N19

Evaluation of Hematologic Parameters in 40 Dogs Receiving Long-Term Cytarabine for Meningoencephalomyelitis of Unknown Etiology

Jessica Reese – Carolina Veterinary Specialists; Peter Early – NC State University Veterinary Hospital; Robert Bergman – Carolina Veterinary Specialists; Lindsay Williams – Carolina Veterinary Specialists; Jennifer Beeman – SAS Institute; Emily Griffith – NC State University

The aim of this retrospective study was to evaluate hematologic parameters in dogs treated long-term (> 6 months) with cytarabine.
N20

Prevalence of Neurologic Worsening Immediately Following Thoracolumbar Hemilaminectomy for Intervertebral Disc Extrusion

Benjamin Williams – Center for Veterinary Speciality + Emergency Care

Intervertebral disc extrusion in the thoracolumbar spine is the most common spinal cord injury in dogs, and surgical treatment often provides a more complete recovery than medical management. While hemilaminectomy is the most common surgical procedure to address these injuries, it is not without potential complications and risks. Previous studies have looked at prognostic factors for neurologic recovery following surgery, long term outcome from surgery, and complications in the early postoperative period. There have been investigations into the possible cause of neurologic worsening in the early postoperative period, which have included anesthetic factors, iatrogenic injury, and natural progression of the injury. Temporary or permanent neurologic worsening is considered a possible early postoperative complication, although previous studies have not identified the prevalence of this problem in the immediate post operative period. The aim of this study was to determine the prevalence of neurologic worsening in the first 24 hours following a thoracolumbar hemilaminectomy for intervertebral disc extrusions.

This retrospective study included ninety-one client owned dogs undergoing thoracolumbar hemilaminectomy at a private specialty hospital, between 2016 and 2018. Surgery and pre/postoperative neurologic exams were performed by a board certified Neurologist (DACVIM - Neurology) or a resident in Neurology / Neurosurgery. Neurologic exam findings were converted from descriptive findings to a modified Frankel scoring system (MFS) and neurologic worsening was defined as a loss of neurologic grade or grades from the preoperative neurologic exam to the 24-hour postoperative exam.

Of the ninety-one cases, twenty-nine (32%) had neurologic worsening. Seventeen had a loss of one neurologic grade, ten had a loss of two grades, and two cases had a loss of three neurologic grades. All cases that neurologically worsened by 2 or more grades were initially graded as weakly ambulatory paraparetic (MFS 4), while cases that worsened by a single grade represented a mix of weakly ambulatory paraparetic, non-ambulatory paraparetic, and one dog that was graded as paraplegic with superficial pain intact (MFS 2) that worsened to paraplegic with only deep pain sensation intact (MFS 1). Forty-one dogs (45%) remained neurologically stable between exams and twenty-one (23%) showed improvement by one or more neurologic grades. This data demonstrates a significant percentage of dogs that neurologically worsen within the first 24 hours of decompressive surgery.

N21

Diagnostic Utility of Inner Ear FLAIR MRI in Dogs with Vestibular Disease

Gibrann Castillo – University of Guelph; Tomas Parmentier – Ontario Veterinary College; Gabrielle Monteith – Ontario Veterinary College; Luis Galtero – Ontario Veterinary College

The inner ear contains endolymph and perilymph. The second is comparable and in continuity with the cerebrospinal fluid (CSF) so it is expected to suppress in fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) if normal. Even though inner ear FLAIR abnormalities have been extensively described in humans with inner ear diseases, its diagnostic value in dogs is yet to be proven. The goal of this study was to investigate the diagnostic utility of FLAIR MRI in dogs with vestibular disease.

Medical records identified 102 dogs that had brain MRI performed because of vestibular signs. Based on the final diagnosis, patients were allocated to 4 groups: otitis media/interna, idiopathic vestibular disease, central vestibular disease and other. Additionally, a control group (n=73) included dogs with normal MRI and without vestibular signs. Inner ears were delineated in Horos viewer as a region-of-interest and its
signal intensity measured in FLAIR and T2-weighted images. The percentage of suppression in FLAIR was calculated and compared between affected and unaffected sides of each individual and between groups using a general linear mixed model. Correlation between suppression and CSF cell count and protein concentration was assessed. Affected inner ears in dogs with otitis media/interna had decreased suppression in FLAIR compared to the unaffected side (P < 0.001), and all the other groups (P < 0.001). No significant correlation was detected between CSF results and suppression. These results show the diagnostic value of FLAIR in otitis media/interna due to lack of suppression in the affected inner ear.

A Retrospective Study of Canine Epilepsy: Etiological Distribution, Therapeutic Outcome, and Survival Time

Yoonsoo Jeong – Chungbuk National University; Ji-Houn Kang – Chungbuk National University; Mhan-Pyo Yang – Chungbuk National University; Byeong-Teck Kang – Chungbuk National University

The purpose of this study was to investigate etiological distribution, therapeutic outcome, and survival time in canine epilepsy. The medical records of 57 epileptic dogs were reviewed for the evaluation of etiological distribution, among which 27 (47%) had idiopathic epilepsy (IdE) and 30 (52%) had structural epilepsy (StE). Twenty-nine dogs (16 IdE dogs and 13 StE dogs) were evaluated for therapeutic outcome and survival time.

The incidence of generalized epileptic seizure (IdE, 56% vs. StE, 44%; P = 0.043) and the median seizure frequency at the time of first presentation (IdE, 2.0/month vs. StE, 13.3/month; P < 0.01) differed significantly between the two groups. Although pre-treatment seizure frequency and duration did not differ significantly, the median duration of seizure in the IdE group (0.5 min) was significantly shorter than that in the StE group (3 min) after treatment (P < 0.01). In addition, the median frequency of seizure was lower in the IdE group (0.25/month) than in the StE group (2.00/month) following antiepileptic therapy (P = 0.053). The median survival time of the IdE group (1.5 years [95% confidence interval (CI), 1.0–2.3]) was significantly longer than that of the StE group (0.4 years [95% CI, 0.2–1.3]) (P < 0.01). The findings regarding etiological data and intracranial lesions may be useful for predicting the treatment response and prognosis of epileptic dogs.

Evaluation of Treatment with Mycophenolate Mofetil and Prednisolone in Dogs with Meningoencephalomyelitis of Unknown Etiology

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Mycophenolate mofetil (MMF) has been considered a comparable alternative option to other published immunosuppressive agents. The purpose of this retrospective study was to describe the use of MMF as an adjunctive immunosuppressive agent with glucocorticoids in client-owned dog with MUE.

Medical records of 86 dogs with MUE and treated with prednisolone and MMF were evaluated regarding response, survival, and drug-related adverse effects. The data collected from the medical records included signalment, the presentation of clinical signs, a time lag between clinical sign onset and initial presentation, neuroanatomical lesion localization, all laboratory evaluations, MRI and CT imaging findings, CSF analysis. Dogs were initially treated with prednisolone 1 mg/kg PO every 12 hours and with MMF 20 mg/kg PO every 12 hours. Details regarding drug tapering were based on neurologic state and adverse drug effects at follow-up.

Finally, eighty-six dogs were included in the study. CR was recorded in 57 dogs (66.28%), whereas 18 dogs (20.93%) had a PR, and 11 dogs (12.79%) were unresponsive to therapy. On a univariate multiple data analysis used to identify the risk factor for CR probability, a significantly higher odds ratio was recorded in dogs that had neurological dysfunctions other than only seizure history before presentation, relative to those that had only seizure history (odds ratio of 3.701; P = 0.013). Of the 75 responders, 34 dogs (45.33%) had a relapse after achieving a treatment response. Of the 34 relapsed dogs, only five dogs failed to achieve re-remission of the relapsed clinical signs. There were no significant differences in CR rates and relapse rates between the acute group and the chronic group.

The median survival time (MST) for all dogs treated with MMF and prednisolone was 558 days (range, 3-2,634 days). There were significant (CRs compared with PRs, P = 0.000; CRs compared with NRs, P = 0.000; PRs compared with NRs, P < 0.001) differences in the MST for dogs that had a CR to treatment during the study (877 days; range, 35-2,634), compared with the MST for dogs that had a PR (122 days; range, 21-879) or a NR (30 days; range, 3-221). The MST was also

| Factor                              | CR, % (n) | Failed CR, % (n) | P     |
|-------------------------------------|-----------|-----------------|-------|
| Sex                                 |           |                 |       |
| Male                                | 66.67 (18) | 33.33 (9)       | 0.789 |
| Female                              | 66.1 (39)  | 33.9 (20)       |       |
| Age                                 |           |                 |       |
| <= 7 years                          | 61.67 (37) | 38.33 (23)      | 0.083 |
| > 7 years                           | 76.92 (20)| 23.08 (6)       |       |
| Lesion distribution                 |           |                 |       |
| Focal                               | 75.56 (34)| 24.44 (11)      | 0.087 |
| Multifocal                          | 56.1 (23) | 43.9 (18)       |       |
| Brainstem involvement               |           |                 |       |
| Negative                            | 73.33 (44)| 26.67 (16)      | 0.179 |
| Positive                            | 50 (13)   | 50 (13)         |       |
| Seizure                             |           |                 |       |
| Negative                            | 53.49 (23)| 46.51 (20)      | 0.393 |
| Positive                            | 79.07 (34)| 20.93 (9)       |       |
| Seizure as the only symptom         |           |                 |       |
| Negative                            | 55.77 (29)| 44.23 (23)      | 0.013 |
| Positive                            | 82.35 (28)| 17.65 (6)       |       |
| Body weight                         |           |                 | 0.758 |
| TNCC                                |           |                 | 0.162 |
| Symptom duration before treatment   |           |                 | 0.568 |
significantly ($P = 0.011$) longer for dogs that did not relapse (993 days; range, 35-2,634) than for dogs that did relapse (410 days; range, 21-2,021). The MST was significantly ($P = 0.01$) longer for dogs that had a focal lesion (690 days; range, 4-2,634), compared with the MST for dogs that had multifocal lesions (401 days; range, 3-1,834). The MST for dogs that had a brainstem involvement was 244 days (range, 3-2,634) and for dogs that did not have a brainstem involvement was 609 days (range, 3-2,502). There was no statistical difference in MST between the two groups ($P = 0.057$). The MST for dogs in the acute group was 401 days (range, 3-2,502) and for dogs in the chronic group was 807 days (range, 3-2,634). There was no statistical difference ($P = 0.125$) in survival time between the acute group and the chronic group.

Univariate multiple Cox regression analysis with 10 variables showed the CR (relative hazard ratio of 4.564, $P = 0.000$) and TNCC (relative hazard ratio of 1.004, $P = 0.032$) at diagnosis to be significant factors in predicting mortality. Age, sex, body weight, the presence of seizure history, the duration of clinical signs before treatment, lesional distribution, brainstem involvement, and TP had no impact on mortality. Treatment-related adverse effects were reported in 39 of 86 (45.35%) dogs. Of these 39 cases, 14 dogs had two or more concurrent adverse effects. The most common adverse effect was gastrointestinal problems ($n = 26$ dogs, 26/86, 30.23%). The second most common adverse effect was sporadic infections ($n = 17$ dogs, 17/86, 19.77%). Pancreatitis was reported in seven dogs (7/86, 8.14%), and one of these dogs
had concurrent pancreatic abscess. The adverse effects attributed to prednisolone therapy were not recorded in this study and improved during tapering. These data suggested that adjunct MMF treatment of MUE appears to be safe and comparable to other immunosuppressive protocols. Treatment should focus on the achievement of CR and preventing the relapse for successful management. Attention to the adverse effects including gastrointestinal upsets, sporadic infections, and pancreatitis, was warranted, particularly in the patient who are treated with high-dose, long-term MMF.

N24

Electrode Scalp Impedance Differences Between Two Electroencephalography Machines In Healthy Dogs

Julia Luca – University of Guelph; Fiona James – University of Guelph; Luis Galtero – University of Guelph; Andrea Sanchez – University of Guelph; Gabrielle Monteith – University of Guelph

This study compared electrode scalp impedance measurements in dogs recorded by wired and wireless electroencephalography (EEG) machines. Seven spayed or neutered adult beagles, weighing between 6 - 10 kg, with normal physical and neurological examinations were used; 1 beagle was recorded twice resulting in 8 recordings. For each recording, impedance was measured using 10 subdermal wire electrodes (SWEs), resulting in 80 impedance readings. Electrodes were placed on specific locations (F7/F8, F3/F4, T3/T4, C3/C4, Fz, and Cz) on the canine head using Propofol sedation. First, the wired EEG machine was connected to the SWEs to measure impedance values. Secondly, after the wireless EEG machine was disconnected from the SWEs, the wireless EEG machine was connected to the SWEs to measure impedance values. The wireless EEG machine recorded higher impedance measurements in comparison to the wired EEG machine for every electrode location on every dog (P < 0.05). The wireless EEG machine’s impedance readings were on average 2.83 kΩ (CI = 95%, SD = 1.42, LLtol = 6.07, ULtol = 0.403) higher than the wired EEG impedance readings. The wired machine’s impedances ranged between < 0.5 kΩ and 9 kΩ (mean = 3.09, median = 2.00, SD = 2.15), whereas the wireless machine’s impedances ranged between 2.688 kΩ and 16.065 kΩ (mean = 5.92, median = 5.05, SD = 2.59). Despite these differences in impedance measurements between the wired and wireless EEG machine, both machines appear to measure the same impedance patterns. Therefore, wireless EEG machines should be acceptable for use in clinical settings.

N26

Effects of Radiotherapy on Seizure Freedom and Survival Time in Dogs with Brain Tumors

Susana Monforte – University of Cambridge, UK; John Rossmeisl – Virginia Maryland Regional College of Veterinary Medicine; Jason Russell – Vanderbilt University; Mark Holmes – University of Cambridge; Annette Wessmann – Pride Veterinary Centre; Jo Morris – University of Glasgow; Jane Dobson – University of Cambridge; An Vanhasenbrouck – University of Cambridge

Dogs with brain tumours commonly present with seizures, which might lead to euthanasia. Both surgical resection and chemotherapy have been reported to allow the control of seizures, whereas the exact role of radiation therapy in seizure reduction is still unclear in both humans and animals. This retrospective study aimed to investigate the efficacy of radiotherapy in controlling epileptic seizures in dogs with brain tumours and to extend their survival time. Thirty-two dogs presented with seizures, resulting from a suspected or confirmed brain tumour. The study group received radiotherapy (n = 18) and was compared with a control group (n = 14). All dogs received medical treatment, consisting of antiepileptic drugs with or without corticosteroids. The effect of radiation therapy on seizure freedom was analyzed. We also investigated whether clinical or MRI characteristics would have influenced seizure frequency following radiotherapy or palliative treatment. Survival times for both groups were described and compared. Minimum follow-up period was 12 months.

The period of seizure freedom was significantly increased in the radiotherapy group (P < 0.0005 using a log rank test), with a mean of 24.0 months (95% CI: 14.3-33.8) versus 1.7 months in the control group (95% CI: 0.5 – 2.9). Nearly halve (44%) of the radiotherapy group were still seizure free at the end of the study, compared with none of the dogs within the control group. Survival time was significantly increased in the radiotherapy group (P < 0.0005 using a log rank test), with a mean of 34.6 months (95% CI: 25.2-44.1) versus 6.2 months in the control group (95% CI: 2.6 – 9.7). In the radiotherapy group, of the 5 dogs that died during the study period, none of them showed recurrence of seizure activity. In the control group, recurrence of seizures was observed before death in all dogs.

A Cox’s proportional hazard analysis found no statistically significant association between clinical (i.e., frequency or severity of seizures) or MRI characteristics (i.e., tumour type, localization or size, edema, mass effect or contrast enhancement) and the length of time that dogs were seizure free.

A longer period of seizure freedom and survival time was observed in dogs with brain tumours following radiotherapy, compared with medical treatment only. Further studies are needed to investigate underlying pathophysiological mechanisms.

N27

Evaluation of Electroacupuncture Treatment in 38 Dogs Presenting Cervical and Cervico-Thoracic Neurological Syndrome

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Thirty-eight dogs presenting cervical pain and/or tetraparesis undergoing electroacupuncture (EA) combined with conservative treatment were evaluated. Neurological score (NS), pain score 0 to 10 (Dynamic Interactive Visual Analogue Scale - DIVAS) and time of recovery of locomotion were analyzed comparing two groups of dogs: Cervical
Degenerative Myelopathy (DM) is a progressive and ultimately fatal neurodegenerative disease of dogs associated with a mutation in the superoxide dismutase 1 gene (SOD1). This mutation results in intracellular aggregation of SOD1 protein and these aggregates are implicated in causing neurodegeneration, making the SOD1 gene a favorable target for silencing therapies. The aim of this study was to determine the safety and efficacy of SOD1 silencing by a novel U1 Adaptor Oligonucleotide targeting canine SOD1 (U1cSOD1) administered intrathecally to healthy dogs.

Six purpose-bred young adult beagles were used, two at a time, so that the intrathecal administration protocol and dosing could be refined between pairs. In the first two pairs, one dog received the U1cSOD1 while the other received an equivalent volume of vehicle. In the last pair, due to successful achievement of SOD1 silencing and streamlining of the technique in the first two pairs, both dogs received 13 mg of U1cSOD1. Five days following administration of either U1cSOD1 or vehicle, dogs were sacrificed and central nervous system tissues were collected for RT-qPCR of SOD1 activity and histopathology.

All dogs tolerated the procedure and test-agent well, experiencing no adverse clinical or histopathological effects. SOD1 expression in the spinal cord for the 4 dogs receiving the U1cSOD1 was reduced to a mean of 30-40% of normal levels, with a gradual increase moving rostrally to 82% of normal levels in the cerebrum. Future directions will include testing safety and efficacy of the U1cSOD1 in DM affected dogs using a long-term dosing schedule.
N30

Long-term Postoperative Pain Evaluation in Dogs with Thoracolumbar Intervertebral Disc Herniation Following Hemilaminectomy.

Natalia Zidan – North Carolina State University; Julia Medland – North Carolina State University; Natasha Olby – North Carolina State University

Chronic neuropathic pain is a common complication in people with spinal cord injury (SCI), but this phenomenon has not been investigated in dogs.

The aims of this study were to determine the reliability of measuring spinal mechanical sensory thresholds (MST) in normal dogs and to compare MSTs in normal dogs and dogs with SCI due to acute thoracolumbar intervertebral disc herniation (TL-IVDH) following hemilaminectomy over a 1-year period postoperatively.

Normal dogs (normal 5-day assessment group and normal Dachshund group), and dogs with surgically treated TL-IVDH (SCI group) were included. MSTs were measured by an algometer applied to the paraspinous muscles at the TL junction and a control site. The normal 5-day assessment group was tested for 5 consecutive days, normal Dachshunds were tested once. SCI dogs were tested on days 7, 14, 28, 42, 180 and 365 postoperatively; MSTs were compared between days in normal dogs, and between SCI and control dogs using mixed effect models. P < 0.05 was considered significant.

Forty SCI dogs, 22 normal Dachshunds and 10 normal dogs were evaluated. Daily algometry for 5 consecutive days in normal dogs did not cause significant MST changes. MST at the incision site of SCI dogs was significantly lower than normal dogs for 1 month postoperatively but not subsequently. However, 3 / 27 dogs had > 50 % reduction in MST 1 year after surgery.

Dogs with SCI have reduced MST for one month following surgical decompression, but this threshold normalizes in most dogs. However, approximately 10 % dogs may develop neuropathic pain.

NM01

Evaluation of Whisker Stress and Eating Habits in Healthy Cats

Taylor Foster – Washington State University; Jennifer Slovak – Washington State University College of Veterinary Medicine

Our goal for this study was to determine if cats fed from a commercially made whisker friendly dish versus their normal food dish, would spend more time at the food bowl, eat more, and drop less food.

Feeding cats from food dishes that are too deep and or too small, is a way that whisker stress may occur via overstimulation or irritation, leading to altered feeding habits, reduced food intake, and unnecessary discomfort. Although whisker stress is a popular cultural and colloquial term, there is no evidenced based clinical research to support its existence. In fact, the term whisker stress has only been used in commentary, personal opinion literature, and in marketing advertisements.

Forty healthy indoor cats that ate dry food out of ceramic or stainless-steel dishes were enrolled; n=38 cats completed the study. Owners fasted their cats for 12 hours and fed them a normal measured amount of food in their normal dish. Owners video recorded their cats eating for up to 5 minutes, or until the cat walked away, via a smart phone, and also recorded how much food the cats had eaten (in cups) and how much food was dropped from the dish (number of kibbles). Owners then switched to feeding their cats from the “whisker friendly” dish (ceramic or stainless steel), and were instructed to feed their cats for one week from the new dish. Following the 7 day transition, owners were instructed to fast their cats for 12 hours and video record them eating as previously described. The following day, the owners offered food in both the normal dish and the whisker friendly dish to determine cat preference.

A linear statistic model was used to relate the change in time spent at the different food dishes, the change in amount of food dropped, and the amount of food eaten. The t-statistic for the intercept was used to assess the hypothesis that there were differences in outcomes between the initial normal food dish and the whisker friendly dish. No evidence was found that eating from the whisker friendly dish increased the amount of time spent eating p = 0.794. No evidence was found to suggest that the amount of food dropped when eating from the whisker friendly dish was less than from the cat’s normal dish p = 0.941. No evidence was found to suggest that the amount of food eaten was greater when using a whisker friendly dish p = 0.737. Finally in regards to food dish preference, the estimated probability to prefer the whisker friendly dish was 0.74 with a 95% confidence interval.

Cats fed from a whisker friendly dish did not spend more time eating, drop less food, or eat more food in a 5-minute period. Some cats seemed to prefer the new whisker friendly dish over their normal food dish. Overall, food dish associated whisker stress did not affect the eating habits of the study cats.

NM02

Impact of Feeding Method On Overall Activity of Indoor, Client-Owned Dogs

Dan K. Su – University of Tennessee Knoxville; Maryanne Murphy – University of Tennessee; Ashley Hand – University of Tennessee; Xiaojuan Zhu – University of Tennessee; Angela Rollins – University of Tennessee

Objective: The aim of this study was to compare the total daily activity time, walking time, and running time using food-dispensing toys versus bowls in a group of client-owned, primarily indoor dogs.

Methods: A two-way, two period, randomized repeated measures mixed-effects crossover study performed on 26 client-owned, primarily indoor dogs.

Results: Toy feeding increased average daily total activity time by 12% (P = 0.028) and walking time by 26% (P = 0.005). The time (daily changes) effect and the treatment by days were not significant on total activity and walking. Age reduced average daily total activity time by 8% and walking time by 7% with each increase in year of age (P = 0.022 and 0.045, respectively). Gender, body condition, and muscle condition had no significant effect on average daily total activity or walking time. Toy feeding, time, their interaction, age, gender, body condition, and muscle condition had no significant effect on average daily running time.

Clinical Significance: Increased total physical
activity, including walking, can be beneficial in maintenance of lean body mass during canine weight loss programs. Based on the results of this study, feeding toys may be helpful during weight loss programs to achieve this goal.

**NM03**

**Less is More? Ultra-Low Carbohydrate Diet and Working Dogs Performance**

Arnon Gal – University of Illinois at Urbana-Champaign; Richard Burchell – James Cook University; Cave Nick – Massey University; Rebecca Owen – Massey University; William Cuttance – VetEnt Te Awamutu; Nicolas Lopez-Villalobos – Massey University; Aaron Herndon – University of Queensland

Low blood glucose concentration may impede performance of professional athletes undergoing strenuous physical activity. Working farm dogs run 37-62 miles per day at 12.5-18.5 mph; however, studies on farm dogs’ nutrition and daily glucose levels are lacking.

Surveys indicated that farmers euthanize seemingly healthy dogs due to reduced performance. Should reduced performance results from correctable, nutritionally-related glucose disturbances, then farmers would benefit from guidelines for nutritionally-improved performance and unjustified euthanasia would be prevented. We hypothesized that, in comparison to a high carbohydrate diet (HCD), an ultra-low carbohydrate diet (ULCD) would decrease glucose concentrations and hence reduce the activity of working farm dogs.

We randomized 22 New Zealand working dogs on four farms to eat either ULCD or HCD for 1 month followed by continuous measurement of interstitial glucose concentration (FreeStyle Libre) and activity (Hyrex® triaxial accelerometer) over 96 hours. We analyzed the data with a linear mixed model fitting random splines to model activity and glucose level over time for each dog within each diet. We calculated the mean ΔAUC/C6 SEM by subtracting AUCULCD from AUCHCD for both glucose and activity. We then tested if these differences were significant.

We found that the carbohydrate dietary content significantly affected the dogs’ activity (329/26 106 arbitrary units; P=0.002) and glucose (−0.93/26 0.06 mmol/L; P<0.001).

In summary, ULCD-fed dogs were more active despite nutritional carbohydrate restriction possibly due to transition to ketogenic metabolism.

**NM04**

**Food with Novel Fiber Blend Improves Clinical Outcomes and Changes Gastrointestinal Microbiome Metabolism in Dogs**

Jennifer M. MacLeay – Hill’s Pet Nutrition; Dale Fritsch – Hill’s Pet Nutrition; Susan Wernimont – Hill’s Pet Nutrition; Matthew Jackson – Hill’s Pet Nutrition; Chun-yen Cochrane – Hill’s Pet Nutrition; Jennifer MacLeay – Hill’s Pet Nutrition; Kathy Gross – Hill’s Pet Nutrition

Recent studies indicate that disruptions in the gastrointestinal microbiome are linked with gastrointestinal diseases. Here, we evaluated the efficacy of a therapeutic food (TF) with select dietary plant fibers known to contain antioxidant and polyphenol compounds in dogs with chronic enterocolitis.
A prospective clinical study was conducted with 31 adult dogs (21.8 ± 15.3 kg, age: 5.4 ± 3.3 years), recruited from private veterinary practices across the United States, diagnosed with chronic enterocolitis (predominantly large bowel diarrhea) and currently experiencing an episode of diarrhea. Dogs were excluded from this study if they had intestinal parasites, systemic diseases, chronic use of colonic motility drugs, received oral antibiotics within past 4 weeks, or consumed a therapeutic food within past 3 months. Dogs qualifying were switched to a complete and balanced dry therapeutic food (TF) including whole grains and fiber sources (ground pecan shells, cellulose, flaxseed, dried beet pulp, dried citrus pulp, pressed cranberries, dried pumpkin, psyllium seed husks, and ginger root) for 56 days. Physical examinations, clinical evaluations and fecal collections were performed on days 1, 2, 3, 14, 28, and 56. Veterinarians evaluated changes in overall clinical signs, recurrence of clinical signs and stool parameters (consistency, blood, mucus, stool frequency) as compared to baseline at days 1, 2, 3, 28, and 56. Pet owners evaluated stool quality on a daily basis and nausea/vomiting, quality of life, and stooping behaviors (straining, unproductive attempts, defecation accidents) at days 1, 14, 28, and 56. Fecal short chain fatty acids (SCFA) were analyzed using liquid-liquid extraction and gas chromatography with flame ionization detection. Statistical analysis was performed using a mixed-effects model. Untargeted metabolomics analysis was performed by a commercial lab and analyzed using repeated measures ANOVA. Results significant at pDiarrhea improved significantly within the first 24 hours of consuming TF. Veterinarians reported that 68% of dogs had complete resolution of their clinical signs and remaining 32% of dogs were improved after 56 days with no dogs having a recurrence of clinical signs (p < 0.05). Additionally, dogs had significant improvement in stool consistency and reductions of blood and mucus in stool. Pet owners reported a significant decrease in nausea/vomiting, and improvements in stooping behaviors and quality of life after consuming TF for 28 days; these changes were sustained through day 56. TF significantly decreased fecal putrefactive metabolites isobutyric, 2-methylbutyric, and isovaleric acids, and decreased fecal ammonium compared to baseline. In addition, TF increased fecal ribulose/xylulose and arabinose, saccharolytic products derived from fiber, compared to baseline. Furthermore, TF significantly increased fecal antioxidant and anti-inflammatory plant compounds such as limonin, nomilin, diosmetin, tangeretin, sinensetin, eriodictyol, secoisolariciresinol diglucoside, vanillate, hesperidin, neoponcirin, and narirutin, as well as postbiotics produced by microbial metabolism such as secoisolariciresinol, hesperetin, poncirin, naringenin, and 4-hydroxycinnamate as compared to baseline. TF rapidly improved stool quality and resolved clinical signs in dogs with chronic enterocolitis and pet owners reported improvement in stooping behaviors and quality of life. TF increased metabolites associated with saccharolytic fermentation, decreased putrefactive metabolites, and increased antioxidant and anti-inflammatory plant compounds and postbiotics. Fiber sources rich in antioxidant and anti-inflammatory compounds may contribute to long term health and contribute to rapid resolution and decreased recurrence of chronic diarrhea.
Evaluation of Visceral Fat Mass in Dogs by Computed Tomography

Itsuma Nagao – the University of Tokyo; Koichi Ohno – the University of Tokyo; Yuka Goto-Koshino – the University of Tokyo; Nozomu Yokoyama – the University of Tokyo; Taisuke Nakagawa – the University of Tokyo; Reina Fujiwara – the University of Tokyo; Kie Yamamoto – the University of Tokyo; Takuro Nagahara – the University of Tokyo; Yukiko Nakazono – the University of Tokyo; Hajime Tsujimoto – the University of Tokyo

In human medicine, visceral obesity, defined as excessive accumulation of visceral fat, has been reported as a risk factor for diabetic mellitus, cardiovascular disease, dyslipidemia, and hypertension. In humans, computed tomography (CT) has been used as the gold standard to measure visceral fat, and it is reported that visceral fat area (VFA) of umbilical slice significantly correlates with total visceral fat mass (VFM). In veterinary medicine, however, few studies have evaluated visceral fat using CT images. The objective of this study was to evaluate visceral fat in dogs using CT images and to determine the slice which significantly correlates with VFM in order to make visceral fat measurement easier.

Ninety dogs which were referred to the Veterinary Medical Center of the University of Tokyo from May to July 2018 and were conducted whole body CT scans for diagnostic purposes were evaluated. In each CT image slice, fat was identified based on an attenuation range of -135 to -105 Hounsfld units (HU) of fat; fat inside the abdominal wall musculature was identified as visceral fat. We calculated VFM as the product of the VFA and thickness, and examined the correlation between VFM and VFA for each lumbar vertebra (L1 to L7). We also calculated visceral fat percentage (VF%) as the ratio of the product of the VFM and fat density to the body weight, and examined the VF% and body condition score (BCS) correlation; VFA% was then calculated as the VFA to body area ratio, and the correlation between VF% and VFA% was examined. Additionally, we examined VF% and abdominal circumference correlation which was compensated for by the body size index characteristics including the ilium wing distance (IWD), femur length (FL), and vertebral length of L6 (VL) in order to predict VFM by body measurement.

The results showed positive correlations between the VFM and VFA at L1 to L7. Among these lumbar vertebrae VFA of L3 showed the highest correlation with VFM (rs=0.968). There was no significant correlation between VF% and BCS (rs=0.517). VF% showed significant correlation with VFA% at L3 ranging from 0.15% - 6.76% in 90 dogs. The abdominal circumference of L3 which was compensated for by the IWD, FL, and VL did not show significant correlation with VF% (rs of 0.466, 0.556, and 0.373, respectively).

This study revealed that VFA of L3 on CT could be used to evaluate canine visceral fat. Moreover, BCS showed no correlation with VF%, indicating that BCS alone is insufficient to evaluate the visceral obesity. Although abdominal circumference is used to estimate visceral fat without anesthesia in human medicine, compensated abdominal circumference did not significantly correlate with VF%. Considering the results, it is difficult to evaluate visceral fat by only morphometric measurement because there were more individual differences here than in humans. Further study is needed to evaluate visceral fat using non-anesthetic methods such as bioelectrical impedance analysis.

A Novel New S-Adenosylmethionine Salt with Increased Bioavailability

David Griffin – Nutramax Laboratories Veterinary Sciences, Inc; Rebekah Strunk – Nutramax Laboratories Veterinary Sciences, Inc; Carolyn Warner, LVT – Nutramax Laboratories Veterinary Sciences, Inc; Robert Gillette – Nutramax Laboratories Veterinary Sciences, Inc

Salts of S-adenosylmethionine (SAMe) contribute to their inherent stability, but this study is first to show an effect on bioavailability. SAMe is the principal biological methyl donor synthesized in all mammalian cells but most abundantly in the liver. SAMe is a compound used by the body to make glutathione which is an antioxidant that protects cells from toxins. When the liver is compromised, production of SAMe can drop, subsequently lowering liver glutathione concentrations in dogs and cats. The objective of this study was to assess and compare the pharmacokinetic profiles of a 225 mg dose of SAMe tosylate disulfate NMXSS75® (Denamarin®, Nutramax Laboratories Veterinary Sciences) and a 82 mg dose of SAMe phytate NMXSS75ATM (Denamarin Advanced, Nutramax Laboratories Veterinary Sciences). These two different supplements were administered once followed by a 7 day washout in a two period crossover design in a laboratory population of beagle dogs n = 18). Dogs were randomly assigned to 1 of 2 groups in which sequence of treatment administration was randomly assigned. Blood samples were drawn at pre-treatment, 30 and 60 minutes; then at 2, 4, 6, 8 and 24 hours after treatment on days 0 and 7. Plasma samples were shipped to and tested at an outside laboratory for SAMe concentrations. Cmax, Tmax, AUC, and half-life means were calculated and analyzed for each group. At the administered dosages there were no significant differences in bioavailability between the two salts. This study shows that the pharmacokinetics of 82 mg of the SAMe phytate compound was comparable to 225 mg of the SAMe tosylate compound. This new salt in Denamarin AdvancedTM allows for lower levels of a SAMe in a product for ease of administration for liver support.

Cats with a Specific AGXT2 Genotype Differentially Respond To Dietary Intervention for Calcium-Oxalate Stone Risk

Dennis E. Jewell – Hill’s Pet Nutrition; Kiran Panickar – Hill’s Pet Nutrition; Laura Heffin-Morgan – Hill’s Pet Nutrition; Jeffrey Brockman – Hill’s Pet Nutrition; Joan Hall – Oregon State University

This study was completed to isolate and evaluate a genotype specific nutritional intervention for reducing the risk of calcium oxalate stones. Metabolomic analysis of 445 cats (metabolomics measured by Metabolon® with scaled imputed data was used to compare the profiles of specific genotypes. A genome wide association study of these
data revealed an association with a variant of alanine--glyoxylate aminotransferase 2 (AGXT2) and 2-oxo arginine (0.45a, 0.91b, 1.26c for AA, AG and GG, respectively). In further analysis it became apparent that there was a significant difference in circulating betaine concentration between the genotypes (1.36a, 1.24a, 1.11b for AA, AG and GG, respectively). A subsequent study used 23 cats (n= 9, 4, 10 for AA, AG and GG, respectively) to evaluate change in stone risk as influenced by dietary inclusion of betaine (included at 0.5%), and botanicals (green tea, fenugreek and tulsi, included at 0.25, 0.025 and 0.0015%, respectively in a test food) compared with a control food without these additions.

Stone risk analysis was completed on urinary samples for struvite relative super saturation (sRSS) and a calcium oxalate titration test (COT). Urine was analyzed for sRSS using the EQUIL 2 program. In brief, this computer program calculates a urine supersaturation ratio (unitless) with respect to the common kidney stone components. The EQUIL 2 program provides an evaluation of the state of urinary saturation based on pH and total concentrations (M/L) of specific analytes. This study measured sodium, potassium, calcium, magnesium, chloride, ammonium, citrate, phosphate, sulfate, and oxalate concentrations. The method uses thermodynamic stability constants to calculate free ion activities for urinary ions. These free ion activities are then used to calculate the supersaturation ratio of urine compared with what would form crystals in pure water. The urine calcium oxalate titrimetric test (COT) was performed using a method whereby the \([\text{Ca}^{2+}] / (\text{added Ox-2})\) ratio is calculated (per L). The ratio represents the concentration of ionized calcium and the amount of oxalate that is added to initiate crystallization. An increasing index value denotes samples at greater risk of calcium oxalate crystallization, whereas decreasing index values denotes those with lower risk. All cats were placed on a pre-trial food for 28 days and then assigned to either control or test food for 28 days. After 28 days food offerings were switched so that after 56 days every cat had eaten both foods for 28 days. There was no change in sRSS with food. All mean values for sRSS were below 0.75 showing a very low risk for struvite stones. There was a significant genotype by food interaction with the control food COTT values (2.66, 2.75, 2.72 for AA, AG and GG, respectively). After consuming the test food, the COT values were 2.91, 2.39, 2.12 for AA, AG and GG, respectively. There was no influence of food on the COTT values for AA whereas the cats with the GG genotype had reduced values compared with either GG cats after consuming the control food or AA cats after consuming test food. These data show that there is a genotype specific benefit for reducing the risk of calcium oxalate stones after feeding a betaine and botanical dietary enhancement.

**NM09**

**Nutrient-Enriched Water Supplements Nutritionally Support Hydration in the Domestic Cat**

*Emma L. Wils-Plotz – Nestle Purina Pet Care; Brian Zanghi – Nestle Purina Pet Care*

This study evaluated the effects of two similar nutrient-enriched water (NW) supplements that differ only in gum content, on water intake and urine measures of hydration of healthy cats. Domestic shorthair cats (N=36; mean age 5.1yrs±SE 0.36; BCS 5-7 on 9-point scale) were separated into three groups (N=12/group) and offered a liquid supplement dose twice daily in a bowl (third bowl option), with a total daily dose of 36ml/kg BW, for 14 days. The three treatment groups consisted of either tap-water (TW) or NW containing 1.2% whey protein and 1% glycerol with 0.11% gums (NW-A) or 1.1% gums (NW-B). Prior to the 14 day treatment period, 7-d baseline established daily TW and food consumption with ad libitum TW and ProPlan Veterinary Diet UR cat dry food portion-fed twice daily to maintain body weight. During the treatment period, all cats always had ad-libitum access to TW. Blood samples were collected on days -1 and 14 for analysis of serum total protein, creatinine, and osmolality. Urine was collected and pooled over 48-hours using inert litter on days -3 to -1 and 12 to 14. Pooled urine samples were collected and analyzed for total protein, creatinine, osmolality (\(\mu\text{g/mL}\)), and urine specific gravity (USG). During the baseline period, tap-water intake, daily urine volume, USG, and total protein, creatinine, and osmolality from urine and serum were not statistically different (P >0.05) between groups. During the treatment period, both the NW-A and NW-B groups had an increase (P osmo, and USG were all significantly (P <0.0001) decreased in cats consuming either the NW-A and NW-B treatments compared to those consuming TW. This study demonstrated that both of the nutrient-enriched water supplements, regardless of gum content and provided with ad libitum access to TW, can increase total daily liquid intake and significantly improve urine measures of hydration in healthy cats based on greater daily urine output and dilution.

**NM10**

**Pharmacodynamic and Pharmacokinetic Analysis of an Oral Sulforaphane Source in Beagle Dogs**

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Sulforaphane (SFN) is a phytochemical produced by the hydrolysis of its precursor glucoraphanin (GFN) by myrosinase (MYR) enzyme, both of which are found in cruciferous vegetables like broccoli. Sulforaphane has been shown to activate the NRF2 pathway which regulates the expression of detoxification and antioxidant proteins. Currently, SFN is being researched in human clinical trials for its cytoprotective properties in a variety of health indications. More recently, veterinary research on SFN is beginning to uncover health benefits for animals. Pharmacodynamic (PD) and pharmacokinetic (PK) studies of an oral GFN and MYR that supports sulforaphane production (Avmavet™, Nutramax Laboratories Veterinary Sciences) were performed in beagle dogs. In an initial PK/PD study, four dogs were administered a tablet containing 15 mg of GFN and active MYR orally once a day for a three day period. PBMC RNA from blood samples collected at pre-treatment, 8 hours (hr), 24 hr, 48 hr, and 72 hr were processed for NQO1.
HO-1, GCLC, GCLM, and IL-6 gene expression analysis. The PD data revealed that all genes examined exhibited an increase in expression following supplementation at all time points examined which was accompanied by an increase in plasma SFN levels. A subsequent PK evaluation was performed to assess if a lower dose resulted in a similar PK profile. A two group cross-over design of a laboratory population of beagle dogs (n = 6) was performed where each dog received one treatment followed by a 7-day washout. Period 1 (Group 1) started on Day 0 where all dogs received 7 mg of GFN orally with food. Period 2 (Group 2) began on Day 7 when dosage was increased to 15 mg of GFN orally with food. For PK evaluations blood was drawn at pre-treatment, 30 minutes, 1 hr, 2 hr, 4 hr, 8 hr and 24 hr time intervals. The PK data revealed an increase in plasma SFN levels in both groups. There were no significant differences between the 7 mg dose and the 15 mg dose in total SFN plasma maximum serum concentration (Cmax), time of maximum concentration (Tmax), and area under the curve (AUC). Both doses revealed a peak SFN plasma concentration between 1-2 hrs post-administration. These studies demonstrate that supplementation with GFN and MYR results in elevated SFN plasma levels in dogs and induces the expression of cytoprotective enzymes. A lower dose of 7 mg of GFN should provide a similar PD response as a 15 mg dose. These findings support further evaluation of how Avmavet™ can be developed and utilized in the veterinary field.

**NM11**

**Inter- and Intra-Rater Reliability of Computed Tomographic Measurement of Feline Epaxial Muscle Area**

Laura Rayhel – The Ohio State University; Jessica Quimby – The Ohio State University Veterinary Medical Center; Eric Green – The Ohio State University Veterinary Medical Center; Valerie Parker – The Ohio State University Veterinary Medical Center; Sasha Bai – The Ohio State University Biomedical Informatics

Non-invasive objective measures of lean body mass in animals are lacking, and measures are needed both for research and clinical cachexia evaluation. In humans and dogs, lean body mass can be estimated from cross-sectional epaxial muscle area on a CT or MRI image, normalized for body size by dividing over a bony landmark like vertebral body height or femur length. This method has not been evaluated in cats. The purpose of this retrospective study was to evaluate intra- and inter-observer reliability of normalized epaxial muscle cross-sectional area measurement on feline CT images. A secondary aim was to determine the relationship between measured normalized cross-sectional epaxial area and subjective muscle condition score (MCS). Feline abdominal and thoracic CT images obtained at the Ohio State University Veterinary Medical Center from 2005-2017 were retrospectively reviewed. On transverse images, the junction of the 13th thoracic vertebra with the heads of the thirteenth ribs was identified. Right and left epaxial muscle circumference and vertebral body height were measured three times each, and epaxial cross-sectional area was calculated using commercial software (ImageJ). The three measurements from each location were averaged to produce one value for the right and left epaxial cross-sectional area and vertebral body height. Then, the average of the right and left measurements was calculated and divided by the vertebral body height to normalize for patient size, giving the overall epaxial area: vertebral height ratio (OAH) for each cat. Measurements and calculations were performed by a board-certified radiologist (EG), a board-certified internist (JQ), and an internal medicine resident (LR). The same measurements were then repeated approximately one month later by all observers. Medical records were reviewed to obtain patient signalment, weight, body condition score, and muscle condition score, if recorded. OAH intra- and inter-rater reliability was assessed with a concordance correlation coefficient (CCC), and Bland-Altman analysis was performed to assess bias and limits of agreement (LoA) between and within observers at different timepoints. OAH for cats with MCS 0-2 (none – moderate atrophy) vs. 3 (severe atrophy) was compared with a Wilcoxon Rank Sum test. Of 167 feline thoracic and abdominal CT scans, 102 met inclusion criteria (sufficient image quality and positioning, cat > 1 year old, no pathology at the thoracolumbar junction). Intra-rater reliability for OAH was good – excellent (CCC 0.889 to 0.989) and bias was minimal (−0.09 to 0.035) within observers at different timepoints. Limits of agreement were narrow within observers [95% LoA: EG (−1.009, 0.930); JQ (−0.646, 0.466); LR (−0.287, 0.356)]. Inter-rater reliability for OAH was also good – excellent (CCC 0.865 to 0.940), but bias was larger (−0.447 to 0.618) when assessed between observers. Limits of agreement were also wider between observers [95% LoA: EG vs. JQ (−1.160, 0.266); EG vs. LR (−0.509, 0.850); JQ vs. LR (0.195, 1.042)]. Mean OAH was significantly lower in cats with subjectively severe muscle atrophy (2.7072) compared to cats with none – moderate atrophy (4.2831) (p < 0.001).

In conclusion, measurement of feline cross-sectional epaxial muscle area on CT showed good – excellent intra- and inter-rater reliability, and mean measurements were significantly lower in cats with severe muscle wasting as assessed on physical examination. Bias and 95% LoA were both larger between observers than within observers implying that comparative studies should be evaluated by the same individual for consistency. Further studies are needed to prospectively correlate this method of muscle mass measurement with subjective MCS and other objective measures of lean body mass.

**NM12**

**Canine Obesity: A Prevalence Study in the City of Sao Paulo, Brazil**

Marcio Brunetto – University of Sao Paulo; Mariana Porsani – University of Sao Paulo; Fabio Teixeira – University of Sao Paulo; Vinicius Oliveira – university of sao paulo; Yara Arakaki – University of Sao Paulo; Vivian Pedrinelli – University of Sao Paulo; Camila Martins – Ponta Grossa State University; Ricardo Dias – University of Sao Paulo; Marcio Brunetto – University of Sao Paulo

Obesity is the main nutritional disease of dogs and it leads to consequences such as decrease in lifespan. The aim of this study was to determine the prevalence of canine obesity in the city of Sao Paulo, Brazil, and to observe factors associated with obesity. Households and areas were selected at random, and number of households was determined according census of dogs and households in Sao Paulo and prevalence of obesity in other countries. A total of 256 households were visited and body condition score (BCS) (scale from 1 to 9) was obtained from 286 dogs. Of these, 25.9% were considered in overweight (BCS
6 and 7) and 14.6% were considered obese (BCS 8 and 9). Factors associated with BCS were gender (p = 0.003), neutering (p < 0.001), frequency of visits to a veterinary clinic (p = 0.026), feeding frequency (p = 0.038), snacks intake (p = 0.011), presence of elderly people in the household (p = 0.006) and owner who ate snacks more than three times per week (p = 0.005). Among obese dogs there was a prevalence of females (73.7%), animals that go to a veterinary clinic (97.4%), animals that eat more than twice a day (52.6%), animals that receive snacks (84.2%), animals that live with elderly people (63.2%) and animals that live with people who eat snacks (73.6%). Prevalence of canine overweight and obesity observed in this study was similar to that observed in other cities (30 to 60%), and factors found to influence BCS were reproductive status, feeding practices and owner habits.

NM13

Frequency and Factors Associated with Canine Overweight and Obesity in a Hospital in Lavras, Brazil
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Obesity is characterized by organic accumulation of body fat that leads to damage, with serious consequences on dogs’ health and quality of life. Currently, obesity is considered the most common nutritional disease among companion animals. An epidemiological survey of canine obesity was carried out in a Veterinary Hospital in Lavras (Minas Gerais, Brazil). From September through December 2013, 117 dogs from reference veterinary hospitals were evaluated, and obesity was diagnosed by body condition score (scale 1–9). Obesity frequency was analyzed regarding breed, age, gender, neutering, feeding frequency, food type, feeding time, reproduction status, living environment, number of pets per household, activity status, exercise duration and exercise type. In this hospital’s population, 35.9% of the assessed dogs were overweight and 18.8% were obese. Canine obesity was correlated (p ≤0.05) with food type (p = 0.05), neutering (p = 0.05), no control of quantity of food (p = 0.006) and feeding behavior (more food motivation) (p = 0.01). In conclusion, with the data obtained regarding characterization of dogs and their owners, it was possible to observe that the frequency and correlated factors of canine overweight and obesity in a veterinary hospital of the countryside city of Lavras, Brazil, were similar to results found in recent studies performed in large urban centers.

NM14

Effect of 2 Urinary Diets on Hematuria in Shelter Cats with Suspected Interstitial Cystitis
Michael R. Lappin – Colorado State University; Natalia Gil – Colorado State University; Lauren Krause – Colorado State University; Deb Greco – Nestle-Purina

There are multiple different veterinary prescription diets that are purposed to aid in the management of struvite and calcium oxalate cystitis and to dissolve struvite cystoliths. Whether positive effects are induced by these diets in cats with suspected feline interstitial cystitis (FIC) that is possibly associated with stress is unknown. The purpose of this pilot study was to determine if there were differences in clinical outcomes in cats with suspected FIC that were fed one of two different veterinary prescription diets.

In this IACUC approved study, cats relinquished to animal shelters in North Central Colorado that were noted to have hematuria and clinical signs of lower urinary tract disease were transferred to the Veterinary Teaching Hospital. All cats had 2 view abdominal radiographs made, were assessed by abdominal ultrasound, and had urine collected by ultrasound guided cystocentesis for urinalysis and aerobic bacterial culture and sensitivity. Cats with hematuria but no other abnormalities (classified as FIC) and cats with radiodense cystoliths were gang housed in 2 different housing chambers and were randomized to be fed one of two veterinary prescription diets (Purina St/Ox or Hill’s c/d Multicare). Cats in the FIC group were housed overnight individually in cages on Days 2, 6, 9, 13, 16, and 20 with Purina Tidy Cat® Litter System without absorbent pads to collect free catch urine for repeat urinalyses. Cats with radiodense calculi were radiographed weekly, with stone removal and analysis planned if the cystoliths did not dissolve after 28 days of dietary management.

At the time of abstract submission, 21 cats had been evaluated. A total of 4 cats either did not meet the entry criteria and were returned to the shelters (2 cats) or had concurrent underlying diseases (IRIS Stage II CKD or development of clinical FIP while on study) and so were excluded. All 4 cats with radiodense stones were fed St/Ox. The cystoliths dissolved in 2 cats and the 2 cats that required surgery had calcium oxalate cystoliths. Of the 13 cats with suspected FIC that completed the dietary trial, 6 were initially fed St/Ox and 7 were fed c/d. Five of 6 cats with suspected FIC fed St/Ox had hematuria resolve in the 28 day observation period. In contrast, 6 of 7 cats with suspected FIC fed c/d had persistent hematuria and were switched to St/Ox. This difference in response to the first diet was statistically significant (p = 0.03). Of the 6 cats with persistent hematuria on c/d, 3 resolved while fed St/Ox and 3 had persistent hematuria. Significantly more cats with suspected FIC had apparent first time responses to St/Ox than to c/d in this mild stress model. The cystoliths that dissolved on St/Ox were presumed struvite and the calcium oxalate stones are not expected to dissolve with dietary management. Continued data should be collected from additional cats to verify the results of this study.

NM15

Beta-Glucan Immune Support Supplement (Imuquint™) Effects on Immune Biomarkers in Beagle Dogs Following Oral Administration
Carolyn Warner – Nutramax Laboratories Veterinary Sciences, Inc; Rebekah Strunk – Nutramax Laboratories Veterinary Sciences, Inc; Robert GilletteEMR – Nutramax Laboratories Veterinary Sciences, Inc; David Griffin – Nutramax Laboratories Veterinary Sciences, Inc

Beta-1,3/1,6-glucans are naturally occurring polysaccharides that can be found in the cell walls of fungi, yeasts, bacteria, and cereals. Beta-glucans have been utilized in humans and animals to modulate immune function and response. There have been multiple studies in dogs that show their beneficial effect on immunomodulation, inflammatory markers, and response to vaccines. The purpose of this study...
was to evaluate the effects of a yeast derived beta-glucan immune health supplement (Imuquin™, Nutramax Laboratories Veterinary Sciences, Inc.) on immune biomarkers in beagle dogs. The 28 day study comprised of a laboratory population of beagle dogs (n = 12) that were randomly distributed into control and supplemented groups. The beta-glucan supplement was delivered orally each morning with food. Blood was drawn on days 0, 7, 14, 21, and 28. Plasma analysis for biomarkers and fecal analysis for metabolomics were completed at outside labs and data was statistically analyzed using repeated measures ANOVA in SigmaStat statistical software (Systat Software, Inc., San Jose, California, USA). Significant differences between control and supplemented groups of dogs were seen in IL-2 (p = 0.013; Figure 1), IL-6 (p = 0.011; Figure 2), phagocytosis (p = 0.013), and Con-A proliferation (p = 0.018). The data demonstrated an elevation of immune biomarker levels over the course of the study in the control group. The supplemented group showed a lesser increase than that of the control group. The findings of this study provide support that beta-glucan supplementation can elicit an immunomodulating effect.

NU01

Urine concentrations of neutrophil gelatinase-associated lipocalin (NGAL), heparin binding protein (HBP), and interleukin 6 (IL-6) can be used in humans to differentiate asymptomatic bacteriuria from urinary tract infection (UTI). Limited NGAL data in veterinary medicine show elevation of urine NGAL in dogs with UTI and/or lower urinary tract signs (LUTS). In this prospective cross-sectional study, samples submitted for urinalysis and aerobic culture were included. Samples were grouped based on clinical signs and culture results: 23 dogs without LUTS and negative culture (control), 23 dogs without LUTS and positive culture (ABU), 15 dogs with LUTS and positive culture (UTI), 16 dogs with LUTS and negative culture (non-UTI) (n = 77 samples total). Urine concentrations of NGAL, IL-6, and HBP were measured using ELISA kits according to manufacturer’s instructions. NGAL and HBP concentrations were not significantly different between groups (Kruskal-Wallis; p = 0.16 and p = 0.84, respectively). IL-6 was significantly higher in samples with positive cultures (Mann-Whitney; p = 0.038). Moreover, dogs with UTI had significantly higher IL-6 concentrations compared to all other groups including ABU (Kruskal-Wallis; p = 0.002).

In conclusion, while a relationship between NGAL or HBP and UTI was not found in this study, urine IL-6 shows promise as a biomarker that is elevated in samples with positive culture as well as clinical signs of UTI, making it useful in differentiating between asymptomatic bacteriuria and UTI.

NU02

Retrospective Evaluation of Characteristics Associated with Urinary Bacterial Growth in Proteinuric Dogs

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Urine cultures are frequently recommended in the evaluation of proteinuric patients to rule out infection as a post-renal cause of proteinuria. This study set out to identify characteristics associated with urinary bacterial growth in proteinuric dogs. Records of dogs presented to the Texas A&M Veterinary Medical Teaching Hospital between January 2008 and January 2018 were retrospectively reviewed. Patients were included if a urine protein-to-creatinine ratio, urinalysis, and quantified urine culture (QUC) were performed within a 2-week period. Cases with a history of recent antimicrobial therapy or with urine collected by any method other than cystocentesis were excluded. Information regarding patient signalment, comorbidities, serum BUN and creatinine, urinalysis findings, and results of QUC were recorded. The association between patient characteristics and presence of urinary bacterial growth was assessed by univariable analysis. A final multivariable model was then constructed using backwards stepwise elimination.

Seven hundred (700) cases met the inclusion criteria, of which, 499 were proteinuric (urine protein-to-creatinine ratio > 0.5). Of these, 32/499 (6.4%) had urinary bacterial growth. In the final
multivariable model, pyuria (odds ratio: 29.8; \( P < 0.0001 \)), bacteriuria (odds ratio: 14.5; \( P = 0.0001 \)), and lower urinary tract disease, including a past history of urinary tract infection or current lower urinary tract signs (odds ratio: 13.7; \( P = 0.0002 \)) were associated with urinary bacterial growth. Of the 32 dogs with urinary bacterial growth, 20 (62.5%) had an active urine sediment.

The proportion of proteinuric dogs with both an inactive urine sediment and urine bacterial growth was low (12/499 dogs: 2.4%), raising questions regarding the necessity of performing QUC in this group of patients. However, our results suggest that an active urine sediment or a history of urinary tract infection or current lower urinary tract signs should prompt QUC for canine patients with proteinuria.

**NU03**

Outcomes, Clinicopathologic and Histopathologic Characteristics of Feline Proteinuric Kidney Disease: 61 Cases

Laura Rayhel – The Ohio State University; Jessica Quimby – The Ohio State University Veterinary Medical Center; Rachel Cianciolo – The Ohio State University Veterinary Medical Center; Andreaonne Cleroux – Ryan Veterinary Hospital - University of Pennsylvania; Toni Franken – The Ohio State University Veterinary Medical Center

In dogs, 48.1% of glomerulonephritides is immune-mediated (ICGN), and therefore may respond to immunosuppressive therapies. Similar data for feline glomerulonephritides are needed to guide treatment decisions and aid in predicting prognosis. The purpose of this study was to describe the types, clinicopathologic features, and prognosis for feline proteinuric renal disease.

Feline biopsy/necropsy samples from proteinuric cats submitted to the International Veterinary Renal Pathology Service (IVRPS) from 2006-2017 were retrospectively reviewed. Referring veterinarians were contacted for follow-up information. Information on signalment and clinicopathologic parameters at time of biopsy and available information on survival time and disease progression were obtained from the medical record. Hypoalbuminemia and hypercholesterolemia were defined according to submitting site reference ranges. Hypertension was defined as systolic blood pressure > 160 mmHg, and anemia as PCV/HCT < 30%. Azotemia was defined as creatinine > 1.6 mg/dl. Descriptive statistics were performed using commercial software (Microsoft Excel, IBM SPSS).

61 feline renal biopsies met inclusion criteria. 6/61 (9.8%) had borderline proteinuria (UPC 0.2 – 0.4), and 55 (90.2%) were proteinuric (UPC > 0.4). 43/61 (70.5%) had primary glomerular lesions, 14 (23.0%) had primary tubular lesions, and 4 (6.5%) had both or had undeterminable primary lesions. 31/61 (50.8%) had ICGN (30) or immune complex arteritis (1); 30/61 (49.2%) had non-immune complex mediated disease. Considering only cats with primary glomerular disease, 31/43 (72.1%) were diagnosed with immune-complex glomerulonephritis (ICGN) or arteritis of some type. The most common primary glomerular histopathologic diagnoses were: membranous glomerulonephritis (GN) (12), membranoproliferative GN (9), podocytopathy (5), and mesangio proliferative GN (4). At time of biopsy or necropsy, median age of cats with glomerular disease was 3.3 years (n = 40, 0.18 – 9.9 years) weight was 3.74 kg (n = 18, 0.81 – 7.02 kg). Median urine protein to creatinine ratio (UPC) was 7.9 (n = 43, 0.45 – 30). Median creatinine was 2.6 mg/dl (n = 42, 0.7 – 16.3 mg/dl). Median BUN was 68.5 (n = 30, 19.6 – 358 mg/dl). Median cholesterol was 249.5 mg/dl (n = 24, 120 – 423 mg/dl). Median albumin was 2.1 g/dl (n = 41, 1.1 – 3.6 g/dl). Median PCV/HCT was 23.7% (n = 31, 12 – 40%). Median systolic blood pressure was 150 mmHg (n = 37, 74 – 240 mmHg). From time of diagnosis to time of death, 22/37 cats (59.5%) became hypertensive, 34/42 (81.0%) became hypoalbuminemic, 30/34 (88.2%) became anemic, 14/25 (56.0%) became hypercholesterolemic, 22/37 (59.5%) developed peripheral edema or cavitary effusion, and 35/43 (81.4%) became azotemic (denominator denotes number of cats with follow up information available for the specific parameter). Mortality date was known for 22/43 cats (51.2%) with glomerular disease; median age at death was 3.64 years (0.41 – 9.62 years). Median time from diagnosis to death was 68.5 days (3 days – 5.06 years).

In conclusion, 70.5% of proteinuric cats with renal biopsies submitted had primary glomerular disease, and 50.8% of those had immune-mediated disease. The majority of cats with glomerular lesions developed hypertension, hypoalbuminemia, anemia, hypercholesterolemia, peripheral edema/cavitary effusion, and azotemia. Given that median survival time following diagnosis of proteinuria for cats with primary glomerular disease was approximately 2 months, and considering the large proportion of cats with immune-mediated disease, further studies are needed to determine the effect of immunosuppression on morbidity and mortality in cats with primary glomerular disease.

**NU04**

An Algorithm Based On Clinical Data Predicts Feline Chronic Kidney Disease Two Years Before Diagnosis

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Advanced machine learning methods, combined with large sets of health screening data, provide opportunities for diagnostic value in human and veterinary medicine. The aim of this study was to derive an algorithm that would predict the risk of cats developing azotemic chronic kidney disease (CKD) using data from electronic health records (EHR) collected during routine veterinary practice.

Data were extracted from EHRs of cats visiting Banfield Pet Hospitals over a period of more than 20 years. This resulted in a data set with 106,251 individual cat EHRs that was split into two parts; 67% of the data was used to build a prediction model, with the remainder used to validate model performance. Feature selection was conducted using cross-validation on a recurrent neural network (RNN) architecture and model performance was evaluated based on Receiver Operator Characteristic (ROC)/Precision-Recall (PR) curves and the F1 score.

The final model was a RNN with 4 features (plasma creatinine, urea nitrogen, urine specific gravity and age). CKD prevalence in the data set was 17% (18,408 cats) and these cats were generally older, had higher creatinine levels and lower USG, compared to cats with “no CKD” status. Model performance is presented in Table 1. When
Table 1: Model performance.

| Time before diagnosis (years) | Accuracy (%) | Sensitivity (%) | Specificity (%) | Positive Predictive Value (%) | Negative Predictive Value (%) |
|------------------------------|--------------|----------------|-----------------|-------------------------------|-------------------------------|
| 0                            | 96.9         | 90.7           | 98.9            | 96.5                          | 97.0                          |
| 0.5                          | 93.4         | 76.7           | 99.0            | 96.2                          | 92.7                          |
| 1.0                          | 90.1         | 63.0           | 99.1            | 96.0                          | 88.9                          |
| 1.5                          | 88.0         | 53.3           | 99.6            | 97.9                          | 86.5                          |
| 2.0                          | 86.0         | 44.2           | 99.6            | 97.4                          | 84.3                          |

predicting CKD near the point of diagnosis, the model displayed a sensitivity of 90.7% and a specificity of 98.9%. In predicting future risk of CKD, the model sensitivity decreased when increasing the time horizon for prediction, with 63.0% 1 year before diagnosis and 44.2% 2 years before diagnosis, but with specificity remaining around 99%. As an example, the specificity of the algorithm, coupled with a sensitivity of 63.0%, means that out of 100 cats with a prevalence of 15%, 93 cases will be correctly predicted as either not developing azotemia or developing azotemia in the next 12 months. Here we present evidence for the use of machine learning to build an algorithm that predicts cats at risk of developing CKD up to 2 years prior to diagnosis with high specificity. The application of this approach can directly support veterinarians in making clinical decisions.

NU05

Open-Label Pilot Efficacy Study of Recombinant Feline Erythropoietin in Client-Owned Cats with Non-Regenerative Anemia

Beasley L. Mason – KindredBio; Stephanie Pierce (Small Animal Internal Medicine) – Kindred Biosciences, Inc.; Tianhua Hu – Kindred Biosciences, Inc.; Melinda Poole – Kindred Biosciences, Inc.

The primary objective of this study was to evaluate the effectiveness of recombinant feline erythropoietin (rFEO) on hematocrit (HCT) and Quality of Life (QoL) in client-owned cats with non-regenerative anemia secondary to International Renal Interest Society (IRIS) Stage 3 Chronic Kidney Disease (CKD).

Twenty-three client-owned cats at least 1 year of age with IRIS Stage 3 CKD and non-regenerative anemia with a 15-30% hematocrit were enrolled. Cats were excluded if they had rapidly progressing CKD defined as a 25% increase in fasting serum creatinine between two consecutive evaluations, had been administered whole or packed red blood cells within 6 weeks of baseline. Cats with any of the following diseases or conditions were excluded: neoplasia, liver disease, feline leukemia virus, feline immunodeficiency virus, diabetes mellitus, uncontrolled hyperthyroidism, HCT < 15%, or systemic blood pressure > 160 mmHg. Owners provided consent prior to any cat being subjected to study-related procedures. On Day 0, rFEO was initiated at 3 μg/kg subcutaneously. Subsequent doses at Weeks 1-5 were tailored to each cat. HCT assessments were performed on-site at all scheduled and unscheduled study visits. Doses were administered no more frequently than weekly. A cat was considered a treatment success if the HCT returned to the laboratory normal reference range by Week 6 or the HCT had improved by 30% compared to baseline. Owners completed a validated, generic, feline Health Related Quality of Life (HRQL) assessment online on Day 0, Weeks 1, 2, 3, 4, 5, and 6.

In this study, rFEO rapidly increased mean HCT, with statistically significant improvement seen as early as Week 1 (P < 0.0001). The effect was sustained at Weeks 2, 3, 4, 5, and 6 (P < 0.0001 at each visit) (Figure 1). Compared to baseline, the mean peak relative improvement in HCT was 55.4%. In addition, 95.5% of the 22 evaluable patients achieved treatment success over the 6-week treatment period. Furthermore, cats treated with rFEO demonstrated statistically significant improvements over baseline (P).

The administration of rFEO was well tolerated. Based on preliminary review, adverse events were mild, and most were consistent with underlying CKD. No cat required early discontinuation of rFEO due to an adverse event associated with treatment. One cat was humanely euthanized due to rapidly progressive CKD. In this study, treatment success was achieved in 95.5% of the 22 evaluable client-owned cats with non-regenerative anemia secondary to IRIS Stage 3 CKD administered rFEO. Based on preliminary review of the safety data, rFEO appears to be well tolerated.

NU06

Double-Blind, Placebo-Controlled-Study To Evaluate Mirtazapine-Transdermal-Ointment in Cats Experiencing Unintended-Weight-Loss: Analysis of Cats with Suspected Renal-Disease

Beasley L. Mason – KindredBio; Valentine Williams – Kindred Biosciences, Inc.; Tianhua Hua – Kindred Biosciences, Inc.; Jessica Lee – Kindred Biosciences, Inc.; Melinda Poole – Kindred Biosciences, Inc.

To evaluate the safety and effectiveness of mirtazapine transdermal ointment in cats with unintended weight loss. This post-hoc analysis
was conducted in cats with suspected renal disease, as there is a potential delayed clearance of mirtazapine in these cats. Client-owned cats > 1 year of age, weighing ≥ 2 kg, with a documented loss (≥ 5%) in body weight (BW) were included. Cats were treated once daily with either 2 mg/cat mirtazapine transdermal ointment (Kindred Biosciences, Inc.) or placebo ointment applied to the inner surface of the pinna for 14 ± 3 days. Mean percent change in BW between the mirtazapine and placebo group was evaluated from Day 1-to-14. A post-hoc analysis was conducted to evaluate the subset of enrolled cats with suspected renal disease (defined as having urine specific gravity < 1.035 and serum creatinine > 122 μmol/L [1.6 mg/dL] at baseline).

A total of 230 cats were enrolled (n = 115 in both groups). Of the intent-to-treat population, suspected renal disease was identified in 49 mirtazapine and 44 placebo cats. The mean percent change in BW was + 3.9% (SE ± 0.8%) in the mirtazapine and + 0.9% (± 0.5%) in the placebo group (P = 0.0022). There was no significant difference between groups in incidence of overall adverse events (AEs) (P = 0.774) or behavioral AEs of vocalization (P = 0.1183) and hyperactivity (P = 0.3637).

Daily topical application of mirtazapine transdermal ointment to the inner pinna of the ear effectively increased body weight within 14 days in client-owned cats with suspected renal disease experiencing unintended weight loss.

NU07

Serum Symmetric Dimethylarginine Concentrations in Greyhound Puppies – Evidence for Breed Specific Physiologic Differences

C. Guillermo Couto – Couto Veterinary Consultants; Rachel Murphy – IDEXX Laboratories Inc.; Michael Coyne – IDEXX Laboratories, Inc.; Sean Hardy – IDEXX Laboratories, Inc.; M. Alexis Seguin – IDEXX Laboratories, Inc.

Serum concentration of symmetric dimethylarginine (SDMA) is a reliable renal biomarker in dogs, and unlike creatinine, is independent of muscle mass. Greyhounds have higher serum creatinine concentrations, often attributed to greater muscle mass and potentially also subclinical renal disease secondary to drugs administered during the dogs’ racing careers. The SDMA reference interval for adult Greyhounds has been reported to be higher than the adult canine reference interval (RI) of 0 – 14 μg/dL. The purpose of this study was to evaluate SDMA concentrations in samples obtained from pre-training Greyhound puppies as measured by Liquid Chromatography Mass-Spectrometry (LC MS) to determine if the higher concentration is a breed attribute, reflects underlying renal damage in adult dogs or from methodology-specific interferences.

Serum creatinine and SDMA concentrations in healthy 48 pre-training Greyhound puppies 3 – 8 months-of-age were measured. The median creatinine concentration in puppies was 0.8 mg/dL, lower than that reported in adult Greyhounds, and similar to adult dogs of other breeds. The creatinine RI for pre-training puppies was 0.5 – 1.2 mg/dL. In contrast, the median SDMA concentration for puppies (14 μg/dL), and the SDMA RI for puppies (11 – 19 μg/dL) were similar to those previously reported for adult Greyhounds (14 μg/dL and 9 – 20 μg/dL, respectively), suggesting that a single reference interval (≤ 20 μg/dL) may be appropriate for the entire Greyhound population. The Greyhound puppy SDMA RI was higher than that for puppies of other breeds (0 - 16 μg/dL). The data suggest a breed-specific physiologic difference is responsible for the higher SDMA concentrations in Greyhounds.

NU08

Analysis of Survival Among Different Categories of Chronic Kidney Disease in Dogs

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In dogs, chronic kidney disease (CKD) results from a variety of different underlying disease processes. Previous studies investigating the prognosis of dogs with CKD have not accounted for these differences. The objective of this study was to determine if disease category confirmed by renal biopsy evaluation was associated with the survival of dogs with CKD.

The International Veterinary Renal Pathology Service (IVRPS) houses a database of renal biopsy reports. The service provides a robust evaluation of renal samples that encompasses light microscopy, electron microscopy, and immunofluorescence. Dogs evaluated by the IVRPS between 2008-2018 were included in the study. Survival data was retrospectively obtained for 23% of cases (275/1191). From this cohort, 19 different disease categories were identified. The 5 most represented categories were: focal segmental glomerulosclerosis (FSGS), renal amyloidosis, membranoproliferative glomerulonephritis, membranous glomerulonephropathy, and juvenile nephropathies. Of note, the majority of dogs evaluated by the IVRPS were biopsied due to significant proteinuria. This likely explains the bias towards dogs with glomerular disease.

A Cox regression analysis was used to analyze the survival of dogs in each disease category. FSGS was chosen as a baseline for comparison since it had the highest number of cases with follow-up (n = 93) and an apparent moderate disease progression (i.e., not particularly slow or rapid progression based on a Kaplan-Meier survival curve). The final adjusted model also accounted for serum creatinine concentration and age at the time of diagnosis. A 1-unit increase in creatinine concentration was associated with an approximately 1.3 times increase in hazard ratio (of death, P < 0.01) while a 1-year increase in age increased hazard ratio by approximately 1.1 times (P = 0.02). Dogs with renal amyloidosis had a significantly increased hazard ratio of 1.9 (P = 0.02). No other disease category had a significantly increased or decreased hazard ratio. The median survival time following biopsy for dogs with FSGS was 238.5 days (range of 1-1460 days). The median survival time for dogs with renal amyloidosis was 30 days (range 1-299 days).

Dogs diagnosed with renal amyloidosis had nearly twice the hazard of death when compared to dogs diagnosed with FSGS. Prospective studies accounting for differences in treatment protocols, end points, and additional confounding variables are needed to further assess the prognostic value of a renal biopsy in dogs with CKD.
NU09

Efficacy of Hemoperfusion and Hemodialysis for Treatment of Carprofen and Ibuprofen Toxicosis in Dogs

Carrie A. Palm – UC Davis School of Veterinary Medicine; Maureen Griffin – UC Davis; Birgit Puschner – Michigan State Veterinary School; Steven Epstein – UC Davis; Larry Cowgill – UC Davis

Charcoal hemoperfusion (HP) and hemodialysis (HD) are used to treat NSAID toxicosis in dogs, but associated studies are lacking. This study aimed to describe outcomes and complications of combined HP/HD (HP/HD) in dogs with NSAID toxicosis. Medical records of dogs with suspected NSAID toxicosis that underwent HP/HD that also had plasma NSAID concentrations measured before, during and after treatment were reviewed. Extracorporeal device NSAID extraction ratios (ER) and NSAID reduction ratios over the course of the treatments were calculated.

Two dogs with ibuprofen and 4 dogs with carprofen toxicosis were included. Pre-treatment plasma concentrations were 179 and 637 μg/ml for ibuprofen and median 65 μg/ml (range: 5.5-160) for carprofen. Dogs were treated with HP/HD for a median of 305 minutes (range: 242-368). Total plasma NSAID concentration decreased 82.7% and 92.1% for ibuprofen and median 55.1% (range: 30.8-81.8%) for carprofen. For ibuprofen, most NSAID removal occurred during the first 60 and 90 minutes of treatment (75% and 68% reduction respectively). Median ER across the HP device was 2% (range: 0-13) for ibuprofen and 7% (range: 0-10) for carprofen. Median dialyzer ER was 12% (range: 2-16) for ibuprofen and 5% (range: 0-19) for carprofen. In one dog, plasma carprofen concentration measured 24-hours after HP/HD discontinuation increased by 2 μg/mL from the post-dialysis value. No serious complications occurred. Combined HP/HD was safe. Extraction ratios across the extracorporeal devices were variable and might relate to HP cartridge saturation. Total NSAID elimination may be due to enterohepatic drug recirculation and endogenous and extracorporeal device removal.

NU11

Dissolution of Feline Struvite Uroliths with a Urinary Calming Diet

Matthew Kornya – The Cat Clinic

Struvite urolithiasis is a common cause of lower urinary disease in domestic cats. In many cats, stress or anxiety related behaviors may occur concurrently with urolithiasis. Urinary + Calm is a commercial diet formulated to dissolve struvite stones while preventing calcium oxalate stones. It is also formulated with hydrolyzed milk protein and L-tryptophan to reduce anxiety. The purpose of this study was to determine the efficacy in a clinic setting of the canned and dry formulations in dissolving struvite stones, and to determine the time to dissolution.

Cats were recruited from primary veterinary clinics presenting for lower urinary signs with radiographically identifiable bladder stones. Based on urinalysis results and radiographic appearance of stones, cats were enrolled if the screening veterinarian had a strong clinical suspicion of struvite urolithiasis. Cats were excluded if they were already being fed a urinary diet or if they had concurrent medical issues that precluded a diet change. Cats were exclusively fed the study diet for the duration of the trial. Owners were allowed to choose to feed either canned, dry, or a combination of the two but could not change partway through. Cats with UTIs were treated with antibiotics as per sensitivity results. Non-steroidal anti-inflammatory drugs (NSAIDs) or opioids were used for short term pain control. Radiographs were re-evaluated at ~7 day intervals. Time to dissolution was recorded as the time from when the stone was diagnosed and the diet started to the first radiographic date at which no evidence of a stone was seen.

Table 1

| Patient | 1 | 2 | 3 | 4 |
|---------|---|---|---|---|
| Bladder: |   |   |   |   |
| Urine culture | - | + | + | + |
| Wall culture | + | + | + | + |
| uclust | - | - | - | - |
| ucyst-B | + | + | + | + |
| Left Ureter: |   |   |   |   |
| Urine culture | + | + | + | + |
| uclust | + | - | - | - |
| ucyst-B | + | + | - | - |
| Right Ureter: |   |   |   |   |
| Urine culture | - | + | - | + |
| uclust | - | - | - | - |
| ucyst-B | + | + | - | + |
Ten cats were recruited in the dry diet group. The median age was 6 years; all cats were female. Mean USG at diagnosis was 1.044 and mean urinary pH was 6.65, and 6 cats had struvite crystals. 4 cats had a concurrent UTI confirmed with a positive culture (1 S. felis, 1 Proteus, 2 E. coli). 3 cats had cocci seen on sediment but no culture performed. 3 cats had a negative urine culture and no bacteria seen on sediment.

Five cats were recruited in the canned diet group. The median age was 5.5 years; 2 cats were male and 3 cats were female. 1 cat had an E. coli UTI. Mean USG at the time of diagnosis was 1.041 and mean urinary pH was 8.0. 3 cats had struvite crystals at the time of diagnosis.

Two cats were fed a mixture of canned and dry. The median age was 5 years; 1 cat was male and one female. Both cats had a negative culture, though one of the two cats had cocci seen on the sediment. At the time of diagnosis, m

All cats who returned for follow-up experienced full dissolution of stones and complete resolution of clinical signs (ie no further peruria, pollakiuria, or stranguria). 3 cats were lost to follow-up, two of which stopped returning for check-ins, the third experienced a lower urinary obstruction a few days after recruitment and the stone was removed by cystotomy.

The median times to dissolution are listed in the included table.

Dissolution times were comparable or less than published dissolution times for other dissolution diets. A larger sample size would be needed to determine the statistical significance of this. In this population of 16 cats, the Urinary + Calm diet was successful in dissolving bladder stones in canned, dry, and combination formulations. Dissolution times were comparable or less than published dissolution times for other dissolution diets. Data collection is currently ongoing and is focused on recruiting additional cats to eat canned or combination diets.

**NU12**

**Comparison of IDEXX SediVue Dx® with Manual Microscopy for Detection of casts in Canine Urine**

Demetria M. Vasilatis – UC Davis College of Veterinary Medicine; Larry Cowgill – UC Davis School of Veterinary Medicine; Murthy Yerramilli – IDEXX Laboratories, Inc.; Giosi Farace – IDEXX Laboratories, Inc.; Sean Owens (Clinical) – UC Davis School of Veterinary Medicine

Urinalysis is one of the most commonly performed tests in clinical reference laboratories and veterinary practices. However, microscopic evaluation of urine sediments is inconsistently performed in veterinary practices as manual microscopic examination is labor-intensive, time-consuming and requires specialized training. The identification of casts in the urine sediment examination is of interest as they may be associated with various disease conditions and have historically been considered fragile, making immediate analysis of urine specimens essential for their discovery. The IDEXX SediVue Dx® Urine Sediment Analyzer may aid in the detection of casts in dogs and cats.

The objective of this study was to compare the performance of the SediVue Dx® with manual microscopy for the detection of urinary casts. Canine urine samples (n=455) from 455 patients submitted to the Clinical Laboratory Service at the UC Davis William R. Pritchard Veterinary Medical Teaching Hospital were used for this prospective study.

For manual microscopy, urine was centrifuged to obtain sediment (average volume of 4.6 ml) and reviewed routinely by a licensed medical technologist. For SediVue Dx® analysis (software version: Neural Network 3.0), 165 μl of mixed, uncentrifuged urine was pipetted into a disposable cartridge and 70 images were captured and evaluated using a proprietary algorithm. Each image was subsequently reviewed independently by a boarded clinical pathologist and resident trainee. To determine sensitivity and specificity of the SediVue Dx® compared with manual microscopy, any sample with any identified cast was considered a positive result.

The number of samples in which casts were identified by the manual method was 112/455 (prevalence = 24.6%). Initially, the number of false positives by the SediVue Dx® method was 46 and the number of false negatives by SediVue Dx® method was 53 as compared to the manual method, respectively. On further review of the SediVue Dx® images, 15/53 false negatives were determined to have true casts missed, and 38/53 did not have any casts present. Furthermore, 25/46 false positives were determined to be true positives establishing an overall prevalence of 137/455 (30.1%) and a corrected number of false positives of 21 compared to the manual method. Overall, the sensitivity of the SediVue Dx® was 72% and the specificity was 94% for the detection of all casts.

These findings suggest the SediVue Dx® analyzer may provide better detection of casts in canine urine when used as an in-clinic instrument. Further enhancement of the SediVue Dx® neural network algorithm for cast detection and classification will be beneficial in decreasing false negatives and identifying dogs with medical conditions.

**NU13**

**The Effect of Dietary Sodium on Urinary Calcium and Calcium Oxalate Relative Supersaturation in Cats**

Yann Quéau – Royal Canin; Esther Bijsmans – Royal Canin; Jeremy Laxalde – Royal Canin; Laurence le Verger – Royal Canin; Vincent Bourge – Royal Canin

Calcium urolithiasis is highly prevalent in cats. Urinary dilution is the primary strategy for prevention, and can be achieved by increased sodium content of pet food to drive water intake. However, humans with CaOx renoliths are advised to decrease sodium intake because of the potential increase in renal calcium excretion, which could increase the risk of the disease. Relative supersaturation (RSS) is a measure of crystallization risk, and lower urinary concentration of CaOx precursors can decrease CaOx RSS. Studies prospectively investigating the effect of dietary sodium on CaOx RSS have been performed with inconsistent results, possibly due to the small group sizes and RSS methodology variation.

| Diet | Number of cats (n) | Med time (d) | Min time (d) | Max time (d) |
|------|--------------------|--------------|--------------|--------------|
| Kibble | 10 | 14 | 7 | 28 |
| Canned | 5 | 10.5 | 7 | 14 |
| Both | 2 | 21 | 7 | 35 |
The aim of this study was to retrospectively investigate the influence of dietary sodium content in a wide variety of dry pet foods (moisture < 10%) on urinary volume, urinary calcium excretion and concentration, as well as CaOx RSS in healthy colony cats. Sodium content in pet food was defined as low (LNa) when < 0.6% and high (HNa) when > 0.9% as fed. For all RSS tests, performed between February 2010 and May 2018, a minimum of 6 cats were housed individually and fed the diets during an adaptation phase of a minimum of 7 days, followed by a minimum of 3 days of urine collection. Urinary calcium and oxalate were measured on the pooled sample using ionic chromatography. CaOx RSS was calculated using SUPERSAT software. A total of 41 different RSS tests including 89 individual cats were used to explore 29 different diets. The impact of dietary sodium on urinary volume, calcium excretion and concentration, as well as CaOx RSS was evaluated by linear mixed models. Animal and diet were included as random terms. Variables were log-transformed in order to meet statistical model assumptions, and significance was set at P < 0.05. Data is presented as median [25th, 75th percentile]. Urinary volume was significantly greater on the high sodium diets (LNa: 10.8 [8.7, 13.1] vs HNa: 19.4 [14.4, 26.1] ml/kg BW/day, P < 0.001). Urinary calcium excretion was increased in the high sodium diets (LNa: 5.9 [4.5, 8.2] vs HNa: 9.2 [7.2, 12.7] mmol/kg BW/day, P = 0.0145), but urinary calcium concentration was not different (LNa: 0.6 [0.5, 0.8] vs HNa: 0.5 [0.4, 0.6] mmol/L, P = 0.12). Urinary oxalate concentration was significantly lower in the high sodium diets (LNa: 2.3 [1.83, 2.86] vs HNa: 0.93 [0.8, 1.13] mmol/L, P < 0.001). CaOx RSS was significantly lower with the high sodium diets (LNa: 3.44 [2.49, 4.78] vs HNa: 1.67 [1.32, 2.10], P < 0.001). The results of this study suggest that increased dietary sodium is associated with an increase in urinary calcium excretion. However, urinary calcium and oxalate concentrations decrease, which can possibly be explained by the increase in urinary volume, thereby decreasing CaOx RSS. An increase in dietary sodium therefore decreases the risk of CaOx urolithiasis in short-term feeding trials, which can mainly be attributed to the effect on urine dilution. Further studies are required to assess the effect of increased dietary sodium when diets are fed for a longer period of time.

NU14

Stability of Urine Specimens Stored with Preservatives at Room Temperature or without Preservatives Under Refrigeration
Harmeet K. Aulakh – Louisiana State University; Karamvir Aulakh – Louisiana State University; Kirk Ryan – Louisiana State University; Chi-Liu – Louisiana State University; Mark Acierno – Midwestern University

To determine the effect of preservative tube storage on urinalysis test results when stored at room temperature compared to refrigerated plain glass tubes. Prospective blinded controlled study

Urine samples from 20 healthy dogs

A urine sample from each dog was divided into 6 aliquots: 3 aliquots in BD Vacutainer®Plus Urinalysis Preservative Tubes (BD UAP) and 3 aliquots in plain glass tubes (PGTs). Preservative tubes were stored at room temperature and PGTs were refrigerated. Urinalysis was performed at 0h, 24h and 72h and results from all tubes were compared with results from a 0h plain glass (reference) tube.

There was high agreement for physical parameters for urine stored in PGTs and preservative tubes, except for color at 24h. There was also high agreement for all chemical and microscopic parameters at all-time points except for WBCs (moderate correlation at 24h and 72h), RBCs (low at 24h, fat droplets (moderate at 24h, negligible at 27h) and squamous cells (moderate at 24h and 72h) for preservative tubes, and RBCs (moderate at 24h, low at 72h), fat droplets (moderate at 24h, low at 72h), and squamous cells (low at 24h, moderate at 72h) for PGTs.

A preservative tube stored at room temperature offers comparable results with urine samples kept in a refrigerator for 24h and up to 72h for the majority of parameters. If a delay in urinalysis is expected, the BD UAP tube is a viable option for storage and transport of canine urine samples.

NU15

Evaluation of Serum Symmetric Dimethylarginine (SDMA) in Dogs Naturally Infected by Leishmania infantum in Brazil
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Canine visceral leishmaniasis (CVL) is a zoonotic disease caused by the protozoa Leishmania sp. Among the clinical alterations observed in dogs infected by the protozoa, symptoms associated with decrease of renal function are often described. Serum symmetric dimethylarginine (SDMA) has recently been evaluated as a renal biomarker that can efficiently detect early renal dysfunction in dogs. The level of serum SDMA has shown a significant relationship with the glomerular filtration rate because it is only excreted through renal clearance in dogs. This study evaluated serum SDMA in 60 dogs naturally infected by L. infantum. The inclusion criteria of the dogs in the study were to have either positive Elisa, Rifi above 1:160 or positive reaction on Snap Leishmania Idexx. All dogs, regardless of their renal staging by the IRIS (International renal interest society), were submitted to the following exams: serum SDMA, complete blood count, reticulocytes, serum creatinine, serum blood urea nitrogen (BUN), serum phosphorus, urinalysis, urinary protein creatinine ratio (UP/C), systemic blood pressure (SBP) and abdominal ultrasonography. The dogs were divided into the IRIS stages: At Risk Stage, Stage 1, Stage 2, Stage 3 and Stage 4. The correlation between renal markers and SDMA was determined by Pearson’s coefficient of correlation analysis. Serum SDMA has shown a 99.9% relationship with serum creatinine (R = 0.89; P = 0.001), serum BUN (R = 0.81, P = 0.001) and serum phosphorus (R = 0.7, P = 0.001). The relationship of serum SDMA with the UP/C was 95% (R = 0.31, P = 0.05). The serum SDMA presented a 99% negative relationship with the hematocrit (R = -0.46, P = 0.01) and urinary density (R = -0.39, P = 0.01). With reticulocytes and and Urinary Creatinine the negative relationship was 95% (R = -0.306, P = 0.05).

Serum SDMA had 60% relationship with BP (R = 0.09, P = 0.47). Of the animals with increased serum SDMA, 13/19 (68.4%) had Systemic Blood Pressure (SBP) above 140 mmHg and 14/19 (73.6%) had UPC.
above 0.5, with no active sediment in the urine test, being classified as proteinuric animals. Dogs in IRIS at Risk stage had a serum SDMA increased on 13.3% (4/30), in IRIS stage 1 31.2% (5/16), IRIS stage 2 50% (3/6), IRIS stage 3 83.3% (5/6) and IRIS stage 4 100% (2/2). In 4 dogs that were classified in IRIS stage 2 and 3, the creatinine is higher than 1.4mg / dl but lower than 1.8mg / dl (reference value at Idexx laboratory). In these animals, the creatinine value may have been influenced by other factors. All animals that had increased creatinine and SDMA within normal range were animals weighing more than 44lbs (20kg). All dogs with hyperphosphatemia showed increased serum SDMA. The 9 animals in the IRIS stage of risk and stage I that presented increased SDMA had repeat the creatinine test after 6 months. In the 2 animals that presented SDMA above 30 μg / dl, creatinine values increased from 1.3 mg / dl to 2.9 mg / dl in the first case and from 0.9 mg / dl to 2.1 mg / dl in the second case. The 7 animals that presented SDMA between 15 μg / dl and 30 μg / dl presented elevation of more than 50% in the creatinine value, but still remained below 1.4 mg / dl. The present data shows that serum SDMA has a strong relationship with several parameters used in the evaluation of renal function, such as serum creatinine, serum phosphorus, serum BUN, hematocrit, UP/C, urinary density and reticulocytes. Serum SDMA had a weak relationship with BP. Serum SDMA increased prior to image alteration on kidney ultrasonography in 13.3% (4/30) of the dogs. Serum SDMA also increased in 15% (9/60) of dogs before values of serum creatinine were above 1.4mg / dl. The differences found in IRIS staging considering serum SDMA were: of the dogs in IRIS stage 2, 33.3% (2/6) had serum SDMA above 25μg / dl, so these dogs were reclassified from IRIS stage 2 to IRIS stage 3. Dogs in IRIS stage 3, 16.6% (1/6) had serum SDMA above 45μg/dl so this dog was reclassified from IRIS stage 3 to IRIS stage 4. So we conclude that serum SDMA is an important tool for the evaluation of renal function and should be used routinely in the evaluation of dogs infected with L. infantum.

**NU16**

Effects of Calcifediol Supplementation on Markers of Chronic Kidney Disease-Mineral Bone Disorder in Dogs

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Chronic kidney disease-mineral bone disorder (CKD-MBD) is affected by complex interactions between creatinine, phosphorus, calcium, vitamin D metabolites, parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23). The effects of supplementation with calcifediol (25-hydroxyvitamin D; 25[OH]D) on these markers in dogs is unknown.

Ten dogs with International Renal Interest Society (IRIS) stages 2 and 3 CKD were included. Dogs received an extended-release calcifediol [25[OH]D] supplement [Rayaldee® - median dose 2.0 μg/kg/dose (range, 1.6-2.7 μg/kg/dose)] for 3 months; it was then discontinued. Dogs were evaluated monthly for 5 months. Data was tested with repeated measures analysis and reported as medians (ranges).

Concentrations of serum creatinine, phosphorus, total calcium, ionized calcium, 25(OH)D, 1,25-dihydroxyvitamin D (1,25(OH)₂D), 24,25-dihydroxyvitamin D (24,25(OH)₂D), and PTH, and plasma FGF-23 are listed in Table 1. All vitamin D metabolites were significantly greater at 3 months. No impact was noted on non-vitamin D metabolite markers of CKD-MBD. Short-term supplementation with extended-release calcifediol appeared to be safe and well-tolerated. Further studies should evaluate the long-term effects of calcifediol supplementation.

**NU17**

Characterization and in vitro Susceptibility o Canine UPEC Isolates to a Novel E. coli Probiotic

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The rise in antibiotic resistance amongst urinary tract infections (UTIs) in dogs underscores the need for non-antibiotic approaches to UTIs. The probiotic Escherichia coli Nissle-1917 (EcN) has many benefits including antimicrobial activity against many human pathogens including uropathogenic E. coli (UPEC). The aim of this study was to phylogenetically characterize UPEC in canine UTI cases and investigate the in vitro susceptibility of these isolates to EcN.

Thirty-eight dogs with positive E. coli urine cultures were included in the study. Characterization of UPEC isolates was performed by clade analysis, serotyping and virulence factor analysis by multiplex PCR testing. EcN effectiveness against UPEC isolates was tested in vitro using microcidin plate analysis.

Clinical signs were consistent with lower urinary tract infection in all dogs. UPEC clades noted in this subset of dogs included A, B1, B2, D, and E. Serogroup and virulence factors correlated with clade analysis.

Table 1. Laboratory variables of CKD-MBD in 10 dogs at baseline, 3 months on calcifediol supplement, and 2 months after discontinuation. (*P < 0.01)

| Laboratory variable & reference range | Baseline | Month 3 | Month 5 (n=9) |
|--------------------------------------|----------|---------|--------------|
| Creatinine (0.6-1.6 mg/dL)           | 2.3 (1.7-4.5) | 2.5 (1.6-4.9) | 3.4 (1.6-6.6) |
| Phosphorus (3.2-8.1 mg/dL)           | 3.8 (2.7-6.5) | 4.4 (2.7-6.9) | 4.6 (3.1-8.5) |
| Total calcium (9.3-11.6 mg/dL)       | 10.6 (9.6-11.9) | 11.3 (9.5-12.5) | 11.2 (9.4-13.2) |
| Ionized calcium (4.9-5.8 mg/dL)      | 5.06 (4.79-5.37) | 5.07 (4.70-5.71) | |
| 25-hydroxyvitamin D (ng/mL)          | 50.2 (31.3-66.0) | 249.9 (149.7-469.9)* | 68.7 (49.4-112.3) |
| 1,25-dihydroxyvitamin D (pg/mL)      | 37.3 (29.3-56.7) | 66.1 (56.9-88.1)* | 34.1 (25.6-50.5) |
| 24,25-dihydroxyvitamin D (ng/mL)     | 20.6 (6.9-40.6) | 83.8 (22.1-151.7)* | 50.5 (16.9-104.4) |
| Parathyroid hormone (0.5-5.8 pmol/L) | 3.9 (1.4-20.1) | 2.8 (0.5-43.2) | 3.1 (2.2-72.4) |
| Fibroblast growth factor-23 (pg/mL)  | 798 (103-4,145) | 1,219 (229-8,824) | 1,555 (234-37,002) |
as reported in human UPEC studies. Sixty-eight percent of UPEC isolates were susceptible to the EcN probiotic in vitro. Average zone of growth inhibition from the EcN probiotic was 5.1 mm (SD 1.79 mm).

UPEC isolates from canine patients were similar to isolates in human patients in pathogenicity, susceptibility, and genetic background. In vitro susceptibility of canine UPEC isolates were frequently susceptible to the EcN probiotic through growth rate characteristics and/or microcin production. Further studies are required to better assess the efficacy of EcN in vivo and to determine the utility of EcN as a UTI prevention tool in dogs.

**NU18**

Smartphone-Based Colorimetric Method for At-Home Urinalysis Dipsticks Reading in Cats: A Pilot Study

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Urine collection is increasingly used by pets’ and especially cats’ owners. Urine dipstick analysis is a convenient method for assessment of several biochemical aspects of urine and can be easily performed by non-healthcare professionals. However, visual reading is less reliable than automated analysis. Dedicated smartphone-based colorimetric methods (SBCM) have therefore the potential to improve the quality and transmission of urinalysis results to the attending veterinarian and hence the level of care and follow-up of small animal patients.

The aim of the present study was to compare the results obtained by a SBCM with those obtained by semi-automated point-of-care (POC) urinalysis as a reference method.

Proteins, bilirubin, “blood”, glucose and ketones reactive pads results were considered. Positive and negative control solutions available from the dipsticks’ manufacturer as well as purposely designed artificial urine samples were used. Briefly, 2 urine reagent strips (URIT 14G) were simultaneously dipped in each sample; one was read by the SBCM and the other one by the POC analyzer at the same time.

Eighty comparisons were obtained for each analyte and for each expected concentration. The overall agreement (exactly the same response) between the two methods was 78.6%. When results were considered either negative or positive (+/− to +), SBCM sensitivity and specificity were 97.3% and 98.7%, respectively. When clinically relevant cut-off where used, SBCM sensitivity and specificity were 99.0% and 100.0%, respectively.

In conclusion, SBCM shows good to excellent intrinsic diagnostic performances and appears suitable for at-home dipstick urinalysis.

**NU19**

Analysis of Bias Between the Sedivue Dx™ and Manual Microscopy in Detecting Urine Sediment Cells

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The IDEXX SediVue Dx™ (SediVue) is an automated analyzer for urine sediment examination in veterinary patients. This study set out to determine the bias between manual microscopy and the SediVue for quantification of red blood cells (RBCs) and white blood cells (WBCs) in urine. Results from urine sediments based on concurrent evaluation of manual microscopy (using a KOVA system) and the SediVue (1.0.1.3) were retrospectively evaluated. Bias was determined using Bland-Altman plots for urine sediment samples containing RBCs (n = 462) and WBCs (n=510). Diplomates of ACVIM and ACVP at Texas A&M were surveyed to quantify bias that might impact clinical decision making. Samples with bias ≥25 cells, or samples with 5-100 cells/high power field (HPF) and bias ≥ 100% were considered to have “high bias.” SediVue-captured images of high-bias samples were then manually evaluated and compared with manual microscopy results.

The median RBC and WBC bias by category were as follows: for RBCs, ≤ 5 RBCs/HPF: 0.3; 5.1-10 RBCs/HPF: 10.1; 10.1-20 RBCs/HPF: 10.6; and > 20 RBCs/HPF: 28.93; for WBCs, ≤ 5 WBCs/HPF: 0.1; 5.1-10 WBCs/HPF: 2.2; 10.1-20 WBCs/HPF: 9.4; and > 20 WBCs/HPF: 26.6.

Ninety-eight samples (21.2%) with RBCs and 77 samples (15.1%) with WBCs were initially considered to have high bias between the methods; however, only 12 (2.5%) RBC- and 16 (3.1%) WBC-containing samples had high bias following manual review of SediVue images. The bias in RBC and WBC counts between manual microscopy and the SediVue was unlikely to impact clinical decisions in a majority of cases. Manual review of SediVue-captured images helped reduce observed bias.

**NU20**

Urinary Tract Infection Escherichia Coli’s Potential to Produce Biofilm and the Effect on Antimicrobial Resistance

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Studies show that between 14 % and 26 % of dogs will develop at least one Urinary Tract Infections (UTIs) during their life. E. coli is widely recognized as the most common cause. While the majority are easily treated, some patients develop UTIs that are resistant. In humans, it has been documented that certain uropathogenic bacteria have the ability to form a matrix of extracellular polymeric substances resulting in a biofilm. This biofilm offers bacteria protection from the immune system as well as antibiotics. The purpose of this study was to identify uropathogenic bacteria capable of producing a biofilm from isolates collected from patients with community acquired UTIs as well as assess the degree of antimicrobial resistance the biofilm provided.

In this study, isolates from 35 canine patients with E. coli UTIs as identified by a diagnostic laboratory were assessed for their ability to produce a biofilm. Biofilm producing ability was measured by the crystal violet assay technique as quantified at an absorbance at 570 nm. Isolates were classified as non-biofilm producing (OD < 0.2) moderate (OD 0.2 - 0.399) or strong (>0.4) producers. Of 35 canine isolates,
7 were moderate and 4 were strong biofilm producers. The degree of antibiotic resistance afforded by the biofilm is currently being accessed by determining the minimal bactericidal concentration of commonly prescribed antimicrobial agents. Results are pending.

NU21

Serum Aluminum Concentrations in Healthy Cats and Cats with Chronic Kidney Disease

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Aluminum is poorly absorbed from the gut and is excreted by the kidneys. The use of aluminum-containing phosphate binders in humans with chronic kidney disease (CKD) has been severely curtailed due to documented aluminum toxicity. Aluminum hydroxide is a first-line treatment in cats with CKD and hyperphosphatemia, but serum aluminum levels have not been investigated in these cats. The purpose of this study was to compare serum aluminum levels between healthy cats, cats in various stages of CKD, and those taking aluminum-containing phosphate binders (aluminum hydroxide).

Inductively coupled plasma mass spectrometry was used to measure serum aluminum concentrations. Serum samples were collected prospectively from healthy cats presented to the clinic for wellness evaluation or routine follow-up for cats with chronic kidney disease. 23 healthy cats, 41 cats with CKD not receiving aluminum hydroxide (20 with IRIS stage II, 12 with IRIS stage III, and 7 with IRIS stage IV), and 9 cats with CKD receiving aluminum hydroxide were included. No significant difference in serum aluminum concentration was found when comparing healthy cats (median 0.061 ppm, interquartile range [IQR] 0.034-0.132) to cats with IRIS stage II CKD (median 0.041 ppm, IQR 0.031-0.047), stage III CKD (median 0.033 ppm, IQR 0.029-0.044), stage IV CKD (median 0.032 ppm, IQR 0.028-0.050) and cats taking aluminum hydroxide (median 0.038 ppm, IQR 0.033-0.041). Aluminum does not appear to increase in cats with CKD compared to healthy cats.

NU22

Serum and Urine Concentration of Amoxicillin-Clavulanic Acid in Cats with and without Chronic Kidney Disease

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Impaired renal clearance of potentiated penicillins is a well-recognized pharmacokinetic observation in human patients with chronic kidney disease (CKD). This has resulted in standard dose adjustments based on renal function to avoid drug accumulation. Similar information about the disposition of amoxicillin-clavulanic acid in cats with CKD is not available in the literature. The purpose of this study was to evaluate the concentration of amoxicillin-clavulanic acid in the urine and serum of cats with azotemic CKD in comparison to cats without CKD. Client-owned cats with azotemic CKD (n = 6; International Renal Interest Society stage II - V) and without azotemic CKD (n = 6) that were prescribed amoxicillin-clavulanic acid for reasons unrelated to the study were enrolled. Cats included in the azotemic CKD group had serum creatinine > 2.0 mg/dL, USG < 1.035 and a clinical diagnosis of CKD. Cats without CKD included in the control group had serum creatinine < 1.8 mg/dL, USG > 1.035 and no clinical diagnosis of CKD. Creatinine > 2.0 mg/dL was chosen to define the group of interest as impaired clearance of amoxicillin-clavulanic acid is more common in humans with greater renal impairment. Serum and urine samples were obtained at steady-state (7 days after initiating drug) and within 1 - 2 hours of receiving an oral dose. Owners completed a survey regarding occurrence of side effects (vomiting, diarrhea, decreased appetite, none). Amoxicillin and clavulanic acid serum and urine concentrations were measured with liquid chromatography coupled to tandem mass spectrometry; concentrations were compared between groups with a Mann-Whitney test. Correlation between serum creatinine and drug concentrations in urine and serum was determined using Spearman rank correlation.

Median dosage of amoxicillin-clavulanic acid was 14 mg/kg (range 12.7 – 15.2 mg/kg) in azotemic CKD cats. Three of 6 cats experienced side effects, including decreased appetite (n = 1), diarrhea (n = 1) and both decreased appetite as well as diarrhea (n = 1). Median dosage of amoxicillin-clavulanic acid was 13.1 mg/kg (range 12 – 13.5 mg/kg) in cats without CKD. Four of 6 cats had side effects which included decreased appetite (n = 2), vomiting (n = 1) and diarrhea (n = 1). Cats with azotemic CKD had significantly lower urine amoxicillin concentrations (median 50,525 ng/ml, range 8,115 – 158,500 ng/ml) in comparison to the cats without CKD (median 328,250 ng/ml, range 33,100 – 576, 500 ng/ml) (p = 0.02), and urine amoxicillin concentrations were negatively correlated with serum creatinine (p = 0.01; r = - 0.71). Cats with azotemic CKD tended to have higher serum amoxicillin concentrations (17,275 ng/ml, range 2,345 – 21,400 ng/ml) than cats without CKD (median 5,590 ng/ml, range 1,150 – 9,995 ng/ml) (p = 0.06), and serum amoxicillin concentrations were positively correlated with serum creatinine (p = 0.03; r = 0.62).

No significant difference between groups or correlation with creatinine was noted for clavulanic acid concentrations in either urine or serum. Serum and urine amoxicillin concentrations were respectively positively and negatively correlated with serum creatinine, suggesting that altered pharmacokinetics exist in cats with CKD (i.e. decreased renal clearance) and may be more apparent as disease severity progresses. However, CKD cats still achieved urine drug concentrations greater than Clinical Laboratory Standards Institute (CLSI) resistance breakpoints for urinay E. coli isolates in cats. Studies with a larger population are required to determine what dose adjustments should be considered for azotemic feline patients.

NU23

C-Reactive Protein in Hypertension and Immune-Complex Glomerular Disease

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Approximately half of dogs undergoing renal biopsy for proteinuria have immune-complex glomerulonephritis (ICGN). Preliminary data
suggests that hypertension may be more common with ICGN. CRP elevations have been shown to correlate with hypertension in people. We hypothesized that CRP would be associated with hypertension and with a diagnosis of ICGN in dogs. The International Veterinary Renal Pathology Service database of previously obtained renal biopsies was used to select patients with documented systolic blood pressure measurement who also had stored serum samples for CRP measurement. Normotension was defined as < / = 150 mmHg systolic blood pressure, while dogs with a documented systolic blood pressure > 150 mmHg were classified as hypertensive. Groups were compared using a t-test. Thirty-nine dogs meeting our criteria have been evaluated. They have been categorized as non-ICGN normotensive (n=10), non-ICGN hypertensive (10), ICGN normotensive (7) and ICGN hypertensive (12). Comparison of CRP concentrations amongst these four categories did not show statistically significant correlations. However, CRP concentration was higher in hypertensive dogs (median 27.65 mg/L) compared to normotensive dogs (median 3.65 mg/L, p < 0.01). A similar correlation was not identified between ICGN and non-ICGN groups, but the study was underpowered to detect a difference. The correlation between hypertension and an inflammatory biomarker such as CRP supports a role for hypertension in inducing inflammatory vascular lesions.

NU24

Short-Term Efficacy of Epidural Pain Management in Dogs Undergoing Routine Cystoscopy
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The effects of epidural anesthesia on pain management in dogs undergoing routine cystoscopy have not been evaluated. Twenty-six dogs undergoing routine cystoscopy were enrolled in a prospective, randomized, blinded observational study. Systemic health status was confirmed prior to enrollment in all dogs. Exclusion criteria included evidence of non-urinary disease, inability to administer protocol medications, behavioral problems, or evidence of orthopedic or neurologic disease. Four dogs were removed from the study due to status unblinding, patient cooperation, or incomplete follow-up. Dogs were assigned to the treatment group which received an epidural [(Atramorph 0.2 mg/kg, 1% ropivacaine 0.2 mg/kg)(total volume delivered, 1 mL/4.5 kg of body weight (1 mL/9.9 lb) to a maximum of 10 mL)] (n=9), or a non-epidural control group (n=13). Vital signs were monitored for 24 hours, and sedation and pain scores, behavioral assessments, and presence/absence of epidural complications were evaluated for 7 days post-procedure. Mann-Whitney test was used to determine differences in outcome variables between groups.

All dogs tolerated the epidural without complications (including urine retention, neurologic status, and pruritis). No significant differences were noted at any timepoint (baseline, 6, 12 and 24 hours, and 5 and 7 days post-procedure) in vital signs, pain scores, sedation scores, or behavioral assessments between groups. Epidural anesthesia was well tolerated by the dogs in this study. Results were unable to document a benefit of the epidural in this cohort of dogs. Further studies are required to better assess the efficacy of epidural anesthesia in cystoscopic procedures associated with more discomfort.

NU25

Changes in Creatinine and Symmetric Dimethylarginine During Pregnancy in the Dog
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In both healthy humans and dogs, glomerular filtration rate (GFR) is known to be increased throughout the course of a pregnancy primarily due to increased cardiac output. Following birth, GFR decreases until it returns to normal levels approximately 8 weeks postpartum.

Figure 1: SDMA (a) and Creatinine (b) concentrations in pregnant dogs over time
In humans, the increase in GFR during pregnancy is matched to a decrease in serum creatinine. Limited human studies have probed the trend of symmetric dimethylarginine (SDMA) in healthy pregnant women. One study found a statistically significant decrease in SDMA by the end of the first trimester which persisted into the 2nd trimester. To our knowledge no similar studies have been performed in dogs. We therefore performed a small pilot study to assess the trend of creatinine and SDMA in pregnant dogs.

Working with a licensed commercial dog breeder, 15 female dogs were enrolled into the study. Blood draws were taken prior to breeding and at days 21, 42 and 73 (this time point being between 1 and 6 days postpartum). Creatinine and SDMA measured using previously validated liquid chromatography mass spectroscopy methods. The protocol was reviewed and approved by the veterinary staff of the breeder.

The SDMA and Creatinine concentrations for each of the 15 dogs together with the population median at each timepoint are shown in figures 1. The medians for days 21, 42 and 73 were compared to day 0 using individual Mann-Whitney test and significance determined at p=0.05 and any comparisons not shown were found to not be significant. These results would seem to mirror the human findings that the creatinine and SDMA do decline during pregnancy. These results do suggest that both creatinine and SDMA values should be carefully evaluated when attempting to probe for kidney disease during pregnancy.

**NU26**

**Intravascular Fluid Volume Assessment of Dogs with Glomerular Disease Using Clinical, Laboratory and Imaging Parameters**

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The assessment of the intravascular volume status of dogs with glomerular disease (GD) lacks evidence on the choice and the use of criteria to differentiate hypovolemic-underfilled from hypervolemic-overfilled patients. A prospective pilot study was designed to evaluate parameters associated with intravascular volume in dogs with GD compared with non-proteinuric chronic kidney disease (CKD).

Clinical assessment was based on heart rate, pulse quality, capillary refill time, acral temperature, and systolic blood pressure; echocardiographic evaluation included LA:Ao and EDVI; laboratory assessment included BUN:creatinine, fractional excretion of sodium, NT-proBNP, renin activity, and aldosterone. Parameters were compared between GD and CKD, and between clinically hypovolemic and non-hypovolemic dogs.

15 dogs with GD (SDMA 41.5 mg/dl, UPC 10.4) were compared to 10 CKD dogs (SDMA 26.5 mg/dl). Hypovolemia was identified clinically in 6/15 dogs; all CKD dogs were estimated normovolemic. 11/15 GD dogs and 3/10 CKD dogs were hypertensive (170 mmHg [160-184]); CKD, 146 mmHg [140-163], P=0.01). LA:Ao and EDVI were within described limits in 14/15 GD and 8/10 CKD dogs. BUN:creatinine, FENa and NT-proBNP were not different between groups, but elevated NT-proBNP was seen without evidence of cardiac disease. Renin (0.44 ng/ml/h [0.29-0.88], P=0.04) and aldosterone (0 pmol/l [0-20], P=0.04) were lower in GD than in CKD. Dogs estimated clinically hypovolemic were not different from non-hypovolemic dogs for all parameters, including RAAS activity. Despite differences in clinical parameters, dogs with GD had only marginal differences in laboratory parameters supposed to reflect their intravascular volume. Unexpected was the inactive RAAS in 13/15 dogs with GD.

**NU27**

**Evaluation of Serum Erythropoietin, Cyanocobalamin, and Iron Concentrations in Dogs with Chronic Kidney Disease**

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The pathogenesis of anemia secondary to chronic kidney disease (CKD) and associated factors of erythropoiesis have not been investigated clearly in dogs. The objective of the present study was to examine whether non-regenerative anemia is related to serum concentrations of erythropoietin (EPO), cyanocobalamin, and iron in dogs with CKD.

Fifty-six dogs with CKD and 20 healthy dogs were enrolled in this study. The dogs with CKD were receiving medications, such as histamine 2 receptor antagonists (H2RAs), proton-pump inhibitors, and/or aluminum-based phosphate binders, over 2 weeks. Thirty dogs with CKD and non-regenerative anemia were divided into four groups according to the International Renal Interest Society classification. Serum concentrations of EPO, cyanocobalamin, and iron were measured by enzyme-linked immunosorbent assay, electrochemiluminescence immunoassay and colorimetric assay, respectively.

 Serum iron concentrations were significantly lower in dogs with CKD and non-regenerative anemia than in healthy dogs and dogs with CKD without non-regenerative anemia, whereas serum EPO concentrations were not significantly different among the groups. Serum iron concentrations were significantly lower in dogs with non-regenerative anemia and CKD receiving famotidine and an aluminum-based phosphate binder than in healthy dogs, whereas serum EPO concentrations were not different among the groups.<p> These results suggest that the development of non-regenerative anemia in dogs with CKD may be related to serum iron concentrations which can be affected by the administration of H2RAs and aluminum-based phosphate binder. Therefore, it is likely to be necessary to monitor the development of anemia in dogs with CKD, particularly after administration of drugs.
Cystatin C as an Early Biomarker for Chronic Renal Failure in Dogs
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Chronic kidney disease (CKD) involves the progressive loss of kidney function over a period of months or years. The glomerular filtration rate (GFR) has long been considered the gold standard for evaluating kidney function. However, direct measurements of GFR are time-consuming and labor-intensive, and therefore are not routinely used in practice. Traditionally, creatinine (CREA) has been the biomarker of choice used by the International Renal Interest Society (IRIS) to evaluate and monitor kidney disease. CREA is the most frequently quantified analyte in human and veterinary clinical chemistry laboratories as an indirect measure of GFR. However, CREA can be mildly increased or often remain within the reference range until approximately 75% of all nephrons are no longer functional. Symmetric dimethylarginine (SDMA) is another renal biomarker that has been reported and is not influenced by muscle mass, but some non-renal changes can affect the accuracy of tests for SDMA. With growing interest in the application of cystatin C (CysC) as a new renal functional biomarker in veterinary medicine, the aim of this study was to evaluate the serum CysC concentrations in several populations of dogs with CKD, and compare it with CREA and SDMA in terms of efficacy as a biomarker of renal function. A total of 41 client-owned dogs were included in this study. They were brought to the Chonnam National University Veterinary Medicine Teaching Hospital from 2017 to 2018 for medical check-ups, new diagnoses, or treatment of current disorders. Of all the dogs with renal disease, 46.3% (19/41) had IRIS stage I CKD, 17.1% (7/41) had IRIS stage II CKD, 34.1% (14/41) had IRIS stage III CKD, and 2.0% (1/41) had IRIS stage IV CKD. The control dogs did not show any evidence of CKD. Serum SDMA and CysC levels were significantly (P < 0.05) elevated in dogs with various IRIS stages of CKD when compared to healthy controls. In particular, plasma CREA and serum SDMA lacked efficacy in detecting early-stage CKD (IRIS stage I), while serum levels of CysC were elevated in the early stage of CKD (IRIS stage II). Furthermore, the serum levels of CysC also demonstrated a strong correlation with plasma CREA (r = 0.668, P = 0.001) as well as serum SDMA (r = 0.708, P < 0.001) levels. Our results showed that serum CysC (sensitivity, 75.6%) was a more sensitive and specific biomarker for detecting CKD in dogs than plasma CREA (sensitivity, 53.6%) and serum SDMA (sensitivity, 68.2%). In particular, serum CysC (area under the curve [AUC] = 0.797, P < 0.001; sensitivity 63.1%) showed a higher diagnostic power for IRIS stage I CKD than plasma CREA (AUC = 0.634, P = 0.198; sensitivity 57.8%) and serum SDMA (AUC = 0.647, P = 0.150; sensitivity 47.3%). It may also be more accurate than other biomarkers for identifying underlying pathophysiological processes associated with CKD. In conclusion, using serum CysC as a biomarker for CKD allows for earlier detection of kidney dysfunction in dogs compared to measurements of plasma CREA and serum SDMA. Earlier detection might be desirable for initiating renoprotective interventions to slow the progression of kidney disease sooner, ultimately extending the life expectancy of dogs with CKD.

Histologic Assessment of the Aging Feline Kidney in Cats Without Kidney Disease
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In humans, renal aging is associated with increased frequency of glomerulosclerosis, interstitial fibrosis, inflammation and tubular atrophy. The presence of similar lesions in cats is less clearly documented. The purpose of this study was to describe renal histopathologic findings in aging cats without kidney disease. Archival paraffin-embedded kidney tissue was examined from 79 cats without kidney disease (serum creatinine < 1.6 mg/dL, USG > 1.035 and no clinical suspicion of kidney disease: 0-4 years (young, n=18), 5-9 years (mature, n=19), 10-14 years (senior, n=36), 15+ years (geriatric, n=6). Exclusion criteria included renal disease/neoplasia, diabetes mellitus, hyperthyroidism, urinary tract infection, proteinuria, or history of NSAID or chemotherapy administration. Three stains (hematoxylin and eosin, periodic acid–Schiff–hematoxylin, and Masson’s trichrome) were scored by a pathologist blinded to clinical data. Glomerulosclerosis was recorded as number of affected glomeruli/25 glomeruli examined. Semiquantitative variables included tubular atrophy, interstitial inflammation and fibrosis were assessed as percentage of affected tissue. Lesions were compared between groups using ANOVA with Dunn’s post hoc analysis. Dichotomous variables included presence or absence of lipid vacuoles in the interstitium, lipid casts within tubules, and vascular lesions. Glomerulosclerosis increased with age (median number of glomeruli affected/25 glomeruli: young 0/25, mature 1/25, senior 1/25, geriatric 2/25). Geriatric cats had significantly more glomerulosclerosis than mature cats (p = 0.03) and young cats (p = 0.004). Senior cats had significantly more glomerulosclerosis than young cats (p = 0.01). Tubular atrophy also increased with age (median percentage of affected tissue: young 0%, mature <5%, senior <5%, geriatric 5-15%). Geriatric cats had significantly more tubular atrophy than mature cats (p = 0.004) and young cats (p < 0.0001). Senior and mature cats had significantly more tubular atrophy than young cats (p = 0.0002 and p = 0.01 respectively). Inflammation increased with age (median percentage of affected tissue: young 0%, mature <5%, senior <5%, geriatric 5-15%). Geriatric cats had significantly more inflammation than senior cats (p = 0.02), mature cats (p = 0.007) and young cats (p < 0.0001). Senior cats had significantly more inflammation than young cats (p = 0.004). Fibrosis increased with age (median percentage of affected tissue: young 0%, mature 0%, senior <5%, geriatric <5%) and geriatric and senior cats had significantly more fibrosis than young cats (p = 0.01 and p = 0.04 respectively). Frequency of tubular lipid (scored as a dichotomous variable) increased with age (young: 28%; mature: 58%; senior: 78%; geriatric: 100%) as did
frequency of interstitial lipid (young: 22%, mature: 58%, senior: 86%, geriatric: 100%) and frequency of fibrointimal hyperplasia (young: 0%, mature: 14%, senior: 19%, geriatric: 50%).

Similar to humans, renal aging in cats without kidney disease is characterized by increasing glomerulosclerosis, tubular atrophy, interstitial inflammation, fibrosis and frequency of fibrointimal hyperplasia. Tubular and interstitial lipid also increase with age, and this lesion may be unique to domestic cats.

**NU30**

**Bun to Creatinine Ratio: How could it Helps to Interpret Azotemia in Dogs**

_Felipe dos Santos Muniz – UniCare; Fernanda Triani Gomes de Knegt – UniCare Laboratory_

Blood urea nitrogen (BUN) and serum creatinine are widely used in veterinary medicine primarily for the evaluation of renal function. However, they can be altered by pre-renal, renal or post-renal factors. In human medicine the BUN to Creatinine Ratio (BCR) has been extensively used to differentiate between these three causes of azotemia or uremia. The aim of this study is to propose the use of BCR in the veterinary clinical routine and report possible causes of decrease and increase. 254 female and male dogs, of different races and ages, were retrospectively evaluated. The survey was performed through clinical chart and laboratory analysis. After performing the tests, we classify all dogs using BCR. The inclusion criteria in the study were only animals with complete history of anamnesis including information on type of feeding, main complaint and adequate description of clinical examination. The normal value for BCR, in this study, was established by calculating the median and standard deviation of the values found in the animals that did not present any clinical alterations. Healthy blood donor dogs were used to calculate normal values. Thus, we defined normal values of BCR between 11 and 21. All 108 dogs that presented stable chronic conditions remained with BCR between 11 and 21. Among the 21 dogs that presented BCR below normal values, 48% (10/21) had a decreased liver function and increased serum bile acids, being the main cause of the decrease in BCR, as found in the literature of human medicine. As other possible causes, we obtained the following results: 33% (7/21) had acute leishmaniasis and 19% (4/21) of the animals showed signs of low protein intake due to unbalanced feeding. In dogs that elucidated a increased BCR, we had as the main cause patients with cardiac disease or acute kidney disease, totaling 48% (59/125). Other causes include: Gastrointestinal bleeding in 25% (31/125); Suspected feeding of excess protein 11/125 (9%); Hyperadrenocorticism 11/125 (9%); Dermatitis with corticoid use 3% (4/125); Fever in 2% (3/125); Diabetes 2% (3/125); Severe Periodontal Disease 1% (2/125); Only 1 animal in this group did not present clinical alterations. He was performing surgical risk for elective neutering. We conclude that healthy or chronic dogs tend to a BCR between 11 and 21. The main causes of low BCR are hepatic dysfunction and malnutrition. The major causes of increase BCR are acute kidney or cardiac disease, excessive protein intake, use of corticoids, hyperadrenocorticism, fever, diabetes or severe periodontal disease. Dogs with AKI tends to have an increased BCR, except in the cases of dogs with leishmaniasis, where due to glomerulonephrititis BCR tends to be decrease. Dogs with chronic diseases tends to have a normal BCR.

Patients, high BCR patients had higher hospital mortality compared with low BCR patients. We suggest that with these data we can contribute to helping veterinarians to distinguish and better classify the types of azotemia between pre-renal, renal and renal.

**NU31**

**Avaliation of the Renal Function: A Quantitative and Multifactorial Index of Kidney Injury**

_Felipe dos Santos Muniz – UniCare; Fernanda Triani Gomes de Knegt – UniCare Laboratory_

The evaluation of renal function is a fundamental procedure in veterinary medicine. Some clinical and laboratories parameters are routinely used for the staging of kidney disease. Currently, the principal staging of chronic kidney disease is the IRIS that is classified in a qualitative way in four stages. Here, we propose a quantitative and continuous index to help the clinician to improve the comprehension of the patient’s renal function. The aim of this study is to present a continuous, quantitative and multifactorial index of kidney function (IKI) in dogs based in laboratory parameters that are used as markers of renal evaluation and System Blood Pressure (BP). 60 Client-owned dogs were avaliated in the study. Currently, serum creatinine is the major reference of renal function. To create the index (IKI), we included the laboratory parameters that had a significant relationship with serum creatinine. To establish this relationship we use the Pearson coefficient of correlation (R). The level of significance established was 95% (x = 0.05). The parameters with R equal or greater than the established significance level were included, to calculate the IKI. The following parameters showed a P = < 0.05: serum symmetric dimethylarginine (SDMA) and phosphorus, urinary protein creatinine ratio (UP/C) and urinary density. Despite the low correlation (P = 0.48) with creatinine, BP was included because of its clinical importance. To calculate the IKI this formula was apply:

\[ IKI = \frac{(Creatinine)(R2*SDMA)+(R2*Phosphorus)+(R2*UP/C)+(R2*BP))/(R2*Urinary\ density) \]

R2 is the coefficient of determination calculated from R value of each parameter in relation to serum creatinine. R2 was included to weight each parameter. As R2 range from 0 to 1, the higher R2, higher is contribution to the index.

The values of Pearson coefficient of correlation and coefficient of determination with serum creatinine were: serum SDMA (R = 0.8996, R2 = 0.8094, P = 0.001), serum phosphorus (R = 0.7791, R2 = 0.6071, P = 0.001), UP/C (R = 0.3109, R2 = 0.0967, P = 0.05), urinary density (R = -0.364, R2 = 0.1367, P = 0.05) and BP (R = 0.0917, R2 = 0.0084 e P = 0.48). The serum SDMA and phosphorus, BP and UP/C is directly proportional to serum creatinine. Urinary density had the negative correlation coefficient (R = -0.364), inversely proportional to creatinine. The IKI ranged from 48 to 977 demonstrating good amplitude. The present data shows that evaluating in a multifactorial way we were able to identify cases where an overestimation or sub-evaluation of the dog’s staging occurred. With a quantitative and multifactorial evaluation we can improve and discriminate the renal injury of dogs. Example: DOG 1 had a 1.3mg/dl serum creatinine, serum SDMA
Kidney disease is common in older dogs, especially chronic kidney disease (CKD), however, kidney disease can occur at all ages, associated with diverse etiologies contributing to acute kidney injury or CKD. The purpose of this study was to use renal biomarkers, symmetric dimethylarginine (SDMA) and creatinine (Cr), to identify the most common dog breeds at risk for kidney dysfunction, categorized by life stage. Canine blood samples submitted for serum chemistry testing between July 2015 through December 2017 were identified and retrospectively collected from U.S. IDEXX Reference Laboratories. SDMA and Cr concentrations were available for 847,886 dogs identified by a single breed, and for 499,684 mixed breed dogs. To estimate concentrations of moderate kidney dysfunction, critical thresholds above the reference intervals were used for both SDMA and Cr. The percentage of dogs with an increase in kidney function markers (SDMA >18 μg/dL or Cr > 1.9 mg/dL) was determined by age group for each breed. The top 25 most popular breeds in our dataset were analyzed and compared to a baseline defined as the average of all breeds. Of the top 25 breeds, 14 breeds had a higher percentage of dogs with increased SDMA or Cr concentrations as compared to the baseline for one or more age groups (P < 0.05). Some breeds had significantly increased SDMA or Cr in multiple age groups, with 3.6 % or greater in the senior age group (7 to 10 years of age). SDMA alone was more useful than Cr alone in identifying the most common dog breeds at risk for kidney dysfunction, categorized by life stage.

**NU34**

**Increase in Circulating Levels of Pro-Inflammatory Markers CCL16 and C5 in Canines with Kidney Dysfunction.**

Selena Tavener – Hills Pet Nutrition Center; Kiran Panickar – Hills Pet Nutrition Center; Selena Tavener – Hills PNC; Dennis Jewell – Hills PNC

Chronic low-grade inflammation is a key contributor to the progression of kidney disease. The release of cytokines and other pro-inflammatory proteins may further contribute to detrimental kidney health by increasing interstitial edema and renal fibrosis. Alteration of glomerular filtration rate (GFR) is also associated with an increase in markers of inflammation. The aim of the present study was to investigate the inflammatory markers in canines which developed renal disease naturally and were diagnosed with renal disease either during life or following necropsy, as assessed by a veterinarian. RNA was isolated using the PreAnalytix PAXgene blood RNA kit from canine blood obtained at necropsy and stored as bioarchived samples from ten canines with renal disease (9.6-14.7 yr) and ten controls (10.1-14.8 yr). At the time of death the mean blood creatinine concentration and BUN were elevated in dogs with renal disease compared to control (both p < 0.01). Following cDNA synthesis, samples were processed for changes in gene expression using the Canine cytokine RT² Profiler PCR Array for inflammation. Using the ΔΔCt method, and HPRT as the housekeeping gene, there was a 1.7 and 1.67-fold increase in Complement C5 and chemokine CCL16 respectively in dogs with renal disease compared to controls (both p < 0.05). In addition, there was a 1.65 and 1.96 fold increase (ns) in CCR3 and IL5RA respectively in renal disease. Modest increases (>1.2 fold) were also observed in the expression of inflammatory markers CCR8, CXCL11, and CXCL12.

**ABSTRACTS**

### NU32

**Prospective Evaluation of 2 Treatment Protocols in Preventing Recurrence in 51 Cats with Obstructive FIC**

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Urethral obstruction (UO) is a common complication of feline idiopathic cystitis (FIC). Robust treatment recommendations to prevent its recurrence are scarce. Prospectively evaluate two treatment protocols for prevention of clinical signs and recurrent UO.

Prospective, randomized clinical trial, including male cats with obstructive FIC. Cats were treated with phenoxybenzamine and alprazolam for 2 weeks, with (study group; 24 cats) or without (control group; 27 cats) low-dose meloxicam (0.025 mg/kg/day PO) and monitored for 6 months.

Heart rate had a good predictive performance for hyperkalemia (ROC curve AUC, 0.86), with cut-off values of 150 bpm and 129 bpm demonstrating a specificity of 100% and a sensitivity of 50% for hyperkalemia (>5.35 mEq/L) and severe hyperkalemia (>7.0 mEq/L), respectively. Cumulative number (%) of cats with rUO at 10 days, 1, 2 and 6 months post-discharge was 1 (2%), 2 (4%), 4 (8%) and 8 (16%), respectively. Twelve (24%) cats experienced recurrent FIC signs within 6 months, with (8 cats) or without (4 cats) concurrent UO. Recurrence was unaffected by treatment protocol or feeding a urinary therapeutic diet (P > 0.4). All cats were alive at 6 months.

Heart rate is a clinically useful tool for predicting hyperkalemia in cats with obstructive FIC. Addition of low-dose meloxicam to phenoxybenzamine and alprazolam treatment for 2 weeks conferred no clinical benefit. Unlike previous reports, recurrence was unaffected by urinary therapeutic diets. However, in both instances, cohort size might have been insufficient to detect potential differences.

### NU33

**Identifying Common Dog Breeds At Risk for Kidney Dysfunction with Symmetric Dimethylarginine and Creatinine**

Jennifer S. Ogeerc – IDEXX Laboratories Inc.; Donald Szlosek – IDEXX Inc.; Donald McCrann – IDEXX Inc.; Laureen Olavessen – IDEXX Inc.; Michael Coyne – IDEXX Inc.; Celeste Clements – IDEXX Inc.

Kidney disease is common in older dogs, especially chronic kidney disease (CKD), however, kidney disease can occur at all ages, associated with diverse etiologies contributing to acute kidney injury or CKD. The release of cytokines and other pro-inflammatory proteins may further contribute to detrimental kidney health by increasing interstitial edema and renal fibrosis. Alteration of glomerular filtration rate (GFR) is also associated with an increase in markers of inflammation. The aim of the present study was to investigate the inflammatory markers in canines which developed renal disease naturally and were diagnosed with renal disease either during life or following necropsy, as assessed by a veterinarian. RNA was isolated using the PreAnalytix PAXgene blood RNA kit from canine blood obtained at necropsy and stored as bioarchived samples from ten canines with renal disease (9.6-14.7 yr) and ten controls (10.1-14.8 yr). At the time of death the mean blood creatinine concentration and BUN were elevated in dogs with renal disease compared to control (both p < 0.01). Following cDNA synthesis, samples were processed for changes in gene expression using the Canine cytokine RT² Profiler PCR Array for inflammation. Using the ΔΔCt method, and HPRT as the housekeeping gene, there was a 1.7 and 1.67-fold increase in Complement C5 and chemokine CCL16 respectively in dogs with renal disease compared to controls (both p < 0.05). In addition, there was a 1.65 and 1.96 fold increase (ns) in CCR3 and IL5RA respectively in renal disease. Modest increases (>1.2 fold) were also observed in the expression of inflammatory markers CCR8, CXCL11, and CXCL12.
in renal disease. Complement activation and subsequent inflammation contributes to progressive kidney disease. Also, given that CCL16 can activate dendritic cells and T cells to further contribute to inflammation in renal disease, blockade of C5 and CCL16 signaling or their receptors may be important in ameliorating the initiation and/or reducing the progression of renal disease.

NU35

Markers of Injury, PLD4 and Rho Gtpase-Activating Proteins, Are Increased in Kidney Tissue From Felines
Kiran S. Panickar – Hills Pet Nutrition Center; Selena Tavener – Hills PNC; Regina Hollar – Hills PNC; Sukhaswami Malladi – Hills PNC; Dennis Jewell – Hills PNC

A progressive decline in kidney function is a key characteristic of kidney disease. Independent of the cause of kidney disease, one of the pathologies associated with kidney disease is fibrosis. Another feature associated with renal dysfunction is the formation of calcium oxalate stones formed from the crystal deposits in the tubular epithelial cells. The aim of the present study was to investigate the gene expression profile in renal cortex obtained from cats at necropsy in order to identify biomarkers of renal dysfunction for better insight into markers of fibrosis and injury. At time of death the circulating levels of creatinine as well as symmetric dimethyl arginine (SDMA), both markers of kidney decline in cats, were significantly higher in cats with renal disease (n=11) or stone-forming cats (CaOx, n=12) when compared to controls (n=19). Using RNAseq in kidney tissue, we found a significant increase in the expression of phospholipase D4 (PLD4), a key glycoprotein involved in renal fibrosis in cats with kidney disease (6.62 fold) and stone formers (6.67 fold) compared to controls (both p < 0.0001). However, some differences between the two groups were also observed including differences in the expression of Rho GTPase activating genes. Rho proteins encoded by ARHGAP family genes are negative regulators of Rho family GTPases. Importantly, RhoA family proteins are essential in the proper functioning of the glomerular filtration barrier under basal conditions, but both, increased or decreased RhoA activity, can result in podocyte dysfunction. While Rho GTPase activating protein 30 (ARHGAP30) and ARHGAP9 were significantly increased in both kidney disease and stone formers compared to controls, ARHGAP45 was decreased in kidney disease but not stone formers and ARHGAP15 was increased in stone formers but not kidney disease. Further, a decrease in NPHS2, a gene that encodes for podocin, a podocyte junction protein, was observed in kidney disease when compared to controls (−3.22 fold; p < 0.05). Podocytes are highly differentiated cells that are important in maintaining glomerular permeability. There was also a 6.5 fold increase in the expression of succinate receptor 1 (SUCNR1) in renal disease but not stone formers when compared to controls (p < 0.001). Activation of SUCNR1 located in the kidney has been hypothesized to play an important role in renovascular hypertension. Our results indicate important differences in the pathogenesis of kidney dysfunction in cats with kidney disease or stone formers. While an important marker of renal fibrosis was observed in both groups, other important differences in gene expression suggest that the optimal nutritional therapy may be different.

NU36

Protective Effects of Antidesma acidum on Endothelial Nitric Oxide Synthase in Doxorubicin-Induced Feline Kidney Cells
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Chronic kidney disease (CKD) is prevalent in cats and is associated with nitric oxide (NO) deficiency caused by a lack of endothelial nitric oxide synthase (eNOS). The present study aims to investigate the protective effects of the ethanol crude extract of Antidesma acidum, a flavonoid-rich extract, on eNOS protein and gene expression in doxorubicin (DOX)-induced feline kidney cells. A. acidum was analyzed the free radical scavenging capacity by using a diphenylpicrylhydrazyl (DPPH) assay. Feline kidney cells were incubated for an appropriate time with a dose of DOX and A. acidum in pretreatment conditions, after which a cytotoxicity assay was conducted and eNOS protein levels and gene expression were measured by using western blot analysis and relative gene expression. A. acidum extract concentrations of 0.1 to 100 μg/ml were not toxic to feline kidney cells, and we next selected treatment of feline kidney cells with 8 μM DOX at 48 hours, which resulted in 50% reduction in cell viability. An appropriate dose and time of DOX. Pretreatment with A. acidum can significantly increase eNOS gene and protein expression in DOX-induced feline kidney cells. A. acidum may protect feline kidney cells from CKD by increasing the eNOS gene and protein production. Further study is needed to investigate the protective effects of A. acidum in cats with naturally occurring CKD.

NU37

Fecal Fatty Acids in Cats with Chronic Kidney Disease and Correlation to Gut-Derived Uremic Toxins
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Cats with chronic kidney disease (CKD) have been documented to have intestinal dysbiosis and elevated serum concentration of gut-derived uremic toxins indoxyl sulfate (IS) and p-cresol sulfate (pCS). Additional metabolites of colonic bacteria that could be disrupted by intestinal dysbiosis are fatty acids. The primary aim was to evaluate fecal fatty acids in cats with CKD. The secondary aim was to correlate fecal fatty acid concentrations to serum IS and pCS concentrations. In this prospective cross-sectional study, voided fecal samples and serum were collected from client-owned cats with International Renal Interest Society (IRIS) stage 2-4 chronic kidney disease (n=30) and healthy older (≥ 8 years) control cats (n=11). Fecal concentrations of
short-chain fatty acids (acetic acid, propionic acid, butyric acid) and branched-chain fatty acids (isobutyric acid, isovaleric acid, valeric acid) were measured using gas chromatography-mass spectrometry. Serum concentrations of IS and pCS were measured by liquid chromatography tandem mass spectrometry. Mann-Whitney U test (CKD vs healthy cats) and Kruskal-Wallis test (IRIS CKD stage 2, IRIS CKD stages 3 & 4, healthy cats) with Dunn’s post-hoc analysis were used to compare fecal fatty acid concentrations between groups. A Spearman correlation coefficient (rho) was computed to evaluate the association between serum IS and pCS concentrations and fecal fatty acid concentrations in CKD cats (n = 10) and healthy control cats (n = 28). A P value < 0.05 was considered significant. No significant differences were found in fecal concentrations of acetic acid, propionic acid, butyric acid, isobutyric acid, and valeric acid. Fecal concentrations of isovaleric acid were significantly increased in CKD cats compared to healthy controls (P = 0.018). When compared between stages of CKD, IRIS CKD stages 3 & 4 cats had significantly higher (P = 0.033) fecal concentrations of isovaleric acid compared to healthy controls, however there was no significant difference between IRIS CKD stage 2 cats and healthy controls (Table). Isovaleric acid (rho, 0.35; P = 0.03) and valeric acid (rho, 0.45; P = 0.005) correlated to pCS, but isobutyric acid (rho, 0.29; P = 0.08) did not. Fecal short-chain fatty acid concentrations did not correlate to pCS. None of the fecal fatty acids correlated to IS. In conclusion, feline CKD is associated with increased fecal isovaleric acid, in particular IRIS CKD stages 3 & 4 cats. Fecal isovaleric acid and valeric acid concentrations were positively correlated to serum pCS concentrations supporting malassimilation of protein in cats with CKD.

**O01**

**Phase I Dose Escalating Study of Oral Cyclophosphamide in Tumor-Bearing Cats**

Catherine M. Chan – University of Missouri / Queensland Veterinary Specialists; Kenneth Rassnick – Veterinary Medical Center of CNY; Angela Frimberger – Veterinary Oncology Consultants; Sandra Nguyen – Small Animal Specialist Hospital; Antony Moore – Veterinary Oncology Consultants

Cyclophosphamide is an alkylating agent used to treat cats with lymphoma, carcinomas and sarcomas. Despite its use, no clear consensus exists regarding appropriate feline clinical dosage of oral cyclophosphamide. Toxicities are rarely reported at current dosages of oral cyclophosphamide (200 to 300 mg/m²).

The purpose of this study was to perform a modified phase I dose escalation study of oral cyclophosphamide to determine the maximum tolerated dose (MTD) in tumor-bearing cats.

Prospective clinical trial involving forty client owned tumor-bearing cats with predominately lymphoma. The cyclophosphamide dosage was escalated by approximately 10% in cohorts of at least six cats (specifically 300, 330, 360, 400, 440 and 480 mg/m²).

The MTD of oral cyclophosphamide in this study appeared to be 440 mg/m² with an inter-treatment interval of two to three weeks, however further data is ongoing to determine if cats can tolerate treatment at 460 mg/m². Hemogram is recommended one, two and three weeks after first cyclophosphamide treatment, and immediately before each subsequent dosage of cyclophosphamide (or any potentially myelosuppressive chemotherapy agent). The DLT was neutropenia (which can be delayed). This higher dosage was safe in combination with prednisolone and L-asparaginase; but has not been evaluated (and thus should not be substituted at this higher dosage) in combination with other chemotherapy agents such as CHOP protocol. Further evaluation of tumor response and cumulative toxicity data to this higher dosage of cyclophosphamide will be of interest.

**O02**

**Adrenergic Receptor Antagonists Decrease Bone Sarcoma Cell Viability and May Enhance Radiosensitivity with Sustained Treatment**

Megan Duckett – University of Minnesota; Shee Kwan Phung – University of Minnesota; Linh Nguyen – University of Minnesota; Ali Khammanivong – University of Minnesota; Erin Dickerson – University of Minnesota; Kathryn Dusenbery – University of Minnesota; Jessica Lawrence – University of Minnesota

Expression of adrenergic receptors (AR) have been demonstrated at several sites of primary and metastatic tumor growth and they may impact biologic processes such as proliferation, survival, metastasis, and angiogenesis. AR antagonists like propranolol and carvedilol inhibit proliferation, induce apoptosis in a concentration-dependent manner, and may synergize with some chemotherapy agents in some cancers. Radiation resistance is mediated in many cells by upregulation of pro-survival pathways, which may be influenced by ARs. Studies evaluating AR antagonists combined with radiation are limited; thus, the purpose of this study was to determine the effect of AR inhibition on radiosensitivity in a panel of biologically aggressive canine and human sarcoma cell lines. The hypothesis was that propranolol and carvedilol would increase radiosensitivity in both canine and human sarcomas, thereby supporting preclinical investigation in a canine model of spontaneous disease.

This study evaluated the *in vitro* effect of propranolol, a non-specific β-AR antagonist, and carvedilol, a dual β-AR and α1-AR antagonist, on viability and radiosensitivity in four primary bone sarcoma cell lines: human osteosarcoma (HOS), canine osteosarcoma (OSCA32, OSCA40) and human Ewing sarcoma (A673).

Immunoblot analysis confirmed AR expression in both human and canine sarcoma cell lines. Single agent propranolol or carvedilol inhibited cell viability in all cell lines in a concentration-dependent manner, measured by MTS assay. Notably, the mean inhibitory concentrations (IC50) for carvedilol in sarcoma cells were approximately 4-fold lower than propranolol and may be clinically relevant in dogs. Clonogenic survival analysis did not demonstrate increased bone sarcoma cell kill when cells were treated with propranolol or carvedilol prior to radiation compared to radiation alone. In contrast, prolonged exposure to low-dose carvedilol significantly decreased clonogenic survival after radiation compared to radiation alone in an aggressive canine osteosarcoma cell line.
Results suggest sustained dual AR inhibition may provide a novel method of increasing sarcoma cell kill when combined with radiation. Further investigation is needed to clarify the mechanistic effects of AR antagonism in neoplastic cells.

O03

Molecular Prevalence of Bartonella, Babesia, and Hemotropic Mycoplasma Species in Dogs with Hemangiosarcoma

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Hemangiosarcoma (HSA), a locally invasive and highly metastatic endothelial cell neoplasm, accounts for over two-thirds of all cardiac and splenic neoplasms in dogs. Infection with Bartonella spp. has been reported in association with both neoplastic and non-neoplastic vasoproliferative lesions in animals and humans, and a previous study documented a high prevalence of Bartonella spp. DNA in splenic tissue from dogs with HSA (26% of cases). However, the presence of Bartonella spp. in splenic tissue could potentially be explained by the spleen’s role in removal of hemotropic parasites from systemic circulation, or by Bartonella spp. bacteremia. Therefore, investigation of the association between Bartonella spp. and HSA in other tissues, as well as in systemic circulation, is needed to clarify the role of this genus of bacteria in dogs with HSA.

The objective of this study was to determine the prevalence of Bartonella spp. in conjunction with two other hemotropic pathogens, Babesia spp. and hemotropic Mycoplasma spp., in tissues and blood samples from dogs with histopathologically diagnosed HSA. The hypotheses were: 1) the prevalence of Bartonella spp. infection in dogs with HSA will be greater than the prevalence of Babesia or hemotropic Mycoplasma spp., and 2) the prevalence of Bartonella spp. infection in dogs with HSA in the spleen will be similar to prevalence in dogs with HSA in other anatomic locations, such as cardiac muscle.

Surgical biopsy or post-mortem fresh frozen tissues and whole blood samples from dogs diagnosed histopathologically with HSA were generously provided by the biospecimen repository of the Canine Comparative Oncology and Genomics Consortium. Each blood and tissue sample was tested by qPCR for the presence of Bartonella spp., hemotropic Mycoplasma spp., and Babesia spp. DNA. For each of the 107 dogs included in this study, two tissue samples and one whole blood sample was tested by qPCR.

Eighty-nine dogs had splenic tumors (10 stage 1, 39 stage 2, and 40 stage 3), 17 had cardiac tumors, and one had a subcutaneous tumor. Tissues collected were not required to be from the primary tumor location, so samples analyzed included 82 spleen, four liver, two kidney, two pericardium, and 17 other or unreported sites. Of the 107 dogs, Bartonella spp. DNA was amplified and sequenced from 79 tissue samples (74%). In contrast, tissues from seven dogs were PCR positive for hemotropic Mycoplasma spp., a significantly smaller proportion (7%, p < 0.0001). Bartonella spp. DNA was not amplified from any whole blood specimen. Hemotropic Mycoplasma spp. DNA was amplified from whole blood of two dogs. Babesia spp. DNA was not amplified from any whole blood or tissue specimens. There was no significant difference in Bartonella spp. tissue prevalence based on the location of the tumor, with 82% of dogs with cardiac HSA PCR positive for Bartonella DNA compared to 72% of dogs with splenic HSA (p = 0.552). Bartonella henselae was found most commonly, in 77 tissue samples (72%); Bartonella koehlerae was found in four samples, three of which also had B. henselae.

While use of archived specimens precluded systematic sampling of particular organs of interest, this study identified a high prevalence of Bartonella spp. DNA in various anatomic organ sources in dogs with HSA. These findings are supportive of the need to further investigate the role of Bartonella in the development of HSA.

O04

Protease-Activated Receptor-2 is Associated with Adverse Outcomes in Canine Mammary Carcinoma

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Protease-activated receptor-2 (PAR-2) is a G protein-coupled receptor that is activated by serine proteases. In humans, PAR-2 is expressed in breast cancer cells and associated with tumor progression and metastasis; however, the significance of PAR-2 in canine mammary carcinoma remains unclear. The purpose of this study was to examine the expression of PAR-2 in dogs with mammary carcinoma and the role in tumor progression.

Mammary carcinoma tissues were obtained from 31 dogs. All tumors were surgically removed at the Veterinary Medical Center of the University of Tokyo from December 2009 to December 2015 and diagnosed based on histopathology. Immunohistochemistry for PAR-2 was conducted on 4-μm thick paraffin-embedded sections. To evaluate the effect of PAR-2 activation on the migration and invasion of tumor cells in vitro, scratch assay and matrigel-coated transwell invasion assay were performed using a canine mammary carcinoma cell line (CIPp), respectively. To evaluate actin polymerization, phalloidin staining and western blotting for cellular F/G actin ratio were performed.

PAR-2 immunoreactivity was not observed in normal mammary glands. In contrast, the PAR-2 immunoreactivity was found in cytoplasm of tumor cells in dogs with mammary carcinoma and the staining intensity was different from each case. Of the 31 dogs with MC, 7 (23%) cases were PAR-2 negative (score 0), 11 (35%) were weak expression (score 1+), 9 (29%) were moderate expression (score 2+), and 4 (13%) were strong expression (score 3+). To investigate the association of PAR-2 expression with clinical characteristics and outcomes in dogs with MC, each case was classified as the high (score 2+ and 3+) or low (score 0 and 1+) PAR-2 expression group. The overall survival of the cases with high PAR-2 expression was significantly shorter than that of the cases with low PAR-2 expression. Moreover, the PAR-2 expression level was significantly associated with the presence of lymph node involvement and advanced clinical stage. PAR-2 activation significantly enhanced migration and invasion activity of CIPp cells. Phalloidin staining showed that F-actin fluorescent intensity was increased in CIPp cells with PAR-2 agonist. Western blotting confirmed that PAR-2 activation increased F/G actin ratio, suggesting actin polymerization.
These results suggest that PAR-2 is expressed in tumor cells and high expression of PAR-2 is associated with adverse outcomes in dog with mammary carcinoma. PAR-2 signal may induce migration and invasion of tumor cells by induction of actin polymerization, facilitating the metastasis in canine mammary carcinoma.

O05

Prognostic Factors in Dogs with Pulmonary Carcinoma Treated with Surgery plus or minus Adjuvant Chemotherapy
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This retrospective study evaluated outcomes and prognostic factors of dogs with primary pulmonary carcinoma (PCa) treated with surgery plus or minus chemotherapy. We reviewed canine medical records, where histopathologically-confirmed PCa was diagnosed in resected lung lobes at the Japan Small Animal Cancer Center between April 2005 and April 2018. Signalment, coughing, tumor size, TNM classification, surgical margins, histopathologic features, and adjuvant chemotherapy administration were prognostic factors. The Kaplan-Meier method measured survival, and statistical significance (P < 0.05) was assessed with the log rank analysis for univariate analysis and the Cox proportional hazard model for multivariate analysis. Thirty-six dogs without distant metastases were evaluated. Thirteen dogs received carboplatin (n = 11), doxorubicin (n = 1), or combination of carboplatin and doxorubicin (n = 1). The median progression-free survival (PFS) and overall survival (OS) were 506 days (95% CI: 222 to 936 days) and 716 days (95% CI: 40 mm), T-stage (stage 1 vs. 2 or ≥ 3), histopathologic invasion and/or satellite nodules determined PFS. Tumor size (40 mm), histopathologic invasion and/or satellite nodules determined OS. Incomplete surgical margins were the sole PFS prognostic factor (P = 0.007) by multivariate analysis. Dogs receiving chemotherapy did not have longer OS compared to those that did not have chemotherapy (1587 versus 506 days; P = 0.237). Our study dogs had longer PFS and OS compared with those of other studies. The efficacy of adjuvant chemotherapy should be evaluated prospectively with larger population.

O06

Outcomes with Conventional Fractionated and Stereotactic Radiotherapy for Suspected Heart-Base Tumors in Dogs
Katherine Hansen – UC Davis VMTH; Michael Kent – UC Davis VMTH; Alain Thean – UC Davis VMTH

Published results of radiation treatment for suspected heart-based tumors in dogs are limited. In this retrospective longitudinal study, eight dogs with either clinical signs attributable to a heart-base mass, or a mass that was progressing in size, were treated with conventional fractionated radiotherapy (CRT) or stereotactic body radiotherapy (SBRT). Two dogs had progressively enlarging masses on echocardiogram but were otherwise asymptomatic. Clinical findings in symptomatic cases included at least one of the following: retching/coughing (4), exercise intolerance (2), collapsing episodes (1), pericardial effusion (2), abdominal effusion (1), dyspnea due to chylothorax (1). Cases treated with CRT received 2.5 Gy X 20 fractions, while SBRT cases were treated with 6 Gy X 5 or 8 Gy X 3 fractions. At analysis, 7/8 dogs were deceased, and one dog was living 230 days (d) post-treatment. The median overall survival (OS) from first treatment was 785 d (95% CI 114-868 d [range 114-1492 d]). Five dogs received 50 Gy via CRT (OS 817 d; (95% CI 155 d – not reached [range 155-1492 d])). Three dogs received SBRT with one alive at analysis (OS 414 d [95% CI 114 d – not reached [range 114-414 d]]). A statistically significant difference was not found between survival for CRT and SBRT. Of the six patients with clinical signs, five showed improvement per the owner or clinician. Five cases had at least one follow-up ultrasound, and four showed reduction in mass size. This study provides preliminary evidence that radiation may benefit clinically affected or progressive cases with heart-base masses.

O07

Identification and Monitoring of Doxorubicin-Induced DNA Modifications as Biomarkers for Improving Veterinary Chemotherapy
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Chemotherapy treatment in veterinary patients aims to maintain a good quality of life during therapy while extending patient survival. A strategy to reach this goal is through the implementation of personalized, patient-oriented treatment protocols, which can be developed by monitoring drug-specific biomarkers. Many commonly used chemotherapeutic drugs, such as doxorubicin (DOX), base their cytotoxic activity on the formation of DNA modifications (adducts), which trigger cell death. Because the degree of interaction of the drug with DNA plays a central role in its mechanism of action, drug-induced DNA adducts may be used as biomarkers to predict patient outcome and toxicity profiles and allow individual dose adaptation. The purpose of this study was to characterize the DNA adducts formed by DOX to identify potential biomarkers for clinical development.

We developed a high-resolution/accurate mass (HRAM) LC-MS-based DNA adductomic approach to screen and monitor for DOX-induced adducts. The approach is based on data-dependent triggered MS2 fragmentation events for the most abundant full scan ions, and confirmation of DNA adduct identities by triggered MS3 fragmentation events upon observation of accurate mass neutral losses of common DNA adduct features. We applied our approach to screen for DNA adducts in DOX exposed purified DNA isolated from calf thymus and in 15N-labeled DNA generated from E. coli.
Our approach enabled detection of 23, 37 and 24 potential adducts induced by DOX alone or in the presence of formaldehyde and paraformaldehyde, respectively. These masses are now being targeted in DNA isolated from DOX-exposed cells and DNA isolated from the blood of dogs treated with DOX. These results will be used for investigating the relationship between DNA adduct levels and cell viability or therapy response with the potential of developing tools to support personalized medicine protocols.

O08

Upregulation of the Hedgehog Pathway and Associated Anti-Apoptotic Factors Can Be Inhibited by Itraconazole in Canine Osteosarcoma Cell Lines (VCS Award Winner)
Dana R. Connell – University of Illinois; Holly Pondenis – University of Illinois; Anne Bärger – University of Illinois; Timothy Fan – University of Illinois

Osteosarcoma (OS) is the most common primary bone cancer in canines, yet therapies for pet dogs with metastatic OS remain clinically ineffective. With the advent of precision medicine, strong scientific and clinical impetus exists to further investigate druggable molecular perturbations that might contribute to canine OS pathology. Dysregulated activity of the hedgehog (HH) pathway has been identified in diverse cancers in people and animals, and leads to overexpression of anti-apoptotic proteins including Bcl-2. Itraconazole is an antifungal agent that can inhibit Smoothened (SMO), a crucial G protein-coupled receptor located on the primary cilium, which initiates HH pathway signaling. We hypothesize that components of the HH pathway are overexpressed in canine OS cell lines with concurrent Bcl-2 overexpression relative to normal osteoblasts. Additionally, itraconazole would inhibit HH pathway signaling by promoting SMO relocation off the primary cilium.

RNA transcript and protein expressions of SMO and Bcl-2 were characterized in 4 canine OS cell lines and normal canine osteoblast cultures. The IC50 concentrations of itraconazole in OS cell lines were determined, and itraconazole’s effects on the HH pathway signaling partners were evaluated at RNA and protein levels. OS cell lines overexpress SMO and Bcl-2 relative to normal osteoblasts. The IC50 of itraconazole in OS cells ranged from 490-790 nM. Itraconazole effectively perturbs protein expressions of the HH signaling pathway and downstream targets. SMO and Bcl-2 are overexpressed in OS cells and itraconazole can disrupt the HH signaling pathway at biologically-relevant concentrations, warranting further investigation of itraconazole as an adjuvant therapy for OS.

O09

A Randomized Double-Blind Trial Investigating the Efficacy and Safety of a A New Polyamine-Vectorized Anticancer Drug (F14512) for Treatment of P-Glycoprotein Overexpressing Non-Hodgkin Lymphoma in Dogs (VCS Award Winner)
Pierre Boyé – Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Roslin, United Kingdom; Franck Floch – Oncovet; François Serres – Oncovet; Quentin Pascal – Oncovet-Clinical-Research (OCR); Juliette Hordeaux – Oncovet-Clinical-Research (OCR); Laurent Marescaux – Oncovet; Nicolas Guibaud – Institut de Recherche Pierre Fabre; Bruno Gomes – Institut de Recherche Pierre Fabre; Dominique Tierny – Oncovet-Clinical-Research (OCR)

Introduction: F14512, a new polyamine-vectorized epipodophyllotoxin core drug (etoposide), has shown antitumor efficacy in multidrug-resistance cancer cell lines, superior to the parent drug etoposide. The objective of this study was to compare the safety and efficacy of F14512 and etoposide phosphate in dogs with spontaneous non-Hodgkin lymphoma (NHL) and investigate the potential benefit of F14512 in P-glycoprotein (Pgp) overexpressing lymphoma.

Methods: Prospective, randomized, double-blinded study. Dogs were stratified regarding prior treatment received and randomly assigned to receive F14512 or etoposide phosphate, over an 8 week-period protocol (1 cycle every 2 weeks). Pgp-expression was assessed using immunohistochemistry adapted from Dhaliwal et al., 2013. Response assessment and adverse events (AEs) were evaluated according to VCOG criteria.

Results: Forty-eight client-owned dogs with intermediate/high-grade NHL were randomized to receive F14512 (n = 25) or etoposide phosphate (n = 23). Twenty-eight (58%) dogs were classified as Pgp-positive (high and moderate); 15 dogs received F14512, 13 dogs received etoposide phosphate. Median PFS were 86 days (4 - 224), 86 days (7 - 224) and 91 days (4 - 202) for all dogs, F14512-group, and etoposide phosphate-group, respectively. F14512 demonstrated a significant improvement in PFS in dogs with Pgp-positive lymphoma compared to etoposide phosphate (139 days [16 - 224] vs. 91 days [4 - 168]; p = 0.049). Hematologic AEs were more frequent in F14512 group (p = 0.001). Digestive AEs were more frequent in etoposide phosphate-group (p = 0.049).

O10

Expression and Genetic Variation of Apoptosis Inhibitor of Macrophage in Canine Histiocytic Sarcoma
Mona Uchida – The University of Tokyo; Kazuyuki Uchida – The University of Tokyo; Hiroyuki Tani – Nippon Veterinary and Life Science University; Makoto Bonkobara – Nippon Veterinary and Life Science University; Shingo Maeda – The University of Tokyo; Tomohiro Yonezawa – The University of Tokyo

Canine histiocytic sarcoma (HS) is a malignant tumor derived from macrophages or dendritic cells. We previously reported that apoptosis inhibitor of macrophage (AIM), a secretory protein of macrophages, induces apoptosis of HS cells in vitro. Hence, we hypothesized that decreased expression and/or dysfunction of AIM occur in HS. All specimens were utilized with the owner’s written consent. Immunohistochemistry and TUNEL staining of HS tissues were performed to observe AIM expression and apoptosis activity. The coding sequence of AIM was analyzed in the tumor and blood samples from the same HS dogs to detect AIM germline variants. The appearance rates of gene variants in the HS dogs were compared to non-HS disease control dogs. The intensity of AIM immunoreactivity was very weak or negative in around 30% of the HS dogs, while it was positive in almost 40% of them. The number of TUNEL positive cells showed no obvious correlation with AIM expression levels. Two major missense mutations of AIM gene were identified. The same sequences were detected from
the tumor and blood samples from the same dogs. The mutations appeared more frequently in the HS dogs than the non-HS dogs. AIM expression intensity was various and not related to apoptosis activity in the HS tissues, suggesting that AIM expression levels do not affect HS progression strongly. Meanwhile, the AIM missense mutations could be the germline variations and they were related to HS occurrence. It is suggested that these variants affect the function of AIM and HS tumorigenesis.

O11

Applicability of Canine Serum Thymidine Kinase 1 and C-Reactive Protein in Healthy and Solid Tumors

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Non-invasive biomarkers are used for diagnosis and prognosis/treatment monitoring for various tumors. The purpose of this study was to evaluate the serum thymidine kinase 1 (TK1) and C-reactive protein (CRP) concentrations in healthy and solid tumor dogs. Additionally, tumor occurrence index (TOI) between the healthy and solid tumor dogs was compared.

Serum samples from healthy dogs (n = 25) and dogs with solid tumors (n = 110) diagnosed histopathological examination included in this study. Dogs with inflammation, infection, and uncontrolled acute concurrent disease were excluded. Signalements, tumor types and locations were collected from the medical record. Solid tumors included epithelial-origin tumors (carcinomas, n = 71), mesenchymal-origin tumors (sarcomas, n = 28), and other-origin tumors (melanoma and mast cell tumors, n = 11). Serum TK1 protein and CRP levels were analyzed and compared among healthy and solid tumor groups. The TOI was calculated using logistic regression of these two biomarkers and evaluated among each group.

Serum TK1 and CRP levels of solid tumor dogs were significantly increased than those of healthy dogs. Among groups, carcinoma groups had the highest TK1 levels followed by other-origin solid tumors, sarcomas and healthy dogs (P = 0.000). Serum CRP value was highest in sarcomas followed by carcinomas, other-origin solid tumors, and healthy dogs (P = 0.000). Serum TK1 value was not related to tumor types within in each tumor groups. TOI was significantly different between healthy and solid tumor dogs (P = 0.000). The area under the receiver-operating characteristic curve was 0.948 for TOI versus 0.863 for TK1 (P = 0.0013) and 0.780 for CRP (P < 0.0000). The sensitivity and specificity for TOI were 81.8% and 100%, respectively. These results strongly suggest that measurement of serum TK1 protein, CRP, and calculating TOI could be an effective method for detection of solid tumors in dogs. The integration of these biomarkers were more effective and accurate detection of solid tumors from healthy dogs than either biomarkers were used alone.

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O12

Overexpression of Prostate Specific Membrane Antigen by Canine Hemangiosarcoma Cells Allows for Microscopic Disease Detection in Blood with Polymerase Chain Reaction (VCS Award Winner)

Matt Dowling – VCA Northwest Veterinary Specialists; Johnathan Samuelson – University of Illinois; Bahaa Fadl-Alla – University of Illinois; Holly Pondenis – University of Illinois; Mark Byrum – University of Illinois; Anne Barger – University of Illinois; Timothy Fan – University of Illinois

Introduction: Canine hemangiosarcoma (cHSA) is a metastatic tumor that disseminates by hematogenous and direct implantation routes. Therapies for cHSA are ineffective, partially due to advanced clinical stage at diagnosis. Sensitive methods for detecting microscopic cHSA could lead to more timely therapeutic interventions and improved outcomes. Prostate-specific membrane antigen (PSMA) is a transmembrane protein overexpressed by prostate carcinoma and tumor-associated endothelium of various solid tumors. Recently, PSMA has been qualitatively demonstrated to be expressed in cHSA, however, quantitative PSMA expressions and the potential utility of PSMA transcript identification in blood for confirming microscopic cHSA burden has not been reported. Therefore, study objectives are to determine if PSMA is quantitatively overexpressed in cHSA, and if PCR could be used to detect low levels of cHSA cells in blood.

Methods: Quantitative protein and gene expressions for PSMA were characterized in 1 normal endothelial and 6 cHSA cell lines by fluorescent microscopy and RT-PCR. Graded expressions of PSMA were determined in spontaneously-arising cHSA tumor samples. The sensitivity of PCR as a molecular diagnostic test to detect PSMA transcripts in blood from healthy and cHSA-bearing dogs were evaluated.

Results: PSMA gene and protein are overexpressed (up to 5-fold) in cHSA cells compared with non-malignant endothelium. PSMA ampli-cons can be identified in blood by conventional PCR, and correlate with the presence or absence of microscopic cHSA disease burden.

Conclusions: PSMA is quantitatively overexpressed in cHSA and detection of PSMA amplicons in blood as a method of detecting microscopic cHSA disease burden appears feasible with PCR methods and warrants further investigation.

O13

Genome-Wide Quantitative DNA Methylation Analysis in Canine Melanoma

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Malignant melanoma is one of the common cancers in the dog. It frequently occurs in the oral cavity with rapid growth, invasiveness, high rate of metastasis and prognosis is poor. Many clinical or pathological studies have evaluated the therapeutic or prognostic factor, but the outcome of treatment has not been improved and exact mechanisms are still unrevealed. Currently, epigenetic mechanisms that are changes in gene expression not involving changes to the underlying DNA sequence, have been focused in human medicine. Among epigenetic processes, DNA methylation that is the conversion of cytosine
to 5-methylcytosine at cytosine-guanine (CpG) dinucleotides, represses gene transcription, and methylation of CpG islands (CGIs) that are CpG-rich regions, located mostly in promoter regions of tumor suppressor-genes, leads to tumorigenesis. The purpose of this study was a genome-wide analysis of the methylation status in canine malignant melanoma.

The methylation specific signatures created by sequential digestion of genomic DNA with Smal and Xml enzymes are quantitatively analyzed by next-generation sequencer. Using 4 normal oral mucosa from healthy dogs and cells obtained from 4 canine melanoma cell lines, approximately 125,000-180,000 CpG sites across the dog genome were analyzed.

We analyzed 89,124 CpG site in common among 8 samples and found 3,275-5,021 hypermethylated sites in CGIs of melanoma cell lines compared with normal oral mucosa. In addition, some of these hypermethylated sites are located adjacent to the genes such as HOXA9 and HOXB13 in that hypermethylation was reported in human malignant melanoma. Next, we analyzed 16 spontaneous cases of canine oral malignant melanoma and identified 461 CpG sites in CGIs that were hypermethylated in more than half of the cases. Greater than 80% of these CpG sites were overlapped with hypermethylated CpG sites of each cell line.

The results of this study suggest that there was a large number of hypermethylated CpG sites also in canine malignant melanoma as in human and could be associated with tumorigenesis. These DNA hypermethylation were observed in common among melanoma cases and cell lines. The methylation status could be a diagnostic or prognostic marker in malignant melanoma.

O15

Biodynamic Imaging of Bone Marrow Aspirates from Tumor-Bearing Dogs as a Predictor of Chemotherapy-Induced Neutropenia: A Pilot Study (VCS Award Winner)

Blake Marcum – Purdue University; Zhe Li – Purdue University; John Turek – Purdue University; George Moore – Purdue University; David Nolte – Purdue University; Michael Childress – Purdue University

Neutropenia is the most common dose limiting toxicity in human and veterinary patients receiving cytotoxic chemotherapy. Biodynamic imaging (BDI) is a novel technology that measures light refraction frequencies to detect motion in tissue samples. It has been used previously in xenograft models to predict sensitivity to chemotherapy agents. We hypothesized that BDI performed on ex vivo samples from bone marrow aspirates would accurately predict neutropenia in dogs undergoing treatment with doxorubicin chemotherapy.

Bone marrow aspirates were obtained from the proximal humerus of patients prior to treatment with doxorubicin. A portion of the aspirate samples was placed in RPMI for biodynamic imaging. Macroscopic bone marrow particles were immobilized in 96-well plates using previously described techniques. BDI was performed with doxorubicin at 0.1 μm, 1 μm, and 10 μm along with DMSO as a control.

Patients were then treated with 30 mg/m2 doxorubicin intravenously over thirty minutes. CBC at the seven day nadir point were recorded. Correlation matrices were utilized to identify discriminatory breakpoints between BDI biomarkers and changes in neutrophil counts.

Ten client-owned dogs were enrolled. BDI was able to accurately predict breakpoints for patients with the largest proportionate change in neutrophils. BDI also predicted all three dogs with neutropenia that was Grade II or higher. BDI can be performed on bone marrow aspirates in order to predict neutropenia in patients receiving doxorubicin chemotherapy. This may be useful clinically to prevent undue adverse events in patients whose bone marrow would be more sensitive to chemotherapy.

O16

Pharmacokinetics of Bleomycin in Dogs Treated with Electrochemotherapy

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Bleomycin is a cytotoxic polypeptide antibiotic produced by Streptomyces verticillus. Its use in veterinary chemotherapy is very limited nowadays, recently a protocol describing its intralesional application for the treatment of achantomatous ameloblastoma in dogs has been proposed with good results, but to our knowledge, no other notable studies have been published. However, bleomycin is one of the two most commonly used cytotoxic drugs with electrochemotherapy (ECT), where it can be administered both intravenously or intratumorally. In electrochemotherapy, electric pulses are applied locally to the tumors to enhance the uptake of the drug due to the increased permeabilization of the cell membrane caused by electric pulses. When bleomycin in dogs treated with ECT is applied intravenously, the dose is 0.3 mg/kg body weight, delivered in slow bolus injection, and electric pulses are applied 8 to 10 minutes thereafter. This dose and timeframe are determined experimentally and result from the data of preclinical studies in rodents and clinical studies in humans, as there are no studies determining the pharmacokinetics of bleomycin in dogs. Therefore, the aim of our study was to determine the pharmacokinetic properties of bleomycin in dogs in order to define an effective therapeutic window for ECT.

In the preliminary part of our prospective study, we determined pharmacokinetic parameters of bleomycin in eight dogs with histologically different tumors that were treated with intravenously administered bleomycin (0.3 mg/kg body weight), followed by ECT. Having received a bolus injection of bleomycin, blood samples were collected in serum-separated tubes at time point 0 (before bleomycin administration), and 5, 10, 20, 30 and 60 or 120 minutes after bleomycin bolus injection. The samples were centrifuged for 10 minutes at 1300 rpm, the serum pipetted, frozen and kept at -20°C. The concentration of bleomycin in sera samples was determined by liquid chromatography coupled to high resolution mass spectrometry.

The results of the pharmacokinetic analysis showed a monophasic serum clearance curve of bleomycin in dogs. Following the intravenous injection (0.3 mg/kg body weight) the mean serum concentration measured 5 minutes after injection was 1.62 ± 0.13 μg/ml. The plasma bleomycin concentration declined with an elimination constant
(k_d) of 0.03 min^{-1} and it reached the mean serum concentration of 0.52 ± 0.06 μg/ml at 60 minutes after the injection. The elimination half-time was 22.56 min. The mean area under curve (AUC) was 78.75 μg min/ml, the mean volume of distribution (Vd) 167.68 ml/kg and the mean total body clearance (CL) 5.23 ml/min/kg. To summarize, the preliminary results of our study showed that bleomycin has a monophasic elimination curve in dogs after a bolus intravenous injection. The determined half-life of the drug was 22.56 min, which justifies the 20-minute interval for pulse delivery, as specified in the Operating procedures of the electrochemotherapy for treatment of tumor in dogs (Tozon et al., 2014). The low volume of distribution could be due to bleomycin’s previously reported difficult passing through the cell membranes and therefore low sequestration within peripheral tissues. However, the relatively short half-life and plasma clearance could suggest sequestration of bleomycin and therefore its prolonged cytotoxic effects in the tumor tissue, that is only in the tissue where electric pulses are applied. This is in accordance with several preclinical studies, where electroporation increases bleomycin uptake up to a thousand-fold into the tumor tissue, which allows excellent local tumor control and lower probability of systemic side effects.

O17

Expression of Hedgehog Signaling and Inhibition at the Level of Smoothened in Canine Osteosarcoma Cells

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Osteosarcoma (OSA) is the most common malignant bone cancer in dogs. Canine and human OSA share several features, including tumor environments, response to traditional treatment, and several molecular pathways. Hedgehog (Hh) signaling contributes to tumorigenesis and progression of various cancers, including human OSA. This study aimed to identify the role of the Hh signaling pathway in canine OSA cell lines, including Abrams, D17, and Moresco, focusing on the signal transducer Smoothened (SMO).

The mRNA and protein expression levels of Hh components in three canine OSA cell lines were compared with those in normal canine osteoblast cells, as assessed by quantitative reverse transcription polymerase chain reaction and western blot analysis. CCK-8 assay, crystal violet staining, and annexin V/PI flow cytometry were performed to determine the effect of SMO inhibitor cyclopamine on cell viability, colony formation, and apoptotic cell death.

Figure 1

Figure 2
mRNA and protein levels of SHH, IHH, SMO, and PTCH1 were aberrant in all examined OSA cell lines compared with canine osteoblast cells. Cyclopamine significantly decreased cell viability and colony-forming ability in the canine OSA cell lines in a dose-dependent manner. Moresco cells, which expressed the highest level of SMO protein, were the most sensitive to the anticancer effect of cyclopamine among the three canine OSA cell lines tested. Hh downstream target gene and protein expression in canine OSA cell lines were downregulated after cyclopamine treatment. In addition, cyclopamine significantly increased apoptotic cell death in Abrams and Moresco cells. In conclusion, this study revealed that Hh/SMO signaling is activated in canine OSA cell lines and cyclopamine suppresses OSA cell survival via inhibition of SMO. The results are expected to be helpful for investigating the etiopathogenesis of canine and human OSA and developing new treatments for canine OSA.

O18

Health-Related Quality of Life of Dogs Treated with Electrochemotherapy and/or Interleukin-12 Gene Electrotransfer

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Quality of life (QoL) in veterinary medicine has many different proposed definitions, but broadly represents the aspects of an animal's life that make that life better or worse for that specific animal. Health-related quality of life (HRQoL) defines the specific effect of a medical condition on an individual's health. In oncology, HRQoL represents the effect of cancer and its treatment on body function and well-being. Obtaining information about HRQoL can aid in decision-making, provide prognostic information, improve owner-veterinarian interaction and evaluate the impact of new treatments in clinical trials. Electrochemotherapy (ECT) is an efficient method for local tumor control that combines the use of chemotherapy and electroproporation. Electroporation can also be used for introduction of plasmid DNA into the cells (gene electrotransfer, GET). In oncology, one of the most promising is GET of plasmid encoding interleukin-12 (IL-12), which has local and systemic effects on various tumors. The purpose of this study was to evaluate the owners' perception of HRQoL of dogs and cats, treated with ECT alone or in combination with IL-12 GET and/or surgery. The owners of 41 dogs with histologically different tumors, treated with ECT alone or in combination with IL-12 GET (non-invasive treatment) and/or surgery (invasive treatment), were offered the "Cancer Treatment Form" full of the very practical questions (58.5%). Majority of the dogs' owners (85.4%) would opt for the therapy again, regardless of the financial costs. In conclusion, our results show that the majority of the owners of dogs assessed their dogs' quality of life as good and improved after the treatment, especially in dogs that were treated with non-invasive treatment (without surgery) and in those that responded to the treatment.

OT01

Molecular Genetically Defined Canine Ehlers Danlos Syndromes

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Background: Ehlers Danlos Syndromes (EDS) are a group of diseases characterized by tissue fragility, skin hyper-extensibility, and articular hypermobility. Objective: Evaluate the clinical presentation, molecular genetics, and ultra-structural abnormalities in dogs with EDS. Methods: Whole genome sequences (WGSs) were generated for 9 dogs with EDS. Light and electron microscopy revealed dermal collagen fibril morphology in a skin biopsy from one affected dog. Results: Causal variant alleles were identified in 4-of-9 dogs with EDS. Three were heterozygous variants in COL5A1: a single-base deletion and frameshift (9:50826530delT) in a Pit Bull Terrier; a single-base deletion and frameshift (9:50830985delC) in a Scottish Terrier; and nonsense mutation (9:50808554C>T) in a Golden Retriever. These variants caused a relatively mild form of EDS with articular hypermobility and lax, hyperelastic, fragile skin that accumulated atrophic scars. A more severe form of EDS resulted from a
homozygous nonsense mutation ADAMTS2 \((11:2408978C>T)\) that occurred in a 2-month-old Doberman with hypermobile carpal, tarsal, and stifle joints with intermittent pain and effusion. The skin was hyper-elastic and extremely fragile. This dog was euthanized after minimal trauma resulted in a severe degloving injury to the dorsum. Conclusion/clinical importance: The identification of the molecular genetic causes for individual canine EDS cases will lead to more precise prognoses, management practices, and breeding recommendations.

**OT02**

**Measurement of Volatile Sulfur Compounds in Canine Saliva Using Gas Chromatography**  
**Julie K. Spears – Nestle Purina; Brittany Vester Boler – Nestle Purina**

Volatile sulfur compounds (VSC), the major component of breath malodor, are produced through microbial fermentation of sulfur amino acids present in saliva, sloughed epithelial cells, remaining food particles, and blood. Salivary putrefaction in vitro assays have been used in humans to evaluate VSC production. The objective of this study was to determine if a saliva putrefaction in vitro assay can be used in canines to evaluate the impact of breath malodor interventions. Fresh unstimulated saliva was collected from healthy adult dogs, pooled, and homogenized. Substrates (honey, spirulina, and a honey:spirulina combination) were anaerobically incubated in duplicate with pooled saliva at 37°C. Tubes containing water only were inoculated with saliva to determine VSC produced with no additional ingredients. Following a 24-hour incubation, headspace samples were collected from each tube for VSC determination (methyl mercaptan, dimethyl sulfide, hydrogen sulfide, and total VSC) via gas chromatography (OralChroma). Average of each VSC produced in water only tubes were subtracted from values of ingredients of interest. Compared to water, more hydrogen sulfide was produced with honey and spirulina alone, while the combination produced less. Spirulina alone produced large amounts of methyl mercaptan, while honey alone and the combination produced less. Dimethyl sulfide levels were lower than water for each ingredient alone, but greater than water with the combination. Overall, total VSC were lowest with honey:spirulina and honey. These results indicate the in vitro saliva putrefaction assay can be used in canines to detect differences in VSC production.

**OT03**

**Morphological Changes in the Round Window Membrane and Cochlea in Chinchillas with Otitis Media**  
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The round window membrane (RWM), semi-permeable to antibiotics, bacterial toxins, and inflammatory mediators, separates middle ear and scala tympani of the cochlear basal turn of inner ear. Changes of RWM can result in hearing loss. The object of this study was to assess any morphological and structural changes of RWM and cochlea by 2D and 3D measurements in acute otitis media (AOM). Study materials consisted of temporal bone specimens of Chinchillas obtained from the Temporal Bone Collection of the University of Minnesota Otopathology Laboratory. Experimental group consisted of 6 animals with AOM induced by intrabullar injection of Streptococcus pneumoniae 7F and then euthanized 7 days after inoculation while control group consisted of 6 healthy animals. The thickness \((mean \pm std)\) of the RWM was significantly \((P < 0.05)\) increased in the experimental group \((42.02 \pm 28.5 \mu m)\) compared to those \((17.05 \pm 3.4 \mu m)\) in the control group by 2D measurements taken from three different points of RWM. Moreover, the volume \((mean \pm std)\) was significantly \((P < 0.05)\) increased in the experimental group \((0.106 \pm 0.038 \text{mm}^3)\) compared to those in the control group \((0.066 \pm 0.008 \text{mm}^3)\) by 3D measurements of RWM. Spiral ligament area was significantly \((P = 0.015)\) decreased in the cochlear basal turn in the experimental group compared to the control group while stria vascularis area was significantly \((P > 0.05)\) decreased in all turns in the experimental group. A significant \((P < 0.05)\) loss of the outer hair cells was determined in all cochlear turns in the experimental group while the loss of inner hair cells was significant \((P < 0.05)\) in the lower and upper basal, and the lower middle turns in the experimental group compared to the control group. The number of spiral ganglion cells in the experimental group was decreased significantly \((P = 0.015)\) compared to the control group. The results of present study indicated that S. pneumoniae 7F induced AOM in Chinchillas leads significant changes in both RWM and cochlea suggesting a possible association between AOM and hearing loss.

**OT04**

The Effect of Client Complaints on Veterinary Support Staff  
**Lisa A. Murphy – Veterinary Specialty Center of Delaware; Charles Rogers – Veterinary Internal Medicine of NE; Colleen Tansey – Inland Veterinary Specialists; Kylee Malouf – Park Animal Hospital; Rachel Natsume – Vet Villa Animal Hospital; Briana Ward – Orrell Animal Hospital; Ruth Murphy – Psychiatric Department of Beaumont Hospital; Reid Nakamura – Idexx Laboratories**

The purpose of this study was to investigate the effect of client complaints on veterinary support staff (VSS) welfare, their job satisfaction and how they perceive such effects on veterinarians affect the way they practice veterinary medicine. An anonymous survey was modified from a previous veterinary study and distributed online via two Facebook groups specific to veterinary technicians. Only veterinary technicians, veterinary assistants, kennel staff and receptionists were eligible to participate. The survey was available from January 15th to March 15th 2019. 170 surveys are currently available for review. 73% of respondents had received a client complaint within the preceding 6 months with the cost of care the most common reason. The majority of respondents (62%) worried to varying degrees about a client complaint being made against them. Almost 65% reported being verbally assaulted by a client within the preceding six months and 33% reported being threatened with litigation.
Similar to a previous study evaluating client complaints on veterinary internists, client complaints are a common source of distress for VSS. However, it appears that VSS report a higher rate of verbal assault than small animal veterinary internists. This study shows that VSS report high levels of stress associated with their career and that the wider veterinary community needs to evaluate ways to mitigate these stressors for VSS going forward.

**P01**

**The Effects of Grapiprant on Acute Pain and Inflammation Following Ovariohysterectomy in Dogs**

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Grapiprant, an EP4 prostaglandin E2 (PGE2) receptor antagonist, is FDA approved only for the management of osteoarthritis pain in dogs. There are almost no oral analgesic options available for dogs that cannot tolerate NSAIDs, especially for acute pain such as that associated with soft tissue surgery. The objectives of this study were to compare the analgesic efficacy of grapiprant to carprofen for the treatment of post-operative ovariohysterectomy (OVH) pain and inflammation in dogs.

12 adult female intact beagle dogs, two to five years of age, were randomly assigned to one of two treatment groups: oral grapiprant (2mg/kg) or carprofen (4.4mg/kg), two hours prior to surgery and every 24 hours for three total doses. An ultrafiltration probe was placed within the OVH incision to collect interstitial fluid (ISF). Pain at the incision site was assessed by masked investigators via mechanical nociception threshold testing (MNT) and the Glasgow Composite Pain Scale (CMPS-sf) before drug administration and at multiple time points for 72 hours following dosing and surgery. ISF samples were collected to assess PGE2 concentrations, measured via ELISA. Data was analyzed using 2-way ANOVA. There were significant differences in both CMPS-sf scores (p < 0.001) and MNT (p = 0.003) over time when compared to baseline, but no significant difference between treatment groups. PGE2 concentrations in ISF were higher in dogs receiving grapiprant compared with carprofen (p < 0.001).

This study supports the use of grapiprant for post-operative pain following OVH in dogs; however, additional studies are warranted to determine its effectiveness in a larger population of dogs.

**P02**

**In Vitro Investigation of Feline Transdermal Gabapentin**

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Pain identification and treatment can be extremely challenging in the domestic cat as they are notoriously food selective, especially when medications are added. Cats also have an exquisite fight or flight mechanism when they are approached with the unwelcome administration of medications. This can consequently harm the human administering the medication and also the human animal bond between the person and the cat. Therefore, non-invasive mechanisms for chronic drug delivery to our feline patients is absolutely clinically necessary. Gabapentin is a popular neuropathic pain medication used in human and veterinary medicine. It is available in various oral formulations and has recently been evaluated as a human transdermal medication; however there have been limited published transdermal studies evaluating its use in the feline species. The purpose of this study was to investigate whether transdermal gabapentin at various concentrations administered in a commercially available and commonly used proprietary base, (Lipoderm®) permeates feline skin in an in vitro setting.

Fresh feline cadaver skin (IACUC ASAF # 6190) approximately 2.5 cm x 2.5 cm obtained from the ears and shaved cervical region was collected and assembled into static Franz-Type Diffusion cells (PermeGear®) in a warm water bath at 102°F. Pre-formulated gabapentin at concentrations of 6% (6 mg), 10% (10 mg) and 20% (20 mg) total dose applied initially and then again at 12 hours, obtained from a USP-FDA pharmacy was applied topically to the different skin samples via the donor chamber of the Franz-Diffusion cell. The experiment was performed in triplicate. One hundred microliters of phosphate buffered saline solution located in the receptor channel was collected for gabapentin quantification at various time points; 0, 2, 4, 12, and 24 hours after transdermal gabapentin application. The samples were immediately frozen at -80 °C and sent for final drug concentration analysis utilizing a validated HPLC mass-spectrometry method.

Results showed sequential increases of gabapentin levels for time points at 2, 4, 12, and 24 hours after transdermal application to both the ear and shaved cervical skin of cats for all concentrations tested. The results of this study suggest that gabapentin penetrates feline ear and cervical skin under experimental in vitro conditions. Future in vivo studies are planned to test this hypothesis in clinical patients.

**P03**

**Evaluation of Multiple Doses of a Long-Acting Oral Opioid Containing an Abuse Deterrent in Dogs**

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The purpose of this study was to assess in dogs a long-acting oral opioid formulation containing a deterrent to human abuse/misuse. We hypothesized opioid effects would be maintained throughout the dosing for both treatments. Previous studies have documented the combination of methadone/-fluconazole/naltrexone provides at least 12 hours of opioid effect when administered as a single dose to dogs previously administered fluconazole. The speed of onset in dogs not pre-administered fluconazole and the effects of multiple doses have not been determined.
Twelve healthy Beagle dogs were divided into two equal groups using a parallel study design. Group 1 received methadone:fluconazole:naltrexone at 1: 5: 0.25 mg/kg repeated ~12 hours later followed by 0.5: 2.5: 0.125 mg/kg approximately q12h PO for a total of 4 doses. Group 2 received methadone:fluconazole:naltrexone at 1: 5: 0.25 mg/kg followed by 0.5: 2.5: 0.125 mg/kg at approximately 4, 10 and 24 hr for a total of 4 doses.

Rectal temperature (previously correlated to analgesia in dogs) was significantly decreased from baseline in both groups (P < 0.05) throughout the dosing (including after the initial dose) and for 12 hours after the last dose (except 1 time point in Group 1). Baseline and subsequent von Frey measurements were variable in both groups, but significant antinociception occurred in both groups including after the first dose. Mean methadone plasma concentrations exceeded 10 ng/mL throughout the dosing.

In conclusion, significant opioid effects and antinociception occurred in dogs after multiple doses of a long-acting oral opioid formulation containing an abuse deterrent.

**P04**

Comparison of the Pharmacokinetics of Two High Doses of Intravenous Ascorbic Acid in Healthy Dogs

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High-dose ascorbic acid is used as alternative treatment in human cancer, but not in veterinary medicine. Additionally, the pharmacokinetics of high-dose intravenous ascorbic acid in veterinary medicine have not been studied. Therefore, the aim of this study was to evaluate pharmacokinetic parameters and safety of high-dose ascorbic acid after intravenous infusion in healthy dogs.

Six healthy beagle dogs were administered intravenous ascorbic acid at two separate doses of 1.5 g/kg and 3 g/kg for 4 hours. The blood and urine samples collected before and after administration were assayed by high-performance liquid chromatography-tandem mass spectrometry. Plasma concentrations in both groups reached peak levels at 3 hours after administration and remained above the minimum therapeutic concentration for 10 hours. Plasma concentration remained higher at 3 g/kg dose than at 1.5 g/kg. The difference in urine pH between the two groups was not significant. High-dose intravenous ascorbic acid was well-tolerated in all dogs. The results support that high-dose intravenous ascorbic acid is safe in dogs and can be beneficial in treating cancer in dogs.

**R01**

Pharmacokinetics of a Modified, Compounded Theophylline Product in Dogs

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Theophylline is a commonly used bronchodilator drug for treatment of chronic canine bronchitis, but no formulations validated in dogs are currently available. An oral, modified, compounded theophylline product (MCT), which could fulfill this need, is available through a USP-compliant, veterinary compounding pharmacy; however, its pharmacokinetic properties are unknown. Therefore, the aim of this study was to determine the pharmacokinetics of MCT and to recommend an appropriate dosing regimen for use in dogs. Plasma drug concentrations were measured in seven healthy, fed dogs after single doses of intravenous aminophylline (IVA, 8.6 mg/kg theophylline equivalent) and oral MCT (10 mg/kg). Plasma concentrations were analyzed by liquid chromatography/tandem mass spectrometry. Administration of IVA best fit a 2-compartment model. The mean ±SD systemic bioavailability of the MCT was 96.2 ±32.9%. MCT mean ±SD time to maximum concentration, absorption time, and terminal half-life were 8.85 ± 3.63, 6.95 ± 3.42, and 8.67 ± 1.62 hours, respectively. Using a 12-hour dosing interval, significant drug accumulation is expected (accumulation ratio 1.62 ± 0.18). Based on simulations of 10 mg/kg q 12 hr dosing, steady state plasma theophylline concentrations are expected to exceed the minimum therapeutic concentration commonly used in dogs (10 mg/mL) for 71.7 ± 35.6% of the dosing interval. Overall, the MCT had high oral bioavailability in most dogs and a time-concentration profile suggesting twice daily dosing at 10 mg/kg may be an appropriate initial therapeutic dosing regimen. Follow up, multi-dose studies are indicated to evaluate plasma drug concentrations at steady state.
Use of Doxycycline with or without Famciclovir in Kittens with Acute Upper Respiratory Tract Disease

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This study aimed to assess effects of antimicrobial administration in kittens with acute upper respiratory tract disease. Twenty-four kittens with clinical signs of acute upper respiratory tract disease were randomly allocated to receive doxycycline (5 mg/kg PO every 12 hours) alone (Group D: n=12) or with famciclovir (90 mg/kg PO every 12 hours; Group DF: n=12) for up to 3 weeks. Clinical disease severity was scored at study entry and daily thereafter. Oculo-oropharyngeal swabs collected at study entry and exit were assessed using quantitative PCR for nucleic acids of feline herpesvirus type 1 (FHV-1), feline calicivirus (FCV), Chlamydia felis, Bordetella bronchiseptica, and Mycoplasma felis. Median (range) age of cats was 1.5 (1-6) months in Group D versus 1.6 (1-5) months in Group DF (P = 0.54). Pathogens detected in oculo-oropharyngeal swabs at entry included FCV (13/24; 54%), M. felis (8/24; 33%), FHV-1 (7/24; 29%), C. felis (7/24; 29%), and B. bronchiseptica (3/24; 12%). Mean (± SD) duration of clinical signs was 12 (± 6.2) days in Group DF and 11 (± 5) days in Group D (P = 0.75). Median (range) total disease score at the end of the study did not differ between groups D [1 (1-1)] and DF [1 (1-3); P = 0.08]. This study revealed no significant difference in response to therapy between cats treated with doxycycline alone or with famciclovir. However, isolation of FHV-1 was relatively uncommon in this study and clinical trials focused on FHV-1-infected cats were warranted to better evaluate effects of famciclovir.

Categorization of Inflammatory Airway Disease in Cats

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Inflammatory airway disease in the cat is sometimes described as asthmatic (eosinophilic) or bronchitic (neutrophilic), however this ignores cats with mixed inflammation and requires collection of airway fluid for characterization. The purpose of this study was to identify clinical features that could aid differentiation of these disease types without need for bronchoscopy or bronchoalveolar lavage (BAL) analysis. Medical records of cats that had bronchoscopy performed between 2011-2018 were retrospectively reviewed. Cats were categorized as having eosinophilic, neutrophilic or mixed inflammation based on BAL differential cytology as follows: eosinophilic (eosinophils >20% with neutrophils 50%); neutrophilic (neutrophils >14% and eosinophils 14% or discordant inflammation from 2 BAL sites). Clinical parameters compared included presenting complaints, signalment, BCS, respiratory rate, complete blood count, bronchoscopy, and BAL (% recovery, total nucleated cell count, differential cell count). Ninety-nine cats had bronchoscopy. Fifty were diagnosed with inflammatory airway disease with 23 eosinophilic, 14 neutrophilic, and 13 mixed. Cough was the predominant presenting complaint in all groups. Respiratory rate and effort did not differ. Cats with eosinophilic inflammation were significantly younger (4.4 ± 3.3 years) than those with neutrophilic (8.0 ± 5.6 years) or mixed inflammation (7.5 ± 4.0 years), P = 0.03. No other clinical parameters were significantly different among groups.</p>

This study found substantial overlap in clinical findings in cats with various forms of inflammatory airway disease. Only age differentiated cats with eosinophilic airway disease from those with neutrophilic or mixed inflammatory airway disease.

Feline Bronchorrhea: a Retrospective Study of 18 Cases (2012–2017)

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Chronic bronchopulmonary diseases in cats with rattling sound and airway hypersecretion through bronchoscopy have often been experienced in respiratory clinic, yet the clinical information is limited. The feline disease is comparable to human bronchorrhea. This study aimed to describe clinical picture of the feline bronchorrhea. We reviewed 18 cats with over 1-month episodes of intermittent rattling, moist cough, respiratory efforts, pulmonary infiltrates or interstitial pattern on chest X-rays, and airway hypersecretion diagnosed with bronchoscopy between 2012 and 2017. We compared the survival time and QOL during the follow-up period (good/fair/poor) among therapies prescribed using a chi-square test, and P < 0.05 was considered statistically significant.

In this study, Russian blue (7/18) and American short hair (5/18) cats were predisposed. The median age was 8.5 (6–13) years. In addition, rattling on admission (11/18), infrequent moist cough (3–10 events/day, 8/18), rapid and labored breathing (13/18), increased breath sounds on chest auscultation (12/18), mild hypoxemia (mean arterial partial pressure of oxygen: 69.5 mmHg) were representative. Chest X-ray densities were predominant on the right posterior lung fields (11/18), including diffuse patchy pulmonary infiltrates (6/18), pulmonary hyperinflation (7/18), and broncholithiasis (4/18). Three of six cats that were undergone chest computed tomography (CT) revealed consolidation within multiple cysts (<3.0 cm in diameter). One cat revealed an enlargement on thoracic lymph nodes on CT and died 2 days after the diagnosis. In all cats, bronchoscopy revealed marked airway secretion (low viscous: 16; thick: 2). Transbronchial lung biopsy samples in eight cats were malignant in two and benign in six. Bronchoalveolar fluid cytology in nine cats revealed mainly foamy macrophages (median: 67.5%), with elevated neutrophils (median: 22.3%). Bacterial cultures in airway samples were negative in 16, insignificant in 2, and positive in none of the cats. Lobectomies including the primary lesions in five
selected cats revealed interstitial pneumonia in two, bronchioloalveolar carcinoma in two, and adenocarcinoma in one. The 60-day survival rate was 72.2%. The median survival time (MST) was 361 days in 16 cats that were prescribed feasible combined therapies, whereas 9 days in 2 cats prescribed no therapy. The MST in cats (n = 5) received lobectomy was significantly longer than that in cats (n = 13) not received lobectomy (596 vs. 222 days; P < 0.05). Furthermore, "Age on admission < 10 years" and "no enlargement on thoracic lymph node on CT" significantly correlated with good or fair QOL (P < 0.05). Feline bronchorrhea has distinguishing clinical characteristics and a relatively poor prognosis. Early identification, initiation of even a feasible therapy, and, if possible, lobectomy could enhance the prognosis. The pathology and etiology of the disease needs to be explored concurrently.