2020 ACVIM Forum On Demand Research Report Program

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Whole genome sequencing to explore genetic risk factors in canine myxomatous mitral valve disease

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Canine myxomatous mitral valve disease (MMVD) is the most common acquired cardiac disease in dogs, with cavalier King Charles spaniel (CKCS) being particularly overrepresented. Almost all CKCS >10 years of age are affected, suggesting that genetic risk factors may be “fixed” in this breed. MMVD is believed to be a polygenic trait, and previous genetic work in canine MMVD has employed a case-control design within individual breeds. However, this design precludes the identification of disease-associated variants that are “fixed” within high-risk breeds and may result in only minimal genetic differences between cases and controls being identified. The aim of the present study was to identify new canine MMVD genes as potential future targets for prevention or treatment of disease. This was achieved by applying a new model for identification of genetic risk variants in complex disease, using whole genome sequencing (WGS) of breeds at relatively high and low risk of disease. Additionally, genetic differences between CKCS with early onset MMVD that died due to congestive heart failure (group C) and CKCS with late-onset MMVD that died due to noncardiac disease (group NC) were explored. Whole genome sequencing (WGS) at 30× coverage was undertaken on 12 CKCS, 6 in group C, and 6 in group NC. Results were compared with WGS from 48 non-CKCS breed dogs, mainly sequenced as part of the Canine Diabetes Genetics Partnership initiative. DNA was extracted from blood samples that were surplus to requirements for clinical purposes. A custom bioinformatics pipeline was used to call, annotate, and prioritize variants for follow-up, based on the Genome Analysis ToolKit, using CanFam3.1 as a reference. Variants were prioritized according to a range of criteria, including their alternate allele frequency (by breed or disease severity status), predicted impact on gene function, or location near a region with a plausible role in cardiac disease. In CKCS, 1993 breed-unique high or moderate impact variants were
A dilated cardiomyopathy (DCM) in dogs

Short-term outcome of a prospective study of diet-associated cardiomyopathy (DCM) after diet change. Dogs with DCM [M-mode fractional shortening (FS) ≤25%, normalized LVId ≥1.8, and normalized LVIDd (nLVIDd) ≥1.2] eating either a nontraditional or traditional diet were enrolled in a 9-month study. Echocardiography, ECG, high-sensitivity cardiac troponin I (cTnI), NT-proBNP, and taurine were measured at baseline. Dogs were treated with cardiac medications as clinically indicated, diet was changed, and echocardiography and biomarker analyses were repeated after 3, 6, and 9 months. As of January, 2020, 59 dogs have been enrolled (36 M/23 F). Median age = 7.7 years (2.6-15.9 years) and weight was 32.0 kg (3.8-97.8 kg). Enrolled breeds included Doberman Pinscher (n = 14), Golden Retriever (n = 7), Pit Bull (n = 6), and Boxer (n = 6), but multiple other breeds were represented. Dogs were ACVIM stage C (n = 45) or stage B (n = 14). Fifty-one of 59 dogs (86.4%) were eating nontraditional diets. Six of 59 dogs had mildly reduced plasma (n = 3) or whole blood (n = 3) taurine concentrations. To date, 41 of 42 dogs still alive were reevaluated at 3 months (23 at 6 months, 16 at 9 months). At 3 months, dogs originally eating nontraditional diets had increased FS (P < .001) and decreased nLVIDs (P = .003), left atrium: aorta (P = .002), NT-proBNP (P = .04), and cTnI (P = .003) compared to baseline. Three in 9 traditional diet dogs were alive at 3 months. Overall, 27 dogs died or were euthanized [12/27 (44.4%)] due to sudden death.

Evaluation of radiographic predictors of left heart enlargement in dogs

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We sought to determine the diagnostic accuracy of vertebral left atrial size (VLAS), vertebral heart size (VHS), and radiologists’ assessment for predicting left atrial enlargement (radLAE) in a large population of dogs with and without echocardiographic left atrial enlargement (echoLAE). Dogs that had an echocardiogram and thoracic radiographs performed within 24 hours were consecutively enrolled provided they did not have pericardial effusion or a cardiac mass. Echocardiographic left atrial enlargement was defined by a left atrium to aortic ratio acquired from short-axis (LA/AoSx) and long-axis (LA/AoLx) >1.68 and >2.54, respectively. Radiographic assessments were blinded to echocardiographic assessments and vice versa. Cutoffs for VLAS and VHS were generated using receiver operating characteristic analyses. Logistic regression was utilized to identify associations with echoLAE. One-hundred eighty-three dogs were enrolled. Dogs were diagnosed with myxomatous mitral valve disease (56%; 35 stage B1, 35 stage B2, and 33 stage C/D), other cardiovascular diseases (25%) or no cardiovascular disease (19%). Prevalence of EchoLAE was 63%. Optimal cutoffs for VLAS (>2.3 vertebral; sensitivity [Sn] 88.9%; specificity [Sp] 73.3%) and VHS (>11.1 vertebral; Sn = 74.6%; Sp = 75.8%) were generated and compared with radLAE (yes/no). Vertebral left atrial size >2.3 (area under the curve [AUC] = 0.81) was more accurate than VHS >11.1 (AUC = 0.75) and radLAE (AUC = 0.76) for predicting echoLAE. Vertebral left atrial size >2.3 and radLAE (both P < .001) were independently associated with echoLAE but VHS >11.1 was not (P = .054). Vertebral left atrial size is a useful radiographic measurement for predicting echoLAE, particularly if echocardiography or a radiologist’s assessment is unavailable.

Short-term outcome of a prospective study of diet-associated dilated cardiomyopathy (DCM) in dogs

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The objective of this study is to evaluate serial changes in echocardiography and cardiac biomarkers in dogs with suspected diet-associated dilated cardiomyopathy (DCM), after diet change. Dogs with DCM (M-mode fractional shortening (FS) ≤25%, normalized LVId ≥1.8, and normalized LVIDd (nLVIDd) ≥1.2) eating either a nontraditional or traditional diet were enrolled in a 9-month study. Echocardiography, ECG, high-sensitivity cardiac troponin I (cTnI), NT-proBNP, and taurine were measured at baseline. Dogs were treated with cardiac medications as clinically indicated, diet was changed, and echocardiography and biomarker analyses were repeated after 3, 6, and 9 months. As of January, 2020, 59 dogs have been enrolled (36 M/23 F). Median age = 7.7 years (2.6-15.9 years) and weight was 32.0 kg (3.8-97.8 kg). Enrolled breeds included Doberman Pinscher (n = 14), Golden Retriever (n = 7), Pit Bull (n = 6), and Boxer (n = 6), but multiple other breeds were represented. Dogs were ACVIM stage C (n = 45) or stage B (n = 14). Fifty-one of 59 dogs (86.4%) were eating nontraditional diets. Six of 59 dogs had mildly reduced plasma (n = 3) or whole blood (n = 3) taurine concentrations. To date, 41 of 42 dogs still alive were reevaluated at 3 months (23 at 6 months, 16 at 9 months). At 3 months, dogs originally eating nontraditional diets had increased FS (P < .001) and decreased nLVIDs (P = .003), left atrium: aorta (P = .002), NT-proBNP (P = .04), and cTnI (P = .003) compared to baseline. Three in 9 traditional diet dogs were alive at 3 months. Overall, 27 dogs died or were euthanized [12/27 (44.4%)] due to sudden death.

Insights from corrected chloride calculation in dogs with congestive heart failure

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Hypochloremia associated with congestive heart failure (CHF) in dogs is likely multifactorial. Loop diuresis causes 1:2 sodium (Na+): chloride (Cl−) loss, whereas free water retention (FWR) causes 1:1 Na+:Cl− dilution. We hypothesized that serum Cl− corrected for Na+ would approximate measured Cl− (mCl−) in most CHF dogs because of loop diuresis, but that Cl− would normalize after correction in some CHF dogs, indicating FWR. Dogs with preclinical heart disease served as controls (no diuretics, no FWR expected) for comparison to CHF dogs. The corrected Cl− (cCl−), mCl−, and their difference were compared between 71 preclinical and 100 CHF dogs, and between stage C (76) and stage D dogs (24), using the formula: cCl− = (mid-reference range Na+/measured Na+) × mCl−. Both mCl− and cCl− were lower in CHF vs preclinical dogs, and stage D vs stage C dogs (both P < .0001). The cCl− was higher than mCl− in CHF dogs (P = .014), but not preclinical dogs (P = .2). Median (10th-90th percentile) difference between cCl− and mCl− was 0.69 mmol/L (−2.16 to 3.4) for CHF dogs. This difference was greater for stage D vs stage C dogs (P = .0006). A diuretic resistance indicator (uNa:uK < 1.0) was found in 5 CHF dogs with >90th percentile increase in Cl− after correction. No hypochloremic CHF dogs had normal cCl−. Serum Cl− remained low after correction in hypochloremic CHF dogs, consistent with loop diuretic effects on electrolytes; however, some showed
notable increases in Cl\(^-\) after correction, supportive of concurrent FWR. Future study correlating cCl\(^-\) to serum antidiuretic hormone concentrations is warranted.

**NEUROLOGY**

A novel ultrasound-guided cerebral biopsy device  
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The objective of this study was the development and validation of a simple, low cost, disposable cerebral biopsy device visible with intraoperative ultrasound (IOUS). The designed biopsy device was first tested on nonanimal tissue, then on a cadaver. Clinical trials were conducted on 8 patients with intracranial lesions to obtain biopsy specimens for microscopic examination. The biopsy obtained from a nonanimal specimen was used to evaluate size of the sample. Cadaver samples were evaluated by microscopic examination. In the final phase of the project, samples were obtained and examined from 8 clinical patients after a standard craniectomy/craniotomy using IOUS guidance. Biopsy samples were successfully obtained from the cadaver and the clinical patients using the device. Microscopic examination of the 8 clinical samples by pathologists resulted in a histopathologic diagnosis representative of the intracranial lesions seen on advanced imaging and intraoperatively. Quality of the biopsy was measured by visual and ultrasound imaging evidence and the ability of pathologists to evaluate the sample and render a diagnosis based on the samples. Microscopic evaluation of formalin-fixed samples revealed representative tissues and were of diagnostic quality in all patients. The novel biopsy device provided high-quality, diagnostic samples with no observed negative effect on the patients either clinically or as determined by IOUS of the site postbiopsy. The device is disposable and offers a good alternative to other cerebral biopsy techniques. The novel cerebral biopsy device coupled with intraoperative ultrasound offers a simple, reliable method of obtaining quality biopsy in patients with intracranial masses.

Activity and sleep quality in idiopathic epileptic dogs compared to age- and breed-matched controls  
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The purpose of this study was to compare activity levels and sleep quality in dogs with idiopathic epilepsy to age- and breed-matched controls. The study population included 67 dogs diagnosed with idiopathic epilepsy between 1 and 9 years old that were receiving at least 1 antiepileptic drug (AED) and a seizure frequency of at least 1 seizure every 3 months. The control population consisted of age- and breed-matched control dogs with no diagnosed medical conditions that were not receiving any medications. Activity was measured using a canine-specific activity monitor (FitBarkTM), which was attached to the dogs' collars at all times. A sleep score was also calculated (as a percentage) by measuring the amount of activity (restlessness) during a specified 4-hour window of sleeping time (decided by the owner). Dogs with idiopathic epilepsy receiving AEDs had, on average, a 20% decreased in activity compared to the control population, which was statistically significant (P value = 1.24e-11). When breed was considered, epileptic Border Collies and German Shepherd Dogs on AEDs had significantly lower activity levels compared to controls (P values of .007 and .011, respectively). Sleep scores were also significantly different between the epileptic and control groups, with the epileptic dogs having better sleep quality (less restlessness) compared to the control population (P value = .033). Breed was not statistically significant for sleep score. In conclusion, epileptic dogs receiving AEDs are statistically less active and have better sleep quality compared to age- and breed-matched controls.

Architectonic-based cortical brain atlas for the canine  
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Rapid advancement of advanced neuroimaging techniques, data processing, and analysis in neuroimaging has allowed researchers to investigate the brain in novel ways. Stereotaxic cortical brain atlases divide the cortex into architectonically distinct regions, which are thought to correlate to function. They have become a vital tool for tissue segmentation and cortical localization and allow for a standardization of processing and analysis in functional, diffusion, and structural MRI techniques. Although architectonically based stereotaxic cortical brain atlases have been created for humans, rodents, primates and felines, to date, none have been made available for the canine. In this study, we created a canine brain atlas from high-resolution T1-weighted MRI data generated in vivo from 30 neurologically healthy, mixed-breed, mesaticephalic dogs. This atlas includes a population template and a cortical atlas. The effect of normalization of brain data from canine populations with differing skull shapes to the population template was tested. The cortical atlas was created by manual segmentation of the cerebral cortex to delineate regions with distinct myeloarchitecture. When dogs with differing skull shapes were normalized to the final population template, brachycephalics demonstrated the highest warping of neural structures. The myeloarchitectonic-based cortical parcellation resulted in generation of 108 cortical regions per hemisphere grouped into frontal, sensory-motor, perisylvian, parietal, cingulate, and occipital lobar areas. This study introduces a foundational tool to the field of veterinary neuroimaging, opening doors for novel advanced MRI techniques to be used to improve our understanding of neuroanatomy and neuropathology in the dog.

Corpus callosum diffusivity alteration is a component of healthy aging in the canine

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The aging brain is known to alter in structure, connectivity, and diffusivity in the human. Most notably the corpus callosum is observed to exhibit alterations in diffusivity values and, associated with this, right-left cerebral connectivity is reduced. The aging canine brain has been observed to reduce in volume; however, the effect of aging on corpus callosum diffusivity has not been evaluated. In this study, we applied the novel technique of voxel-based analysis to identify the extent and location of significant diffusivity abnormalities in the brain of healthy aged canines. Diffusion tensor imaging data were acquired from 20 neurologically and behaviorally normal mesaticephalic canines grouped into young (aged between 2 and 6 years) and old (aged between 10 and 11 years) cohorts. The data were skull stripped, corrected for noise, phase distortion, motion, and eddy-current distortion. Tensor metrics FA and MD were calculated and voxel-based statistics applied a 2-sample t test to identify significant differences between age groups. Family-wise error correction was used to correct for multiple comparisons. There were significant decreases in FA within the body and dorsal fibers for the corpus callosum in FA in the aged canine group when compared to the younger group. Similarly, there were significant decreases in MD in the corpus callosum in the aged group though these decreases were less widespread. Healthy aging is associated with decreases in FA and MD within the corpus callosum. This is similar to the diffusivity changes present within humans and may reflect alterations in structural connectivity between hemispheres.

ONCOLOGY

Application of the International Myeloma Working Group response criteria in dogs with multiple myeloma
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Current recommendations for monitoring disease progression and response to treatment of immunoglobulin producing tumors in humans includes evaluation of serum or urine paraprotein (M-protein) concentration, involved free light chains and tumor size. Densitometry, the preferred method for qualifying M-proteins in human medicine, has recently been validated for use in the dog and was shown to outperform Ig-class-specific radial immunodiffusion and ELISA-based methods. A retrospective pilot study was performed to evaluate the applicability of the human based International Myeloma Working Group (IMWG) response criteria in dogs with multiple myeloma. Archives from 2016 through 2019 were reviewed for canine cases with a diagnosis of multiple myeloma. M-protein documented by serum protein electrophoresis (SPE) and immunofixation (IF) initial sample, and subsequent electrophoretic evaluation electrophoretic evaluation of serial samples. M-proteins were quantified densitometrically from the electrophoretic study. When M-protein was not discernable on serial samples by SPE alone, repeat IF was performed, according to IMWG recommendations. Change in M-protein concentration was calculated and case progression was characterized using the IMWG response criteria. Available clinical history was reviewed. Survival was calculated as the time from initial electrophoretic evaluation to death or last known contact. Fifteen cases met inclusion criteria. This included a total of 69 SPE evaluations. A pretreatment sample was evaluated in 10 cases. All cases received some form of chemotherapy but this was not standardized. One case had a presumed solitary lesion with attempted surgical excision, but histopathology documented incomplete excision and chemotherapy was instituted. Total protein was within normal limits in 39 of 69 samples. Biochemically derived globulins were measured in 27 samples and were within normal limits in 13 samples. Complete response (CR, lack of M-protein by SPE and IF) was documented in 1 case. Six cases achieved very good partial response (VGPR, M-protein decreased by >90% but still detectable by IF) and an additional 4 cases met the criteria for a partial response (PR, 50%-90% M-protein reduction). The cases that attained CR, VGPR, or PR survived longer (median 630 days) than those that did not meet partial response criteria (M-protein change did not attain 50% decrease, median 333 days), log rank P = .013 (Figure 1). During the treatment, 4 cases had M-protein defined progressive disease (>25% and minimal 0.5 g/dL M-protein increase) which correlated with concurrent or subsequent clinical deterioration. The majority of canine cases met IMWG criteria for a VGPR or PR although it is notable that only 1 dog achieved a CR. Preliminary data indicate that evaluation of electrophoresis-based M-protein detection and quantification using the human response criteria is possible in dogs with multiple myeloma and suggests that response measured in this manner correlates with survival. Evaluation of a larger cohort of cases in a prospective study is warranted.

The selective inhibitor of nuclear export verdinexor exhibits biological activity against canine osteosarcoma cell lines
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Osteosarcoma (OS) is the most common malignant bone tumor in dogs. Despite aggressive surgical management and systemic chemotherapy, 90% of dogs still die due to chemotherapeutic-resistant metastatic disease. As chemotherapy remains the backbone for treatment of OS, the development of combinational treatments with novel targeted molecular therapeutics and conventional chemotherapy represents a potential strategy to enhance therapeutic response. Exportin 1 (XPO1, also known as CRM1) is a chaperone protein responsible for the export of >200 target proteins out of the nucleus. Dysregulation of XPO1 activity is documented in a number of human cancers and XPO1 expression has been associated with the development of chemotherapy resistance. Prior studies in canine melanoma and mammary carcinoma cell lines have demonstrated that XPO1 is a relevant target for therapeutic intervention and recent phase I and phase II clinical trials evaluating a novel, orally bioavailable Selective Inhibitor of Nuclear Export (Verdinexor) in dogs with spontaneous cancer demonstrate antitumor activity against non-Hodgkin lymphoma. In the present study, we sought to characterize the expression of XPO1 in primary canine OS tumor samples, OS cell lines and normal osteoblasts and evaluate the in vitro efficacy of Verdinexor alone or in combination with doxorubicin in canine OS cell lines. Real-time PCR and Western blotting were performed to assess XPO1 transcript and protein expression in normal canine osteoblast cells, canine OS cell lines and primary OS tumor tissues. Canine OS cell lines and a subset of primary OS tumors had increased expression of XPO1 mRNA and protein compared to normal canine osteoblasts. All canine OS cell lines exhibited dose-dependent growth inhibition and increased caspase 3,7 activity in response to low nanomolar concentrations of Verdinexor (IC50 concentrations ranging from 21 to 74 nM). Notably, growth inhibition of normal canine osteoblast cell lines treated with Verdinexor was only observed at high micromolar concentrations (IC50 21 µM). The combination of Verdinexor and doxorubicin resulted in potent inhibition of cell viability and demonstrated synergetic activity in 3 canine OS cell lines. Concordantly, OS cell lines showed increased DNA damage as assessed by γH2AX immunofluorescence following combination treatment with Verdinexor and doxorubicin compared to either single agent. These findings demonstrate that Verdinexor has biologic activity against canine OS cell lines at physiologically relevant doses and suggest that XPO1 inhibition in combination with standard doxorubicin treatment offers promising potential for chemotherapeutic intervention in canine OS.

SMALL ANIMAL INTERNAL MEDICINE

Effect of dilution on specificity of saline agglutination tests for diagnosis of immune-mediated hemolytic anemia

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Saline agglutination tests (SATs) are commonly used in the diagnosis of canine immune-mediated hemolytic anemia (IMHA), but frequent false positive results have been reported using a 1:1 saline to blood ratio. To determine if increased saline to blood ratios improve specificity, SATs were performed at a saline to blood ratio of 1:1, 4:1, 9:1, and 49:1 for 104 dogs with hematocrits ≤30%. SATs were considered positive if at least 1 of 5 randomly selected ×40 objective fields contained ≥1 aggregate of ≥4 erythrocytes or ≥2 aggregates of 3 erythrocytes. Of the 104 dogs, 5 were classified as affected by IMHA based on a positive direct antiglobulin gel test and clinical evidence of hemolysis. These criteria were not met in 96/104 dogs classified as unaffected by IMHA and 3/104 were excluded because IMHA status was unclear. Specificity for IMHA was 32% (95% confidence interval 22-43) for 1:1, 56% (45-66) for 4:1, 70% (60-79) for 9:1, and 95% (88-98%) for 49:1. Sensitivity was 100% (40-100) for 1:1 and 4:1 and 80% (30-100) for 9:1 and 49:1. Positive predictive value increased from 7% (6-8) for 1:1 to 44% (23-68) for 49:1. Negative predictive value was 100% at 1:1 and 4:1 and 99% at 9:1 and 49:1 (92-99). Diagnostic accuracy was 35% (25-46) for 1:1, 58% (47-67) for 4:1, 70% (60-79) for 9:1, and 94% (88-98) for 49:1. Based on these results, SATs should be performed at a dilution of at least 49:1 to improve specificity for diagnosis of IMHA.

Development and implementation of a hemovigilance program at a university veterinary teaching hospital

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Patient blood management (PBM) is an evidence-based multidisciplinary approach to optimizing the care of patients who may require a transfusion. One aspect of PBM is hemovigilance, which focuses on recognizing and reporting transfusion reactions. The potential for fatal consequences make the prevention, rapid recognition, and treatment of transfusion reactions a critical requirement for the safety of veterinary patients receiving blood products. The objective of this study was to describe the development and implementation of a small animal hemovigilance program at a university veterinary teaching hospital. A hemovigilance working group composed of veterinary specialists in clinical pathology, internal medicine, and emergency and critical care was established. This group developed evidence-based definitions of transfusion reactions, reaction classification systems, and a transfusion reaction reporting form. The reporting form contained sections for patient information, transfusion information, administration details, and reaction details. Reaction events were classified by reaction type, severity grade, and imputability to transfusion. Following implementation of the hemovigilance program, transfusion reaction data were collected and examined for the period spanning October 2014 and March 2019. During the study period 718 canine transfusions (4 whole blood, 400 packed red blood cell [pRBC], 300 fresh frozen plasma [FFP], 7 platelet rich plasma, 7 cryoprecipitate) and 124 feline transfusions (5 whole blood, 95 pRBC, 24 FFP) were administered. There were 32 total reactions (27 canine and 5 feline), with the most common reaction being febrile nonhemolytic transfusion reactions (19/32, 59%). The incidence rate of transfusion reactions was found to be 3.8% in dogs and 4.0% in cats. For the confirmed reactions, classification criteria for case definition, reaction severity grade, and imputability were able to be
Feline pneumonia: A 10-year retrospective
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Pneumonia is a poorly described disease in cats. Objectives of this study were to describe clinicopathologic, radiographic, and microbiologic features of affected cats. Medical records at the UC Davis Veterinary Medical Teaching Hospital were searched to identify cats with a clinical diagnosis of bronchopneumonia (BP) or aspiration pneumonia (AP). Results extracted from the record included signalment, exam findings, pertinent historical details, and presence of conditions suspected to be risk factors for aspiration. Diagnostics were collated including CBC, BALF analysis, and microbial culture. Radiographs were scored by a single, masked radiologist using a published rubric. Sixty cats with identified including 32 with AP and 28 with BP. Airway samples were obtained in 32 cats with BP and 6 with AP. Forty-four cats with inflammatory airway disease (IAD) were used as controls. Duration and prevalence of cough were significantly less in cats with AP (13 days, 8/32) compared to cats with BP (240 days, 27/28); P = .02, P < .0001, respectively) and respiratory rate was higher (43 vs 26, P = .007). Gastrointestinal disease was the most common risk factor associated with AP. Radiographically, 57 cats had evidence of disease. Cats with AP were more likely to have an alveolar pattern and higher total score than cats with BP or IAD and less likely to have a bronchial pattern. Mycoplasma (12) spp. were the most frequently cultured organisms from BALF in cats with BP. These results suggest that bacterial pneumonia should be suspected in cats with cough and severe radiographic infiltrates.

Canine influenza virus: Dog and person factors likely influencing transmission in canine show settings
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Canine influenza is an important disease-affecting dogs, especially in situations where many dogs come together (e.g., boarding, shows). Recent outbreaks of canine influenza have involved dog shows in the United States with anecdotally high levels of dog-dog transmission of canine influenza virus (CIV), resulting in dog illness and death, and wide-spread transmission into the general dog community. Despite the importance of this disease, little is known of modifiable factors linked to CIV spread in dogs participating in shows. Study objectives were to describe the knowledge, attitudes, practices (KAP) and observations related to CIV transmission and disease prevention by individuals participating in dog shows in the United States and Canada and pilot a voluntary surveillance tool to serve as an early warning mechanism to identify disease events linked to dog shows. Two questionnaires were developed, widely distributed through national and regional kennel club organizations and administered (May-Nov 2019) online to a large convenience sample of individuals that had ever shown at least 1 dog in the past, with a focus on those showing in Canada and the United Sates. The KAP questionnaire was completed by 2301 respondents, while the illness surveillance questionnaire was completed by 449 respondents (had participated in a show within the past 14 days). Overall, respondents were very/somewhat concerned (64%) that 1 of the dogs they show will become infected with CIV at a show. Despite this concern, respondents' knowledge of CIV was lacking in several key areas (eg, routes of transmission, infectious period) and high-risk practices frequently reported (eg, rarely/never immediately disinfect high contact items brought back from a show). Although a high proportion (>93%) of respondents reported all of their show dogs had received core vaccines (or had titers performed, as appropriate) in the past 3 years, a minority had received "lifestyle" vaccines in the past year (CIV [51%], Bordetella [60%], leptospirosis [40%], Lyme [20%]). Of the illness surveillance respondents, a minority reported that since the event 1 or more of the dogs they showed had developed cough (2%), diarrhea (7%), or vomiting (2%). Of these 44 ill dogs, 8 (18%) required veterinary care at which time Bordetella (n = 4) and CIV (n = 1) were diagnosed. A high proportion (39%) of the respondents that reported ill dogs shortly after their most recent event, stated that since the dog became ill additional dogs at their facility not at the event had also become ill, suggesting further transmission. This study suggests several concerning CIV knowledge gaps and high-risk practices among the dog showing community, but also a high level of motivation to make changes in practices. Although not definitive evidence for show-associated transmission, these findings are suggestive of such an occurrence and highlight the potential utility of this surveillance tool. Together, these findings can be used to inform veterinary recommendations for CIV prevention.

Canine leptospirosis in Canada, test-positive prevalence, and risk factors (2009-2018)
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In dogs, the incidence and seroprevalence of Leptospira spp. appears to be increasing, particularly in North America where it is considered a reemerging disease. Research on leptospirosis regional distribution and dog-related risk factors is limited in Canada and does not include currently used clinical testing methodology such as PCR. Study objectives were (a) evaluate the temporal and spatial distribution of canine leptospirosis in Canada, and (b) describe dog, geographic, and temporal risk factors for leptospirosis. This repeat cross-sectional study evaluated canine leptospirosis PCR testing (n = 10 437 dogs) submitted by Canadian veterinarians between July 2009 and May 2018. Logistic regression was used to identify significant risk factors for test-positive dogs. One or more positive leptospirosis PCR test results were reported from 880 dogs (8.4%). Number and proportion positive varied by year;
number of dogs positive ranged from 12 (2010) to 367 (2017), while proportion positive ranged from 4.8% (2011) to 14.2% (2017). There was regional variation with the proportion positive ranging from 1.0% (3/307; Alberta/Saskatchewan/Manitoba) to 18.5% (104/562; Nova Scotia). The final multivariable model identified male (OR = 1.3), young (≤ 1.0 year; OR = 2.1), toy breed (OR = 3.3), urban (OR = 1.3) dogs to be at significantly increased odds for leptospirosis. Significant 2-way interactions of season with both province and year highlighted the complex spatial and temporal influences on leptospirosis occurrence. This research builds on prior studies on canine leptospirosis and provides important new disease distribution and risk factor information for Canada. Our findings will inform veterinary recommendations for leptospirosis diagnosis and prevention in Canada.

Evaluation of antimicrobial prescriptions in dogs with suspected bacterial urinary tract disease
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Antimicrobial stewardship is getting increasing attention in veterinary medicine and assessment of antimicrobial use practices is a core component of stewardship activities. A retrospective review of antimicrobial prescriptions for dogs diagnosed with infectious urinary tract disease in veterinary practices in the United States and Canada was performed to evaluate antimicrobial prescribing. A total of 7803 antimicrobial prescriptions from 7387 dogs were evaluated; 7127 dogs from 673 clinics from the United States and 260 dogs from 50 clinics in Canada. The main clinical concerns were acute cystitis (n = 6582), recurrent cystitis (n = 428), and pyelonephritis (n = 326). Amoxicillin/clavulanic acid (2702, 41%), cefpodoxime (1024, 16%), and amoxicillin (874, 13%) were the most commonly prescribed antimicrobials for acute cystitis. The median duration was 12 days (range 3-60 days, interquartile range [IQR] 4 days). There was a significant difference in duration between 2016 and 2018, and 2016 and 2018 (both P < .001), with lower duration in 2018. Fifty-five percent (3634) dogs were treated with a recommended first line drug, rising from 52% (736/1410) in 2016, 53% (1348/2536) in 2017 and 59% (1550/2636) in 2018 (P < .0001). There were significant differences in prescription patterns and overall use of “highest priority critically important antimicrobials” between Canada and the United States, and between different U.S. regions. Amoxicillin/clavulanic acid (146, 33%), marbofloxacin (95, 21%), and cefpodoxime (65, 14%) were most commonly used for recurrent cystitis, with a median duration of 14 days (range 3-77, IQR 10.5). Amoxicillin/clavulanic acid (86, 26%), marbofloxacin (56, 17%), and enrofloxacin (36, 11%) were most commonly prescribed for pyelonephritis; however, 91 (28%) dogs received 2 antimicrobials and 2 (0.6%) 3 antimicrobials. The median duration of treatment was 14 days (range 3-77 days, IQR 11 days). Fluoroquinolones were prescribed significantly more often (P < .0001) to dogs with pyelonephritis compared to dogs with acute cystitis, while first (P = .018) and third generation cephalosporins (P = .009) and penicillins (P = .0007) were more commonly prescribed to acute cystitis cases. Decreases in duration and increased use of recommended first-line antimicrobials over the study period were encouraging but room for interventions to optimize antimicrobial use remains.

Carriage of extended spectrum beta-lactamase-producing E. coli and K. pneumoniae in healthy dogs and cats
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Antimicrobial resistance is a widely recognized concern in companion animal medicine. Fecal, oral, and aural culture swabs were obtained from 77 dogs and 32 cats at the time of routine spay/neuter surgery. Each swab was processed utilizing sequential selective media broths (MacConkey + cephalothin followed by MacConkey + cefalothin + ceftiofur) followed by MacConkey agar plating. Colonies phenotypically and morphologically consistent with Escherichia coli or Klebsiella pneumoniae were positively identified to species level with PCR, and a multiplex PCR protocol was utilized for identification of extended spectrum beta-lactamase (ESBL) genes. Numerous ESBL genes, including those from the ACC, CIT, CTM, EBC, FOX, OXA, TEM, and VIM families, were detected. Kirby Bauer antimicrobial susceptibility testing was performed on all isolates. Multidrug resistance was common in E. coli isolates as indicated by high resistance rates for tetracycline (76.8%), trimethoprim sulfamethoxazole (78.6%), enrofloxacin (37.5%), gentamicin (30.4%), and chloramphenicol (32.1%). No isolates were resistant to imipenem. Resistance to cefpodoxime was detected in 100% of E. coli and K. pneumoniae isolates. Escherichia coli and K. pneumoniae with confirmed ESBL characteristics were detected in 39% and 11.7% of dogs, and in 28.1% and 25% of cats, respectively. In total, an ESBL-producing E. coli and/or K. pneumoniae was detected in 53.2% of dogs and cats presenting for routine surgery. This alarmingly high prevalence of ESBL-producing E. coli and K. pneumoniae in apparently healthy dogs and cats suggests that there is an urgent need for improved antibiotic stewardship guidelines and compliance in the veterinary community.

Pharmacokinetics of gabapentin in cats with chronic kidney disease
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Gabapentin (20 mg/kg orally once to 2 hours prior to visit) has become popular as a medication prescribed to reduce stress and improve compliance in feline patients during veterinary visits. However, this dose may be inappropriate for elderly cats, specifically those with chronic kidney disease (CKD) as gabapentin is cleared only by renal excretion. Therefore, the purpose of this study was to assess the pharmacokinetics of gabapentin in cats with CKD in comparison to clinically healthy cats using a limited-sampling approach. Apparently, healthy cats and cats with stable IRIS stages 2 and 3 CKD were enrolled. Diagnostic tests required before enrollment included serum biochemistry profile, complete blood count, urinalysis, blood pressure, and serum total
The half-life was determined by calculating the rate of gabapentin elimination between the 3- and 8-hour time points. Comparison of half-life between the CKD and clinically healthy cats was performed with Mann-Whitney test and correlation between serum creatinine and gabapentin half-life was performed with the Spearman rank. Five apparently healthy and 9 CKD cats (4 IRIS stage 2, 5 IRIS stage 3) completed the study. CKD cats had a median age of 15 years (range 5-18) and a median serum creatinine 2.6 mg/dL (2.4-3.3). Apparently, healthy cats had a median age of 1.6 years (range 1-7) and a median serum creatinine 0.9 mg/dL (0.9-1.3). After administration of a single oral 10 mg/kg dose of gabapentin (median 10, range 9.6-10.3 mg/kg), CKD cats had a significantly longer median calculated half-life (13.1 hours, range 7.3-19.9) in comparison with normal cats (4.3 hours, range 3.9-6.9)(P = .001). Serum creatinine was significantly correlated with calculated half-life (P = .0002, r = 0.86). CKD significantly delays the clearance of gabapentin, and this information should be taken into account when prescribing to these patients.

Role of angiotensin II and prostaglandins in the renal function following an oral protein load

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Glomerular proteinuria can be a primary manifestation of kidney disease as well as an important factor involved in the secondary progressive parenchyma damage and functional impairment. Although there is relevant evidence for understanding the pathophysiology and therapeutic intervention, some questions are still waiting for answers. The objective of this study was to evaluate the effects of oral protein load on the glomerular and tubulointerstitial functions, considering the roles of renal prostaglandin and angiotensin II (AII), by means of pharmacological inhibition of the mechanisms involved. Seven intact healthy dogs (3 males and 4 females) were enrolled. They were medium-sized, adult, multi-mixed-breed dogs, with age (years) and body weight (kg), expressed as median (range), of 5 (3-6) and 13 (10-22), respectively. All of them were adapted to the experimental management. Three consecutive pairs of experiments were conducted at 7-day intervals. The first pair included a baseline assessment (control) and oral protein load (OPL) consisting of powdered egg white at 4.7 g/kg of body weight (partially rehydrated for consumption). For the second pair, the dogs were treated with flunixin meglumine, a nonsteroidal anti-inflammatory drug (NSAID), at 1.1 mg/kg, q 12 hours, for 2 days, and OPL on the second day (NSAID + OPL). For the third pair of experiments, the dogs were treated with enalapril maleate, an angiotensin-converting-enzyme inhibitor (ACEi), at 0.5 mg/kg, q 12 hours, and OPL on the second day (ACEi + OPL). Endogenous creatinine clearance (eCcr), urine protein-to-creatinine ratio (UP/C), urine output (UOP), sodium fractional excretion (NaFE), and serum and urine osmolality (sOsm; uOsm) were obtained during a 4-hour period of urine collection, after overnight 12-hour fasting followed by OPL when indicated. Water ad libitum was available during fasting and evaluation. Oral protein load increased (P < .05) glomerular filtration rate (GFR), even when subjects were under the effect of NSAID or ACEi, although, at a lower rate (P < .05). Urine output and NaFE increased (P < .05) as well, even under the ACEI effect. With NSAID the GFR did not change significantly, suggesting that prostanooids would not participate in the regulatory mechanism of glomerular filtration. However, renal hydrosaline regulation was modified, although OPL addition was effective in inducing a different response. The increase of GFR following ACEI inhibition evidenced a possible role of AII in maintaining a low GFR during fasting as observed in the baseline control. The association with the OPL, unexpectedly, resulted in a statistically nonsignificant increase in UP/C (range 0.1-0.4), that reached a pathological level, although the GFR increase was lower (P = .015) than obtained previously (only ACEi). The study presented, despite having a small n, raises a possible basis for further investigations that may explain and confirm the benefits of protein restrictions in CKD.

**TABLE 1** Variables of renal function from 7 healthy dogs under evaluated by Wilcoxon matched pairs signed rank test (basal vs each treated).

| Basal control data | median (range) | treatments          | OPL | NSAID | NSAID + OPL | ACEI | ACEI + OPL |
|--------------------|---------------|---------------------|-----|-------|-------------|------|------------|
| eCcr (mL/min/kg)   | 2.0 (1.2-2.8) | ↑                   | ns  | ns    | ↑           | ↑    | ↑          |
| UOP (μL/min/kg)    | 6.8 (3.1-21)  | ↑                   | ↓   | ns    | ↑           | ↑    | ↑          |
| NaFE (%)           | 0.16 (0.06-1.0)| ↑                   | ↓   | ↑     | ns          | ns   | ns         |
| UP/C               | 0.12 (0.06-0.2)| ns                  | ns  | ns    | ns          | ns   | ns         |
| uOsm/sOsm (%)      | 362 (180-627) | ns                  | ↑   | ↑     | ns          | ns   | ns         |
| sOsm (mEq/L)       | 301 (285-304) | ns                  | ↑   | ns    | ns          | ↑    | ns         |

Note: ↑, Significantly increased (P < .05); ↓, significantly decreased (P < .05).

Abbreviations: ACEi, angiotensin-converting-enzyme inhibitor; eCcr, endogenous creatinine clearance; FENa, fractional excretion of sodium; NSAID, nonsteroidal anti-inflammatory drug; OPL, oral protein load; sCr, serum creatinine; sOsm, serum osmolality; UOP, urine output; uOsm, urine osmolality; UP/C, urine protein-to-creatinine ratio.
Cats with early stage chronic kidney disease may benefit from moderate restriction in dietary phosphorus
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Dietary phosphorus (P) restriction for the long-term management of feline chronic kidney disease (CKD) significantly improves clinical parameters and prolongs life expectancy of cats. Here we describe responses in a cohort of cats diagnosed with early-stage CKD to different dietary regimens and levels of phosphorus restriction. The study was designed as a 45-month observational study of a cohort initially consisting of 19 adult domestic shorthair cats, age range 1.8-9.25 years, 10 males and 9 females, all neutered. CKD and IRIS stage was based on creatinine, urine specific gravity, and ultrasound structural changes to kidney tissue and renolith detection. CKD progression was monitored by approximately yearly ultrasounds and 6-monthly blood and urine analyses. Cats were initially fed a renal dry diet (P 0.96 g/1000 kcal; Ca:P 1.75; protein 60 g/1000 kcal) for 17 months. Subsequently, they were transitioned onto a mix of a renal wet (P 0.79 g/1000 kcal; Ca:P 1.59; protein 69 g/1000 kcal) with the dry diet for 4 months, and finally a further transition to more moderately restricted wet (P 1.60 g/1000 kcal; Ca:P 1.32; protein 101 g/1000 kcal) and dry (P 1.35 g/1000 kcal; Ca:P 1.55; protein 78 g/1000 kcal) diets for 24 months. Nine and 10 cats were diagnosed with CKD IRIS stage 1 and nonazotaemic stage 2, respectively. Four cats were euthanized, 1 each during months 12, 15, 20, and 40. Serum urea, creatinine, phosphorus, vitamin D, and parathyroid hormone did not significantly change over time while on the renal diet, while after 6 months of fibroblast growth factor 23 (FGF-23) and blood total and ionized calcium had started to increase. By month 17, FGF-23 above 700 pg/mL was observed in 3 cats (17.6%, all IRIS stage 2) and 88% had developed hypercalcemia (15/17 iCa > 1.35; 5/17 tCa > 2.95 mmol/L). An increase in renolith and urolith prevalence from 1/17 to 7/17 cats was also observed. Upon transition to the moderately restricted phosphate diets, FGF-23, total and ionized calcium decreased, with 1 cat remaining hypercalcemic (6%), and prevalence of renoliths and uroliths decreasing as the end of the study period approached. Cats with early CKD fed a dry therapeutic renal diet with P 0.96 g/1000 kcal for 17 months developed hypercalcemia, some also renoliths and uroliths, which were largely resolved following transition onto a diet regime with P content of 1.35-1.60 g/1000 kcal. Other parameters of kidney function remained stable for the 24 months on the moderately restricted phosphorus diet. While the pathophysiology of hypercalcemia associated with feeding renal diets remains to be determined, these results suggest that this may occur more frequently in cats with early stage, nonazotaemic CKD. In these cases, feeding a less phosphate restricted diet may be a more appropriate long-term management strategy.

EQUINE

Equine 3D electroanatomical mapping in sinus rhythm elucidates the electrocardiogram and cardiac electrical activation pattern
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Understanding the depolarization pattern of the equine heart under normal physiological conditions, and its relationship to the surface electrocardiogram (ECG), is of uppermost importance for research on pathophysiology of complex arrhythmias. The purpose was to use a 3D electroanatomical mapping system under general anesthesia to evaluate the qualitative and quantitative atrial and ventricular depolarization patterns and correlation with the surface ECG in 7 healthy horses in sinus rhythm. Bipolar activation maps of the endocardium were analyzed. The first atrial activation was located at the height of the crista terminalis. Only 1 interatrial conduction pathway was recognized. The first and second P wave deflections represented the right and left atrial depolarization, respectively. His bundle electrograms could be recorded in 5 out of 7 horses. Left ventricular activation (Figures 1-3) started at the mid septum, right ventricular activation apically from the supraventricular crest. This was followed by separate depolarizations at the height of the mid free wall. Further ventricular depolarization occurred in an explosive pattern. Electrically active tissue could be found in all pulmonary veins. In contrast to findings of previous studies, all parts of the ventricular depolarization contributed to the surface ECG QRS complex. This
study provides a reference for the normal sinus impulse endocardial propagation pattern and for conduction velocities in equine atria and ventricles. This information allows to improve ECG reading and develop better recording techniques, such as new 12-lead ECG configurations, in horses.

MARCKS is a novel target in equine asthma syndrome
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The goal of this study was to investigate the myristoylated alanine-rich C kinase substrate (MARCKS) protein as a potential novel therapeutic target for equine asthma syndrome (EAS). Our lab, and others, previously established a role for MARCKS in both neutrophil and macrophage inflammatory functions. In the current study, we hypothesized that MARCKS protein is upregulated in BAL cells in horses with asthma; and that inhibition of the MARCKS protein with a MARCKS-specific inhibitor peptide known as MANS, will diminish the zymosan-induced respiratory burst of equine alveolar macrophages, and peripheral blood neutrophils, ex vivo. Bronchoalveolar lavage (BAL) was performed as previously described using a 3-m cuffed Bivona tube and 300-mL sterile saline. BAL cytology (400 cell count differential), physical, and rebreathing exam were used to classify horses as normal, mild/moderate, or severe EAS. Total MARCKS protein expression in BAL cell lysates was quantified using an equine-specific MARCKS ELISA (MyBioSource, San Diego, California) and normalized to total protein (BCA assay) in cell lysate. Equine alveolar macrophages were isolated by adherence. Peripheral blood neutrophils were isolated by ficoll density-gradient centrifugation. Zymosan-induced respiratory burst was measured as luminol-enhanced chemiluminescence. Cells were pretreated with 0, 10, 25, 50, or 100 μM MANS (MARCKS-specific inhibitor peptide), or 100 μM RNS (control peptide). Normalized MARCKS protein expression was significantly higher in BAL cell lysates from horses with mild/moderate (n = 14) and severe asthma (n = 5), compared to BAL cell lysates from horses with normal lower airway cytology (n = 6) (1-way ANOVA, P < .05). MANS peptide pretreatment, but not RNS, significantly attenuated zymosan-induced respiratory burst of equine alveolar macrophages and peripheral blood neutrophils (1-way ANOVA, P < .05). These findings determine that MARCKS protein expression is altered in BAL cells from horses with EAS, indicating a possible role for MARCKS in the pathophysiology of EAS, and support MARCKS inhibition as a potential therapeutic strategy to alter the inflammatory response of airway cells including alveolar macrophages and neutrophils.

FOOD ANIMAL

Near-infrared spectroscopic analysis of biofluids for rapid detection of Mannheimia haemolytica infection in cattle
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Mannheimia haemolytica (MH) is a leading cause of bovine respiratory disease (BRD). Diagnosis of BRD traditionally relies on visual assessment, which can be untimely, insensitive, and nonspecific; more rapid and accurate diagnostic tests are needed. Near-infrared spectroscopy (NIRS) allows fast, noninvasive assessment of biofluids, whereby the chemical profile is described by the intensity and structure of sample reflectance or transmittance spectra in the NIR band (780-2500 nm). We hypothesized that NIRS evaluation of breath condensate, nasal secretions, saliva, plasma, and/or serum (biofluids) could profile signatures differentiating cattle before and after MH infection. Biofluids were collected from five 6-month-old Holstein steers over 4 days prior to and 23 days after intrabronchial challenge with MH. Clinical signs (rectal temperature, heart and respiratory rate, and respiratory score) and complete blood counts (CBC) were assessed prechallenge and postchallenge. By day 2 postchallenge calves had signs of acute MH infection with significantly increased (P < .05) rectal temperatures, heart rates, and respiratory rates. Complete blood counts revealed significantly increased (P < .05) leukocyte and neutrophil counts. Due to severe disease calves were treated with a 5-day course of antimicrobial (AM, ceftiofur hydrochloride). Biofluid samples were grouped into 4 categories: (a) baseline; (b) infected, pre-AM; (c) infected, post-AM; and (d) recovered. Spectral signatures of biofluids (n = 3800) collected via ASD FieldSpec3 portable spectrometer were analyzed with multivariate statistics using UnscramblerX v. 10.5. Chemometrics were applied to the wavelength range 1300 to 1600 nm using a balanced randomly selected subset of samples (n = 150) with an 80/20 calibration/validation distribution for linear discriminant analysis (LDA-PCA). Analysis discriminated samples according to category; LDA-PCA showed correct classification between 89.6%-100% and 81.7%-100% for the calibration and validation process, respectively. NIRS with multivariable analysis rapidly discriminated MH infection states in cattle, establishing a basis for further development of NIRS as a BRD diagnostic tool.

Gastrointestinal pharmacokinetics and phenotypic persistence of antimicrobial resistance over time after florfenicol administration in steers
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Antimicrobial resistance (AMR) is a global threat to both human and veterinary medicine leading to recommendations to use lower tier antimicrobials such as florfenicol, a common antibiotic used in cattle. Our objective was to quantify co-selection of enteric AMR bacteria in cattle treated with 2 labeled dosing regimens of florfenicol. In addition, we wanted to gain insight on the gastrointestinal pharmacokinetics of florfenicol and how it correlates with AMR. We hypothesized the prevalence of AMR isolates would be higher and persist longer in the steers administered the repeated, lower dose of florfenicol. We also hypothesized that the gastrointestinal pharmacokinetics of florfenicol for the lower, repeated dose would be below a therapeutic level for a longer period of time. Twelve 6-month-old steers underwent gastrointestinal surgery to facilitate placement of an ultrafiltration probe in the ileum and colon. An interstitial probe was placed in the region of
the withers. Jugular vein catheterization allowed for blood to be collected. Ultrafiltrate, interstitial fluid, and blood were collected for the pharmacokinetic analysis. Twenty-four hours after surgery, the steers were either administered 20 mg/kg florfenicol intramuscularly (IM) (n = 6) 48 hours apart or 40 mg/kg florfenicol subcutaneously (SC) (n = 6) once. Feces were collected manually until day 38. To determine the pharmacokinetic parameters of florfenicol administration, HPLC with UV detection will be utilized. To assess the phenotypic resistance changes over time, 2 different methods were used. Feces were serially diluted and plated on selective media to ensure growth of Escherichia coli and Enterococcus. The pure colonies were grown on blood agar plates and used on gram-positive and gram-negative NARMs plates to assess a wide variety of resistance to antibiotics. In addition, the serially diluted feces were plated on either plain MacConkey, plain Enterococcus or MacConkey or Enterococcus plates infused with either tetracycline (16 μg/mL), ampicillin (32 μg/mL, E. coli: 16 μg/mL, Enterococcus), or ceftiofur (8 ug/mL) in triplicate. After incubation, plates were counted and averaged to determine the concentration (CFU/g) of either E. coli or Enterococcus at each time point. To determine the prevalence AMR isolates, the log CFU/g of the resistant isolates was divided by the log CFU/g of wild-type isolates at each time point. These were averaged across steers at each time to compare over time within the dosing group and between the dosing groups using student T tests with appropriate Bonferroni correction to account for multiple testing. Pharmacokinetic analysis will be completed using Phoenix to determine pharmacokinetic parameters. There was no significant difference from baseline log growth for E. coli or Enterococcus in either dosing group. Based upon the antibiotic infused media, there was a significant increase in log growth of tetracycline resistant E. coli compared to baseline in the IM group (P = .007). No other comparisons were significant. We anticipate that these findings will provide guidance to veterinarians on the most appropriate dosing regimen of florfenicol in cattle in order to minimize selection of AMR enteric bacteria.