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**European Society of Comparative Gastroenterology**

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**Society of Comparative Hepatology**

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ORAL RESEARCH COMMUNICATIONS

ESCG-O-1

Gastrointestinal protectants in clinical practice: Evaluation of prescription patterns among general practitioners in Portugal

A. R. Nova Baptista, B. S. Bora, R. A. Oliveira Leal


Gastrointestinal protectants (GIP) are indiscriminately prescribed among general-practitioners. Proton pump inhibitors (PPIs) have become one of the most commonly prescribed acid suppressants and concerns about its use has been raised. In 2018, an American College of Veterinary Internal Medicine consensus statement was published, discussing the evidence-based and rational use of GIP. The aim of this study was to evaluate the current prescription trends of GIP, assessing if the consensus guidelines are being applied. An observational cross-sectional study was conducted using an online uploaded survey, promoted through social media posts in Portuguese online veterinarian forums. General-practitioners answered comprehensive questions related to the most frequently prescribed GIP and the underlying clinical context for its use. PPIs were detailed with questions assessing a possible preference comparing to other compounds, frequency of administration and relation with food intake, length of therapy, withdrawal practices and whether they are prescribed in monotherapy or in association. A total of 124 answers were obtained. PPIs were the most frequently prescribed GIP accounting for 62% of the answers. Histamine type-2 receptor antagonists (H2RAs) were preferred in 16% while sucralfate was the option in 22%. The most common reasons evoked for PPIs prescription were: gastrointestinal erosion or ulceration treatment (98%), prophylaxis of gastric lesions in animals with nonerosive gastritis (89.5%), prevention of reflux oesophagitis (80%), prophylaxis of steroid-induced ulceration (70%) and pancreatitis (61%). Eighty-three veterinarians (67%) mentioned they prefer omeprazole mainly due to a more scientific basis and a better perception of efficacy. 69% prescribe omeprazole once-daily while 24% recommend a twice-daily administration. 91% mention it should be administered short-term before or with meal and 7% do not respect it. Concerning length of therapy, while 51% of general-practitioners do not extend treatment for more than three weeks, 49% prescribe it for longer periods. Among them, 59% stop it abruptly while 41% gradually reduce it. 34% of the veterinarians associate omeprazole with H2RAs. Results obtained on this survey support that PPIs, namely omeprazole, are the preferred choice among Portuguese general-practitioners. GIP are widely prescribed in gastrointestinal erosion or ulceration, in accordance with ACVIM consensus recommendations. However, they are still used prophylactically, when its therapeutic value is discussable. Once-daily therapy, the association with H2RAs and an abrupt withdrawal after 3 weeks of use are incorrectly recommended, stressing the need for a better awareness about GIP use in daily clinical practice. Further studies are needed to extrapolate these conclusions to other European countries.

Disclosure

Conflicts of Interest Study funded by: Project UIDP/CVT/00276/2020 (funded by FCT) (overall, there is no Conflict of interest but a disclosure to report).

ESCG-O-2

Evaluation of omeprazole use for the treatment of dysrexia and vomiting in cats with chronic kidney disease


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Cats with moderate to advanced chronic kidney disease (CKD) often display gastrointestinal (GI) signs such as nausea, vomiting, and decreased appetite. Acid suppressants such as proton pump inhibitors (PPIs; e.g. omeprazole) are one of the most commonly prescribed medications for the treatment of such clinical signs. There is no evidence that PPI administration and, in particular omeprazole, will improve the GI signs associated with CKD in cats. The aim of this study is to evaluate the effect of oral omeprazole administration on appetite or vomiting in cats with moderate to advanced CKD. Thirteen client-owned cats with moderate to advanced CKD showing signs of inappetence or vomiting were recruited at three academic institutions and one referral hospital in the United States. A multi-institutional, prospective, double-blinded, randomized, cross-over study was performed. Vomiting frequency and appetite were compared in cats with CKD treated with either omeprazole or placebo. All cats were randomized to receive omeprazole (1mg/kg PO q24hr) or placebo (lactose gel capsule PO q24hr) for 14 days. Cats underwent a 14-day washout between treatments. A daily log was completed by the owner assessing appetite and vomiting during and for 14 days before and after each treatment. Appetite was assessed by scoring system (decreased, unchanged, or increased) and percentage of food consumed in a day (0%, 25%, 50% and 75%). An ANOVA was performed to determine if average daily percentage food consumed, average appetite score, vomit percentage, and total number of times vomited different between treatments. The average age of the 13 cats was 12.8 years. IRIS staging was as follows: 46% (6/13) stage II, 46% (6/13) stage III, and 8% (1/13) stage IV. All cats included had signs of inappetence as noted by owners. A statistically significant difference was observed for the percentage of food consumed between treatments (P = 0.0322). Post-hoc analysis revealed that on average, using least squares means, approximately 3% more food was consumed with omeprazole treatment compared to placebo. There was no significant difference in vomiting frequency between treatments.
Overall, this study showed that hypoxemic cats with moderate to severe CKD consumed a small but statistically significantly greater amount of food with omeprazole compared to placebo; in contrast, no difference in vomiting was seen between the two treatments.

Disclosures

The mentoring author, Dr. Tolbert, is a paid consultant for TriviumVet and a paid speaker for Kindred Biosciences. However, neither company paid for this study nor do they have an interest in the study objectives or outcome.

ESCG-O-3

**Endoscopic features of Feline Gastrointestinal Eosinophilic Sclerosing Fibroplasia: A series of 4 cases**

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Feline Gastrointestinal Eosinophilic Sclerosing Fibroplasia (FGESF) is an uncommon inflammatory condition of poorly defined etiology. The entity is characterized by a densely fibrous and eosinophil-rich intramural mass mainly involving the gastro-duodenal or the ileo-colic junctions. To our knowledge, endoscopic features of FGESF have not yet been reported. The aim of this retrospective study was to describe the endoscopic lesions of FGESF. Inclusion criteria were histological diagnosis of FGESF in cats in which an abdominal ultrasonography and a digestive tract endoscopy had been performed. Two domestic shorthair cats and two Main Coon were included. The mean age was 3.9 ± 1.3 years. Animals initially presented with vomiting (n = 3), diarrhea (n = 3) and weight loss (n = 2). A firm abdominal mass was palpated in 2 cats. Perforation and obstruction were identified in 3 cats. One cat showed hypocobalaminemia and two cats had moderate hypoalbuminemia. Ultrasonographic examination revealed a mass in the proximal duodenum (n = 3) or at the ileo-colic junction (n = 1). A focal thickening of the intestine wall including a loss of stratification was identified in all cases. Moreover, an isolated or multifocal abdominal lymphadenomegaly was noted in 3 cats.

Endoscopically, the main findings included a dysplastic and proliferative aspect of the mucosa (n = 4), a large ulcer with a yellow to green discolored surface (n = 3) and the identification of trichobezoars (n = 2). Diagnosis of FGESF was achieved after histopathological analysis of per-endoscopic biopsies in 2 cats or full-thickness surgical biopsies in 2 cats. Typical histological findings were associated to bacterial intracellular or intralesional fungi (consistent with phycomycetes) in two and one cat respectively.

Our results suggest that FGESF has a characteristic appearance endoscopically with a dysplastic and proliferative aspect of the digestive mucosa, sometimes associated with ulcerative lesions displaying yellow-green discoloration. Therefore, FGESF should be part of the differential diagnosis of proliferative and ulcerative lesions localized in the gastro-duodenal or ileo-colic junction during endoscopy. Moreover, endoscopy could guide the therapeutic management and it may even influence whether a surgical resection is practicable.

Disclosures

No disclosures to report.

ESCG-O-4

**Diarrhea has a greater impact on the fecal metabolome of dogs than does dietary intervention**

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Diet can impact the canine gut microbiome, and is often investigated in the context of novel ingredients (e.g., vegetable-sourced protein, VEG) and/or significant changes in macronutrient content (e.g., raw-food diet, RAW). However, the gut microbiome is intrinsically redundant, with many bacterial species occupying the same niche, or performing the same functions. It is still unknown how those diets, and the microbial changes they cause, impact the fecal metabolome, and how they compare to those induced by gastrointestinal disease. In this study, we describe the fecal metabolome in healthy dogs fed one of three types of diet and in dogs with chronic (CE), acute non-hemorrhagic (AD), or acute hemorrhagic (AHDS) diarrhea. Fecal samples were collected from healthy dogs fed commercially available traditional dry dog food (CON; n = 27), VEG (n = 9), or RAW (n = 12) exclusively for at least 30 days prior to sampling. Fecal samples from dogs with CE (n = 7), AD (n = 8), and AHDS (n = 10), obtained at the time of diagnosis, were used from previous clinical trials.

Metabolites were extracted and untargeted liquid chromatography with high resolution accurate mass spectrometry analysis was performed. Statistics were performed using MetaboAnalyst 4.0. Significance of FDR corrected P-values was set at q < 0.05.

A total of 145 named compounds were identified. Of those, only 3 were significantly different between the diet groups, including solanidine (q < 0.001) and N-acetyl-D-galactosamine (q < 0.001). When dogs with CE were included in the comparison, a phytochemical, sinapine, was found to be higher in dogs with CE compared to dogs fed either or the 3 diets. However, dogs with acute diarrhea showed a strong shift in their fecal metabolic profiles, with 38 metabolites significantly altered in AD, and 67 in AHDS when compared to CON, VEG, and RAW. Changes included increases in amino acids such as tryptophan (AD q = 0.001, AHDS q < 0.001) and citrulline (AD and AHDS q < 0.001), tryptophan degradation metabolites such as kynurenic acid (AD and AHDS q < 0.001) and indoleacrylic acid (AD q = 0.002, AHDS q < 0.001), and compounds such as N-acetyl-L-tyrosine (AD q = 0.003), which affects tryptophan metabolism by inhibiting the production of melatonin.
These results indicate that the impact of diet on the fecal metabolome, and consequently in gut function, is smaller than the effect of gastrointestinal disease. Interestingly, AD cases had been classified as uncomplicated, as they were mild and without any detectable underlying pathology. These findings reveal that diarrhea, and in particular AD, has a bigger impact on the fecal metabolome than previously acknowledged.

Disclosures
RP, JMS, JAL and JSS’s salaries are paid by the GI Lab, which offers diagnostic tests on a fee-per-service basis.

ESCG-O-5
Differential microRNA expression-profiles in feces and serum of dogs with chronic inflammatory enteropathy and with gastrointestinal cancer

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Differentiating chronic inflammatory enteropathy (CIE) and gastrointestinal (GI) cancer in dogs is challenging due to the similarities in clinical presentation. Fecal and serum microRNAs show potential as biomarkers of human colorectal cancer. We hypothesized that dogs with GI cancer had a different fecal and serum microRNA expression profile than healthy dogs or CIE. Twenty-one dogs, 6 healthy, 9 with CIE and 6 with GI cancer, were prospectively recruited based on detailed diagnostic work-up. Dogs were excluded if they had co-morbidities or immunomodulatory medications. Fecal and serum samples were frozen within 1 hour of collection. RNA was extracted and smallRNAseq was performed. Processed reads were mapped to the canine genome (CanFam3.1)

Age and gender were not significantly different comparing groups, but cancer dogs were larger breeds and had a higher body weight (P = 0.04). Differential expression (DE) of miRNAs comparing GI cancer and CIE were, in feces: miR-451 (2.25 fold change (FC), P < 0.01); and in serum, miR-1 (1.58 FC, P = 0.02), miR-122 (2.38 FC, P = 0.04), miR-133a (3.8 FC, P < 0.01), miR-133c (3.56 FC, P < 0.01), miR-143 (2.07 FC, P = 0.02) and miR-145 (3.38 FC, P < 0.01). Comparing GI cancer and healthy dogs the same DE miRNAs as above and miR-194 (-3.65 FC; P < 0.01) and miR -320 (-2.14 FC; P = 0.04) were seen in feces. No DE miRNAs were found comparing CIE and healthy dogs.

In conclusion, we identified DE miRNAs associated to CIE and GI cancer. Confirmations using qPCR on a larger study population is warranted to confirm the applicability of these miRNAs as diagnostic biomarkers.

Disclosures
This study was funded by the Independent Research Fund Denmark.

ESCG-O-6
Evaluation of anamnestic and clinicopathologic factors that might explain the poor correlation between pancreatic lipase concentrations (DGGR-lipase and Spec cPL) and ultrasonographic evidence of pancreatitis in dogs

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Pancreatic lipase concentrations and pancreatic ultrasound (US) are the two cornerstones for a clinical diagnosis of pancreatitis in dogs. Multiple studies have shown that results of both modalities are poorly correlated, which complicates the diagnosis pancreatitis.

We therefore investigated which anamnestic and clinicopathologic factors affect the pancreatic US evaluation, and which clinicopathologic parameters correlate best with an ultrasonographic diagnosis of pancreatitis in dogs.

In this retrospective study, data from dogs presenting with gastrointestinal clinical signs between 2016-2020 were included if a DGGR-lipase and a full abdominal ultrasound examination were performed within 24h of each other. Spec cPL results were included when taken from the same blood sample as the DGGR-lipase. Dogs pretreated with corticosteroids were excluded. Spearman correlation was used for measuring relationships. Cohens kappa (k) was used as measure of agreement of two categorical variables. Mann-Whitney U test was applied to compare metric variables between US categories. Differences between pancreatic US diagnosis and categorical variables were assessed using Chi-square Test. All tests were performed two-tailed at a 5% level of significance. Data from 234 dogs were available for analyses, of which 102/234 dogs also had a Spec cPL measured. DGGR-lipase and Spec cPL correlated significantly (p = 0.916, P < 0.001). There was only a slight agreement for Spec cPL>400 mcg/l and US positivity (k = 0.251, 95% CI+/- 0.190, and fair agreement for Spec cPL >216 U/l and US positivity (k = 0.251, 95% CI +/-. 0.125).

Median DGGR-lipase, segmented neutrophils, alkaline phosphatase, and alanine aminotransferase values were significantly higher in dogs with US positivity compared to dogs with a normal pancreas, however the results of the latter 3 laboratory values were within reference range. If the radiology submission form contained “suspection of pancreatitis” or “increased lipase”, the final US diagnosis was significantly more often positive than expected. The presence of irregular or rounded contours, a hypoechoic, mixed-echoic or hyperechoic pancreas, an enlarged pancreas, hyperechoic mesentery, peripancreatic effusion, gastric wall thickening, corrugated duodenum, a painful
pancreatic area, and patient age >6 years were also significantly associated with US positivity. DGGR-lipase and Spec cPL were significantly higher when rounded contours, an enlarged pancreas, hyperechoic mesentery, and peritoneal effusion were present. Only DGGR-lipase was significantly higher when a hyperechoic pancreas, and significantly lower when a normal pancreatic echogenicity was present.

Our findings might be useful when designing future studies assessing diagnostic performances of lipase assays in the absence of a gold standard.

Disclosures

No disclosures to report.

ESCG-O-7

Hepatobiliary disorders and elevated blood urea nitrogen during the treatment are possible prognostic factors for feline pancreatitis

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Feline pancreatitis (FP) has been increasingly diagnosed using the feline pancreas-specific lipase (Spec-fPL) test in recent years. There are several reports on the prognostic factors for FP; however, the information on the utility of those factors or the association of the prognostic factors with other diseases is limited. In this study, we evaluated the prognostic impact of the laboratory findings in FP. The medical records of patients with FP between September 2014 and July 2019 were reviewed. Seventy-eight patients hospitalized with FP were included in this study. The diagnostic criteria for FP included clinical signs, Spec-fPL results (>3.5 μg/L), and ultrasonographic findings. The presence of other gastrointestinal disorders or neoplastic diseases was ruled out via ultrasonography. Fisher’s exact test was performed to determine the correlation between the mortality and the clinical signs, the findings of the physical examination, laboratory examination, or imaging. P values less than 0.05 were considered statistically significant.

All patients underwent treatment; 68 patients eventually survived, while 10 died. Significant correlations were found between mortality and the following variables: hypothermia (P = 0.04), icterus (P = 0.025), or hyperechoicogenicity and enlargement of the liver (P = 0.004) at the first visit; increase in the value of alkaline phosphatase (P = 0.007), γ-glutamyltransferase (P = 0.015), or total bilirubin (P = 0.006) 3-5 days after the treatment; and elevated white blood cell (P = 0.006), total bilirubin (P = 0.03), or blood urea nitrogen (P = 0.03) during the treatment. There were no significant differences in the findings of blood examination between survivors and nonsurvivors at the first visit.

These results suggest that the increase in the value of hepatic enzymes during treatment is a possible prognostic factor and hepatobiliary disorders such as extrahepatic biliary obstruction, cholangiohepatitis, or hepatic lipidosis have an influence on the prognosis of FP. However, it is difficult to predict the prognosis of FP by blood examination at the first visit. Therefore, it is important to detect hepatobiliary disorders via ultrasonography and monitor hemodynamics and inflammation by continuous blood examination to predict the prognosis of FP.

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ESCG-O-8

The effect of assisted enteral feeding on treatment outcome in dogs with inflammatory protein-losing enteropathy

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Assisted enteral feeding can help improve perioperative outcome in humans with inflammatory bowel disease. The effects of assisted enteral feeding on treatment outcome have not been previously investigated in dogs with inflammatory protein-losing enteropathy (PLE). Therefore, the aim of this study was to determine if dogs with inflammatory PLE that had an enteral feeding tube placed had a better outcome to treatment compared to dogs with inflammatory PLE that did not have an enteral feeding tube placed.

A retrospective study design at a UK referral teaching hospital included 20 dogs with inflammatory PLE that had enteral feeding tube placement within 5 days of gastrointestinal biopsy. The comparison group consisted of 37 dogs with inflammatory PLE that did not have an enteral feeding tube placed. The minimum follow-up time required after date of diagnosis was 4 months. Positive outcome was defined as survival time greater than 4 months or death unrelated to PLE. Negative outcome was defined as death related to PLE less than 4 months after diagnosis.

Dogs in the enteral feeding tube group had a significantly higher canine chronic enteropathy clinical activity index (CCECAI) compared to dogs in the non-enteral feeding tube group (P < 0.001, median range): enteral feeding tube group = 14 (7-19); non-enteral feeding tube group = 7 (4-13) and significantly lower appetite scores (P < 0.001, enteral feeding tube group = 5 hyporexic, 14 anorexic, 1 unknown; non-enteral feeding tube group = 19 unchanged, 10 hyporexic, 7 anorexic, 1 unknown) at diagnosis. In the enteral feeding tube group, 75% (15/20) had a positive outcome compared to 46% (17/37) in the non-enteral feeding tube group. Assisted enteral feeding was associated with increased odds of a positive outcome in dogs with inflammatory PLE (OR 3.2, 95% CI 1.0-10.5), with a trend towards significance (P = 0.054).

Despite statistical significance not being reached with treatment outcome between the two groups, a greater proportion of dogs in the enteral feeding tube group had a positive outcome compared to dogs in the non-enteral feeding tube group, despite the former group having a significantly higher CCECAI at diagnosis. Therefore, this study suggests assisted enteral support in dogs with inflammatory PLE could be associated with improved clinical outcome and hence should be
actively addressed in these cases. However, larger studies that account for confounding variables such as disease severity and appetite are needed to definitively quantify the impact of assisted enteral feeding on treatment response and outcome in dogs with inflammatory PLE.

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ESCG-O-9

Short-term feeding with high-fat diet induces dysbiosis-associated changes of fecal metabolites consistent with changes in serum metabolomics in dogs

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Many chronic diseases leading to high morbidity and mortality in today's western societies, including diabetes mellitus, inflammatory bowel disease, and colorectal cancer have epidemiologically been linked to the consumption of high fat diets (HFD). Of the large animal models used in translational research, the dog is especially relevant because canine gut anatomy, physiology and diet have adapted to that of humans during domestication. Consequently, the composition of the gut microbiota is strikingly similar between dogs and humans, with 60% taxonomic and functional overlap. We have previously shown that repeated exposure to HFD triggers dysbiotic changes of the gut microbiome in dogs, concomitant with changes in the serum metabolome. However, changes of the fecal metabolome after HFD feeding have not been reported so far.

Fecal samples were collected before (33% fat) and after (47% fat) HFD feeding for 2 weeks in 8 healthy adult Beagle dogs. Untargeted metabolomics analysis was performed by the West Coast Metabolomics Center at the University of California. Analytes were separated using an Agilent 6890 gas chromatograph and mass spectrometry was performed on a Leco Pegasus IV time of flight mass spectrometer. Differences in the abundance of serum metabolites between study groups were evaluated using a Mann-Whitney test in Prism 5. For multivariate analysis, data were normalized to the sum of the total spectral integral, log transformed, mean centered, and divided by the standard deviation of each variable prior to multivariate analysis. PCA was performed and a Random Forest Analysis was generated using MetaboAnalyst 4.0.

A total of 684 metabolites were detected. Of those, 188 were identified metabolites, while 496 lacked full structural identification. Twenty-five metabolites differed significantly between pre- and post-HFD treatment. Of these, 9 were identified metabolites, namely zymosterol, gamma-tocopherol, tocopherol-acetate, levoglucon, glycerol, dihydrocholesterol, D-erythrosphingosin, beta-sitosterol, and cholesterol. The Random Forest analysis identified the compounds with the highest degree of separation between groups to be cholesterol, zymosterol, dihydrocholesterol, beta-sitosterol and gamma-tocopherol. Pathway analysis revealed that 85 and 46 of differential metabolites were implicated in the steroid and the primary bile acid biosynthesis pathways, respectively. These results concur with our previous findings of reduced lipid metabolites in the serum after HFD in dogs.

This is the first report correlating fecal and serum metabolome in dogs after HFD. Our data show clear effects of HFD-induced fecal metabolome changes in the cholesterol and primary bile acid biosynthesis pathways, leading to related lipid metabolism changes in the serum.

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ESCG-O-10

Histopathological concordance of concurrent duodenal and ileal biopsy specimens in dogs

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Dogs with gastrointestinal signs frequently undergo investigation via collection of endoscopic or surgical gastrointestinal biopsy. The current evidence in canine chronic enteropathy (CE) supports concurrent sampling of duodenal and ileal biopsy via endoscopy to prevent oversight of ileal lesions. However, previous studies have not been performed in dogs with non-CE diagnoses or contrasting the concordance between both sites in full-thickness biopsy specimens with those of endoscopic biopsy specimens. Therefore, our objective was to compare histopathological concordance in concurrent duodenal and ileal biopsies collected via endoscopy or surgical biopsy in all dogs undergoing this procedure. A second aim was to determine if signalment, clinicopathological variables and ultrasound abnormalities helped to distinguish discordant from discordant samples at these two sites.

The electronic medical record database was searched for all canine cases that had both sites biopsied. One hundred and forty-one dogs were included, 128 underwent concurrent endoscopic biopsy of the duodenum and ileum and 13 underwent concurrent surgical biopsy of both sites. The histopathological concordance of inflammatory cell type (intraepithelial lymphocytes, lamina propria lymphocytes, plasma cells, eosinophils and neutrophils), neoplasia and severity (mild, moderate, severe) between the duodenum and ileum was assessed for each case. A board-certified veterinary pathologist reviewed all suitable cases. Comparison of signalment, clinicopathological variables and ultrasound abnormalities between concordant and discordant cases was performed using logistic regression.

Our results showed that 5 out of 13 (38%) full-thickness biopsy specimens were fully concordant in both cell type and severity compared to
62 out of 128 (48%) endoscopic biopsy specimens. Full discordance for both cell type and severity was demonstrated in 2 out of 13 (15%) full-thickness biopsy specimens and in 26 out of 128 (20%) endoscopic biopsy specimens. Four out of 13 (31%) full-thickness biopsy specimens demonstrated discordance in severity alone, whereas 2 out of 13 (15%) had discordant cell type alone. Nineteen of the 128 (15%) endoscopic biopsy specimens were discordant in severity alone, whereas 21 out of 128 (16%) cases were discordant in cell type alone; including 3 neoplasia cases solely in the duodenum (1 adenocarcinoma and 2 lymphoma) and 1 lymphoma case solely within the ileum. There were no significant signalment, clinicopathological variables or ultrasound abnormalities that distinguished concordant from discordant samples.

In conclusion, concordance was comparable for full-thickness and endoscopic biopsy specimens at both sites. However, the frequency of discordance seen in both full and endoscopic biopsy specimens necessitates sampling of both sites.

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Dr Sarah Caulfield has no disclosures to report.

ESCG-O-12

Canine acute hemorrhagic diarrhea syndrome: A retrospective evaluation of data for the identification of possible prognostic markers
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Acute hemorrhagic diarrhea syndrome (AHDS) describes a syndrome of bloody, sometimes life-threatening diarrhea. However, there is currently no marker to distinguish uncomplicated from severe cases. Therefore, the aim of this retrospective study was the identification of prognostic factors from signalement, history, clinical symptoms (AHDS-score), laboratory results and therapeutic regime in order to predict the disease-course. 191 AHDS-cases from 2017 to 2018 were enrolled in this retrospective study. Inclusion criteria consisted of an acute onset of bloody diarrhea, no previous treatment and no other diagnosed condition causing bloody diarrhea. For statistical analysis, the SPSS statistic program was used. P < 0.05 was considered statistically significant.

The incidence of AHDS was higher during winter and springtime. Middle aged and small dogs were overrepresented. Significantly longer hospitalization was observed in patients with decreased body temperature (P < 0.001), higher AHDS-score (P = 0.042), increased hematocrit (P < 0.001), reduced leukocyte concentration (P = 0.009) and toxic neutrophils (P < 0.001) at presentation. Older animals (P = 0.005), lower body temperature (P = 0.015), higher hematocrit (P = 0.001), lower leukocytes (P = 0.031) and a higher AHDS-score (P = 0.007) triggered antibiotic treatment. A plasma transfusion was more likely required in patients with lower bodyweight (P = 0.010), lower body temperature (P < 0.001), and haemconcentration (P < 0.001).

Older age and higher AHDS score at time of presentation predicted antibiotic use. Hypothermia, haemconcentration and leukopenia are associated with more severe courses of AHDS and might serve as useful negative prognostic markers.

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No disclosures to report.
**ESVC-O-1**

The Mitral Insufficiency Echocardiographic (MINE) score: A severity classification of myxomatous mitral valve disease in dogs

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The ACVIM guidelines are commonly used for the clinical classification of dogs with myxomatous mitral valve disease (MMVD). From an echocardiographic point of view, the evaluation of MMVD severity is based on cardiac remodeling, quantification of mitral regurgitation and estimation of left ventricular filling pressure. The aim of this study was to propose an easy-to-use echocardiographic severity classification of mitral insufficiency in dogs.

This retrospective, multicenter observational study included dogs with MMVD imaged between 2011 and 2019, of which an updated follow-up was available in December 2019. The proposed severity classification was based on four echocardiographic parameters: left atrium-to-aorta ratio (LA/Ao), left ventricular end-diastolic diameter normalized (LVIDDn), fractional shortening (FS%) and E-wave transmitral peak velocity (E-vel). Specific echocardiographic cut-offs were defined based on previous prognostic studies on MMVD, and severity scores were assigned as follows: mild (score: 4-5), moderate (score: 6-7), severe (score: 8-12), end-stage (score: 13-14). Clinical usefulness of this score was tested by evaluating the association with survival. Long-term outcome was assessed by telephone interviews with the owners. Survival was analyzed using Kaplan-Meier curves, logrank tests and Cox's proportional hazards. The ROC curve analysis and the Youden index were used to define the best cutoff score to predict cardiac mortality.

A total of 647 dogs with MMVD were included, 296 in ACVIM stage B1, 189 in ACVIM stage B2 and 162 in ACVIM stage C-D. Of these, 181 died for cardiac-related causes and 104 died for no cardiac-related causes. Median survival time was significantly different (P < 0.05) between all the proposed severity classes: mild [2344 days, 95% confidence interval (CI) 1877-2810 days], moderate (1852 days, 95% CI 1145-2559 days), severe (710 days, 95% CI 597-819 days) and end-stage (171 days, 95% CI 107-235 days). A total score > 8 was predictive of cardiac death (AUC = 0.83, 95% CI 0.79-0.87; P < 0.0001; sensitivity 70% and specificity 82%). According to multivariable analysis, the independent predictors of cardiac mortality were LA/Ao [hazard ratio (HR) = 2.22; 95%CI 1.45-3.38; P = 0.0002], LVIDDn (HR = 2.56; 95%CI 1.34-4.89; P = 0.0005), FS% (HR = 1.02; 95%CI 1.01-1.04; P = 0.0008) and E-vel (HR = 4.39; 95%CI 2.52-7.64; P < 0.0001).

In conclusion the MINE score, proposed as an echocardiographic severity classification of MMVD, has proven to be clinically effective since it is associated with survival. This classification provides prognostic information and could be useful for an objective echocardiographic assessment of MMVD.

**Disclosures**

No disclosures to report.

**ESVC-O-2**

The longitudinal outcome of canine myxomatous mitral valve disease (MMVD) registry (LOOK-Mitral) study: Baseline characteristics

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The longitudinal outcome of canine myxomatous mitral valve disease (MMVD) registry (LOOK-Mitral registry) was established to describe the natural history and predictors of outcome in affected dogs. This study is aimed to describe the baseline characteristics of dogs in the LOOK-mitral registry. Dogs with echocardiographic evidence of MMVD were prospectively enrolled by thirteen referral centers. A total of 6,102 with MMVD were included. Median age was 13 years (2-22 years) and mixed breed was most common breed (n = 1,360, 22%). Concomitant diseases were reported in 2,459 dogs with chronic respiratory diseases occurring most frequently (34%), followed by presence of azotemia (15%) and orthopedic diseases (13%). With regard to disease severity, 65% of the dogs were in ACVIM Stage-B1, 15% in Stage-B2 and 20% in Stage-C. Dogs in Stage-B1 were younger (P < 0.0001) than dogs in other stages. Murmur intensity, heart rate during physical examination and vertebral heart score were positively correlated with severity of the disease. Dogs in Stage-C were more likely to have tachypnea (P < .0001), dyspnea (P < .0001), cough (P < .0001), syncopal episodes (P < .0001) and tachyarrhythmias (P < .0001) compared to dogs in Stage-B1 and B2. Echocardiographic indices of size and function were respectively correlated, positively and negatively with severity of disease. Interestingly, 243 dogs out of the 333 with an increased normalized end-systolic left ventricle internal diameter (>1.26) weighed <20 kg. Concomitant diseases occurred more frequently in dogs in Stage-B than in dogs in Stage-C (P < .0001). This study describes MMVD in a large population of dogs and provides new findings of clinical relevance.

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**ESVC-O-3**

The prognostic value of clinical, radiographic, echocardiographic variables and biomarkers levels for assessing risk of the onset of heart failure or cardiac death in dogs with preclinical myxomatous mitral valve disease: The DELAY Study

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Introduction: The preclinical phase of myxomatous mitral valve disease (MMVD) includes a heterogeneous group of dogs and the identification of dogs at higher risk of developing heart failure (HF) or cardiac death is of clinical relevance. Objectives: to identify the prognostic value of clinical, radiographic and echocardiographic variables, as well as cardiac biomarkers N-terminal pro brain natriuretic peptide (NT-proBNP) and cardiac troponin I in dogs with preclinical MMVD. Animals: 168 dogs with pre-clinical MMVD and left atrium to aortic root ratio (LA:Ao) > 1.6 and normalized left ventricular end-diastolic diameter (LVEDDn) > 1.7. Methods: Prospective, randomized, multicenter, single-blinded, placebo-controlled study. Clinical parameters radiographic and echocardiographic variables and cardiac biomarkers levels were compared at different time points. Using receiving operating curves analysis, best cut off for selected variables were identified and risk to develop the study end point at 6 months interval was calculated. Results: LA:Ao > 2.1 (hazard ratio [HR] 3.2 confidence interval [CI] 1.9-5.6), normalized left ventricular end-diastolic diameter > 1.9 (HR 6.3, CI 3.3-11.8) early transmitral peak velocity (E peak) > 1 m/sec (HR 3.9 CI 2.3-6.7) and NT-proBNP 1500 μmol/L (HR 5.7 CI 3.3-9.5) were associated with increased risk of HF or cardiac death. Dogs with LA:Ao > 2.1, E peak > 1 and NT-proBNP > 1500 μmol/L have a higher risk to reach the combined end-point at 12 months. Conclusions: a model including echocardiographic variables and NT-proBNP can be used to identify dogs with preclinical MMVD at higher risk to develop HF or cardiac death at 12 month.

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ESVC-O-5
Polymorphisms in the serotonin transporter gene do not associate with myxomatous mitral valve disease or circulating serotonin levels in Cavalier King Charles Spaniels
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The neurotransmitter serotonin has an impact on valvular degeneration and function, and alterations in serotonin signaling have been reported in dogs with myxomatous mitral valve disease (MMVD). In Maltese dogs, three single nucleotide polymorphisms (SNPs) in the serotonin transporter (SERT) gene have been suggested to associate with MMVD. The aim of this study was to investigate if MMVD severity and/or serum serotonin concentrations are associated with the SERT polymorphisms in Cavalier King Charles Spaniels (CKCS). Furthermore, ELISA and HPLC measurements of serum serotonin were compared. The study included 72 CKCS (42 females and 30 males; 7.8 [4.8:10.0] years (median [Q1:Q3]) prospectively enrolled and parentally unrelated, allocated in the following American College of Veterinary Internal Medicine (ACVIM) groups: A (n = 19), B1 (n = 20), B2 (n = 20), another study. Eligible dogs were older than 1 year of age, without concomitant cardiac disease, or heart murmurs > 2/6. All dogs underwent cardiac auscultation and if a murmur ≤ 2/6 was heard, or there was no murmur, an echocardiogram was performed. The severity of MR was subjectively assessed by color Doppler echocardiography as trivial, trace, mild, moderate, or severe. 138 dogs were examined. On screening examination, murmur distribution was as follows: 95 = no murmur, 5 = 1/6, 20 = 2/6, 16 = 3/6, and 2 = 4/6. 120 CKCSs met inclusion criteria, 119 tolerated echocardiography and two of these had small, inaudible patent arterial ducts. MR severity distribution in the remaining 117 CKCSs was as follows: 40 = no MR, 4 = trivial, 6 = trace, 64 = mild, 3 = moderate. Intensity of murmur was significantly associated with severity of MR (P = 0.002) and age (P = 0.0004). 79% (37) of dogs older than 5 years of age and 94% (16) of dogs older than 7 years of age had more than trivial MR despite no or low-grade murmurs. 55 (59%) CKCSs with no murmur had more than trivial MR. In conclusion, we report a high prevalence of echocardiographically detected MR in CKCSs with no or low-grade murmurs.

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C (n = 13). TaqMan genotyping assays were designed for the following polymorphisms: c.814insG; c.1192delT; c.1323A/G in the SERT gene. Serum serotonin concentration was determined using ELISA and HPLC. Multivariable regression analysis was used to assess the influence of genotype, ACVIM group, sex and age on serum serotonin concentration. Spearman correlation, paired t-test and difference plot were used to compare serotonin ELISA and HPLC measurements. Taqman analyses revealed no polymorphisms in any of the selected locations of the SERT gene in the CKCS. ACVIM group, age and sex did not influence serum serotonin concentration except for males (432.5 [358.2-535.2] mg/mL), who had higher serum serotonin concentrations, measured by HPLC analysis, compared to females (342.7 [250.7-454.3] mg/mL) (P = 0.03). Serum serotonin concentration measured by ELISA correlated with HPLC measurements (ρ = 0.87 (P < 0.0001)) but was lower (mean difference = -22.00; P = 0.02). The difference was independent of sex (mean difference (95% CI) = 0.071(0.7%), 0.166(1.5%), [-0.773(-6.44%), 1.010(8.64%]), #2- 0.238(2.09%), 0.435(3.62%), [0.193(1.71%), 0.284(2.47%)], -0.605 (-5.0%), 1.081(9.18%), #3- 0.12 (0.99%), 0.152(1.22%), [0.104(0.86%), 0.136(1.12%)], [-0.179(-1.40%), 0.418(3.38%)].

In conclusion, the selected SERT SNPs associated with MMVD in Maltese dogs were not found in CKCS dogs and are therefore unlikely to impact MMVD pathophysiology or serum serotonin concentration in this breed. ELISA and HPLC serum serotonin measurements indicated good correlation but ELISA underestimated serotonin concentrations (constant systematic error) compared to HPLC.

Disclosures

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ESVC-O-6

Accuracy of deep learning enabled software to measure vertebral heart size in dogs with myxomatous mitral valve disease

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Clinicians routinely assess cardiomegaly by measuring vertebral heart size (VHS) from a right lateral radiograph. Although this method has limitations, many clinicians consider it a first line diagnostic test for staging myxomatous mitral valve disease (MMVD). One of these limitations is related to the skill of the clinician in consistently identifying the appropriate landmarks for measurement, both on the cardiac silhouette and on the vertebrae. Deep-learning-enabled software has been developed and validated in many medical imaging fields including veterinary medicine and in some fields it is recommended over human measurements due to improved accuracy and repeatability. Thus, such software may be capable of measuring VHS for clinicians without the need to identify the landmarks.

The objective of this study was to assess the accuracy of novel deep-learning-enabled software (DL, MetronMind) to measure VHS in dogs with MMVD.

One trained observer measured VHS from right lateral radiographs of 349 dogs with MMVD of varying severity using two methods. The first method was the same as that reported in many clinical studies and annotated as the traditional method (VHS-T), the second method was modified to mimic the method utilized by the DL and annotated as modified (VHS-M). The modification involved using a single measurement of 5 thoracic vertebrae starting at the cranial aspect of the 4th vertebra, which was then divided by 5 to establish the average length of 1 vertebra. This vertebral average length was used to scale the length of the long and short axis dimensions and summed to yield the VHS-M. All other landmarks were identical between these two VHS measurement methods. The VHS measured by DL is annotated as VHS-DL and utilized the same landmarks as the VHS-M method.

Agreement was assessed between methods as follows: #1 VHS-DL versus VHS-M, #2 VHS-DL versus VHS-T, #3 VHS-T versus VHS-M. Results are expressed as bias, bias standard error, [95% bias confidence interval] and [95% agreement limits]. #1- 0.119(1.1%), 0.455 (3.85%), (0.071(0.7%), 0.166(1.5%)), [-0.773(-6.44%), 1.010(8.64%)), #2- 0.238(2.09%), 0.435(3.62%), [0.193(1.71%), 0.284(2.47%)], -0.605 (-5.0%), 1.081(9.18%), #3- 0.12 (0.99%), 0.152(1.22%), [0.104(0.86%), 0.136(1.12%)], [-0.179(-1.40%), 0.418(3.38%)].

These results demonstrate good agreement between VHS-T and VHS-M performed by a human. There was also good agreement between the VHS-DL method and both the VHS-M and the VHS-T. MetronMind DL software measurement of VHS in dogs with MMVD represents a novel clinically useful tool. Enlargement of the teaching set of radiographs used by the DL software may improve agreement between methods.

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ESVC-O-7

Correlation between radiographic vertebral heart size and vertebral left atrial size and echocardiographic measurements of left heart size in Cavalier King Charles Spaniels with preclinical myxomatous mitral valve disease


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Thoracic radiographs are often utilized as a screening tool to assess heart size in dogs, with vertebral heart size (VHS) and vertebral left atrial size (VLAS) reported as objective measurements of global heart size and left atrial size, respectively. Cavalier King Charles Spaniels (CKCS) are predisposed to developing myxomatous mitral valve disease (MMVD), with radiographs frequently used to screen for evidence of left-sided cardiomegaly secondary to MMVD. Normal VHS in CKCS (10.8 +/- 0.5) is reportedly higher than non-breed-specific cut-offs (9.7 +/- 0.5). Breed-specific VLAS cut-offs have not been reported in CKCS. If available, use of breed-specific radiographic cut-offs is recommended by the 2019 ACVIM MMVD consensus statement.
The objective of this study was to determine how echocardiographic measurements of left ventricular and left atrial size correlate to VHS and VLAS in preclinical CKCS not receiving cardiac medications. Two-hundred and thirty asymptomatic CKCS were prospectively enrolled and staged according to the 2019 ACVIM MMVD consensus statement after undergoing a standard echocardiogram. There were 14 CKCS in Stage A, 169 in Stage B1 (22 of which had echocardiographic left ventricular or left atrial enlargement but not both) and 44 in stage B2. A right lateral thoracic radiograph was obtained for measurement of VHS and VLAS in all dogs. Spearman rank-order correlation coefficients ($r_s$) were calculated to determine the strength of associations between echocardiographic and radiographic measurements. The VHS was positively, but not strongly, correlated with normalized left ventricular internal diameter at end-diastole (LVIdDn) ($r_s = 0.59$; CI:0.49-0.67; $P < 0.0001$), left atrial to aortic root ratio (LA:Ao) ($r_s = 0.52$; CI:0.40-0.61; $P < 0.0001$) and left atrial volume indexed to body weight (LA Vol/BW) ($r_s = 0.60$; CI:0.50-0.68; $P < 0.0001$). The VLAS was positively and weakly correlated with LVIdDn ($r_s = 0.40$; CI:0.28-0.51; $P < 0.0001$), LA:Ao ($r_s = 0.41$; CI:0.29-0.51; $P < 0.0001$) and LA Vol/BW ($r_s = 0.43$; CI:0.31-0.53; $P < 0.0001$). Correlation between VHS and a combination of LVIdDn+LA:Ao ($r_s = 0.59$; CI:0.49-0.7; $P < 0.0001$) and LVIdDn+LA Vol/BW ($r_s = 0.60$; CI:0.50-0.28; $P < 0.0001$) were also explored given the global nature of the VHS measurement, with correlations found to be similar to those derived from individual echocardiographic variables. These findings suggest that in CKCS, neither VHS nor VLAS correlate strongly with selected echocardiographic measurements of left ventricular or left atrial size. The lack of strong correlation between VHS and VLAS to the echocardiographic measurements of interest for MMVD staging may impact the performance of thoracic radiographs as a screening tool in CKCS.

Disclosures

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ESVC-O-8

A prospective multicenter study to determine the accuracy of history, physical examination, biochemical parameters and biomarkers to identify dogs with stage B2 degenerative mitral valve disease: The HAMLET study

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Treatment is indicated in dogs with preclinical degenerative mitral valve disease (DMVD) and cardiomegaly (stage B2). This is best diagnosed using echocardiography, however relying upon specialist imaging limits access to accurate diagnosis. This study aimed to evaluate whether cardiac biomarker concentrations could be analyzed alongside other clinical data to accurately identify dogs with echocardiographically confirmed stage B2 DMVD.

The study was prospective and cross-sectional. Client owned dogs ($n = 1887$) were assessed by veterinary cardiologists in Germany, the UK and the USA. All patients had a history taken, underwent physical examination, had a blood sample collected for biochemistry and cardiac biomarker concentrations and underwent echocardiography. A proportion ($n = 175$) underwent thoracic radiography. Clinical observations and bloodwork parameters were entered in explanatory (multivariable logistic regression) and predictive models with echocardiographically confirmed stage B2 disease as the outcome. Predictive models were developed using a subset of data and tested on the remainder. The ability to identify stage B2 dogs was assessed by ROC analysis.

Age, appetite, alanine aminotransferase (ALT) activity, body condition score, creatinine concentration, murmur intensity and N-terminal propeptide of B-type natriuretic peptide (NT-proBNP) concentration were independently associated with the risk of having stage B2 disease. The discriminatory ability of this model (AUC, .84; 95% CI, .82 - .87) was superior to using NT-proBNP (AUC, .77; 95% CI, .74 - .80) or vertebral heart score alone (AUC, .76; 95% CI, .69 -.83). A predictive logistic regression model could identify dogs likely to be in stage B2 (AUC test set, .86; 95% CI, .81 -.91). The findings suggest that widely accessible parameters could be used to screen dogs with preclinical DMVD. Encouraging at risk patients to seek further evaluation may result in a greater proportion of dogs being appropriately managed.

Disclosures

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Pulmonary hypertension (PH) can be categorized as precapillary or postcapillary, or combined. Recent publications in humans have identified indices that can discriminate between pre- and postcapillary PH by using echocardiographic surrogates for the transulmonary gradient (TPG) obtained by indexing tricuspid regurgitation velocity (TR) to indices of left atrial pressure. This study aimed to determine if selected echocardiographic variables would prove discriminatory between pre- and postcapillary PH and combined PH.

This retrospective study evaluated the following echocardiographic variables: transmitral E wave velocity (E vel), the ratio of E vel to the isovolumic relaxation time (E:IVRT), the ratio of E vel to the Tissue Doppler e’ waves (Ee’ lat and Ee’ sep). TPG surrogates were created by indexing TR to indices of left-sided filling pressures (TR/E:IVRT, TR/E:e’ at and TR/E:e’ sep). These were compared between the groups (PH vs control, precapillary vs postcapillary vs control). Receiver operating characteristic (ROC) curves were generated to evaluate the ability of each variable to differentiate between precapillary and postcapillary PH and between pre/postcapillary PH and combined PH.

There were 270 dogs with PH (142 postcapillary, 128 precapillary). 61 control dogs without PH and 12 dogs with suspected combined PH.

La:Ao, E vel, Ei:IVRT, Ee’ lat and Ee’ sep were higher in dogs with postcapillary PH compared with controls and precapillary PH (all \( P < 0.001 \)), but did not differ between precapillary PH and control dogs. TR/Ei:IVRT was lower in postcapillary PH compared with precapillary PH and control dogs (all \( P < 0.001 \)) and lower in control than precapillary PH (\( P < 0.001 \)). TR/E:e’ lat was higher in dogs with precapillary PH than postcapillary PH and control dogs (both \( P < 0.001 \)). TR/E:e’ sep was higher in dogs with precapillary PH than postcapillary PH and control dogs (both \( P < 0.001 \)) and higher in dogs with postcapillary PH compared with control dogs (\( P = 0.04 \)).

ROC curves showed similar area under the curve (AUC) for E vel (0.979), Ei:IVRT (0.979) and TR/Ei:IVRT (0.975) for distinguishing precapillary from postcapillary PH. ROC curves showed similar AUC for E vel (0.825), Ei:IVRT (0.778) and TR/Ei:IVRT (0.778) for distinguishing precapillary PH from combined PH. When differentiating postcapillary PH from combined PH, ROC curves showed a higher AUC for TR/Ei:IVRT (0.890) compared with E vel (0.783) and E:IVRT (0.836).

Surrogate measures of TPG were not more accurate than existing measures of left-sided filling pressures for differentiating between pre- and postcapillary PH. Surrogate TPG measures may be of use in differentiating postcapillary from combined PH.

**Disclosures**

No disclosures to report.
Area under the receiver-operating characteristic curve for PHS indicated a cut-off value of 5 discriminated G2 from G1 with a sensitivity of 77% and a specificity of 100% (AUC: 0.944; \( P < 0.001 \)). Moderate to severe PCPH can be detected with good accuracy by non-cardiologists using a 10 point CV-POCUS PHS score.

Disclosures
No disclosures to report.

ESVC-O-11

Rapid atrial ectopic firing in dog: a retrospective study in 10 cases
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Rapidly focal atrial activity is one of the main mechanisms responsible of atrial fibrillation (AF) onset in human medicine. The pulmonary veins (VPs) are the main source of focal activity, although other sites of focal activity have been described. These foci trigger AF by a burst or firing of rapid discharges. Multiple electrophysiologic mechanisms have been proposed as the basis for focal PV firing, including abnormal automaticity, triggered activity, and micro-reentry. Autonomic tone and atrial stretch play an important role on PV activity. The aim of our study was to describe electrocardiographic appearance of rapid atrial ectopic firing in the dog. A total of 10 Holter tracings recorded in dogs with ectopic atrial firing were retrospectively analyzed. The sample group included dogs of different breeds with an average age of 9.7 years (range 2-14), M/F 7:1 and an average body weight of 32.7 Kg (range 7-50). Five out of ten dogs presented underlying structural heart disease with variable grade of cardiac remodeling and congestive heart failure (suspected myocarditis, degenerative mitral valve disease, dilated cardiomyopathy and heartworm disease) while the remaining had normal cardiovascular system. All 24-hour tracings showed a sinus rhythm interrupted by numerous episodes of self-limiting or sustained runs of focal atrial tachycardia (FAT) throughout the recording (average 451 episodes; range 18-2502). Focal atrial tachycardias were characterized by an average duration of 183 beats (range 3-364) and an average cycle length of 233.3 ms (range 190-250 ms). Episodes of paroxysmal firing atrial activity (range 1 – >800 per 24 hours) with an average duration of 67.16 seconds (range 0.65-2460) were identified in all recordings. These episodes were characterized by a rapid atrial activity with an average atrial cycle length duration of 150.18 ms (range 90-260), short RP’ (average 116.9 ms; range 50-250), different atroventricular conduction ratios (range 1:1 to 12:1), variable atrial depolarization morphology in the orthogonal system (prevalent positive in all leads) and irregular cycle length. Episodes were triggered by a sinus beat, premature ventricular ectopic beat or a supraventricular ectopic beat. Three episodes induced AF. AF was persistent in one case and paroxysmal in 2 cases (6 and 59 min respectively). Electrophysiologic mapping was available in one case, showing the site of firing at the level of left superior pulmonary veins. The present study described, as it was reported previously in human medicine, a rapid ectopic atrial firing as possible mechanism of AF onset in dogs.

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ESVC-O-12

Comparison of temporary pacing techniques in dogs undergoing permanent pacemaker implantation
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Temporary cardiac pacing is commonly performed in bradycardic dogs immediately prior to permanent pacemaker implantation. This maintains adequate cardiac output under general anesthesia. Transvenous temporary pacing (TVTP) starts before anesthetic induction and requires fluoroscopy. Transthoracic temporary pacing (TTTP), after anesthetic induction, does not need fluoroscopy, is less invasive, and is technically simpler but may be less reliable in larger dogs. Although well described in dogs, there are no published studies comparing TVTP and TTTP directly. We hypothesized that TTTP decreases procedure and fluoroscopy times compared with TVTP, but also increases risk of failure to capture in larger dogs.

A total of 60 dogs were included for analysis. One third of these had TTTP (n = 20, 33.66%) compared to two thirds with TVTP (n = 40, 66.33%; \( P = 0.013 \)) and the TTTP dogs were lighter (TTTP 9.2 kg (6.38-12.02); TVTP 26 kg (17.1-34.9); \( P < 0.001 \)). However, temporary pacing events were similar for both groups (TTTP, 5/20 (25%); TVTP, 8/40 (20%); \( P = 0.65 \)). Although TTTP decreased premedication to induction time (TTTP 65 min (43.5-86.5); TVTP 105 min (86.5-123.5); \( P < 0.001 \)) and the total procedure duration (TTTP 200 min (161-239); TVTP 235 min (203.5-266.5); \( P = 0.015 \)), it did not decrease fluoroscopy time (TTTP, 269.5 min (117.5-421.5); TVTP, 295 min (157-433); \( P = 0.302 \)).

Both forms of temporary pacing are reliable. Compared to TVTP, TTTP reduces the time it takes to implant a pacemaker in a dog.
This is mainly because it reduces the delay between pre-medication and anesthetic induction rather than the duration of fluoroscopy. However, this advantage is offset by increased risk of anesthetic events. Importantly, though, in this retrospective study, the potential influence of bodyweight cannot be discounted.

Disclosures

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ESVC-O-13

Noninvasive electrocardiographic parameters to assess interventricular dyssynchrony in dogs with bundle branch blocks

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Bundle branch block (BBB) is an anomaly of the conduction of the electrical impulse along the intraventricular specialized conduction system. The electrocardiographic diagnosis of BBB in dogs is based on the abnormal duration (>80 ms), axis and morphology of the QRS complex. In humans, the analysis of R-peak time (RPT) in precordial leads has been used to differentiate right from left BBB. In case of BBB, interventricular dyssynchrony (interD) is present and can be assessed using ECG parameters. The aims of this study were to define electrocardiographic features of complete BBB in dogs and the utility of RPT to evaluate interD.

12-lead electrocardiographic tracings of 40 privately-owned dogs (20 with right BBB and 20 with left BBB) were retrospectively reviewed. Each ECG was recorded using the Wilson’s precordial lead system modified by Santilli et al. For each tracing, duration, morphology and QRS complex mean electrical axis (MEA) on the frontal plane and RPT duration in precordial leads (V1-V6) were analyzed using three randomly selected consecutive beats. To evaluate interD, the interD index (IDI) was calculated using the formula V2RPT-V1RPT/QRS duration (%) as previously reported in human medicine. Normality was tested using the Shapiro-Wilcoxon W-test. To evaluate differences between leads, nonparametric analysis of variance was performed by Kruskal Wallis test, as data were not normally distributed. Data are expressed as median and range.

In right BBB, QRS complex duration was 104 ms (81-139), MEA was -111° (-73.3 - +142), the commonest morphology in lead II was qrS, and RPT (ms) in V1 was 61 (48-82), V2 25 (12-45), V3 25 (12-45), V4 24 (11-44), V5 25 (11-42), V6 25 (11-43). In left BBB, QRS complex duration was 107 ms (81-135), MEA was 75.6° (+23.5 - +86.3), the commonest morphology in lead II was qrR, and RPT (ms) in V1 was 17 (10-39), V2 49 (17-73), V3 48.5 (23-77), V4 52 (31-78), V5 53 (30-78). V6 55 (30-78). In right BBB, RPT was significantly longer in V1 than in V2-V6 (P < 0.05) and in left BBB RPT was significantly longer in V2-V6 than V1 (P < 0.05). The IDI was significantly increased: -33% in case of right BBB and 32% in case of left BBB.

This study defines ECG features and RPT in dogs with right and left BBB. Like in human beings, RPT and IDI can be used to define interD in dogs.

Disclosures

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ESVC-O-14

Comparison of three two-dimensional echocardiographic methods of assessing left ventricular size in Doberman Pinschers

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Dilated cardiomyopathy (DCM) commonly affects Doberman Pinschers and leads to systolic dysfunction of the heart. The reference standard for diagnosing DCM includes measuring left ventricular end-systolic and end-diastolic volumes using Simpson’s method of disks (SMOD), though linear left ventricular end-diastolic (LVIDd) and end-systolic (LVIDs) measurements via short-axis M-mode (SA-MM), long-axis M-mode (LA-MM), and short-axis 2D (SA-2D) have been reported. While it is suspected that these measurements are similar, no study has directly compared all of these measurements in the same dog. The purpose of this study was to directly compare linear dimensions in a population of Doberman Pinschers. One hundred and forty screening echocardiograms between 2017-2019 were analyzed. The LVIDd and LVIDs were evaluated using leading edge to leading edge technique from the right parasternal short axis view at the level of the papillary muscles (SA-MM and SA-2D). These measurements were also evaluated using the right parasternal long axis four chamber view using either M-mode or anatomic M-mode (LA-MM). Finally, monoplane Simpson’s SMOD was used to determine end-diastolic and end-systolic left ventricular volumes from the right parasternal long axis view. All measurements were repeated over three cardiac cycles. Data were tested for normality using Shaprio-Wilk and a Kruskal-Wallis test with Dunn’s multiple comparisons was used to compare the mean rank difference between modalities. Bias was evaluated via Bland-Altman and correlation was evaluated using Spearman’s correlation. The averages of three linear measurements were compared with SMOD and sensitivity and specificity for diagnosing occult DCM were determined.

The mean rank differences were significantly different between all linear dimensions in both diastole and systole. Short-axis 2D measurements had significant bias compared with SA-MM (diastole +1.19 mm, systole +1.65 mm) and LA-MM (diastole +4.36 mm, systole +3.87 mm) as did SA-MM compared with LA-MM (diastole +3.17 mm, systole +2.22 mm). All linear dimensions had moderate positive correlation with SMOD in both diastole and systole. The sensitivity and specificity of each linear measurement to detect DCM was as follows: SA-2D (sensitivity 72.0%, specificity 88.5%), SA-MM (sensitivity 80.0%, specificity 88.5%), LA-MM (sensitivity 78.6%, specificity 88.5%), SMOD (sensitivity 74.3%, specificity 88.5%).
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ESVC-O-16

A dog’s dinner: Evidence of metabolic derangement in dogs with naturally occurring valvular heart disease and congestive heart failure

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The myocardium requires continuous ATP production and when healthy, this is predominantly derived from β-oxidation of lipids. Cardiovascular disease affects flux through metabolic pathways and conditions of stress or ischemia may favor less oxygen demanding pathways. This manifests as reduced β-oxidation and increased dependence upon glucose as a substrate. This shift is maladaptive; decreasing ATP production and increasing concentrations of toxic lipid species that can exacerbate myocardial dysfunction. It is hypothesized that similar processes occur in canine degenerative mitral valve disease, so the aim of this study was to evaluate changes in the lipidome in the context of worsening disease severity.

Patients (n = 40) had been longitudinally evaluated at a research clinic (2004–2017) and paired residual serum samples were selected from visits in ACVIM stage B1: asymptomatic disease without cardiomegaly, and stage C: congestive heart failure (CHF). Samples were processed using ultra-performance liquid chromatography mass spectrometry (UPLC-MS) and lipid profiles compared using linear mixed effects models.

Using an adjusted P value (Q < 0.05), 169 UPLC-MS features were associated with disease progression. From these, 20 molecules were annotated by comparing tandem-MS data to reference databases. In CHF, quantities of some circulating acylcarnitines, lysophosphatidylethanolamines, phosphatidylethanolamines, phos- phoinositols and sphingomyelins increased. Concentrations of some ceramides and lysophosphatidylcholines decreased.

These results are consistent with altered myocardial metabolism in advanced disease and certain findings may reflect an accumulation of acyl-coAs secondary to reduced β-oxidation. There is a need for further research, as therapeutic interventions could be used to ameliorate metabolic derangement prior to the onset of CHF.

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ESVC-O-15

ECG abnormalities in Irish wolfhounds

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Irish wolfhounds (IW) are commonly affected with dilated cardiomyopathy (DCM) and atrial fibrillation (AF). The aim of this study was to evaluate the incidence and clinical significance of abnormalities: ventricular premature depolarizations (VPDs), notched QRS complexes, fascicular and bundle branch block in this breed.

Data from cardiovascular examinations performed in 1673 IW, including echocardiography with simultaneous ECG recording and a 3 min, six-lead ECG registered in right lateral recumbency, were retrospectively evaluated. Dogs were longitudinally followed, and owners instructed to report date and circumstances of death.

VPDs (as singles up to 50/min, with bigeminy, as pairs, multifocal, or as runs) were recorded in 57/433 IW (13.2%) with DCM diagnosis, in 3/60 (5%) with AF, and in 55/1180 (4.7%) IW without DCM. Out of those IW with information on time and cause of death, sudden cardiac death (SCD) was reported in 13/47 (27.7%) of IW with DCM and SVPDs as singles in 12/433 (2.8%) IW with DCM with later development of AF and 6/375 (1.7%) of IW with DCM without VPDs, in 3/43 (7%) of IW with AF, and in 2/37 (5.4%) of IW with VPDs but without DCM, compared to 0/543 (0%) of IW without DCM or VPDs. Runs of supraventricular (SV) tachycardia were detected in 9/433 (2.1%) and SVVPDs as singles in 12/433 (2.8%) IW with DCM with later development of AF in 3 and 6 of these dogs, respectively. Notched QRS complexes (Rr, RR, Rr, rr) were present in 37/433 (8.5%) of IW with DCM, in 6/60 (10%) of IW with AF, and in 100/1180 (8.5%) of IW without DCM. Left anterior fascicular block was seen in 2/433 IW with DCM (0.5%), and in 10/1180 (0.85%) without DCM, while right bundle branch block was detected in 7/433 (1.6%) IW with DCM and in 12/1180 (1%) without DCM. One IW without DCM had left bundle branch block, and 5/433 (1.2%) had AV-block I.

While fascicular and branch block abnormalities were more commonly seen within certain families, there was no apparent association of any of the observed conduction abnormalities with cardiac morbidity and mortality. While most dogs had multiple (up to ten) cardiac examinations, an important limitation of this study is that 24 h monitoring was not performed which would have permitted the best assessment of VPDs.

However, this study suggests that IW with DCM and VPDs during routine echocardiography and 3 min ECG recordings, have an even higher risk to die from SCD than DCM dogs without VPDs.

Disclosures
No disclosures to report.
Differences in left ventricular remodeling secondary to chronic volume loading between English Springer Spaniels and two other similar athletic breeds

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English springer spaniels (ESS) have larger and rounder ventricles than other breeds. How this geometry impacts responses to volume overload is not well understood. Therefore, we compared left ventricular geometry and remodeling between ESS and two similarly sized athletic breeds (Border collies, BC, and Labrador retrievers, LR) in naturally-occurring chronic left ventricular (LV) volume loading conditions (mitral regurgitation, MR, and patent ductus arteriosus, PDA).

We searched records at four large cardiology referral centers in the UK for cases of MR (due to either congenital or acquired valve disease) and PDA in the three breeds. We recorded age, gender, presence of congestive heart failure (CHF), body weight and specific echocardiographic variables. We then compared echocardiographic normalized measures of left ventricular size between ESS vs. Non-ESS dogs. To account for the possible confounding effects of different disease status, cases with MR were normalized to left atrial size (by dividing LV measures by the short-axis left atrium to aortic ratio (LA:Ao)). Cases with CHF were further examined as a separate group. Medical treatment was not recorded. Data were assessed for normality and groups were compared with t-tests or Mann-Whitney-U tests, as appropriate.

165 dogs were included: 129 had MR (46 ESS, 37 BC, 46 LR) and 36 had PDA (12 ESS, 16 BC, 8 LR); 57 cases had CHF. All measures of LV size (internal dimensions and Simpson’s derived volumes, normalized to body size) were larger in ESS than Non-ESS in MR cases (P < 0.0001 for all comparisons), whereas PDA cases did not differ. When considering only cases of CHF, the differences remained in the MR cases (P < 0.0001 for all comparisons). In dogs with CHF secondary to PDA, diastolic dimensions and volumes did not differ between ESS and non-ESS but ESS had larger systolic dimensions and volumes than non-ESS dogs (P < 0.05).

ESS have greater LV dimensions when exposed to chronic MR, compared with BC and LR, but not when exposed to volume overload from a PDA. However in PDA cases with CHF, ESS have greater LV systolic dimensions than Non-ESS dogs. These data suggest that ESS as a breed have a different LV remodeling phenotype compared with two other similarly sized athletic breeds, supporting the idea that the ESS has a unique cardiac morphotype.

Disclosures
No disclosures to report.
Diagnosing infective endocarditis can be challenging as the non-specific clinical signs and subtle echocardiographic lesions may mimic those of immune-mediated disease (IMD) and myxomatous mitral valve disease (MMVD), respectively. We hypothesized that serum cardiac troponin I (cTnI) concentration would differentiate dogs with endocarditis from these two control groups.

Records from two referral centers were reviewed for dogs diagnosed with endocarditis, MMVD or IMD. Dogs were included if it was possible to measure serum cTnI at time of diagnosis. Dogs with MMVD were excluded if they had ever experienced CHF and dogs with IMD were excluded if they were non-steroid responsive. Comparisons were explored using Kruskal-Wallis and Mann-Whitney U tests. Receiver operator curve analysis was performed to investigate cTnI cut-off values. Cox proportional hazards was used to investigate the association between cTnI and survival.

Seventy-two patients were included (endocarditis n = 29; MMVD n = 27; IMD n = 16). Serum cTnI measurements were significantly higher in the endocarditis group (0.69 ng/mL) than the MMVD (0.05 ng/mL) and IMD groups (0.05 ng/mL, P < 0.001). There was no significant difference in serum cTnI concentration between the control groups (P = 0.28). Increased cTnI could differentiate endocarditis from MMVD and IMD with moderate accuracy (AUC 0.857, P < 0.001). A cut-off of 0.6ng/mL had 100% specificity and 52% sensitivity, which was considered practically useful. There was no association between cTnI concentration and survival time (P = 0.201) in patients with endocarditis.

Our study suggests that in patients with a compatible clinical presentation, a cTnI concentration ≥0.6ng/mL should increase the suspicion of endocarditis.

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ESVC-O-20

CRISPR/Cas9 genome engineering to model the R820W mutation effects in iPSC-derived cardiomyocytes
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Hypertrophic cardiomyopathy (HCM) is the most common heart disease in cats, often leading to congestive heart failure and death. Although some causative mutations, such as MYBPC3/R820W in Ragdoll cats, have been identified, the underlying pathological processes driving disease remain unclear. Using primary cardiomyocytes as a disease model is limited as they survive poorly in culture. This has been overcome by the differentiation of induced pluripotent stem cells (iPSC) into cardiomyocytes (iPSC-CM). CRISPR/Cas9 based gene editing of iPSCs allows comparison between isogenic cell lines therefore controlling for background genetic factors. These disease models have led to significant advancements in the understanding and treatment of human HCM. The development of similar models would represent a substantial benefit for feline medicine.

Our aim was to study the effects of the R820W mutation using CRISPR/Cas9 engineering in human iPSC-derived cardiomyocytes. iPSCs were nucleofected with Cas9 protein, template DNA and guide RNA. In total 576 single cells were cloned, expanded and genotyped. Next-generation sequencing identified 34 cell clones that were homozygous (R820W+/+) and 35 clones that were heterozygous (R820W+/-). Six clones (three R820W+/+ and three R820W+/-) were differentiated into cardiomyocytes (iPSC-CMs). Homozygous (R820W+/+) iPSC-CMs had a larger cell area compared to WT cells (2804 ± 120 μm² vs. 2170 ± 112 μm², mean ± SEM, P < 0.001), indicating hypertrophy of mutant cells. Immunocytochemistry showed cells in both groups expressed cMyBPC at the protein level and this was incorporated into the A-band of sarcomeres as expected. Interestingly motion analysis of contracting cardiomyocytes showed that mutant cells had a longer relaxation phase (580.5 ± 23.5 ms vs. 472.1 ± 17.8 ms, P < 0.001), longer contraction duration (753.9 ± 24.7 ms vs. 642.4 ± 18.4 ms, P < 0.001) and lower contraction amplitude (10749 ± 758.2 a.u. vs. 16434 ± 1139.0 ms, P < 0.001) compared to WT cells. The time to peak contraction was not different between the two groups (174.4 ± 5.6 ms vs. 169.9 ± 5.7 ms, homozygous vs. WT, P = 0.576). This indicates that the R820W+/+ mutant cells have a normal initiation of contraction, but cells have impaired relaxation and reduced contractile distance. These results show that CRISPR mediated knock in of R820W+/+ in iPSC-CMs effectively recapitulates functional features characteristic of HCM. This provides new evidence for the direct effects of the R820W mutation in isogenic cell lines, and creates a platform to screen novel therapeutics for feline HCM.

Disclosures
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ESVC-O-21

Pharmacodynamics of ACE inhibitors in dogs with cardiac disease, proteinuria or hypertension: When size matters. A retrospective study of 326 cases
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Angiotensin converting enzyme inhibitors (ACEi) are standard-of-care medications for the treatment of cardiac disease, systemic hypertension and proteinuria in veterinary medicine; however, no consensus exists regarding the ideal dose of ACEi in dogs. The objective of this retrospective analysis was to determine the effect of varying dosing schedules of ACEi (i.e. dose amount and/or frequency) on short-term and long-term outcome variables in dogs.
A total of 326 dogs presented to a veterinary teaching hospital between 2005 and 2018 were included in the analysis: 148 dogs received ACEi for management of cardiac disease; and 178 dogs for treatment of non-cardiac diseases (hypertension [N = 50], proteinuria [N = 45], or both [N = 83]). The study included 3 consecutive visits: (i) initial ACEi prescription (Visit 1); (ii) next follow-up (~15 days after Visit 1); and (iii) long-term follow-up for dogs with cardiac disease. Dogs receiving ACEi treatment prior to Visit 1 were excluded. Data were analyzed using linear regression methods for continuous variables, logistic regression for categorical variables, and Cox proportional hazards models for survival data. P < 0.05 were considered statistically significant.

The majority of dogs received enalapril (>80%) and B.I.D dosing (58%) at inclusion, with a median initial daily dose of 0.65 mg/kg ACEi. Study results showed a mild dose-response effect of ACEi on BP in dogs, with higher doses (and/or B.I.D dosing) resulting in an average ~15mmHg decrease in BP (P < 0.04), compared to ~5mmHg decrease for lower doses (and/or S.I.D dosing). This effect was most pronounced in proteinuric dogs. In the subgroup of hypertensive dogs, B.I.D dosing was associated with a greater decrease in urine protein: creatinine ratio compared to S.I.D dosing (P = 0.03). In dogs with cardiac disease, both ACEi dose and frequency had a significant effect on prolonged survival from Visit 1 to all-cause mortality. This retrospective study also confirmed the safety and tolerability of ACEi across a wide dosing range, with only 4.3% incidence of dose decrease or discontinuation due to side effects.

Overall, these results suggest that higher dose and/or more frequent dosing of ACEi are associated with positive short-term and long-term outcomes in dogs with cardiac and non-cardiac diseases. Further research is warranted to determine whether these effects are primarily driven by an increase in ACEi dose amount or frequency.

Disclosures
No disclosures to report.

ESVCN-O-1

A long term feeding trial to aid in establishing No Observed Adverse Effect Levels (NOAELs) for different sources of phosphorus in feline diets

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High dietary phosphorus (P), particularly added as soluble salts, may contribute to the development of chronic kidney disease (CKD) in cats. There is currently no guidance for safe maximum limits of P in cat foods. Consequently it is important to establish safety for different dietary P sources. The aim of this study was therefore to establish the safety of P supplied at 1g/1000kcal from a highly soluble P salt, at two different total P levels, in feline diets.

Seventy-five healthy adult cats (n = 25/group) were fed either a low P control diet with no P salt added (1.4g/1000 kcal; Ca:P 0.97) or a test diet formulated with either moderate (Test 1 - 4g/1000 kcal; Ca:P 1.04) or high (Test 2 - 5g/1000 kcal; Ca:P 1.27) total P for a period of 30 weeks in a randomized parallel design. Both test diets contained sodium tripolyphosphate (STPP) providing 1gP/1000 kcal of total P. Blood, urine and faces samples were collected, glomerular filtration rate (GFR) determined, and renal ultrasound and bone density (DXA) scans performed at baseline and at regular time points to assess health. Following transition back to a commercial diet (total P ~ 2.34g/1000 kcal; Ca:P 1.3), further blood and urine samples were collected after 4 weeks. Responses were analysed via linear mixed effects models with fixed effects of diet group, time point (weeks), and their interaction, including cat as a random effect.
There was no significant effect of diet on GFR, DXA or ultrasound data and all blood and urine parameters remained within physiological reference ranges. However, urea, creatinine and fibroblast growth factor-23 (FGF-23) significantly increased in cats fed test diets from week 2 onwards (P < 0.001, P ≤ 0.041 and P ≤ 0.028, respectively). P and calcium balance were increased in the test diet groups at week 27 (P < 0.001). On transition back to a commercial diet, data indicated that plasma urea, creatinine, FGF-23 and parathyroid hormone (PTH) levels were all influenced by the diet change.

The present data suggest that feline diets containing 1gP/1000kcal from STPP and providing a total phosphorus level of 4 and 5g/1000kcal were safe to feed for 30 weeks. Differences in physiological parameters observed during the trial were most likely regulatory responses needed to maintain mineral homeostasis and/or differences in dietary factors other than P levels. These data will assist industry, regulators and professional bodies in developing guidance on safe maximum dietary levels of P for healthy adult cats.

Disclosures
All authors were employed or contracted by Mars Petcare at the time of this study.

ESVCN-O-2

Maintenance energy requirements of cats with obesity after a period of controlled weight reduction
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Weight regain after successful weight loss is a well-known phenomenon both in humans and companion animals with obesity, and a possible reason for this is that energy requirements after weight reduction are less than they were. The aim of the current retrospective observational study was to estimate post-weight reduction energy requirements (MER) in a group of pet cats with obesity that had successfully lost weight and whose weight remained stable during the subsequent weight maintenance phase.

The study protocol was approved by two different Research and Ethics Committees from two institutes, and all owners gave informed written consent. For inclusion, at least 2 months of follow-up data (including bodyweight and food intake via owners’ diary records) had to be available for review, and weight had to remain stable during the weight maintenance period (within ±2% of the weight recorded at the end of weight reduction). Post-weight-loss MER was indirectly calculated with the use of bodyweight and energy intake at different stages of weight management, whilst simple and multiple linear regression was used to identify factors associated with post-weight-reduction MER.

The median (interquartile range) duration of weight maintenance was 179 days (119-408 and, during this time, MER was 159 kJ (134-178; 38 [32-42] kcal) per kg ideal bodyweight (IBW) per day. The average MER during the weight maintenance phase was greater than energy intake at the end of the weight reduction period (134 [126-151] kJ per kg IBW; 32 [30-36 kcal per kg IBW, P < 0.001), but not energy intake at the start of the weight reduction period (142 [130-151] kJ per kg IBW; 34 [31-36] kcal per kg IBW, P = 0.148). Using simple and multiple linear regression, the only factor that was significantly associated with MER during the maintenance period was the mean energy intake during weight reduction (R² = 0.349, P = 0.008).

Maintenance energy requirements after weight reduction in cats with obesity, are typically only 20% greater than energy intake at the end of the weight reduction period, and not significantly different from energy intake at the start of weight reduction. As a result, it is advisable to feed a diet with a lesser energy density to provide low energy requirements during this phase.

Disclosures

ALEX GERMAN Current academic post financially supported by Royal Canin (2002 to current). This author has also given talks related to the topic for Royal Canin, Mars Petcare, BSAVA, Hills, NAVC/VMX, BVA, Nestle Purina, Pfizer/Zoetis, ICC/ISFM, AAFF, FEDIAF, and PFMA. This author’s research relating to the topic has been funded by Royal Canin, Mars Petcare, BSAVA, and Dogs Trust. VINCENT BIOURGE is both employees of Royal Canin.

ESVE-O-1

Urinary liver-type fatty acid binding protein and neutrophil gelatinase-associated lipocalin in hyperthyroid cats before and after radiodiode treatment
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Progressive azotemia can occur in cats after radioactive iodine treatment of hyperthyroidism. This process can contribute to reduced survival times in cats with iatrogenic hypothyroidism. Effective parameters to predict progressive azotemia in feline hyperthyroidism are lacking. Liver-type fatty acid binding protein (L-FABP) and neutrophil gelatinase-associated lipocalin (NGAL) are promising biomarkers for detection of early kidney insult in humans and cats. The objective of this study was to assess urinary L-FABP (uL-FABP) and urinary NGAL (uNGAL) in hyperthyroid cats before and after radiodiode treatment.
Blood and urine samples from 49 cats at 3 different time points i.e. before (T0) (n = 49), 1 month after (T1) (n = 49) and 11-29 months (T2) (n = 26) after radiiodine treatment were analysed. Urinary L-FABP-to-creatinine ratio (uL-FABP/Cr) and urinary NGAL-to-creatinine ratio (uNGAL/Cr) were compared between T0, T1 and T2 using Wilcoxon signed-rank test using median [min-max] values. Correlations between uL-FABP/Cr or uNGAL/Cr and serum creatinine (sCr), total thyroxine (TT4) and glomerular filtration rate (GFR) using plasma exogenous Cr clearance test were determined for all time points together using Spearman’s correlation.

Urinary L-FABP/Cr significantly decreased from T0 to T1 (n = 49; 0.88 [0.03-896.00] versus 0.20 [0.03-2.40] μg/g, respectively; \( P < .001 \)) and from T0 to T2 (n = 26; 0.68 [0.03-896.00] versus 0.19 [0.02-76.41] μg/g, respectively; \( P < .001 \)). Urinary L-FABP was detectable in 40/49 cats at T0, 11/49 at T1 and 14/26 cats at T2. There was no significant change of uNGAL/Cr between time points. Of 26 cats followed from T0 to T2, only 2 cats were azotemic at T2. The first of these cats had markedly high uL-FABP/Cr at T2 (76.41 μg/g), while the second azotemic cat had relatively low uL-FABP/Cr both at T0 (0.88 μg/g) and T2 (0.17 μg/g). There were a moderate and significant correlation between uL-FABP/Cr and TT4 (\( r_s = .33; P < .001 \)) and between uL-FABP/Cr and GFR (\( r_s = .49; P < .003 \)). Serum Cr was negatively correlated with uL-FABP/Cr (\( r_s = -.28; P = .001 \)). Urinary NGAL/Cr was not correlated with sCr, TT4, and GFR.

Urinary L-FABP is upregulated in hyperthyroid cats suggesting ischemic/oxidative stress in feline kidneys during hyperthyroidism. However, uL-FABP is not present in all hyperthyroid cats and resolved when euthyroidism was obtained. Therefore, the ability of uL-FABP to predict progressive azotemia in hyperthyroid cats remains unclear. Also, uNGAL is not a reliable biomarker for the detection of early kidney injury in hyperthyroid cats.

**Disclosures**

No disclosures to report.

**ESVE-O-3**

*The effect of the ghrelin-receptor agonist capromorelin on glucose metabolism in healthy cats*

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Somatostatin secretion from islet delta-cells is important in maintaining low glycemic variability (GV) by providing negative feedback to beta-cells and inhibiting insulin secretion. Capromorelin is a ghrelin-receptor agonist that activates the growth hormone secretagogue receptor on delta-cells. We hypothesized that in cats, capromorelin administration will result in decreased GV at the expense of reduced insulin secretion and glucose tolerance. Seven healthy cats were treated with capromorelin from days 1-30. After the first day, fasting blood glucose increased (+13 ± 3 mg/dL, \( P < 0.0001 \)), insulin decreased (+128 ± 122 ng/dL, \( P = 0.03 \)) and glucagon was unchanged. Blood glucose was increased throughout an intravenous glucose tolerance test on day 1 with blunting of first phase insulin response (\( \text{FPIR} \) 4931 ± 2597 ng/L/15min) compared to day -3 (17437 ± 8302 ng/L/15min, \( P = 0.004 \)). On day 30, FPIR was still blunted (9993 ± 4285 ng/L/15min, \( P = 0.045 \)) but glucose tolerance returned to baseline. Mean interstitial glucose was increased (+19 ± 6 mg/dL, \( P = 0.03 \)) on days 2-4 but returned to baseline by days 27-29 (\( P = 0.3 \)). On days 2-4 GV was increased (SD = 9.7 ± 3.2) compared to baseline (SD = 5.0 ± 1.1, \( P = 0.02 \)) and returned to baseline on days 27-29 (SD = 6.1 ± 1.1, \( P = 0.16 \)). In summary, capromorelin caused a decline in insulin secretion and glycemic control and an increase in glucose variability early in the course of treatment, but these effects diminished towards the end of 30 days treatment.

**Disclosures:** The senior author has served as a consultant to Aratana Therapeutics. This study was partially funded by Elanco Animal Health

**ESVE-O-4**

A novel once-a-week feline recombinant insulin for the treatment of diabetes mellitus in cats

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Treatment of diabetes mellitus (DM) in cats typically requires q12h-q24h insulin injections, posing a major compliance issue for pet owners. Novel treatments enabling decreased frequency of injection while maintaining safety are highly desirable. In this pilot study, we assessed an ultra-long-acting novel formulation of recombinant fusion protein of feline insulin and feline Fc (AKS-267c) administered subcutaneously weekly. Five cats with spontaneous DM (and normal IGF-1) that were controlled on stable doses of standard q12h insulin were transitioned to AKS-267c. The dose of AKS-267c was titrated for 7 weeks as needed based on continuous flash glucose monitoring (FGM). Clinical signs, body weight, fructosamine and mean interstitial glucose (IG) concentrations were used to compare glycemic control at baseline (week zero, on standard insulin therapy) vs. week 7 (after 7 weeks of once-weekly AKS-267c therapy). Data were assessed for normality and compared using parametric or non-parametric (as appropriate) paired tests. After 7 weeks of once-weekly injection, compared to baseline, there were no changes in clinical signs and body weight (mean ±SD body weight gain = 0.2 ± 0.3 Kg, \( P = 0.3 \)). There were no significant changes in fructosamine (-61 ± 199 mmol/L, \( P = 0.5 \)) and mean IG concentrations (median [range] change = -6.2 [-9.3 - +3.1] mmol/L, \( P = 0.2 \)). There were no adverse reactions associated with AKS-267c administration. In conclusion, successful maintenance of glycemia was achieved with this once-weekly novel insulin
therapy. The efficacy and safety of this novel formulation should be further assessed in a large clinical trial.

Disclosures: Akston Biosciences has donated materials for this study and has funded another study performed by the authors.

ESVE-O-5

Performance of a flash glucose monitoring system in cats
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The FreeStyle Libre® (Abbott Laboratories) is a flash glucose monitoring system (FGMS) that measures interstitial glucose (IG) and stores this data onboard until scanned by the reader. The sensors are factory-calibrated, and the system is user-friendly, inexpensive, and could be extremely useful for monitoring diabetes but has not yet been validated for use in cats. We compared blood glucose (BG) concentrations (measured by AlphaTrak®) to IG (measured by the FreeStyle Libre®), during 16 IV glucose tolerance tests (IVGTT) in seven purpose-bred laboratory cats, across the eu- and hyperglycemic range (184 samples). Correlation between BG and IG was assessed using Pearson’s r and the accuracy was assessed against BG as the reference standard using the Bland-Altman method. In the immediate 30 minutes following intravenous bolus of glucose when BG concentrations were declining rapidly (@ 2%/min), IG increased slowly, resulting in a difference of as much as 32.2 mmol/L (579 mg/dL), and no positive correlation between BG and IG. At baseline and between minutes 45-180 of the IVGTT, when BG was stable or rate of decline slow (@ 0.5%/min), there was a strong positive correlation between BG and IG (r = 0.97 [95% CI = 0.96 – 0.98]) with a consistent bias (across the BG range) towards underestimating BG by the Libre (mean ± SD bias 1.3 ± 1.0 mmol/L [23.3 ± 18.1 mg/ml]). In conclusion, the FreeStyle Libre® may be appropriate to monitor diabetic cats, but consideration should be made for the lag between IG and BG during periods of rapid change.

Disclosures
No disclosures to report.

ESVE-O-6

A novel once-a-week canine recombinant insulin for the treatment of diabetes mellitus in dogs
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Treatment of diabetes mellitus (DM) in dogs typically requires q12h-q24h insulin injections, posing a major compliance issue for pet owners. Novel treatments enabling decreased frequency of injection while maintaining safety are highly desirable. In this pilot study, we assessed an ultra-long-acting novel formulation of recombinant fusion protein of canine insulin and canine Fc (AKS-218d) administered subcutaneously. Four dogs with spontaneous DM, controlled on stable doses of standard q12h insulin, were transitioned to AKS-218d. The dose of AKS-218d was titrated for 8 weeks based on continuous flash glucose monitoring (FGM). Clinical signs, body weight, fructosamine and mean interstitial glucose (IG) concentrations were compared between baseline (week zero, on standard insulin therapy) and week 8. Data were assessed for normality and compared using parametric or non-parametric (as appropriate) paired tests. After 8 weeks of once-weekly injection, compared to baseline, there were no changes in clinical signs and body weight (mean [±SD] body weight change = 0.05 ± 0.96 Kg, P = 0.9). There were no changes in fructosamine (-34 ± 145 mmol/L, P = 0.9) and mean IG concentrations (-0.7 ± 10.0 mmol/L, P = 0.9). Low IG (<3.9 mmol/L) was recorded in 2 dogs at week zero (1% and 23% of readings) and 2 dogs at week 9 (5% and 6% of readings). There were no adverse reactions to AKS-218d. In conclusion, successful maintenance of glycemia was achieved with this once-weekly novel insulin therapy. The efficacy and safety of this novel formulation should be further assessed in a large clinical trial.

Disclosures: This work was funded by Akston Biosciences.

ESVE-O-7

RNA transcriptomic analysis as a novel in vitro hypothesis: Generating tool to unravel the pathogenesis of feline hyperthyroidism
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Feline hyperthyroidism is highly prevalent amongst geriatric cats. Causal mechanisms in this disease are not fully understood. Identification of key driving factors in thyroid hyperplasia and autonomous production of thyroid hormone has the potential to reveal novel treatment targets.

RNA-sequencing (RNA-seq) is an unbiased technique for examining the transcriptome of a tissue, to identify genes that are differentially expressed between tissues. We hypothesised that thyroids from hyperthyroid and euthyroid cats would have a different transcriptome from euthyroid cats, one from a medically treated hyperthyroid cat, and one from an untreated hyperthyroid cat. After a ribosomal RNA depletion step, a barcoded RNA-seq library was prepared from each sample and the pooled libraries were sequenced on a single lane of an HiSeq4000.
Sequencing reads were mapped to the feline reference genome (FelCat9.0) and bioinformatic differential expression analysis was undertaken using a bespoke pipeline and high-performance computing cluster. Principal component analysis (PCA) revealed that the 2 euthyroid cats clustered together, separately to the two hyperthyroid cats. The treated and untreated hyperthyroid cats were also separated by the PCA. In total, 1068 genes were significantly differently expressed between hyperthyroid and euthyroid cats. Differentially expressed genes included those related to thyroid hormone synthesis (e.g. SLC5A5, PSMP8), general cell growth (e.g. VEGFA, CNTN1), cell activity (e.g. G6PD, SRXN1) and calcium metabolism (e.g. CYP27B1, CAMK1G).

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

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ESVE-O-8

Clinical features and outcome associated with functional thyroid tumors in 70 dogs


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Due to the historically low incidence of tumor-associated hyperthyroidism in dogs, literature describing the presentation and treatment of this subset of thyroid tumors is limited. The purpose of this study was to review cases of dogs presenting with thyroid masses and concurrent hyperthyroidism in order to describe the clinical behavior of functional thyroid tumors. A secondary objective was to identify any prognostic factors affecting outcome in this population. In this multi-institutional retrospective study, 70 dogs diagnosed with a thyroid mass and concurrent hyperthyroidism were included. Clinical data regarding presentation, treatment, outcome, and functional thyroid status were retrieved. Clinical variables were assessed for effect on survival. Overall median survival of dogs with functional thyroid tumors was 35.1 months (95% Confidence Interval (CI) 23.3 – not reached (NR)), and 1- and 3-year survival rates were 83% (95% CI 74 - 93) and 49% (95% CI 35 - 69), respectively. Histopathologically-confirmed metastasis was identified in 3% of dogs. The magnitude of initial thyroxine elevation had no effect on the development of post-treatment hypothyroidism (P = 0.53) but was associated with decreased survival (Hazards Ratio (HR) 2.73, CI 1.05 - 7.32, P = 0.040). Median survival time of dogs treated with surgical excision (72.6 months, 95% CI 34.1 - NR) was significantly longer than that of dogs who did not receive surgery (15.7 months, 95% CI 4.8 - NR) (P = 0.014). Of the 50 dogs treated with surgery for which thyroid status was known following treatment, 64% developed hypothyroidism post-operatively. This study suggests that dogs with functional thyroid tumors may attain good long-term survival with surgical excision, although post-operative hypothyroidism is common. Higher initial thyroid hormone elevation is associated with reduced survival.

Disclosures

No disclosures to report.

ESVE-O-9

Expression of proteins involved in iodine uptake in canine thyroid tumors and tumor-derived organoids


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Research on modulation of iodine uptake by thyroid cells could help improve radioiodine treatment of dogs with thyroid tumors. It is therefore important to be able to evaluate the expression of proteins involved in iodine uptake (Thyrotropin Receptor (TSHR), Sodium Iodide Symporter (NIS), Pendrin and Thyroid Peroxidase (TPO)) in canine tissue. Organoids derived from canine thyroid carcinoma could provide a unique model for this research. The aims of this study were to establish immunohistochemistry (IHC) protocols for TSHR, NIS, Pendrin and TPO on canine tissue and to evaluate their expression in canine thyroid tumors and tumor-derived organoids.

Antibodies directed against human proteins were selected according to epitope homology to the canine proteins. Antibody specificity was confirmed by Western blot (WB) using lysates of the HTori-3 thyroid human cell line and healthy canine thyroid gland. IHC was validated using formalin-fixed paraffin-embedded (FFPE) micro-cell-blocks of HTori-3 cells and the following canine FFPE tissues as positive controls: thyroid gland for all markers, complementary salivary gland and stomach for NIS and skin for TSHR. Healthy canine skin, liver, brain, lymph nodes and small intestine served as negative controls. IHC was performed in 25 canine follicular cell thyroid carcinomas (FTC) (follicular, follicular-compact, compact), 8 medullary thyroid carcinomas (MTC) and 3 organoid lines derived from FTCs. A scoring system (staining intensity x distribution) was used to evaluate IHC in canine thyroid tumors and tumor-derived organoids.

WB revealed specific bands for TSHR, NIS, Pendrin and TPO in HTori-3 cells and healthy canine thyroid gland. IHC in HTori-3 cells and healthy thyroid gland showed specific cytoplasmic staining in follicular cells for all antibodies. NIS also showed basolateral membranous staining in follicular cells. NIS was expressed in salivary gland by ductal epithelial cells and in stomach by mucous neck epithelial cells. TSHR was expressed in skin by epidermal and hair follicle
keratinocytes. In FTCs, Pendrin was more expressed in follicular tumors while NIS was less expressed in compact tumors. Expression of all four proteins was higher in tumors from hyperthyroid dogs compared to those of euthyroid dogs. All four proteins were highly expressed in organoids derived from FTC. All four proteins were expressed in MTC.

In conclusion, human antibodies for thyroid proteins involved in iodine uptake are suitable to identify the canine orthologues by IHC. TSHR, NIS, Pendrin and TPO are highly expressed in thyroid tumors causing hyperthyroidism. Organoids derived from canine FTC conserve the expression of these proteins.

Disclosures

No disclosures to report.

ESVE-O-10

Urine cortisol/creatinine ratio (UCCR) can potentially identify patients with Addison disease: A pilot study

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Addison disease (hypoadrenocorticism) occurs from a deficiency of mineralocorticoid and/or glucocorticoids secretion from the adrenal glands. Young animals seem to be more susceptible to the disease. Clinical signs may vary from life threatening collapse (Addisonian crisis) to milder clinical signs such as vomiting, diarrhea and weakness. Diagnosis is based on identifying low serum cortisol levels on a pre and post adrenocorticotropin hormone (ACTH) stimulation test.

The aim of the study was to assess if UCCR can be a useful marker of Addison disease. Our hypothesis was that UCCR will be low in patients with Addison disease.

This was a prospective study between 3 different institutions (2 private clinics and one national laboratory). Three groups of dogs were included in the study and each included 4 animals. One group with confirmed Addison disease (hypoadrenocorticism group), one control group of healthy animals (healthy control group) and a second control group of sick animals with diseases other than hypoadrenocorticism (control sick group). The groups were closely matched for sex, weight and age. Residual urine, from a free catch sample, from all sick patients was used to measure UCCR.

All animals from the hypoadrenocorticism group had low UCCR with mean value of 2.67 and median value of 2.55 (range from 1.8 - 3.8 and laboratory ref. range 7.8 - 38.8). UCCR was high on both the healthy control group and the control sick group, with mean value for the healthy control group of 19.075 (range 13.2-27.5) and a mean value of 96.45 (range 22.3-180.4) for the sick control group. The median value for the healthy control group was 17.8 and for the control diseased group was 91.55.

UCCR could potentially be used for the diagnosis of Addison disease and could perhaps be useful for early diagnosis in stable animals. Further, larger, prospective studies are necessary to confirm this.

Disclosures: A grant was taken from the Veterinary Laboratory La Vallonea. Magda Gerou-Ferriani is a consultant for the same Laboratory. Walter Bertazzolo also works as clinical pathologist and he is the scientific director for the same laboratory.

ESVE-O-11

Hypophysectomy as successful treatment of feline hypersomatotropism: A case series of 25 cats

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Hypersomatotropism is caused by a functional growth hormone (GH)-secreting pituitary adenoma and is recognized with increasing frequency as a cause of diabetes mellitus in cats. Successful treatment by transphenoidal hypophysectomy has been described, however larger cohort studies are lacking. In this retrospective study the hormone analysis and survival of 25 cats with hypersomatotropism that underwent hypophysectomy between 2008 and 2020 are described. Diagnosis of hypersomatotropism was based on clinical signs, plasma insulin-like growth factor-1 (IGF-1) levels (median 1600 ng/mL, range 780-4387) and computed tomography or magnetic resonance imaging of the pituitary gland (median height 5.1mm, range 3.6-13.3). Median age at presentation was 9.7 years (range 4.6-12.5). Most cats (n = 21) were neutered males and the remainder neutered females. At presentation, 24/25 cats were diabetic and treated with exogenous insulin. Cats were hospitalized in the Intensive Care Unit for preoperative regulation of their blood glucose levels and hydration status. Hypophysectomy was performed as described previously and histopathology confirmed a somatotroph adenoma in most cases. Postoperative care consisted of regulation of electrolyte and fluid balance, tight monitoring of the patient’s blood glucose levels and tapering of insulin doses accordingly. Postoperative hormone substitution consisted of cortisone acetate, levothyroxine and desmopressin. GH and IGF-1 levels were measured repeatedly in the immediate postoperative period and after discharge.

Postoperative mortality, defined as death within 4 weeks after surgery irrespective of the cause, was restricted to 1 cat (4%). Cats were discharged from the hospital after a median of 7 days (range 3-18). Postoperative complications consisted of decreased tear production (n = 11), clinical signs of hypoglycemia after discontinuation of
Burmese cats in Europe and Australia are predisposed to developing diabetes mellitus (DM), but the DM susceptibility genes are yet to be identified. Whole genome sequencing (WGS) enables comprehensive interrogation of genome-wide variation and has been useful in identifying DM susceptibility variants in people. In cats, WGS has been successful in understanding monogenic disorders, but it has not yet been employed to investigate complex diseases such as DM.

The aim of this pilot study was to identify candidate genetic DM susceptibility variants in the Burmese breed using WGS. DNA was extracted from EDTA blood from 4 diabetic Burmese cats with no evidence of hypersomatotropism, 4 non-diabetic elderly Burmese and 2 non-diabetic elderly DSH cats. Samples were surplus to clinical requirements and obtained with owner consent and ethical permission. Paired-end WGS was performed (30x coverage on Illumina HiSeqX) and the data were analysed using a bespoke GATK-based bioinformatics pipeline. Variants were called against the FelCat9.0 reference genome and annotated according to predicted impact on gene function. Allele frequencies (AF) of 282 Burmese and non-Burmese cats included in the 99Lives database were used to help prioritize selected exonic variants identified in the WGS. Selected exonic variants were further filtered based on AF in diabetic and non-diabetic Burmese cats, as well as predicted impact. In addition, DM-associated variants within genes known to be involved in glucose homeostasis or DM in humans were prioritized.

In total, over 30 million variants were identified; 9.9 million were Burmese-exclusive, of which ca. 20,000 were exonic. Using filtering based on AF, predicted impact and gene function, a final list of 130 DM candidate susceptibility variants was established. This included variants within genes coding for transcription factors, growth factors, receptors and signaling cascade molecules. For some variants, a plausible functional link to DM was immediately obvious. However, several novel candidate DM genes were identified by the presence of high or moderate impact variants segregating with affected Burmese cats in genes not previously associated with DM. Genotyping of 100 additional diabetic and non-diabetic cats at the 130 candidate susceptibility variants further refines the list of regions associated with DM risk in Burmese cats. This approach illustrates the potential value of WGS in feline complex disease genetics and provides new insights into the genetic basis of DM in Burmese cats. All the variants identified by WGS in this group of cats represent a valuable resource for future genetic studies.

Disclosures
No disclosures to report.

ESVE-O-13

Insulin glargine 300 units/ml for the treatment of feline diabetes mellitus

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Insulin glargine (IGla) 300U/ml (IGla-U300) is longer acting and associated with less risk of hypoglycemia in comparison with IGla-U100 in people. IGla-U300 was evaluated in healthy cats, resulting in a flat time-action profile. This prospective study aimed to evaluate the efficacy and safety of IGla-U300 in cats with diabetes mellitus.

Client-owned cats diagnosed with DM were enrolled. Cats treated with corticosteroids during the previous 60 days or with relevant concurrent disorders (e.g., hypersomatotropism, neoplasia) were excluded. Subcutaneous IGla-U300 was administered with an insulin dosing pen at a starting dose of 0.5 U/kg q12h. All cats were fed the same low-carbohydrate diet for the study period. Owners performed a 16-hour blood glucose curve (BGC) once a week at home for 8 weeks, and assigned a score from 1 to 10 for each clinical sign (polyuria, polydipsia, polyphagia, weight loss, general demeanor, and lethargy) related to DM. In-clinic evaluations, including serum fructosamine concentrations, were scheduled within 3 days of the 1st, 3rd, 6th and 8th BGCs. Remission was defined as the absence of clinical signs and glycemia <160 mg/dL for at least 4 weeks after cessation of insulin therapy. Glycemic variability was assessed by calculating the standard deviation of each BGC. The data were analyzed using nonparametric tests. Twelve cats were recruited; 4 were subsequently excluded with IGF-1 >1000 ng/mL. Eight cats (5 newly diagnosed) completed the study. At the 8th BGC, improved or absent polyuria, polydipsia, polyphagia,
weight loss, general demeanor, and lethargy) were reported in 7/8 (87%), 7/8 (87%), 6/8 (75%), 6/8 (75%), 7/8 (87%) and 6/8 (75%) cats, respectively. Two cats achieved remission after 29 and 53 days. Another 2 cats went into remission after the end of the study (day 82 and 96). All cats that achieved remission (4/8; 50%) were newly diagnosed diabetics (4/5; 80%). Median glucose of BGCs and serum fructosamine concentrations significantly decreased when comparing the 1st and the 8th week of treatment (P < 0.01 and P = 0.02, respectively). Glycemic variability was significantly lower at the 5th BGC when comparing cats that achieved remission with cats that did not achieve remission (P = 0.03). Hypoglycemia (nadir <60 mg/dL) was detected in 10/64 (16%) BGCs, while clinical signs consistent with hypoglycemia were not reported. Median (range) IGla-U300 dose in cats still receiving insulin at the 8th BGC was 1.14 (0.17-4.00) U/kg/day.

IGla-U300 is effective and safe for the treatment of feline diabetes.

Disclosures

Disclosures Federico Fracassi Financial support: Dechra, MSD Speaking & consultancies: Boehringer Ingelheim, Dechra, Royal Canin, Hill’s, Nestlé Purina, La Vallonea. Linda Fleeman: No pertinent financial support Chen Gilor pertinent financial support: Akston Biosciences, Elanco. Guido Linari: No pertinent financial support. Lucia Giacomelli: No pertinent financial support.

ESVE-O-14

Glycemic variability in diabetic cats with and without concurrent diseases

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Glycemic variability (GV) refers to glycemic excursions with episodes of hypoglycemia and hyperglycemia throughout the day or on different days and has become an important marker of glycemic control in people. Increased GV is linked to the development of multiple diabetic complications and therefore treatment aims to minimize glycemic fluctuations in humans.

It is currently assumed that any concurrent disease may have a negative impact on diabetes mellitus (DM) management in pets. However, the influence of concurrent diseases on GV has not been studied yet. The objective of this study therefore was to evaluate GV in diabetic cats with and without concurrent diseases.

Blood glucose curves from newly diagnosed diabetic cats between 2010 and 2019 were retrospectively evaluated for GV during three phases (1 = 0-3 weeks, 2 = 4-8 weeks, 3 = 9-12 weeks) after starting therapy. Standard deviations (SD) as a marker for GV were calculated and compared between cats with and without concurrent diseases. Furthermore, comparisons were made between diabetic cats with chronic kidney disease (CKD), chronic gingivostomatitis (CGS), pancreatitis and without any other obvious disease. Data was analyzed using non-parametric tests.

Fifty-two cats with newly diagnosed DM were included, 29 of which had at least one concurrent disease with associated clinical signs (6, 6 and 7 cats with CKD, CGS and pancreatitis, respectively) and 23 were without concurrent disease. In the non-concurrent disease group, GV decreased over time (mean SD in phase 1: 3.5 mmol/l; phase 2: 2.8 mmol/l; phase 3: 2.4 mmol/l) and reached significance in phase 3 compared to phase 1 (P = 0.03), while GV did not decrease in the non-concurrent disease group (mean SD in phase 1: 2.6 mmol/l; phase 2: 2.7 mmol/l; phase 3: 2.5 mmol/l). The comparison between diabetic cats without concurrent disease and with concurrent diseases in general and CKD, CGS and pancreatitis, revealed no significant difference in any of the three time phases.

In conclusion, diabetic cats without concurrent disease show improvement of GV after at least 9 weeks of therapy in contrast to diabetic cats with concurrent diseases. In this rather small cohort of diabetic cats, concurrent CKD, CGS and pancreatitis do not seem to increase GV in the first 12 weeks after diagnosis. We hypothesize that GV is increased in newly diagnosed diabetic cats and differences only become apparent later in the course of the disease.

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ESVE-O-15

Combined treatment of trilostane and retinoic acid in dogs with pituitary-dependent hypercortisolism

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Pituitary-Dependent Hypercortisolism (PDH) is a severe metabolic disorder and represents 80-85% of cases of spontaneous hypercortisolism in dogs. Trilostane is a potent inhibitor of an early stage of adrenal steroidogenesis, but has no therapeutic effects on pituitary tumor. Retinoic acid (RA) inhibits proliferation, invasion and tumor growth, reducing ACTH production and tumor size. The aim of this study was to evaluate the combined effect of trilostane and RA to control hypercortisolism and tumor growth. In this prospective study, 8 dogs with PDH with "partial response" to trilostane - persistence of clinical signs and biochemical abnormalities- were included. The diagnosis of PDH was made according to: clinical signs, urine cortisol creatinine ratio (UCCR), low-dose dexamethasone suppression test, plasma ACTH, abdominal ultrasound and MRI. Dogs received RA (2 mg/Kg/day) for 6 months, while continuing with trilostane (3-4 mg/Kg/12hrs). The concentrations of ACTH, cortisol, UCCR and
Pituitary-dependent hypercortisolism (PDH) is one of the most common endocrine disorders in dogs, and is caused by an ACTH-secreting pituitary adenoma. Hypophysectomy and radiation therapy are good treatment options, but can only be performed in a limited number of specialized clinics worldwide. Many patients therefore rely on medical treatment with trilostane, which inhibits cortisol production but not pituitary adenoma growth. Pituitary adenoma-targeting medical treatment options have shown unsatisfactory results so far. Finding and testing new pituitary adenoma-targeting drugs is hampered by the lack of suitable in vitro models that contain species-specific proliferating pituitary adenoma cells.

The aim of this study was to establish canine pituitary adenoma organoid culture. Organoids are miniature three-dimensional (3D) structures grown from stem cells, that closely resemble the organ or tumor they originate from, and could therefore be used to reliably and efficiently identify pituitary adenoma-targeting drugs. Pituitary adenomas were collected from five dogs that underwent hypophysectomy at our University Clinic. All dogs were diagnosed with PDH based on clinical signs, endocrine testing, and diagnostic imaging. Pituitary adenoma cells were cultured in ice-cold basement membrane extract (BME) with previously established anterior pituitary organoid medium, which was refreshed twice a week. The canine organoids were characterized with histopathology and RT-qPCR.

This study shows that organoids can be cultured from pituitary adenomas, something not previously published in any species. Because organoids closely resemble the primary tumor in many aspects, we expect that this culture model will pave the way to reliably and efficiently identify new pituitary adenoma-targeting drugs for dogs with PDH.

Disclosures

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ESVE-O-16

Canine pituitary adenoma organoids

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Pituitary-dependent hypercortisolism (PDH) is one of the most common endocrine disorders in dogs, and is caused by an ACTH-secreting pituitary adenoma. Hypophysectomy and radiation therapy are good treatment options, but can only be performed in a limited number of specialized clinics worldwide. Many patients therefore rely on medical treatment with trilostane, which inhibits cortisol production but not pituitary adenoma growth. Pituitary adenoma-targeting medical treatment options have shown unsatisfactory results so far. Finding and testing new pituitary adenoma-targeting drugs is hampered by the lack of suitable in vitro models that contain species-specific proliferating pituitary adenoma cells.

The aim of this study was to establish canine pituitary adenoma organoid culture. Organoids are miniature three-dimensional (3D) structures grown from stem cells, that closely resemble the organ or tumor they originate from, and could therefore be used to reliably and efficiently identify pituitary adenoma-targeting drugs. Pituitary adenomas were collected from five dogs that underwent hypophysectomy at our University Clinic. All dogs were diagnosed with PDH based on clinical signs, endocrine testing, and diagnostic imaging. Pituitary adenoma cells were cultured in ice-cold basement membrane extract (BME) with previously established anterior pituitary organoid medium, which was refreshed twice a week. The canine organoids were characterized with histopathology and RT-qPCR.

This study shows that organoids can be cultured from pituitary adenomas, something not previously published in any species. Because organoids closely resemble the primary tumor in many aspects, we expect that this culture model will pave the way to reliably and efficiently identify new pituitary adenoma-targeting drugs for dogs with PDH.

Disclosures

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ESVE-O-17

Calcium and phosphate homeostasis in dogs with naturally occurring hypercortisolism

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Hyperphosphatemia is a common finding in dogs with hypercortisolism and was reported as a negative prognostic factor for survival. Previous studies observed that dogs with hypercortisolism frequently show hyperphosphatemia, increased serum parathormone (PTH) concentration, decreased phosphaturia, and increased calciumuria. However, concurrent evaluation of all these variables was never performed. In humans, hypercortisolism increases both calcium and phosphaturia, thus differing from dogs.

The aim of this study was to evaluate calcium and phosphate metabolism in dogs with hypercortisolism. Serum PTH (sPTH), 25-hydroxyvitamin D (25D) and calcitriol (1,25D) concentration along with urinary fractional excretion (%) of phosphate (FEP) and calcium (FECa) were concurrently evaluated in dogs with hypercortisolism.

Dogs with newly diagnosed hypercortisolism (pituitary or adrenal-dependent), before treatment, without severe comorbidities (e.g. diabetes mellitus, other neoplasia), were enrolled (December 2018-January 2020). Age and body weight matched-healthy dogs were included as control group (CG). Dogs receiving drugs or diets (e.g. renal diet) affecting calcium and phosphate balance were excluded. Hematology and chemistry profile including ionized calcium...
Cortisolism remain unclear. The mechanisms causing hyperphosphatemia in dogs with hypercortisolism is not fully understood. Elevated sPTH concentration could increase vitamin D-hydroxylation rate, as suggested by the increased serum 1,25D concentration did not differ between groups. Dogs with hypercortisolism had higher FECa and sPTH concentration compared with CG (4.7[0.2-18] vs 1.2[0.5-2.8], P < .03 and 6[2.6-54] vs 2.7 [0.6-8.4] pmol/L, P < .004, respectively). No significant correlation was found between serum phosphate and sPTH concentrations. Serum 25D concentration was lower in hypercortisolism compared with CG (159[31-434] vs 269[134-391] nmol/L, P < .02), while serum 1,25D concentration did not differ between groups (396[156-527] vs 397[230-507] pmol/L). The 1,25D/25D ratio was higher in hypercortisolism dogs with increased sPTH concentration (2.8[1.1-13.4]) compared both with ones having sPTH concentration within reference interval (1.4[0.8-8.0], P < .01) and with CG (1.3[1-3.4], P < .01).

Hypercortisolism seems to influence vitamin D metabolism in dogs, as described in humans treated with corticosteroids or affected by spontaneous hypercortisolism. Elevated sPTH concentration could increase vitamin D-hydroxylation rate, as suggested by the increased 1,25D/25D ratio, thus explaining normal serum 1,25D concentration. The mechanisms causing hyperphosphatemia in dogs with hypercortisolism remain unclear.

**Disclosures**

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**ESVE-O-18**

**Gut microbiome evaluation in dogs with naturally-occurring hyperadrenocorticism**

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Gut microbiome has a vital role in the host physiologic and immunologic processes. As shown in humans, lipopolysaccharides can stimulate the production of ACTH, questioning its role on the hypothalamic-pituitary-axis. In veterinary medicine, little is known about the influence of naturally-occurring canine hyperadrenocorticism (NO-HAC), on gut microbiome. This study aims to assess if fecal microbiome is modified in dogs with NO-HAC in comparison with healthy dogs. A cross-sectional study was performed, including healthy dogs and dogs recently diagnosed with NO-HAC and not yet submitted to medical treatment (Ethical approval CEIE 01/2019).

NO-HAC was diagnosed taking into account clinical and laboratorial signs, ultrasonographic findings and at least one positive confirmatory test (low-dosage dexamethasone suppression test and/or ACTH stimulation test). Dogs were excluded if they were treated with antibiotics and probiotics over the last six months, if they were hospitalized or vaccinated during the previous month or even if they had a concurrent gastro-intestinal disease.

A total of 18 dogs were enrolled: 9 healthy and 9 dogs with NO-HAC. A fecal sample was obtained from all dogs. DNA extraction was performed using PowerSoil Isolation Kit. The V4 region of the 16S rRNA gene was amplified (primers 515F-806r), followed by sequencing (Illumina MiSeq). Sequencing data analysis was conducted using the platform QIIME2. For statistical purpose, the exact Fisher the ANCOM tests were performed.

When compared to healthy dogs, NO-HAC dogs showed a significant increase on the relative abundance of the phylum Proteobacteria (P < 0.001), namely Enterobacteriaceae family, Pseudomonadaceae family and the genus Campylobacter (P < 0.001, W = 1, W = 1, respectively). These dogs also showed a significant decrease in the phylum Bacteroidetes (P < 0.001), particularly the genus Prevotella (P = 0.011) and Bacteroides (P < 0.001). Within the Firmicutes phylum, there was a relative decrease of the families Erysipelotrichaceae (P = 0.009) and Clostridiaceae (P = 0.010), and from the genus Robinsoniella (P = 0.011) and Ruminococcus (W = 30). In this same phylum, a significant increase of the genus Streptococcus (from the class Bacilli) occurred (P < 0.001).

This study highlights that dogs with NO-HAC display significant changes on their gut microbiome, when compared to healthy dogs. The identified changes of the phylum Proteobacteria, Bacteroidetes and the family Erysipelotrichaceae are in agreement with what has been observed in other endocrine diseases, namely human Diabetes Mellitus. Further studies are needed to better understand why Streptococcus and Clostridiaceae are modified, correlating these findings with metabolomic data.

To the author's knowledge, this is the first study that describes gut microbiome changes in dogs with NO-HAC.

**Disclosures**

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**ESVE-O-19**

**Efficacy and safety of tightly controlled hyperadrenocorticism in dogs treated with trilostane in general practice**

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There is no agreement about ideal control of hyperadrenocorticism in dogs treated with trilostane. Many veterinarians select sub-optimal
control of hyperadrenocorticism to limit the possibility of clinical hypothalamic-pituitary-adrenal (HPA) axis dysregulation. The aim of this study was to assess clinical responses in dogs treated in general practice with tight control as defined by an ACTH-stimulated cortisol concentration of <70 nmol/L (Advia Centaur) when test performed 4-6h after trilostane.

Between August 2017 and February 2019, dogs with confirmed hyperadrenocorticism that had a minimum 12 month period of tight control were identified. Owners were provided with a published questionnaire relating to the period(s) of tight control. Data from the laboratory submissions and owner questionnaires were analysed for age, sex, breed, duration of treatment, duration of tight control, adverse effects and clinical scores. For each period of tight control, the percentage of basal and ACTH-stimulated cortisols within the following a priori categories were determined: <14, 14-29 nmol/L.

There were 44 periods of tight control in 43 dogs. Age at end of tight control was 5.5-17.5 years (median 12.5). All dogs were neutered (23 female, 20 male). Twenty five (58%) were purebred dogs and 11 (25%) were Maltese dogs and their crosses. Tight control period ranged from 12-36 months (median 19). Total treatment time was 16-60 months (median 30). Median survival time was not reached.

Dogs were treated once daily (32), twice daily (10) both (1) or other (1). Mean interval between ACTH stimulation tests was 3.8 months. Twenty four percent (51/216) and 46% (100/216) basal cortisols were <14 and <30 nmol/L respectively. Six percent (15/269) and 36% (98/269) post-stimulation cortisols were <14 and <30 nmol/L respectively. There was no association between frequency of "sickness or diarrhea" and dogs' percentages of results <14 or 14-29 nmol/L.

Clinical score was excellent (4-11) in 28, reasonable (12-16) in 13 and poor (17) in 2; median score 10. Median scores for drinking, urinating, appetite and skin/coat were 1 (normal). Median score for general assessment was 2 and median score for exercise was 3. Clinical score was impacted by the exercise assessment which many owners considered unrelated to hyperadrenocorticism or its treatment.

Tight control of hyperadrenocorticism assessed by an ACTH stimulation test 4-6h after trilostane resulted in excellent clinical scores in most dogs. Tight control did not impact negatively on dogs’ health. An ACTH-stimulated cortisol of 30-70 nmol/L on this assay appears a safe and efficacious monitoring goal in general practice.

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ESVE-O-21

MicroRNAs as liquid biomarkers for canine Cushing’s syndrome

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Cushing’s syndrome, also known as hypercortisolism, is a common endocrine disorder in dogs. In 80-85% of cases, it is caused by an ACTH-secreting pituitary adenoma (pituitary-dependent hypercortisolism; PDH). PDH can be treated surgically or medically. However, the pituitary adenoma can recur after surgery. During medical treatment with trilostane, the pituitary tumor can continue to grow and induce space-occupying neurological abnormalities and significant deterioration of life quality. Additionally, finding the correct trilostane dose remains challenging. Non-invasive biomarkers that could detect early recurrence, correlate with pituitary adenoma size and assess efficacy of medical therapy would therefore greatly improve patient care.

MiRNAs show great potential as liquid biomarkers because their expression patterns have been shown to change during disease, and they have been found to be relatively stable in circulating blood.

The aim of this study is to identify miRNAs correlating with steroidogenesis and tumorigenesis of PDH, which would be differentially expressed between dogs with or without PDH.

Serum/EDTA samples of dogs with PDH (n = 3), cortisol-secreting adrenocortical tumors (csACT: n = 5), hormonally silent ACTs (n = 3) and healthy dogs (n = 3), were analyzed in leftover samples, for the expression levels of 40 target miRNAs and 5 reference miRNAs. The miRNAs were selected based on previous miFinder experiments and literature.

Seven miRNAs were expressed at higher levels in dogs with PDH compared to the healthy dogs (miRs 18-5p, 21-5p, 122-5p, 132, 141, 375 and 483-3p), while three were expressed at lower levels (miRs 18-5p, 21-5p, 122-5p, 132, 141, 375 and 483-3p), and three were expressed at lower levels (miRs 18-5p, 21-5p, 122-5p, 132, 141, 375 and 483-3p), while three were expressed at lower levels (miRs 18-5p, 21-5p, 122-5p, 132, 141, 375 and 483-3p). Of these miRNAs, miR-18-5p and miR-132 were expressed at higher levels in dogs with both PDH and csACTs, compared to healthy dogs and dogs with hormonally silent ACTs, potentially indicating hypercortisolism. Additionally, miR-218-5p and miR-503 were also expressed at lower levels in dogs with both PDH and csACTs, compared to dogs with ACTs and could therefore be PDH-specific.

This study shows that several miRNAs are differentially expressed between dogs with PDH, csACTs and healthy dogs, which can be combined into a unique canine PDH miRNA profile. This profile has the potential to assist in the monitoring of medical treatment of hypercortisolism, to assist in early detection of recurrence after hypophysectomy, and might even be linked to pituitary adenoma size.

Disclosures
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diagnosis of Cushing’s syndrome, there is no single highly accurate test. Novel methods to aid the diagnosis of Cushing’s syndrome are warranted. Use of machine learning-based classification algorithms for disease prediction are increasingly reported and offer the potential to aid the diagnosis of Cushing’s syndrome. This study aimed to use machine learning methods utilizing clinical variables collected from primary-care veterinary electronic patient records (EPRs), to predict a later confirmed diagnosis of Cushing’s syndrome at the point of first suspicion in dogs.

A nested case-control study design used records from a VetCompass™ population of 905,544 dogs. Confirmed CS diagnoses were highly correlated (r = 0.8, P < 0.01). Results between both qPCR tests in the diagnosis of canine sinonasal aspergillosis (SNA) has not yet been assessed. The aim of this study was to evaluate the presence of fungal material using qPCR targeting Aspergillus spp (PanAsp) and A. fumigatus (Asp fum) in samples obtained from nasal cavities of dogs with various nasal diseases and healthy dogs.

In SNA dogs, Asp fum and PanAsp were positive in 13/20 and 14/20 dogs with a mean cycle threshold (Ct) of 30.6 [range 23.2 - 33.3] and 28.3 [24.3 - 34.5], respectively. The PanAsp was also positive in 3 non-SNA dogs: one with cured SNA, one with LPR and one with nasal tumor, but at very low load (Ct>33). Results between both qPCR were highly correlated (r = 0.8, P < 0.01). For Asp fum and PanAsp, the sensitivity was 65% and 70% and the specificity was 100% and 94%, respectively.

Disclosures

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ESVIM-O-2

Aspergillus qPCR testing on nasal swab: A useful tool for diagnosis and follow-up of sinonasal aspergillosis in dogs?

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Polymerase chain reaction (PCR) testing either for Aspergillus spp or for Aspergillus fumigatus is now available; however, the interest of such tests in the diagnosis of canine sinonasal aspergillosis (SNA) has not yet been assessed. The aim of this study was to evaluate the presence of fungal material using qPCR targeting Aspergillus spp (PanAsp) and A. fumigatus (Asp fum) in samples obtained from nasal cavities of dogs with various nasal diseases and healthy dogs.

In SNA dogs, Asp fum and PanAsp were positive in 13/20 and 14/20 dogs with a mean cycle threshold (Ct) of 30.6 [range 23.2 - 33.3] and 28.3 [24.3 - 34.5], respectively. The PanAsp was also positive in 3 non-SNA dogs: one with cured SNA, one with LPR and one with nasal tumor, but at very low load (Ct>33). Results between both qPCR were highly correlated (r = 0.8, P < 0.01). For Asp fum and PanAsp, the sensitivity was 65% and 70% and the specificity was 100% and 94%, respectively.

Disclosures

No disclosures to report.

ESVIM-O-1

Relationship between bronchial collapse and heart size in coughing dogs with heart murmur studied by computed tomography

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Heart disease has long been regarded as a common cause of coughing in dogs, due to dilated left atrium (LA) or cardiomegaly compressing the bronchi. However, some authors suggested that coughing might be more due to an underlying respiratory disease (ie, bronchomalacia or chronic bronchitis) than to LA enlargement causing airway collapse.

The study aim was to evaluate the association between LA enlargement/cardiomegaly and bronchial collapse in coughing dogs with heart murmur using computed tomography (CT).

Twenty-four client-owned coughing dogs with heart murmur were prospectively recruited over 4 months. Tracheal and thoracic radiography, echocardiography, and thoracic CT were performed in enrolled dogs without general anesthesia. Fourteen historical control dogs, with normal thoracic CT and no history of heart murmur and coughing, were searched from the Radiology database. Bronchial to aorta (Ao) ratio was blindly measured by 3 radiologists, and the bronchi that were significantly narrowed in dogs with murmur compared to controls were identified. The relationship between degree of collapse and LA/Ao and Vertebral Heart Scale (VHS) was evaluated in dogs with murmur via mixed-effects regression model.

The left-sided bronchi and caudal most right-sided bronchi were significantly narrower in dogs with murmur than in controls. Increasing LA size was only associated with accessory lobe bronchus narrowing. However, increasing VHS was significantly associated with narrowing of all left-sided bronchi and accessory and right caudal lobe bronchi. Results indicate an association between cardiomegaly and airway collapse in dogs with heart murmur, and support heart size exacerbation of cough in these dogs.

Disclosures

No disclosures to report.
Asp*um qPCR test on deep blinded nasal swabs appears highly specific but only moderately sensitive to diagnose canine SNA. In some dogs, fungal plaques are exclusively found in the frontal sinus and are probably not reached by blinded sampling. Since A. *fumigatus* is the most common etiological agent of canine SNA (96.7% of isolates), Asp*um* testing appears appropriate; however, PanAsp testing is a non-negligible tool to detect the small percentage of SNA cases related to other Aspergillus species. Results also show that healthy predisposed dogs do not seem to be carriers and confirm that *A. fumigatus* does not appear to have a major role in LPR. The negative results found in cured SNA dogs show a good correlation with clinical and rhinoscopic findings. In conclusion, Asp*um* and/or PanAsp (qPCR testing) on deep nasal blinded swabs can be useful for detection of SNA at diagnosis and after cure.

Disclosures

No disclosures to report.

ESVIM-O-3

Characterization of the bronchoalveolar lavage fluid by single cell gene expression analysis in healthy dogs: a promising technique.

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Single-cell mRNA-sequencing (scRNA-seq) is a technique which enables unbiased, high throughput and high-resolution transcriptomic analysis of the heterogeneity of cells within a population. This recent technique has been described in humans, mice and other species in various conditions to cluster cells in populations and identify new subpopulations, as well as to study the gene expression of cells in various tissues, conditions and origins. In dogs, a species for which markers of cell populations are often limiting, scRNA-seq presents with elevated yet untested potential for the study of tissue composition.

As a proof of principle, we used scRNA-seq to identify cellular populations of the bronchoalveolar lavage fluid (BALF) in healthy dogs (n = 4).

Cells in suspension isolated from fresh BALF were loaded into the Chromium™ and were directly encapsulated with unique barcoded primers using the drop-sequencing method. Cells were lysed and reverse transcription of mRNAs took place into vesicles. cDNA was amplified after the breakage of the vesicles and sequenced on an Illumina NextSeq500. The analysis of the results and statistical analysis were performed using Cell Ranger software (v1.2.0), Seurat package in RStudio (v3.1.2) and the gene set enrichment analysis tool (GSEA-P).

A total of 5710 cells were obtained and analyzed. Fourteen distinct clusters of cells were identified, further identified as alveolar macrophages (AMs) (3 clusters) and monocytes-derived macrophages (1 cluster). The first cluster of AMs composed by the majority of cells exerted functions involved in immune defense and response, the second cluster exerted functions involved in immune response regulation and the third exerted functions involved in metal ions homeostasis. Clusters of CD8+ and CD4+ T cells were also found (1 cluster each) as well as clusters of mature and immature dendritic cells (1 cluster each) and clusters of ciliated or non-ciliated epithelial cells (1 cluster each). Finally, subpopulations of B cells, neutrophils, basophils and cycling cells were also identified (1 cluster each).

We used for the first time in dogs the scRNA-seq to investigate cellular subpopulations of the BALF. This study hence expands our knowledge on dog lung immune cell populations, paves the way for the investigation at single-cell level of lower respiratory diseases in dogs, and establishes that scRNA-seq is a powerful tool for the study of dog tissue composition.

Disclosures

No disclosures to report.

ESVIM-O-4

Intradermal testing in dogs with eosinophilic bronchopneumopathy

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The etiology of canine eosinophilic bronchopneumopathy (EBP) has not been clarified to date, however, underlying allergic disease has been suggested. The aim of this prospective study was to investigate positive reactions on intradermal testing (IDT) in dogs with EBP and healthy control dogs.

The study included 20 dogs diagnosed with EBP and 22 healthy control dogs. Inclusion criteria for EBP patients were typical clinical signs, eosinophilic inflammation in the lower airways with more than 14% eosinophils in the bronchoalveolar-lavage-fluid and exclusion of pulmonary parasites. IDT was performed with 43 allergens in both groups of dogs. Positive IDT reactions after five and 15 minutes were compared between groups using Pearson’s Chi-squared test. The level of significance was ≤0.05.

While dogs in the EBP group showed a mean of 8.9 positive reactions to all allergens tested, the control group had a mean of 7.6 positive reactions. Dogs with EBP showed significantly more overall positive reactions than dogs in the control group (P = 0.001). In addition, dogs with EBP had significantly more positive reactions for single allergens. These included tabanus (P = 0.001), plantago lanceolata (ribwort) (P = 0.004), acarus siro (P = 0.003), lolium (ryegrass) (P = 0.031), and beech (P = 0.005).

The results of the study suggest that EBP might be associated with allergen-specific IgE-production compatible with allergic disease in some dogs.
Disclosures
No disclosures to report.

ESVIM-O-5
Effects of calcitriol on oxidative burst, phagocytic function, and cytokine production in shelter dog leukocytes
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Calcitriol, the hormonally active metabolite of vitamin D, has been shown across many species (e.g., humans, dogs, mice, chickens, cows) to augment innate immune responses and dampen aberrant proinflammatory cytokine production. Community acquired infections including canine infectious respiratory disease complex are common in shelters and consume limited shelter resources, impact adoption rates, and can result in unnecessary euthanasia. Prophylactic oral vitamin D supplementation decreases the incidence and severity of upper and lower respiratory tract infections in humans. Before a clinical trial investigating the clinical benefit of oral vitamin D supplementation in shelter dogs can be pursued, an in vitro study evaluating the immunomodulatory effects of calcitriol in blood from shelter dogs is needed. Therefore, the objective of this study was to determine if incubation of whole blood obtained from healthy shelter dogs housed in a shelter for ≥ 7 days with calcitriol would alter granulocyte/monocyte (GM) oxidative burst and phagocytic function as well as pathogen-associated molecular pattern (PAMP)-stimulated leukocyte production of tumor necrosis factor (TNF)-α, and interleukin (IL)-6, and IL-10.

Ten healthy dogs housed in a shelter for ≥ 7 days were enrolled in a prospective cohort study. Whole blood from these dogs was incubated with calcitriol (10^{-7} M) or ethanol (control) for 24 h. Subsequent to this incubation phagocytosis of opsonized-Escherichia coli (E. coli) and E. coli-induced oxidative burst were evaluated via flow cytometry. In addition, leukocyte production of TNF-α, IL-6, and IL-10 were measured using a canine-specific multiplex assay. Two-way repeated measures analysis of variance and paired t-tests were used to assess leukocyte cytokine production and phagocytosis/oxidative burst, respectively. Calcitriol significantly decreased leukocyte TNF-α production (P = 0.009) but not IL-6 (P = 0.12), irrespective of type of PAMP exposure. Calcitriol significantly increased IL-10 when cells were exposed to LTA (P = 0.002). Tumor necrosis factor-α-to-IL-10 ratio was significantly decreased with calcitriol when cells were exposed to LPS (P < 0.001) or LTA (P = 0.004). Calcitriol did not significantly affect GM oxidative burst (P = 0.16) or phagocytic function (P = 0.25).

These data indicate that calcitriol attenuates proinflammatory immune responses without affecting GM oxidative burst or phagocytic function in vitro in whole blood obtained from healthy dogs housed in shelters.

Disclosures
No disclosures to report.

ESVIM-O-6
Immune function and serum 25-hydroxyvitamin D in shelter dogs
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There is a high prevalence of infections in shelter dogs that consume a substantial amount of limited resources, impacts adoption rates, and can result in euthanasia. The cause for this high rate of infections is largely unknown including if immune dysfunction or vitamin D could be contributory factors. This study had two objectives: 1) to establish a baseline understanding of several immune function parameters in shelter dogs and 2) to determine if serum vitamin D concentrations are associated with immune function.

Ten apparently healthy shelter dogs and 10 healthy, non-shelter, age, breed, and sex-matched control dogs were included. Serum 25-hydroxyvitamin(OH)D, the major circulating vitamin D metabolite was measured using high performance liquid chromatography. Whole blood samples were stimulated with lipopolysaccharide (LPS), lipoteichoic acid (LTA), or phosphate buffer solution (negative control) and tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10 were measured with a canine-specific multiplex bead-based assay. Granulocyte/monocyte (GM) phagocytosis of opsonized-E. coli and E. coli-induced oxidative burst were evaluated with flow cytometry. Serum 25(OH)D concentrations, GM phagocytic and oxidative burst capacities were compared between shelter dogs and non-shelter dogs using two-tailed, unpaired t-tests. Two-way repeated measures analysis of variance (ANOVA) was performed to assess leukocyte cytokine production and simple linear regression analysis was used to investigate if serum 25(OH)D concentration could predict immune function outcomes.

Shelter dogs had significantly decreased percentage of GM that had phagocytized opsonized-E. coli (P = 0.019) and performed E.coli-induced oxidative burst (P = 0.011) compared to non-shelter control dogs. There was not a significant difference in TNF-α, IL-6, IL-10, or 25(OH)D concentrations between shelter and non-shelter dogs. Serum 25(OH)D concentrations had a weak positive significant association with the intensity of GM E. coli-induced oxidative burst (r^2 = 0.23, P = 0.03). There was a moderate inverse significant association between serum 25(OH)D concentration and LPS-stimulated production of TNF-α in shelter dogs (r^2 = 0.40, P = 0.04).

These results demonstrate dogs housed in a shelter have immune dysfunction. While serum 25(OH)D concentrations did not differ between shelter and non-shelter dogs, significant associations between 25(OH)D concentration and immune function parameters were identified.

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No disclosures to report.
Clinical, clinicopathological and imaging differences between dogs with non-associative, associative and precursor immune-mediated haemolytic anemia

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A retrospective assessment was made of dogs with immune mediated hemolytic anemia (IMHA) from 2015 to 2019. The study compared clinical, clinicopathological and imaging findings between dogs with either non-associative, associative or precursor-mediated IMHA to determine if there were significant differences between the 3 groups of disease, and identify any potential predictors of mortality. We hypothesised that we were more likely to have observed imaging pathology in cases of associated anemia. Age, breed, sex, physical examination at presentation, haematology, biochemistry, blood type, imaging type and findings, concurrent diseases, number of blood transfusions, survival time and cause of death were recorded.

One hundred and thirty dogs, out of 304 diagnosed with IMHA, were included. Seventy five were diagnosed with non-associative IMHA (group 1), 18 with associative (group 2), and 36 with pIMHA (group3). There were statistically significant breed dispositions, with Cocker spaniels overrepresented in group 1 (P = 0.01) and Labrador retrievers in group 3 (P = 0.012). Dogs diagnosed with concurrent thrombocytopenia had a significantly increased mortality rate in all 3 groups (P = 0.006). In all groups, increased urea, bilirubin and AST activity was associated with decreased survival (P = 0.01, 0.008 and 0.04, respectively).

Urea and AST activity were both significantly higher in group 2 (P = 0.005 and P = 0.03); bilirubin was higher in group 1 over groups 2 and 3 (P = 0.02) and platelet count was lower in group 2 and higher in group 3 (P = 0.0001). No statistically significant difference was seen between groups comparing ultrasonographic and radiographic findings. Hepatosplenomegaly was the most common imaging finding in all groups.

Neoplasia was diagnosed in 38.8% of cases in group 2, with 28.5% of these localized to the liver. Seventy seven percent of dogs survived beyond 30 days (56% group 1, 11% group 2 and 32% in group 3). Of the dogs not surviving to 30 days, a mean survival of 16.7 days was identified (73% euthanized, 27% natural death). No significant difference was found between PCV on presentation and mortality (P = 0.19). Number of blood transfusions given had no influence on survival (P = 0.65).

This study provides further evidence of the poor prognostic indicators previously described in the literature. A lower mortality rate was noted in this study than is often reported; a reason could not be identified. Our hypothesis was rejected. Changes detected on imaging alone could not predict to which group a dog would be diagnosed.

Disclosures
No disclosures to report.

ESVIM-O-8
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Point-of-care (POC) Feline Leukemia Virus (FeLV) screening tests are routinely performed in veterinary practice because of their wide availability, high sensitivity and specificity, and rapid results. FeLV testing is often performed in cats with immune mediated hemolytic anemia (IMHA) as part of the investigation into comorbidities or triggering factors. Here we report a number of cats with IMHA where POC FeLV testing was positive but concurrent polymerase chain reaction (PCR) results did not identify FeLV proviral DNA.

Between 2018 and 2019 eight cats diagnosed with IMHA had positive results using POC FeLV test. All cats included had severe anemia, positive direct agglutination test and no other triggering factors for IMHA were identified on screening including hemitropic Mycoplasma PCRs and abdominal imaging.

POC tests using bidirectional flow p27 antigen enzyme-linked immunosorbent assay (ELISA) were performed according to manufacturer instructions. Additional quantitative real time DNA PCR testing was performed. In 6 out of 8 cats (75%) FeLV proviral DNA PCR was negative. POC test errors were excluded by repeat testing from different lots. POC test were replicated in 4 patients using left-over samples of serum, plasma and whole blood. A concurrent p27 ELISA was performed at a reference laboratory in one cat with a positive result. Besides severe anemia (median hematocrit 9.8%, range 3.7-19.4%) mild to moderate thrombocytopenia was present in all patients (median 109K/μL, range 42-182K/μL) and hyperbilirubinemia was present in 4 of 6 patients. Five of 6 patients responded to immunosuppressive treatment and one case was euthanized due to economic constraints. Six months after diagnosis, two patients (including the cat that had a positive result using the external ELISA) were re-tested using the same POC test. Both of them had negative results in the POC and direct agglutination test.

In conclusion, this study identified false positive results using a POC test in cats with IMHA. These results reaffirm the recommendation that cats testing positive using a POC FeLV test should be re-tested using further methods, especially if IMHA is present.

Disclosures
No disclosures to report.

ESVIM-O-9
Clinical, clinicopathological and imaging differences between cats with non-associative, associative and precursor immune-mediated haemolytic anemia
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A retrospective assessment was made of cats with immune-mediated hemolytic anemia (IMHA) from 2015 to 2019. The study compared clinical, clinico-pathological and imaging findings between cats with either non-associative, associative or precursor-mediated IMHA (pIMHA), to determine if there were significant differences between the 3 forms of disease and identify significant predictors of mortality.

Thirty one cats, out of 60 diagnosed with IMHA, were included. Ten achieved a diagnosis of non-associative IMHA, 7 associative, and 13 pIMHA. Age, breed, sex, physical findings at presentation, hematology, biochemistry, blood type, imaging type and findings, concurrent diseases, number of blood transfusions, survival time and cause of death were recorded.

No statistically significant difference was seen between type of IMHA and ultrasoundographic and radiographic findings. Splenic extra-medullary hematopoiesis was identified by cytology most commonly in all forms of IMHA. No significant breed disposition of this cohort was found ($P = 0.57$).

Of all cases, 40% were Domestic Short Hair, 16.7% British Short Hair and 36.7% other pure breeds.

Of patients diagnosed with associative IMHA, neoplasia, specifically lymphoma, was diagnosed in 42.8% of cases. Other causes seen included infection (42.8%), such as FeLV, FIP, and Mycoplasma felis. Cats diagnosed with concurrent thrombocytopenia did not have a significantly increased mortality rate ($P = 0.29$). No significant difference was found between PCV on presentation and mortality ($P = 0.20$), number of blood transfusions given and mortality ($P = 0.07$), blood type and mortality ($P = 0.88$) or number of blood transfusions and IMHA type ($P = 0.89$).

Of the biochemical findings, an increased AST and creatinine kinase were associated with decreased survival ($P = 0.01$ and 0.04, respectively). A lower mean survival time (MST) was observed if younger than median age of 8 years (603 versus 800 days, respectively). MST in cats with non-associative IMHA was 1190 days, with associative IMHA 225 days and pMHA 567 days. Seventy one percent of cats survived beyond 30 days (90% non-associative, 57% associative and 69% with pIMA). Of the cats not surviving to 30 days, a mean survival of 7 days was identified (62.5% euthanized, 37.5% natural death).

A mean of 4.78 hospitalization days was seen, with 4.7 days if non-associative, 7 if associative and 4.1 if pIMA, and a mean of 59.5 days for PCV to reach 30% after treatment initialization was observed (69.45 and 21 days, respectively).

This study provides further evidence of poor prognostic indicators previously described in the literature and provides further assessment of the various forms of hemolytic anemia.

Disclosures

No disclosures to report.

**ESVIM-O-10**

Clinical, diagnostic findings and short-term outcome in 27 cats with non-regenerative anemia due to bone marrow disorders

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Little information is available about clinical picture, diagnostic findings and outcome of feline immune-mediated (IM) and (pre-)neoplastic (PN) non-regenerative anemia (NRA).

This retrospective study aimed to describe and compare the clinical and diagnostic features, and the short-term outcome between cats with IM-NRA and PN-NRA.

Our database was searched for cats diagnosed with NRA (PCV <24% and reticulocyte count <50,000 mL) between March-2011 and October-2019. Inclusion criteria were available bone marrow cytology results and known FIV-FeLV status. Included cats were classified in two groups: PN-NRA [myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)], and IM-NRA [pure-red cell aplasia (PRCA), non-regenerative immune-mediated hemolytic anemia (NRIMHA), and secondary dysmyelopoiesis (SD)]. Cats with myelophthisis resulting from extra-medullary neoplasia were excluded. Signalment, clinical signs, diagnostic investigations, treatments, short-term survival (within 30 days after the first presentation) and remission (resolution of anemia within 2 months) rates were recorded and compared between groups. The level of significance was set at $P < 0.05$.

Twenty-seven cats were included (21 with IM-NRA and 6 with PN-NRA). Definitive diagnoses were: NRIMHA (16), PRCA (3), MDS (4), SD (2), and AML (2). The median age was 2 years. History revealed lethargy (96%), hyporexia (63%), weight loss (22%), digestive (15%) and respiratory (11%) signs, and pica (7%). Physical examination showed systolic heart murmur (63%), respiratory abnormalities (46%), abnormal body temperature (44%), and icterus (15%). The overall median PCV was 10% (ranging from 4.1 to 23.3). Concomitant CBC findings included macrocytosis (63%), severe thrombocytopenia (12%) and leukopenia (12%). Six cats were found positive for infectious agents [FeLV (2), FIV (1), coronavirus (1) and Mycoplasma haemofelis (2)]. Biochemical revealed increased ALT (62%), hyperbilirubinemia (43%) and hypoalbuminemia (35%). Imaging findings included cardiomegaly (57%), severe lymphadenopathy (58%), splenomegaly (42%), hepatomegaly (33%) and abdominal effusion (29%). No difference was found between groups about frequency of these clinical and diagnostic findings. Cats were managed with whole blood transfusion (17), prednisolone (27), cyclosporine (2) and/or cytarabine (2). Median duration of hospitalization (DH) was 4 days. DH was negatively correlated with PCV ($\rho = -0.55; P < 0.05$). Sixty-one % experienced remission within a median of 14 days. Remission rate was more frequent in cats with IM-NRA (67%) compared to PN-NRA (0%) ($P < 0.05$). Short-term survival rate (89% and 20%, respectively) was also higher in cats with IM-NRA ($P < 0.05$).
In this study, IM-NRA and PN-NRA presented with similar clinical and diagnostic findings. However, the remission and short-term survival rates were higher among cats with IM-NRA.

Disclosures
No disclosures to report.

ESVIM-O-11

Comparison of Clinical examination, laboratory findings, prognosis and long-term follow-up between client-owned ill cats naturally infected either by Mycoplasma haemofelis or Candidatus Mycoplasma haemominutum in a single practice

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Feline Hemoplasma chronic carriage has been largely described. We lack information about clinical examination, laboratory findings, prognosis, relapse and long-term follow-up in naturally infected ill cats. We hypothesized they would differ between cats infected either by Mycoplasma haemofelis (Mhf) or by Candidatus Mycoplasma haemominutum (Mhm).

In order to compare both groups, we retrospectively included 63 ill client-owned cats from 2008 to 2020, with positive hemoplasma PCR, BCC, biochemistry and FeLV/FIV status at diagnosis. Treatment was based on saline infusion, dexamethasone (0,2 mg/kg/d/IV/3d) and doxycycline (10mg/kg/d/3d).

Mhf positive cats (28 cases) had acute anorexia (96,4%), digestive symptoms (35,7%), syncopea (14,3%), ataxia (14,3%), dyspnea (7,1%), urinary incontinence (7,1%); most were young (median 2yr [0,75-13]) non-medicalized males (85,1%), with jaundice (64,3%), pale mucous membrane (32,1%), and systolic heart murmur (2-4/6, 39,3%). Few were infected by FeLV or FIV (14,3% each, 1 coinfection). Mean Hemoglobinemia was 5,1g/dl (NR 9-15). ALT always was in normal range (P < 0,001). ALT always was in normal range (P < 0,001). ALT always was in normal range (P < 0,001). ALT always was in normal range (P < 0,001).

Compared to Mhf cats, Mhm positive cats (28 cases, 67,8% males, 32,1% FeLV+, 7,1% FIV+, 1coinfection) were acutely ill (67,8%), emaciated (32,1%) older (median age 7yr, P < 0,001) with weakness (39,3%), fever (median 40°C, P < 0,05). Mean Hemoglobinemia was 8,2g/dl (low, but higher than Mhf, P < 0,001). ALT always was in normal range (P < 0,001). Severe comorbidities were present in 35,7% of cases (leukemia, lymphoma, hyperthyroidism, stomatitis, panleukopenia, pericardial effusion, cardiomyopathy, lipidosis). All cats were discharged from hospitalization. The 7 cats that died within 1-7 months without clinical response to treatment were cachetic at diagnosis. Thirteen cats died thereafter from another cause (mean follow-up 3,5yr). Five cats were still alive at study end (follow-up 5,7yr), (3 lost to follow-up).

Seven other cases had coinfection (Mhf+Mhm).

Mhf and Mhm induced anemia in naturally infected cats, with more severe anemia in the former, whatever FeLV/FIV status. ALT often were increased in Mhf cats. Severe comorbidities were frequent in Mhm cases, cachexia being a predictive factor of death, but a large majority of affected cats were long-term survivors without relapse in both groups.

Disclosures
No disclosures to report.

ESVIM-O-12

Clinical and diagnostic findings and outcome in 58 dogs with immune-mediated polyarthritis

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Paucity of information exists about differences in features and outcome between dogs with idiopathic (i-) and reactive (r-) immune-mediated polyarthritis (IMPA).

The aim of this retrospective study was to compare the clinico-biological picture and outcome between dogs with i-IMPA and r-IMPA.

Our database was searched for dogs diagnosed with both erosive and non-erosive IMPA between June-2004 and January-2020. Diagnosis of IMPA was based on the presence of aseptic neutrophilic inflammation of ≥2 joints at cytology. Idiopathic IMPA was diagnosed after exclusion of underlying infectious, inflammatory or neoplastic diseases and/or based on positive response to immunomodulatory therapy. Dogs with systemic lupus erythematosus, polyarthritis-polymyositis syndrome, and steroid-responsive menigitis-artritis were excluded. Signalment, clinical signs, diagnostic findings and outcome were recorded and compared between dogs with i-IMPA and r-IMPA (level of significance set at P < 0.05).

Fifty-eight dogs (median age 4.7 years) were included (43 dogs with i-IMPA and 15 dogs with r-IMPA). Dogs with r-IMPA had leishmaniasis (7), digestive disorders (3), eosinophilic bronchopneumopathy (3), bacterial infection (1) and gossypiboma (1). Dogs with r-IMPA were more frequently large breed dogs (P = 0.02). Overall, common clinical and physical abnormalities included lameness/stiffness (79%), hyperthermia (72%), peripheral adenomegaly (72%), and lethargy (67%). Joint swelling, pain and/or heat were present in all dogs, involving carpi (87%), tarsi (53%), stifles (54%), elbows (47%), and hips (14%). No significant differences were found between dogs with i-IMPA and r-IMPA. Frequent biological abnormalities were leukocytosis (47%),
anemia (30%), hypoalbuminemia (61%), hyperproteinemia (28%) and increased C-reactive protein (CRP; 90%). Overall, a significant negative correlation was observed between serum albumin concentration and the number of affected joints (rho = -0.31; P = 0.04). Hyperproteinemia (P = 0.02) was more frequent in dogs with r-IMPA, while leukocytosis (P = 0.002) was more frequent in dogs with i-IMPA. Forty-seven dogs were treated with immunomodulatory drugs [prednisolone (45), leflunomide (5), cyclosporine (3), and mycophenolate mofetil (1)]; their administration was more frequent among dogs with i-IMPA (P < 0.001). Clinical follow-up was available for 42 dogs (30 i-IMPA and 12 r-IMPA). Seventy-four% experienced complete remission (CR; no physical abnormalities), while 21% experienced partial remission (persistance of physical abnormalities). The mean time to CR was 38 days and it was significantly shorter in dogs with i-IMPA (29 days) compared to those with r-IMPA (50 days). There was no correlation between CRP-to-albumin ratio and time-to-remission.

Our results suggest that clinico-biological picture and outcome are similar in dogs with i-IMPA and r-IMPA. The duration to achieve CR was shorter in dogs with i-IMPA.

Disclosures

No disclosures to report.

ESVIM-O-13

The mercury challenge: Feline systolic blood pressure in primary care practice, a European survey

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Systemic hypertension is an important condition in cats, but there is an paucity of data on systolic blood pressure (SBP) values measured in cats attending primary care practices. This convenience survey was designed to collect SBP data from a large number of cats across numerous European countries.

From June 2018, Ceva Santé Animale invited primary care clinics to record data from cats aged ≥7 years who had SBP measured as part of their routine veterinary care. Owners gave permission for anonymised information from the cats to be recorded on a central database (mercurychallenge.ceva.com), including basic demographic data, information on concomitant disease and/or medications, SBP values, and any antihypertensive therapy given.

By March 2020, data was available from >9100 cats from 17 countries. Of these data, there were 5262 unique entries from cats ≥7 years of age that were receiving no concomitant prescribed therapies. Analysis of these data revealed that according to ACVIM criteria, 36% of the cats were normotensive (SBP < 140 mmHg), 28% were pre-hypertensive (140-159 mmHg), 17% were hypertensive (160-179 mmHg) and 18% were severely hypertensive (>180 mmHg). 172 cats (3.3%) had hyperthyroidism, 780 (14.8%) had CKD and 28 (0.5%) had both. Median SBP in cats with CKD (157 mmHg), hyperthyroidism (157 mmHg) or both (167 mmHg) were significantly higher than in cats without these conditions (146 mmHg). Excluding the data from cats with CKD or hyperthyroidism, there was a small but significant correlation between SBP and age (Spearman r = 0.261, P < 0.0001), a very low negative correlation with bodyweight (Spearman r = -0.033, P = 0.038), but sex had no significant effect on median SBP values. SBP values measured by oscillimetry (median 151 mmHg) were significantly higher (P < 0.0001) than those measured by Doppler (median 145 mmHg). Subjective assessment of stress was significantly (P < 0.0001) related to SBP with nervous/aggressive cats (median SBP 160 mmHg) having higher values than anxious (153 mmHg) or calm cats (140 mmHg).

SBP measurement was reported to take <5 minutes in 51%, 5-10 minutes in 41% and >10 minutes in 8% of cases. These data suggest SBP can be readily measured in primary care practice and demonstrate a high prevalence of cats with potential hypertension. Cats reported to have CKD or hyperthyroidism had significantly higher median SBP values and both methodology and subjective assessment of stress may assist interpretation of SBP values.

Disclosures

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ESVIM-O-14

A proteomic evaluation of greyhound meningoencephalitis using quantitative mass spectrometry highlights the consideration of viral triggers

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A unique form of breed-restricted meningoencephalitis has been previously reported in Irish greyhounds. This condition typically affects multiple littermates, consistent with an underlying genetic, infectious or environmental trigger, or a combination thereof. However, attempts to identify a cause using genome-wide association and sequencing studies, PCR- and serology-based infectious disease screening, and environmental assessment, have been unrewarding. The aim of this study was to characterize the cerebral inflammatory response on the proteomic level in dogs with greyhound meningoencephalitis (GHME) using mass spectrometry.

Samples were collected from young greyhounds with GHME (n = 7) and age- and breed-matched controls (n = 7). Samples were collected within 30 minutes of euthanasia. Control dogs were euthanized for reasons other than neurological disease and had unremarkable brain histopathology. Proteins from each cerebral tissue were extracted, quantified and digested. A label-free, quantitative proteomic analysis was conducted on 1μg of the purified peptide from each sample using high resolution-accurate mass spectrometry. The relative fold change (RFC) in protein expression was determined. Principal component analysis (PCA) was conducted to identify outliers and clusters of sam-
Prospective evaluation of urinary alkaline phosphatase and γ-glutamyl transpeptidase as diagnostic and prognostic biomarkers of acute kidney injury in dogs

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Urinary alkaline phosphatase and γ-glutamyl transpeptidase activity, normalized to urinary creatinine (uALP/uCr and uGGT/uCr, respectively), increase in naturally-occurring acute kidney injury (AKI). Previous studies, mostly retrospective, included mainly azotemic dogs. We aimed to examine the performance of these markers at presentation for predicting AKI development in hospitalized, non-azotemic dogs, at risk for AKI, including, among others, cases of acute pancreatitis, left congestive heart failure, sepsis, SIRS and surgery (ASA status ≥ 3) within 24 hours of enrollment.

The study included 20 healthy controls, and 120 hospitalized dogs, of which 15 (12.5%) developed AKI. Twenty-three dogs (19%) died, including seven with AKI. Median (IQR) uALP/uCr of the AKI, non-AKI ill and healthy control groups were 0.573 (0.692), 0.312 (0.535) and 0.033 (0.044), respectively (P < 0.001), and for uGGT/uCr, 1.990 (1.304), 1.155 (1.129) and 0.280 (0.279), respectively (P < 0.001). In post-hoc analysis, urinary levels of both uALP/uCr and uGGT/uCr significantly differed between the healthy controls and either AKI or ill, non-AKI groups (P < 0.001 for both), but only uGGT/uCr level differed between the AKI and, ill, non-AKI groups (P = 0.029). The area under the ROC curve (AUC) for uGGT/uCr for predicting AKI was 0.68 (95% CI, 0.55–0.80).

Both biomarkers significantly differentiated healthy and ill dogs. Nevertheless, neither was a good predictor of outcome or development of AKI, notwithstanding statistically significant group differences.

Disclosures

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ESVNU-O-2

Laboratory variation of feline urinary protein: Creatinine ratio

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Proteinuria is an important prognostic factor and therapeutic target in cats with chronic kidney disease. Nevertheless, studies on analytical factors that could affect feline urinary protein: creatinine ratio (fUPC) results are scarce.

The current study aimed to quantify the inter- and intra-laboratory variability of fUPC and to additionally assess the agreement between laboratories with respect to the proteinuria substage according to the International Renal Interest Society (IRIS) classification in cats. Urine samples were collected by cystocentesis from 60 cats (30 healthy, 30 diseased) and aliquoted. To assess inter-laboratory variability, urine from each cat was analysed in four of nine collaborating laboratories. Two of these laboratories received two aliquots per cat, in order to determine intra-laboratory variability. The analytical method for urine protein determination was either turbidimetry based on benzethonium chloride (n = 5) or colorimetry based on pyrogallol red molybdate (n = 4). Urine centrifugation before analysis was performed in five laboratories.

The fUPCs showed good interclass correlation (ICC-inter = 0.90) and excellent intraclass correlation (ICC-intra = 0.99). The fUPCs obtained with turbidimetry versus colorimetry did not significantly differ, nor did centrifugation of urine samples before analysis affect fUPC results.
significantly. Agreement on IRIS substages was moderate (Fleiss’ \( \kappa \) coefficient = 0.55) with cats being classified in the same proteinuria stage in 75% of cases.

The present study shows that choosing a different laboratory to assess feline urine samples does not significantly affect UPC values, but it can lead to a different classification of proteinuria according to IRIS guidelines in 25% of cases.

**Disclosures**

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**ESVNU-O-3**

Liver-type fatty acid binding protein and neutrophil gelatinase-associated lipocalin in feline chronic kidney disease and feline hyperthyroidism

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Liver-type fatty acid binding protein (L-FABP) and neutrophil gelatinase-associated lipocalin (NGAL) are potential biomarkers for early detection of pathophysiological changes in feline kidneys. The detection of chronic kidney disease (CKD) in hyperthyroid cats remains challenging. Early renal biomarkers, in particular in feline hyperthyroidism, are still lacking. The aim of this study was to evaluate L-FABP and NGAL in cats with CKD or hyperthyroidism.

Serum and urine samples from 103 cats, of which 9 azotemic CKD cats (serum creatinine > 2.3 mg/dL and urine specific gravity < 1.035), 49 non-azotemic hyperthyroid cats and 45 healthy cats, were included in this cross-sectional study. Serum L-FABP (sL-FABP), serum NGAL (sNGAL), urinary L-FABP (uL-FABP), and urinary NGAL (uNGAL) concentrations were measured by commercial ELISAs validated for use in feline serum and urine samples. Concentrations of uL-FABP and uNGAL were reported as a ratio to urinary creatinine (uL-FABP/Cr and uNGAL/Cr). The biomarkers were compared between the three groups by Wilcoxon rank sum test and reported as median values. Correlations between both serum and urinary biomarkers and urinary protein-to-creatinine ratio (UPC), and between serum and urinary biomarkers were assessed in all cats using Spearman’s correlation. The sensitivity and specificity of L-FABP and NGAL for the detection of azotemic CKD were determined based on receiver operating characteristic (ROC) analysis.

CKD cats had significantly higher sL-FABP (13.50 ng/mL; \( P = .013 \)) and uL-FABP/Cr (4.90 \( \mu \)g/g; \( P < .001 \)) than healthy cats (4.26 ng/mL and 0.09 \( \mu \)g/g, respectively). Hyperthyroid cats had significantly increased uL-FABP/Cr (0.88 \( \mu \)g/g; \( P < .001 \)) and sNGAL (38.24 ng/mL; \( P < .048 \)) compared to healthy cats (sNGAL: 31.33 ng/mL). There was no significant difference of uNGAL/Cr between groups (CKD cats: 3.34 \( \mu \)g/g; hyperthyroid cats: 2.98 \( \mu \)g/g; healthy cats: 3.19 \( \mu \)g/g). Sensitivity and specificity for the detection of azotemic CKD were 55.6% and 88.9% for sL-FABP (cutoff 13.50 ng/mL) and 100% and 90.9% for uL-FABP/Cr (cutoff 0.18 \( \mu \)g/g). The correlation between sL-FABP and uL-FABP/Cr was moderate and significant (\( rs = 0.36; P < .001 \)), but there was no correlation between sNGAL and uNGAL/Cr (\( rs = -0.02; P = .81 \)). A strong and significant correlation was observed between uL-FABP/Cr and UPC (\( rs = 0.78; P < .001 \)).

In conclusion, L-FABP rather than NGAL seems a potential biomarker for early detection of CKD in cats and may also be a promising early renal biomarker in hyperthyroid cats.

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No disclosures to report.

**ESVNU-O-4**

Evaluation of cystatin B as a marker of acute kidney injury in dogs and cats

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Early diagnosis of AKI and CKD is challenging. Our aim was to evaluate the diagnostic utility of urinary Cystatin B (uCysB) as a kidney injury biomarker. Surplus urine samples were collected from dogs and cats divided into 4 groups: AKI, CKD, healthy-controls, and lower urinary tract diseases [urinary tract infection (UTI) in dogs or urethral obstruction (UO) in cats]. Eighty-eight dogs and 76 cats were included. In dogs, uCysB was higher in the AKI and CKD groups compared with the control and UTI groups (both, \( P < .002 \)). Receiver operator characteristic curve (ROC) analysis of uCysB for predicting AKI in dogs had an area under the curve (AUC) of 0.91 (95%CI, 0.82-0.99, sensitivity 89%, specificity 100%). In dogs with AKI, non-survivors had higher uCysB compared with survivors (\( P = 0.007 \)). ROC of uCysB as an AKI outcome predictor had an AUC of 0.77 (95%CI, 0.61-0.94), and a 1200 ng/mL cut-off point corresponded to sensitivity of 78% and specificity of 67%. Cats with AKI had higher uCysB compared with control (\( P < .001 \)), CKD (\( P = .001 \)) and UO (\( P = .004 \)). ROC analysis of uCysB for predicting AKI had an AUC of 0.92 (95%CI, 0.84-1.0, sensitivity 90%, specificity 92%). uCysB in cats was higher in non-survivors compared with survivors of AKI. ROC analysis of uCysB as an AKI outcome predictor had an AUC of 0.84 (95%CI, 0.56-1.0), and 469 ng/mL corresponded to a sensitivity of 100% and specificity of 75%. In conclusion, uCysB is a sensitive and specific marker as well as a promising marker for AKI in both species.

**Disclosures**

Sarah Peterson and Murthy Yerramilli are IDEXX employees. The analysis of Cystatin B was performed at IDEXX Laboratories, Inc as a part of a research collaboration.
ESVNU-O-5

Soluble alpha klotho in senior cats
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Soluble alpha klotho (sαKl) correlates positively with eGFR in humans. This study aimed to examine the relationship between plasma sαKl and chronic kidney disease (CKD) in senior cats. Clinicopathological information from cats ≥9 years old (n = 143), was sourced from the records of two first opinion practices (2011-2016). Inclusion criteria were availability of stored EDTA plasma and a concurrent plasma FGF23 measurement. Cats with chronic systemic disease or plasma thyroxine > 40nmol/L were excluded. Cats were categorized into four groups: healthy (n = 54), IRIS CKD Stage 1 if plasma SDMA >14µg/dL (n = 21) and IRIS Stages 2 (n = 42) and 3 (n = 19). Stored samples were used to quantify sαKl using a commercially available ELISA that was validated as part of the study. Group comparisons were made by Mann-Whitney U tests and relationships between numerical variables were evaluated using Spearman’s correlation. Data are presented as median [25th, 75th percentile].

Intra and inter assay variability were <10% and dilutional parallelism was observed. Plasma FGF23 concentrations increased with IRIS stage (healthy: 195 [134, 271] pg/mL, Stage 1: 234 [177, 430] pg/mL, Stage 2: 498 [261, 872] pg/mL, Stage 3: 1357 [538, 4692] pg/mL) and were significantly different between all groups (P < 0.05). Plasma sαKl concentration did not differ between groups. There was no correlation of plasma sαKl with plasma creatinine or FGF23.

The lack of association between CKD and sαKl requires further investigation to elucidate whether this finding is due to species differences in pathophysiological mechanisms or methods of sαKl quantification.

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ESVNU-O-6

Risk factors associated with disturbances of calcium homeostasis following the initiation of phosphate-restricted diet in cats with chronic kidney disease
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Dietary phosphate restriction improves survival in cats with chronic kidney disease (CKD). However, feeding a phosphate-restricted diet may contribute to development of hypercalcemia. This study aimed to identify risk factors associated with increasing plasma total calcium concentrations (TCa) following transition onto a phosphate-restricted diet in CKD cats.

Records from two first-opinion practices were reviewed to identify cats with CKD transitioned onto a renal diet. Change in TCa, 200 days following diet change, were assessed using linear regression and dichotomized into “uptrend” (regression gradient >0) and “non-uptrend” (gradient ≤0) groups. Clinical data are presented as median [25th, 75th percentile]. Baseline variables were compared (Mann-Whitney U and logistic regression) to explore risk factors for increasing TCa.

Seventy-one euthyroid cats (IRIS CKD stages 2 [n = 54] and 3 [n = 17]) were enrolled. Forty cats had “uptrend” TCa and 31 were “non-uptrend”. Significantly different variables at baseline (uptrend vs. non-uptrend) included potassium (3.72 [3.46, 4.01] vs. 4.08 [3.82, 4.29] mmol/L; P = 0.003), phosphate (1.20 [1.09, 1.47] vs. 1.39 [1.23, 1.54] mmol/L; P = 0.034) and sodium (150 [148, 153] vs. 152 [150, 155] mmol/L; P = 0.036). No significant differences in TCa, ionized calcium, creatinine, FGF23 and PTH were found. In multivariable logistic regression, baseline potassium (OR = 1.19 per 0.1 mmol/L; P = 0.003) and phosphate (OR = 1.15 per 0.1 mmol/L; P = 0.014) remained independent risk factors for uptrend calcium status.

A lower baseline potassium and/or phosphate concentration is independently associated with increasing TCa in CKD cats fed renal diet, suggesting involvement of these analytes in renal calcium reabsorption may contribute to hypercalcemia in some cats.

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Cats with chronic kidney disease (CKD) have an increased prevalence of positive urine cultures (PUC). To date there is limited information available regarding the prognosis of cats with CKD and concurrent PUC. The aim of this study was to determine the effect of PUC on survival time and disease progression in cats with CKD.

Client owned cats with azotemic CKD were retrospectively identified by searching medical records between 1997-2018. Selected cats were classified as having “no-PUC” or “PUC” based on serial urine culture results. PUC cats were further classified as having one or multiple PUC, and were also classified based on the presence or absence of clinical signs of urinary tract infection (UTI). All cats with PUC received standardized antibiotic treatment irrespective of the presence or absence of clinical signs of UTI. CKD progression was defined as a plasma [creatinine] increase of ≥ 25% within 365 days of CKD diagnosis; PUC also had to have occurred within this timeframe. Survival time and frequency of CKD progression were compared between groups.

There was no significant difference in survival time between cats with no-PUC and cats with any number of PUC (P = .908), or between cats with no-PUC, one PUC and multiple PUC (P = .367). There was also no significant difference in the frequency of CKD progression between the PUC and no-PUC cats (P = .504), or between no-PUC, one PUC and multiple PUC cats (P = .22). When assessing cats with clinical signs of UTI, there was no significant difference in the frequency of CKD progression between cats with true UTI, subclinical bacteriuria and no-PUC (P = .797).

This study demonstrated that when treated with antibiotics, PUC in cats with CKD do not affect disease progression or survival time.

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Renal AA-amyloidosis in shelter cats: A retrospective study based on clinico-pathological data, light microscopy and ultrastructural features

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Systemic AA-amyloidosis is a protein misfolding disease characterized by extracellular deposition of non-soluble fibrils arising from the acute phase protein serum amyloid A; the kidney is one of the target organs. In cats, systemic AA-amyloidosis is described in the familial form in Abyssinian and Siamese breeds, and rarely in the reactive form in domestic shorthairs. Recently, a prevalence of systemic AA-amyloidosis ranging from 46.1-71.4% has been reported in shelter cats. Proteinuria and azotemia are hallmarks of renal AA-amyloidosis in dogs and humans; similarly, chronic kidney disease (CKD) is observed in Abyssinian cats with AA-amyloidosis. Whether shelter cats with renal AA-amyloidosis have CKD is unknown. Hence, aims of this study are to describe kidney laboratory, histopathological and ultrastructural findings in shelter cats with renal AA-amyloid deposits. Cats from one shelter were considered if necropsy was performed within 6 hours from death and kidney samples were collected. Routine histochemistry was used to diagnose and score amyloid deposits and characterize tubulointerstitial damage, electron microscopy to differentiate glomerular lesions. Cats were included if renal-AA amyloidosis was diagnosed and had laboratory data available within 5 weeks before death.

Eleven cats were included. All were domestic shorthairs, 5 were male and 6 females. The mean age was 7.9 years (range: 2-13). All cats had blood analyses available, 9 urinalyses. Mean serum creatinine concentration was 3.1 mg/dL (range: 1.5-7.0); 1 cat was in CKD IRIS stage I, 5 in stage II, 3 in stage III, and 2 in stage IV. SDMA concentration was 32.5 mg/dL (range: 17-69). Ten cats had anemia (hematocrit: 20.9%; 15.8-29), 9 hypoalbuminemia (2.3 g/dL; 1.2-2.9). All urinalyses showed proteinuria (urine protein-to-creatinine ratio: 3.66; 0.5-10.6); in 6 cats proteinuria was >2. One cat repeatedly presented normoglycemic glycosuria. Urinary specific gravity was 1.020 (1010-1046). Ten cats had amyloid deposits in the glomeruli and all in the tubulo-interstitium. Seven cats had concurrent interstitial nephritis, one renal lymphoma and one membranoproliferative glomerulonephritis. Interestingly, two cats with interstitial nephritis and proteinuria >2, had severe tubulo-interstitial amyloidosis and only mild glomerular amyloidosis.

In conclusion, renal AA-amyloidosis is associated with azotemia and proteinuria in shelter cats. Additionally, amyloid deposits are observed both in the glomeruli and tubulo-interstitium. Of note, some cats with prominent tubulo-interstitial lesions may have severe proteinuria. Whether tubulo-interstitial amyloid justifies glycosuria in one cat remains unclear. Renal AA-amyloidosis should be included in the differential diagnoses of shelter cats with CKD.
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ESVNU-O-9

Survival rate and prognostic factors in dogs with acute on chronic kidney disease
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Chronic kidney disease (CKD) is the most common urinary tract disease in small animals, with estimated canine prevalence of 0.5%-7%. Acute exacerbation of CKD (ACKD) is common, however, its etiologies, risk-factors and prognosis have not been described. The aims of this study were to characterize the etiology, clinical and laboratory findings, short- and long-term prognosis of dogs with ACKD. The study included 100 dogs with ACKD, diagnosed and hospitalized in a veterinary teaching hospital. Median age was 144 months (range, 18-288 months). There was no difference in age between survivors (155 months, range, 24-225 months) and non-survivors (132 months, range, 18-288 months) (P = 0.349). Pylonephritis was the most common identified etiology. There was no difference in mortality rate among etiologies (P = 0.464). Median hospitalization time was 5 days (range, 2-29 days) and was significantly longer in survivors (6 days, 2-29 days) compared with non-survivors (4 days, range, 2-20 days) (P < 0.001). IRIS AKI grade was associated (P = 0.009) with the short-term survival. In a multi-variable analysis, increased respiratory rate (P = 0.012), CK activity (P = 0.005) and serum creatinine concentration (P = 0.041) at presentation were associated with outcome. The median survival time of dogs discharged was 105 days (95%CI, 25-184), with 35 and eight dogs surviving up to 6 and 12 months respectively. The etiology (P = 0.16) and serum creatinine concentration (P = 0.59) at discharge were not predictors of long-term survival. In conclusion, the short-term outcome of dogs with ACKD is comparable to AKI, however, long-term prognosis is guarded. IRIS AKI grade is a prognostic indicator of the short-term outcome.

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ESVONC-O-1

Prognostic impact of time interval between surgery and initiation of adjuvant chemotherapy following limb amputation in dogs with appendicular osteosarcoma without distant metastases
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Adjuvant chemotherapy should be initiated in a timely manner following surgery to maximally impact residual neoplasia. However, optimal timing remains unknown. The aim of this retrospective study was to examine whether there was a measurable prognostic impact of the time interval to adjuvant systemic chemotherapy (TI) in dogs with appendicular osteosarcoma following limb amputation. The objectives of the study were to evaluate whether any relationship existed between TI and prognosis and, if so, whether there was an optimal TI or a TI after which the benefit of treatment decreases.

Dogs were included if they underwent limb amputation for a histologically-confirmed appendicular osteosarcoma; had no evidence of distant metastases; had a body weight >15 kg; received at least 4 cycles of adjuvant dose-intensive chemotherapy, and had complete clinico-pathologic and follow-up data. The following clinico-pathological factors were analyzed: breed; age; sex; weight; symptom duration; site of osteosarcoma; lymph node metastasis; distant metastasis; alkaline phosphatase; monocytes; lymphocytes; type of imaging; chemotherapy protocol; number of cycles; chemotherapy-related toxicity, and cause of death.

Dogs were classified into 8 groups based on whether they received chemotherapy 3, 5, 7, 10, 15, 20, 30 days or >30 days after surgery. Survival analyses was performed to identify potential prognostic factors.

One-hundred and thirty dogs were included. The median TI was 14 days (range, 1-70). TI of 7 days was associated with the greatest survival benefit: dogs receiving chemotherapy 8 or more days after surgery had a risk 1.8 times higher for death from osteosarcoma-related causes (P = 0.008).

Median time to progression (TTP) for dogs receiving chemotherapy within 7 days [440 (95% CI, 334-546)] was significantly higher than that for dogs being treated after 7 days [243 (95% CI, 200-286)]; P = 0.026]. Median overall survival (OS) for dogs receiving chemotherapy within 7 days [533 (95% CI, 397-668)] was significantly higher than than for dogs being treated after 7 days [281 (95% CI, 248-314)]; P = 0.007].

No other potential prognostic factors were associated with TTP or OS.

Findings from our study indicate that the timing of chemotherapy initiation is an important prognostic variable. Based on these data, we recommend that great efforts should be made to minimize postsurgical recovery time to enable starting adjuvant chemotherapy within 7 days post-surgery. TI of 7 days was associated with a significant survival benefit in our population of dogs with non-metastatic appendicular osteosarcoma.

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Phase I dose escalation study of 12b80: Hydroxybisphosphonate linked doxorubicin—in dogs with naturally occurring osteosarcoma

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Comparative oncology has revealed that spontaneous occurring osteosarcoma in dogs provides a singular opportunity for preclinical modelling. The molecule 12b80 is a new antineoplastic compound, combining doxorubicin to a bone targeting hydroxybisphosphonate vector using a pH-sensitive linker, designed to specifically trigger doxorubicin release in acid bone tumor microenvironment. In in vivo study, 12b80 displays a stronger antitumor activity on rodent orthotopic osteosarcoma compared with doxorubicin/zoledronate combination. This phase I study was aimed to determine the safety and toxicity profiles of 12b80 in dogs with spontaneous occurring osteosarcoma, with the objective to translate findings from dogs to humans.

Ten client-owned dogs with naturally occurring osteosarcoma were enrolled in a prospective, open-label, phase I dose escalation study using an accelerated dose-titration design followed by 3+3 design. Four dose levels were evaluated: 4 mg/kg (n = 1), 6 mg/kg (n = 2), 8 mg/kg (n = 3), 10 mg/kg (n = 4). The protocol consisted of three cycles of 12b80 intravenous (IV) injections, administered every three weeks. After completion of the three cycles, dogs underwent a complete end-staging (day 56) including whole-body computed tomography and bone tumor biopsy. Endpoints included safety, tolerability, maximum tolerated dose (MTD), and dose-limiting toxicity (DLT) evaluated according to VCOG criteria. Preliminary antitumor activity of 12b80 was also evaluated. The MTD of 12b80 was 8 mg/kg (i.e. equivalent calculated dose of doxorubicin of 110 mg/m², range: 93 – 126). No DLT was observed at this dose level. Most adverse events included grade ≤ 2 gastrointestinal disorders and hypersensitivity reactions. No hematologic DLT were observed at any dose level tested. No cardiac DLT was reported on follow-up echocardiogram (day 56) and postmortem cardiac biopsy. Stable disease without evidence of metastatic disease was reported in two (2/10, 20%) dogs at D56. Histopathology analysis of the bone tumor biopsies following three cycles of 12b80 revealed a stable mitotic index in two dogs and increased necrosis in bone tumor biopsy in five dogs. The median survival for six (6/10, 60%) dogs who completed the three cycles of 12b80 was 157 days (range: 56 – 766).

This study suggests that 12b80 is overall well tolerated in dogs, expanding the therapeutic index of doxorubicin up to four times the standard dose of 30 mg/m² in dogs without associated DLT. The results show potential translational relevance for further clinical development of 12b80 in dog and human osteosarcoma.
Disclosures
No disclosures to report.

ESVONC-O-4

Prevalence of reproduction pathologies and associated death with survival analysis among bitches over 6 years of age in a single practice
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Very few data is available concerning reproduction pathologies (RP) morbidity and mortality among bitches in a context of low sterilization rate. We hypothesized that RP morbidity and mortality would be higher among unspayed compared to spayed females, and looked for RP effect on life expectancy.

Female medical records born from 2000 to 2003 were reviewed in a single practice. Cases were included if at least one complete visit was done from 6 years of age, with known sterilization status (< or >2 years). Ovariectomy was systematically advised at puberty and unilateral complete mastectomy with ovariectomy as soon as any mammary tumor (MT) was discovered. Among 602 included cases (IC), main RP (pyometra, MT) were statistically compared regarding sterilization status. 293 females were followed up until death (UD) with a last clinical examination. Females considered dead due to RP presented either with invasive MT or pulmonary metastasis confirmed with X-ray, or severe pyometra with poor general condition. The age at the last consultation was used for the survival analysis, with data considered right censored for individuals lost to follow-up (n = 309).

The effects of early spaying and of mammary tumors on survival was analyzed with Kaplan-Meier and Cox-model analysis.

Among IC, 8,3% were spayed before-2, 40,5% later and 51,2% weren’t. Seventy-nine females (13,1%) were presented with a pyometra, of which 15 died. Hundred and sixty-three females (27,1%) developed MT (median age 10), 60 had surgery (36,8%, median age 9). Among UD group, 36,2% presented MT during their life of which 18,4% deceased from MT, 4,4% were euthanized without identified cause of death and 13,3% died from another cause.

Early spaying was significantly associated with lower incidence of MT and associated mortality (Fisher’s exact test, respective odds ratio = 0,1P < 0,001; odds ratio = 0,0P = 0,016). The survival analysis did not show any significant effect of the diagnosis of MT or early spaying on survival (P = 0,47 and P = 0,92 respectively). Among females presenting MT, undergoing surgery significantly increased the survival by a factor of 2,6 (cox model, P < 0,001). The age at which MT was discovered had a significant negative effect on survival, with each additional year decreasing the survival by a factor of 1,35 (cox model, P < 0,001).

Reproduction pathologies caused mortality in 23% of females in this low-sterilization rate cohort. Early spaying was statistically associated with lower mammary tumors incidence and mortality but did not change life expectancy.

Disclosures
No disclosures to report.

ESVONC-O-6

Prevalence of peripheral blood and bone marrow infiltration in canine extranodal lymphoma
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Extranodal lymphomas (LSA) account for less than 20% of canine lymphoma cases; this group includes heterogeneous forms classified as stage 5 based on the WHO clinical staging system, regardless of peripheral blood (PBI) and bone marrow involvement (BMI).

Most recent studies have focused on the evaluation of PBI/BMI in multicentric LSA cases but its prevalence and prognostic role in canine extranodal LSA has not been documented in detail. The aim of our study was therefore to evaluate PBI/ BMI in canine extranodal LSA cases. For inclusion into the study, patients were required to have cytological or histological diagnosis of LSA, complete blood count, biochemical examination, chest x-ray study, abdominal ultrasound and flow cytometry evaluation on peripheral blood (PB) and bone marrow (BM). A cut-off of 0,56% (PBI) and 2,45% (BMI) was used in case of large B or large T immunophenotype; no cut-off was adopted in case of aberrant T immunophenotypes.

Seventeen cases were enrolled in the study, including 4 alimentary, 3 spinal, 3 cutaneous, 2 mediastinal, 2 splenic, 1 renal, 1 intra-abdominal and 1 nasal. Sixteen cases were intermediate-large cell LSA and 1 was a small cell LSA. Eight out of the 12 immunophenotyped cases were T-LSA (66,7%) and 4 were B-LSA (33,3%); in 5 cases the immunophenotype of neoplastic cells was not available and PBI and BMI were defined on the presence of atypical and/or large lymphoid cells. At the time of diagnosis, 3 cases (17,7%) showed both BMI and PBI (1 alimentary, 1 cutaneous, 1 intra-abdominal), 2 (11,8%) had only BMI (1 spinal, 1 mediastinal) and 1 (5,9%) only PBI (cutaneous). All these patients except the last one showed one or more hematologic abnormalities. No BMI or PBI were detected in 11 cases (64,7%); hematologic abnormalities were present in 6 cases. Due to the moderate prevalence of BMI/ PBI, our preliminary results suggest that a complete staging including BM aspiration could not be always necessary for canine extranodal LSA. Unfortunately, hematologic abnormalities do not appear to be predictive of the BMI. Further studies to define the prognostic value of BMI are needed to clarify the role of BM evaluation in extranodal LSA.

Disclosures
No disclosures to report.
ESVONC-O-7

A preliminary immunohistochemical study of signal transducer and activator of transcription 3 (STAT3) expression and its prognostic significance in 57 canine anal sac adenocarcinomas
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Prognostication in canine anal sac adenocarcinomas (ASACs) is difficult due to conflicting evidence regarding metastatic rates and median survival times (MST). The transcription factor signal transducer and activator of transcription 3 (STAT3) is a prognostic predictor in several human cancers. The aim of this retrospective study was to assess STAT3 expression in ASAC and explore its association with clinical presentation and outcome. We hypothesized that STAT3 expression would distinguish tumors with early versus late metastasis.

Records from “Institute A” were searched for dogs diagnosed with ASACs from 2008 to 2019. Immunohistochemical expression of phosphorylated STAT3 (pSTAT3) was assessed in primary tumors (n = 52) and metastatic lymph nodes (n = 31) and MST were calculated for cases with low and high pSTAT3 expression.

Of the 57 cases assessed, 27 presented with primary tumors with no metastasis and 30 with primary and local metastatic disease. Nuclear pSTAT3 expression occurred in a minority of neoplastic cells of 55/57 cases, mainly in the tumor periphery. Expression of pSTAT3 showed no significant difference between metastatic cases at presentation and cases that metastasized after 6 months or never metastasized. There was no significant difference between MST in cases with high and low pSTAT3 expression. Cases that presented with metastatic disease had shorter MST (395 days) than those with primary tumors alone (623 days).

pSTAT3 is variably expressed in primary and metastatic ASACs cells, however in this study pSTAT3 did not provide prognostic information for canine ASACs.

Disclosures
No disclosures to report.

ESVONC-O-8

Unravelling tumor-driving mutations in canine mast cell tumors and metastatic lymph nodes by next generation sequencing
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Mast cell tumors are one of the most common canine neoplasms. Although progress has been made understanding this neoplasm, mast cell tumors can still behave unpredictably and at times be a clinical challenge. In addition the presence or absence of metastatic disease can be difficult to conclude as the differentiation between reactive and neoplastic mast cells based on cytology and histology is challenging. It has been shown that some mast cell tumors carry mutations in the KIT oncogene in exon 8, 9 or 11 however little is known about additional somatic variants driving oncogenesis and metastasis.

In order to investigate this further, with the view to identify somatic variants which could shed light on the biological behavior of mast cell tumors and potentially be used for prognostication or detection of metastasis, we performed exome sequencing on paired tumor and normal DNA from 18 mast cell tumors and 11 paired metastatic lymph nodes based on achieved FFPE tissues and peripheral blood samples.

All samples consisted of surplus diagnostic material stored with owner consent. Data was aligned to CanFam 3.1 following the GATK best practices and somatic variants called using Mutect 1, MAC and Strelka with appropriate filtering of normal variants and false positive calls.

After filtering for quality and sequencing coverage, data was available for 13 primary mast cell tumors and (9 cutaneous, 3 subcutaneous, 1 intramuscular) and 10 lymph nodes.

The most commonly mutated gene detected in the mast cell tumors was SETD2, which carried loss of function mutations in 4 tumors (31%). SETD2 is a known tumor suppressor gene, which has previously been shown to be implicated in canine osteosarcoma and in high-grade human mast cell neoplasia. Only 3 (23%) tumors carried mutations in the KIT gene in exon 8, 9 and 11 respectively. In general very few coding mutations where identified in the metastatic lymph nodes which could be related to low tumor cellularity in the sequenced lymph nodes. More sensitive methods should be applied for detection of mast cell tumor specific mutations in lymph nodes.

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ESVONC-O-9

Alternative lengthening of telomeres in canine histiocytic sarcomas of Bernese Mountain dogs and other breeds is infrequently used as telomere maintenance mechanism
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Some human sarcoma subtypes use the telomerase-independent telomere maintenance mechanism (TMM) alternative lengthening of telomeres (ALT) more frequently than other cancers to overcome senescence. Previously, ALT activity was identified in 6/64 various canine sarcomas including 2/5 (40%) histiocytic sarcomas (HS). The aim of this retrospective study was to analyze the prevalence of ALT in a larger canine cohort of HS including a sub-cohort of Bernese Mountain dogs (BMDs).

Sixty-seven dogs with HS including 50 BMDs from two centers were evaluated. ALT-positive canine and human tumor samples served as controls. DNA was extracted by commercial-kit (Nexttec) from...
archived formalin-fixed paraffin-embedded (FFPE) samples submitted for routine diagnostic and quantified by fluorescence dye. ALT activity was examined through extrachromosomal telomeric DNA-circles using radiolabel C-circle (CC) assay. Following published recommendations, after background correction and normalization on input DNA signals of abundant Alu-elements, levels above 5fold background were defined as ALT-positive and between 2-to-5fold as borderline. Samples of two BMDs showed weak ALT activity 5.1 and 5.5fold above background compared to 232fold above background detected from control human osteosarcoma cell line U2OS. Two non-BMD-samples revealed borderline ALT-positive signals of 2.2fold above background each. Other samples were ALT-negative. Positive controls showed CC-signals in expected ranges, important to exclude false-negatives. The results indicate contrary to the previous study that HS seem to use weak ALT activity with prevalences of 4% in BMDs and 59% in other breeds. Future studies may demonstrate high prevalence of the other TMM telomerase activity with potential as prognostic marker and therapeutic target.

Disclosures
No disclosures to report.

ESVONC-O-10

Effect of low dose rate half body irradiation on the remission and survival times for dogs with multicentric, substage a, B cell lymphoma treated with multilagent chemotherapy
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Multiagent chemotherapy has proven highly effective at inducing remission in dogs with multicentric, B cell lymphoma but, despite efforts to expand response durations, current literature suggests average first remission durations are less than one year. Several published studies of dogs treated with half body irradiation show longer survival times but they lack control groups to confirm these results. The aim of this study was to investigate the benefit of half body irradiation to dogs with substage a, B cell lymphoma being treated with chemotherapy.

Nine dogs with stage 3 or higher, substage a, B cell lymphoma who achieved complete remission after the first cycle of a chemotherapy protocol were enrolled in the study prospectively. All cases treated at the same institution with the same inclusion criteria from the preceding 5 years were enrolled retrospectively and from this retrospective cohort 9 individuals were selected as case controls by a blinded, independent, European-boarded specialist in oncology based upon signalment and common influences on prognosis.

All dogs in the study were treated with the same chemotherapy protocol (UW-19 without prednisolone) while the prospective study dogs had the second cycle of chemotherapy substituted with two low dose rate half body irradiation treatments two weeks apart and separated by a single dose of L-asparaginase. The cranial half body irradiation was administered two weeks after the first dose of doxorubicin with L-asparaginase one week later and the caudal half body irradiation delivered the following week. This was followed by two further cycles of chemotherapy. The primary outcome was first remission duration and the secondary outcome was overall survival. The patients were censored at 2 years within our study design.

Dogs in the control group had a median remission time of 261 days and a median survival of 286 days with 0/9 dogs remaining in first remission at 2 years and only 1/9 dogs surviving >730 days. Within the study group 5/9 dogs were in first remission at 2 years and 7/9 dogs were still alive at 2 years resulting in median remission and survival times both >730 days. The differences in remission and survival times were statistically significant with P values of P = 0.011 and P = 0.015 respectively. This study suggests that there is a significant extension in remission duration and survival time for dogs when low dose rate half body irradiation is included in their treatment protocol.

Disclosures
The study received funding from the Australian Animal Cancer Foundation which was paid directly to an external biometrician for input to study design and statistical analysis.

ESVONC-O-11

The use of a combined prebiotic and probiotic oral product and its impact on stool consistency in dogs undergoing radiotherapy
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Diarrhea is a common complication in canine radiotherapy patients unrelated to the radiation treatment. Stress due to the hospitalization most likely contributes to the development of diarrhea in these patients. Probiotics have been shown to mitigate stress-induced diarrhea in cats and dogs. A symbiotic (combination of pre- and probiotic) preparation might have a similar effect in dogs undergoing radiotherapy.

The aim of this prospective, double blinded randomized placebo-controlled study was to evaluate the effect of once daily administration of an oral symbiotic preparation (Enterococcus faecium NCIMB 10415 4b1707, fructo-oligosaccharide, gum Arabic, mannan-oligosaccharide and beta-glucans) on diarrhea, in dogs undergoing radiotherapy. Clinical parameters evaluated included stool consistency, body weight, appetite and vomiting frequency. Dogs receiving radiotherapy to the pelvic area and those with intestinal parasitism identified on an in-house faecal flotation test were excluded. Dogs were fed a bland commercial diet unless their owners requested a specific diet, or the dog required an alternative diet for health reasons.
Data were assessed for normality using Shapiro-Wilk test, and differences between groups analyzed using chi-squared test, T-test or Mann Whitney U test where appropriate. Thirty-one dogs were recruited. There were 16 dogs within group A which received the symbiotic and 15 dogs in group B which received the placebo. The duration of treatment ranged from 11 to 30 days, and there was no difference between groups (median for group A 22 days vs median for group B 22 days, \( P = 0.49 \)). There was no significant difference between stool scores, (mean for group A 5 vs mean for group B 10, \( P = 0.11 \)), nor percentage of days of abnormal stools (median for group A 18 days vs median for group B 17 days, \( P = 0.40 \)). There was no difference between groups for the frequency of vomiting (\( P = 0.682 \)), weight loss (\( P = 0.432 \)) or appetite score (median for group A 1 vs median for group B 0, \( P = 0.47 \)).

The use of the symbiotic product did not provide a clinical benefit compared to a placebo in dogs undergoing radiotherapy.

Disclosures

No disclosures to report.

ISCAID-O-1

Canine vaccination in Germany: A survey of owner attitudes and compliance

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Vaccination is the most important measure for protection against canine infections. There are no studies on vaccination compliance of dog owners in European countries, except UK. Aims of the study were to determine the compliance of German dog owners towards vaccination and identify influencing factors.

Data were collected from August 2018 to February 2019 using an online survey targeted at German dog owners. Owners \( \leq 16 \) years of age, of dogs <8 weeks of age, and veterinarians were excluded. A total of 3,881 questionnaires were evaluated. Factors influencing the vaccination status of dogs were determined by a linear logistic regression model. McNemar’s test and Cohen’s kappa statistics were used to evaluate correspondence between the questionnaire and 340 voluntarily submitted vaccination passports.

In total, 46.8% (n = 1,818/3,881; 95% confidence interval (CI): 45.3-48.4) of the dogs were vaccinated with core vaccines according to current vaccination guidelines. Young age (16 weeks to 15 months) (n = 294/3,881; odds ratio (OR): 3.08; 95%CI: 2.05-4.68), using the symbiotic (n = 221/3,861; OR: 0.08; 95%CI: 0.04-0.18), purpose of keeping the dogs, trips abroad and vaccination recommendations by veterinarians are decisive.

Disclosures

No disclosures to report.

ISCAID-O-2

Comparison of four commercially available point-of-care tests to detect antibodies against canine parvovirus in dogs

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Measuring antibodies to determine immunity in dogs against canine parvovirus (CPV) is a useful tool to avoid unnecessary re-vaccinations. Since recently, 4 point-of-care (POC) tests for detection of CPV antibodies are available in Europe but their performance has not been compared. The aim of this study was to evaluate quality and practicability of these 4 POC tests in the field.

Sera of 198 dogs were included. For antibody detection, virus neutralization (VN) was performed as reference standard (VN titer \( \geq 10 \) was considered positive). Sensitivity, specificity, positive (PPV), negative predictive values (NPV), and overall accuracy (OA) were determined. To assess agreement among POC tests, Cohen’s kappa statistic was performed.

Prevalence of CPV antibodies in VN was 97%. The FASTest® and CanTiCheck® were easiest to perform. The Immunocomb® Canine Vaccicheck had a sensitivity, specificity, PPV, NPV, and OA of 70%, 50%, 98%, 5%, and 70%; the FASTest® CPV/CDV of 95%, 33%, 98%, 18%, and 93%; the TiterCHECK® CDV/CPV of 63%, 67%, 98%, 5%, and 63%; the CanTiCheck® of 80%, 83%, 99%, 12%, and 80%, respectively. Agreement in the number of positive results between all tests was poor (kappa: TiterCHECK®/FASTest® 0.124; TiterCHECK®/Immunocomb® 0.162; CanTiCheck®/Immunocomb® 0.285; CanTiCheck®/TiterCHECK® 0.344; CanTiCheck®/FASTest® 0.267; FASTest® and Immunocomb®: 0.052).

The CanTiCheck® would be the POC test of choice when considering specificity as most important. However, differences in the number of false positive results were minimal between the 4 POC tests due to the high CPV antibody prevalence in the field population.

Disclosures

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Detection of pathogens implicated in canine infectious respiratory disease complex in dogs without respiratory signs hospitalized in a veterinary teaching hospital

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Canine infectious respiratory disease complex (CIRDC) is a major cause of respiratory signs and morbidity due to various pathogens. All are contagious and can be harbored by healthy or recovering carriers. Hospitals represent a potential transmission source, especially in large teaching institutions with high density and constant animals and students’ turnover.

This study aimed to evaluate the detection rate of pathogens implicated in CIRDC, in hospitalized dogs without respiratory signs, at a veterinary teaching hospital.

 Conjunctival and oropharyngeal swabs were prospectively sampled from 125 dogs between February and June 2019. Samples were evaluated for CIRDC agents’ detection using multiplex PCR. All positive results were verified by simplex PCR. Descriptive statistics were used. Detection rate by multiplex testing was 31.2% for influenza A (FluA), 22.4% for canine parainfluenza, 20.8% for Streptococcus equi subsp. zooepidemicus (Sz), 16% for Mycoplasma cynos (Mc), 14.4% for canine adenovirus 2 (CAV2), 11.2% for Bordetella bronchiseptica (Bb), 3.2% for canine pneumovirus (CPnV), 0.8% for canine distemper (CDV) and canine herpes virus 1 (CHV1) and 0% for canine respiratory coronavirus, FluA H3N2 and H3N8. Detection was confirmed in less cases by simplex PCR, leading to a detection rate of 13.6% for FluA, 12.8% for Sz and Mc, 1.6% for CAV2 and 0.8% for CPnV. Simplex PCR did not confirm the detection of CDV, CHV1 and Bb. The non-confirmed detections were close to the sensitivity threshold of the multiplex test. 32.8% of dogs tested positive for at least 1 pathogen. Among them, 1, 2 and 4 agents were isolated respectively in 70.7%, 24.9 and 4.9%.

The detection rate of traditional agents implicated in CIRDC was low, except for Mc; while unusual agents (Sz and FluA) were frequently found. Whether these detections could be associated with mechanical transient carriage or true infection and possible persistent carriage remains to be elucidated. The FluA detected in our cohort was different from those previously isolated during canine outbreaks (specific canine FluA types H3N2 and H3N8) and was only detected during winter months, concurrently to flu outbreak in humans. Further studies are required to determine if owners or hospital members could be the source of infection. This study is also the first to report isolation of Sz in a large cohort of dogs without clinical signs of CIRDC. Sz can be carried by horses. Equine dedicated services are present in our teaching hospital, raising concerns about potential Sz transmission through hospital members.

Disclosures

This research was funded by bioMérieux S.A. (France) and its affiliate BioFire Diagnostics LLC (USA), a private company which, among others, develops molecular testing tools. Three of the authors (M. Baldasso, J. Yugueros Marcos and P. Graieux) are current employees of bioMérieux S.A. and the PCR experiments were run at bioMérieux S.A., Centre Christophe Mérieux. Travel grants for the ECVIM 2020 congress will be granted to A. Brunet and E. Krafitt by bioMérieux S.A. Audrey Brunet also received travel grants from Royal Canin SA.

Outbreak of acute hemorrhagic diarrhea in dogs in Norway: Is Providencia alcalifaciens involved?

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Acute hemorrhagic diarrhea syndrome (AHDS) is characterized by sudden onset of profuse hemorrhagic diarrhea, often accompanied by vomiting, lethargy, hyporexia, hyperthermia and/or hemoconcentration. Young to middle-aged, small- and toy breeds seem prone to the disease. The etiology is often unidentified.

During the autumn of 2019, an unusually high occurrence of severe AHDS, also affecting atypical breeds, was reported by veterinary hospitals in Oslo and Southeastern Norway.

The aim of this retrospective study was to compare AHDS in dogs at our hospital from August to October in 2018 and 2019, describe the clinical presentation and investigate a possible common cause in the 2019 cases.

There were 127 cases in 2019 compared to 19 cases in 2018, but no differences in age, sex, body weight or breeds were found. In the 2019 outbreak, clinical signs were observed a few hours to nine days before presentation. In some dogs, an abrupt aggravation occurred after days with moderate disease. Diarrhea, vomiting, hyporexia and hypothermia were the most common signs. Hematemia was observed in 16.7%. Twenty-four percent were critically or severely ill. English Setters, Irish Setters and Dachshund (rabbit size) constituted 57.0% of the critically ill. Hemocoagulation and increased CRP were the most common changes on blood examination. Diagnostic imaging demonstrated various degrees of functional ileus. Three dogs developed severe acute gastric tympany. Treatment was symptomatic. Intensive monitoring was often necessary. One dog died and five were euthanized due to poor prognosis and/or economic considerations. Necropsy revealed diffuse hyperemia and variable mucosal necrosis in the small and large intestine.
A range of possible infectious causes and intoxications were ruled out based on clinical and pathological findings, microbiological culture and molecular diagnostics. The most common observation was the isolation of *Providencia alcalifaciens* (Pal) (62.0 %) and *Clostridium perfringens* (Cpe) (62.0 %). Co-presence of Pal and Cpe was found in 36.9 %. There was no correlation of presence of Cpe alpha-toxin, enterotoxin, net F/E and severity. Pal was cultivated from all critical cases except two. Mucosal invasion by Pal was confirmed by FISH analysis. Pal is associated with diarrhea in humans and dogs, and a putative cytolethal distending toxin may be involved in virulence. Connections between the diarrheal cases have not been found, but preliminary whole genome sequencing (WGS) of Pal isolates gives suspicion of common source. The presence of Pal may play a role in disease outcome in dogs with AHDS.

**Disclosures**

No disclosures to report.

**ISCAID-O-5**

Demographic risk factors for canine leptospirosis in the UK

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Leptospirosis is an important global disease with wide ranging clinical presentations and diagnostic interpretation challenges. Additionally, risk factors associated with canine leptospirosis in the UK are not well understood. This study aimed to explore demographic risk factors for disease diagnosis in dogs in the UK.

A retrospective case-control study compared 243 confirmed cases of leptospirosis based on samples submitted to reference laboratories between 2013-2019 against a VetCompass denominator population of 905,543 dogs. Of the cases, 106 (44%) were confirmed using the microscopic agglutination test (MAT) and 137 (56%) using PCR. Multiple variable logistic regression was used to evaluate age, breeds with >5 cases, Kennel Club (KC) breed group, sex and neuter status as risk factors for leptospirosis diagnosis (P < 0.05).

All age categories >1 year old had increased odds when compared to dogs <1 year old, with dogs >8-<20 years having nearly 3 times the odds of leptospirosis (OR 2.84, 95% CI 1.02-2.00) Four KC breed groups had increased odds compared to non-KC recognized dogs: Toy (1.27 OR, 95%CI 0.74-2.17), Terriers (24.32 OR 95%CI 3.34-175.71), Utility (2.42 OR, 95%CI 1.14-5.13) and Working (1.18 OR, 95%CI 0.50-2.75). When compared to crossbreeds, all purebreed types examined had reduced odds of infection. Neutered dogs had 1.43 higher odds of infection than entire dogs (95%CI 1.02-2.00). Sex was not significantly associated with infection (P = 0.30).

This research identifies risk factors for leptospirosis specific to the UK dog population, which will aid veterinarians to develop a better index of suspicion for potential cases. The increased odds of diagnosis in older and certain dog breed groups provides further evidence to help inform the index of suspicion and encourage increased use of confirmatory diagnostic testing. Although no individual breeds were identified of high risk, the high odds seen with toy breeds here may reflect reduced vaccine uptake or increased rodent contact in urban settings.

**Disclosures**

PhD student co-funded by MSD and BBSRC.

**ISCAID-O-6**

Ecological niche modelling to explore probability of presence of canine leptospirosis in Great Britain

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Leptospirosis is an important global zoonotic disease that affects a wide range of mammalian species. Although outbreaks are common after flooding, and therefore clear environmental drivers exist, risk factors for canine leptospirosis in Great Britain (GB) have not been examined in this context. Using a presence-only machine learning algorithm (MaxEnt), this study explored the contribution of temperature, rainfall, livestock density (horses, cattle, pigs, sheep), land coverage and urban-rural classification to the probability of presence of canine leptospirosis in GB. Cases were positive test submissions to a reference laboratory between 2009-18 (n = 322). Each variable’s contribution was used to create a map of probability of presence of leptospirosis. We then conducted further separate analysis of cases seropositive to the three most frequent serogroups amongst submissions: Australis, Icterohaemorrhagiae and Saxkoebing.

Our overall final leptospirosis model had a high predictive accuracy (area under the curve, AUC = 0.81), as did our final models for Australis (AUC = 0.92), Icterohaemorrhagiae (AUC = 0.89) and Saxkoebing(AUC = 0.84). For most models, land coverage classification and temperature were the greatest contributors to predictive accuracy. Within these two explanatory variables the factors that increased probability of leptospirosis the most were: urban/suburban land coverage, temperatures between 6.5 and 10.5 °C and higher densities of livestock species. However, for the Australis model, increased horse density from 0 to 400 heads/km² was the most important variable.

There was wide-ranging variation in probability of leptospirosis presence around GB and distribution of areas of high probability of presence differed between the individual serogroup models. Highest probability of presence was seen in the South East, West Midlands and the South West regions of GB. Additionally, areas of the north of England were identified as suitable for the Icterohaemorrhagiae and Saxkoebing serogroup.

Identification of environmental risk factors for different serogroups can potentially be used to inform vaccination strategies at a local level. Urban/suburban land types being the most important factor for the presence of leptospirosis adds further support to the notion that...
the demographic background and location of cases is shifting from a historically rural bias. Additionally, the association between increased probability of presence of leptospirosis with increasing density of horses may indicate their potential importance in the transmission of canine leptospirosis.

Disclosures
PhD student co-funded by MSD and BBSRC.

ISCAID-O-7

Increased frequency of exercise intolerance, coagulation and hematological abnormalities in Angiostrongylus vasorum infected vs. non-infected dogs

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Canine angiostrongylosis may cause severe disease and death. Diagnosis may be challenging due to the spectrum of non-specific clinical signs in infected dogs. A prospective study was conducted in highly endemic areas of Denmark (16 clinics) and Italy (14 clinics) to further characterize the clinical signs and risk factors of A. vasorum infection in dogs. The clinical signs and/or abnormal laboratory findings and possible risk factors were collected from dogs tested for A. vasorum at the selected clinics. The distributions of the risk factors and diagnostically relevant clinical findings were compared in infected vs non-infected animals using Fisher’s exact test.

During the 14 month study, 1628 dogs were tested by fecal and/or serological methods for A. vasorum infection. In total, 1000 dogs (61.6%) were tested because they showed clinical signs consistent with A. vasorum infection while the remaining dogs were tested because they were considered at risk of infection. From the 198 (12.2%) positive dogs, 171 (86.4%) showed clinical signs. Coughing, dyspnea and tachypnea were among the most commonly reported clinical signs in 58.1%, 30.8% and 24.2% of the dogs that tested positive and in 48.9%, 20.8% and 17.0% of the dogs that were positive, respectively. While the proportions of dogs showing each of these signs was significantly greater in the infected dogs (P < 0.019), the difference was not large vs the non-infected population. Twice as many infected dogs had exercise intolerance (50.5%) and abnormal lung auscultation (39.9%) vs. non-infected dogs (24.5% and 19.4%, respectively) (P < 0.0001). Bleeding disorders, pale mucous membranes, petechia/ecchymosis were reported in three to six times as many (P < 0.0001) infected dogs (15.7%, 27.3% and 12.6%, respectively) vs. non-infected dogs (3.7%, 7.4% and 2.1%, respectively). Hematological abnormalities (most often anemia, eosinophilia, thrombocytopenia, basophilia) were reported in more than three times as many positive (27.3%) dogs than in negative (8.8%) dogs (P < 0.0001).

Among the risk factors evaluated, significantly more positive dogs had previous history of A. vasorum infection in their household (19.7%) vs. non-infected dogs (8.6%) (P < 0.0001).

It is concluded that while respiratory signs may be the most commonly reported clinical abnormality in A. vasorum infected dogs, these may be not considered to be discriminative enough because non-infected dogs often show similar signs. In contrast, exercise intolerance, coagulation and hematological abnormalities were reported several times more frequently in infected vs. non-infected dogs, suggesting that these may be more specific to A. vasorum infection, particularly when coupled with respiratory signs.

Disclosures
The study was funded by Zoetis. The presenting authors role was local study monitor. Co-authors are employed by Zoetis.

ISCAID-O-8

Virulence factors might be implicated in clinical presentation of urinary tract infections caused by Escherichia coli in dogs and cats

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Urinary tract infection (UTI) is mainly caused by Escherichia coli. Asymptomatic UTI is frequent in pets, but retrospective studies failed to identify factors associated with clinical presentation. Purposes of this prospective study were to investigate putative risk factors on clinical presentation of E. coli bacteruria and on antimicrobial resistance. Dogs and cats diagnosed with E. coli UTI in 3 French veterinary hospitals were included. Prior exposure to antimicrobials, hospitalization, urine catheterization, and clinicopathological data were recorded. Animals with confounding factor(s) preventing the allocation to symptomatic or asymptomatic group were excluded. Multiplex PCR were used to determine presence of the followed virulence factor genes : siderophores (fyuA, iutA, iroN), adhesins (papG allele I, II and III, sfa/foc, uclD), toxins (sat, hlyA, cnf1, pks, mcmA/mchB). E. coli were stored and standardized antimicrobial sensitivity testing were performed. Logistic regressions were used to assess relationship between risk factors, clinical presentation and antimicrobial resistance.

Ninety-seven animals (72 dogs, 25 cats) were recruited; 48% were asymptomatic; 6.2% and 1% of E. coli, respectively, were MDR and had extended spectrum beta-lactamase. None of the historical, epidemiological or clinicopathological factors was associated with clinical presentation. UclD gene (F17-like adhesin) was the only factor that
Extended-spectrum-beta-lactamases- and carbapenemase-producing Enterobacteriaceae (ESBL) isolates from the gut of sick companion animals in Portugal

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Abstract

Extended-spectrum-beta-lactamases- and carbapenemase-producing Enterobacteriaceae (ESBL) isolates from the gut of sick companion animals in Portugal

Objective: To describe the occurrence and characteristics of ESBL and carbapenemase-producing Enterobacteriaceae (CP)-producing isolates from the gut of companion animals (CAs) in Portugal.

Methods: Feces were collected from animals with and without signs of gastrointestinal infections admitted to 6 referral veterinary hospitals in Portugal between October 2017 and March 2018. The presence of ESBL and CP was screened by disk diffusion and microdilution tests. Bacterial identification was performed by 16S rRNA gene sequencing. ESBL producers were characterized by using the extended-spectrum-beta-lactamases- and carbapenemase-producing Enterobacteriaceae (ESBL) isolates from the gut of sick companion animals in Portugal

Results: A total of 212 CAs were enrolled. ESBL and CP were detected in 11% (24/212) and 4% (9/212) of the CAs, respectively. The most frequent ESBL producers were K. pneumoniae (21/24, 88%), E. coli (18/24, 75%), and Citrobacter freundii (3/24, 13%). The most frequent CP were K. pneumoniae (5/9, 56%). The ESBL producers were associated with ESBL genes blaCTX-M-14 (20 isolates), blaCTX-M-15 (2 isolates), and blaCTX-M-15 (2 isolates). The CP were associated with genes blaKPC (2 isolates), blaVIM-1 (1 isolate), and blaIMP (1 isolate).

Discussion: This study highlights the diversity and importance of ESBL and CP in the gut of CAs in Portugal. The identification of ESBL and CP in CAs emphasizes the need for careful management of antimicrobial use in animal hospitals to prevent the spread of these microorganisms.
β-lactamase genotypes (blaTEM-1 and blaTEM-1 + blaSHV-18). Nine E. coli isolates ESBL producers were obtained from different UTIs HDs and harbored the genes: blCTX-M-9group (3 isolates, in one case associated with blaTEM-1, in other with blCMY-2), blCTX-M-12group (five isolates, in one case associated with blTEM-122) and blCMY-2 (one isolate). Of that E. coli, 66.67% (6/9) presented MDR profiles. One UTI cat presented simultaneously an ESBL-producing Klebsiella pneumoniae and an MDR E. coli, they carried the blaTEM-1 gene and blCMY-2 + blaTEM-1 + blaSHV-12 genes, respectively.

These findings highlight that sick cats are frequent carriers of multidrug-resistant Enterobacteriales harboring mostly ESBLs but also Carbapenemase genes. These results represent an emerging problem and are crucial to demonstrate the importance of interventional antimicrobial stewardship measures to avoid antimicrobial therapy selective pressure and antimicrobial resistance transmission between pets and humans.

Disclosures
This work was supported by JPIAMR/0002/2016 Project - PET-Risk Consortium and FEDER funds through the Programa Operacional Factores de Competitividade - COMPETE and by National funds through the FCT - Fundação para a Ciência e a Tecnologia- CIISA Project (UID/CVT/00276/2020). Adriana Belas holds an FCT PhD grant SFRH/BD/113142/2015. Juliana Menezes holds an FCT research grant supported by the JPIAMR/0002/2016 Project.

ISCAID-O-11
Plasmid-mediated colistin resistance mcr-1 gene harbored on multidrug resistant isolates from companion animals in Portugal
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The global spread of human nosocomial multi-drug resistant Gram-negative bacteria has led to the use colistin for patient treatment in ICUs. Hence, the report of different plasmid-mediated colistin resistance worldwide raises a serious concern. Little is known on colistin resistance in companion animals (CA). Here, we report the screening of fecal samples from CA in Portugal to determine the prevalence of colistin resistant bacteria (Ethical approval CEBEA 027/2018). Between February 2018 and March 2020, fecal samples were collected from dogs and cats. Samples were plated after prior pre-enrichment in peptone water onto SuperPolymyxin medium. Isolates were identified by PCR. Minimal inhibitory concentrations (MICs) for colistin were confirmed, by broth microdilution (Sensititre™ FRCOL, Thermo Fisher Scientific, Wesel, Germany) for species without intrinsic resistance. All isolates were screened by PCR for the presence of five colistin resistance genes (mcr-1 to mcr-5) and Sanger sequencing. MIC determination for other antibiotics was done with MicroScan® Neg MIC Panel Type 44 (Siemens, Sacramento, CA, US).

In total, 102 CAs belong to 80 households were included for this study: 42 healthy dogs and 20 cats; 21 dogs and 1 cat with skin and soft tissue infection (SSTI); 15 dogs and 3 cats with urinary tract infection (UTI). Of these, 33 fecal samples (healthy-17, SSTI-9 and UTI-6) were positive for Proteus spp. in the SuperPolymyxin medium; 15 samples (healthy-12, SSTI-1 and UTI-2) for Enterococcus spp.; 83 samples (healthy-52, SSTIs-18 and UTI-13) were positive for Escherichia coli; 2 samples (SSTI-1 and UTI-1) for Klebsiella pneumoniae and 2 fecal samples from healthy dogs were positive for Pseudomonas aeruginosa. Broth microdilution confirmed colistin resistance, with MICs between 2-8 mg/L, for 7.23% (6/83) of the E. coli isolates (healthy-2, SSTIs-3 and UTI-1) from all dogs, and for 1 out of the 3 P. aeruginosa from a healthy dog. Molecular analysis revealed that four of these E. coli isolates carried the mcr-1 gene (healthy-1, SSTIs-2 and UTI-1). This four E. coli also presented a multidrug resistance phenotype profile. The remaining isolates showing resistant phenotype but lacking the studied resistance genes should be screening for other mcr-gene variants (mcr-6 to mcr-9) to determine the exact spread of these genes in companion animals. There is also the possibility of having chromosomal mutations leading to resistance. These results raise great animal and public health concerns since companion animals may act as reservoirs of plasmid-mediated colistin resistance for humans, highlighting the need of a careful monitoring.

Disclosures
This work was supported by JPIAMR/0002/2016 Project - PET-Risk Consortium and FEDER funds through the Programa Operacional Factores de Competitividade - COMPETE and by National funds through the FCT - Fundação para a Ciência e a Tecnologia- CIISA Project (UID/CVT/00276/2020). Adriana Belas holds an FCT PhD grant SFRH/BD/113142/2015. Juliana Menezes holds an FCT research grant supported by the JPIAMR/0002/2016 Project.

SCH-O-1
Clinical and clinicopathological findings in dogs other than Scottish Terriers with idiopathic vacular hepatopathy
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Idiopathic vacular hepatopathy (IVH) is a well described syndrome in Scottish Terriers (ST) but the etiology, progression and specific treatment for this condition remain unknown. Less is known about the IVH in other dog breeds. The aim of this retrospective study was to describe clinical and clinicopathological features of IVH in breeds other than ST.

Medical records of dogs with cytologically or histologically confirmed hepatic vacular changes were searched from 2014-2019. To be included in the study, a complete history, physical examination, a full blood analysis (including adrenal function testing), urinalysis, and abdominal imaging were also required. Dogs with systemic diseases and history of receiving medication previously associated with development of vacular hepatopathy (VH) were excluded. ST were excluded as well.
Fourteen dogs fulfilled the inclusion criteria. Breeds included were West Highland White Terrier (n = 5), Border Terrier (n = 1), Jack Russell Terrier (n = 1), Siberian husky (n = 1), Shetland sheep dog (n = 1), Doberman (n = 1), Labrador (n = 1), Cocker Spaniel (n = 1), Bichon Frise (n = 1) and Japanese Spitz (n = 1). There were 9 spayed females, 3 sexually intact males, 1 intact female, and 1 neutered male. The median age for all dogs at the time of presentation was 10 (range, 7-13 years). Four dogs (4/14) presented with PU-PD, 1/14 had polyphagia, and the rest were asymptomatic. Blood pressure (SBP) measurement was available for 9/14 dogs, which were all hypertensive (median 180 mm Hg; range, 160-210 mm Hg). All dogs had increased median serum ALP (755; range 298-2840 U/L) and AST concentrations (33; range, 22-82 U/L). Ten dogs (10/14) had increased cholesterol (9.49, range 5.6-15.5 mg/dL) and 5/14 dogs had increased triglycerides serum concentration (1.4; range 0.8-5.01 mg/dL). Urinalysis revealed persistent proteinuria (UPCR > 0.5) in 12/14 dogs (median 2.4; range 0.12-5.9), without azotaemia and normal SDMA. All dogs presented with mild to moderate amount of mobile gall bladder sludge and heterogeneous and hyperechoic liver parenchyma based on abdominal ultrasound.

The results of this small study revealed that IVH with associated hyperphosphatasemia, lipodosis, proteinuria and hypertension can affect different dog breeds, predominantly terrier breeds. In view of VH progression and high incidence of hepatocellular carcinoma in ST, close monitoring of dogs with this syndrome is strongly encouraged.

Disclosures
No disclosures to report.

SCH-O-2

Comparison of lactulose, metronidazole and hepatic specific diet in controlling clinical signs in dogs with congenital extrahepatic portosystemic shunts: A randomized clinical trial

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Current medical management of dogs with congenital extrahepatic portosystemic shunt (cEHPSs) comprises the use of hepatic specific diet (HSD), lactulose and/or antibiotic therapy, aiming to control clinical signs of hepatic encephalopathy (HE). Meta-analysis of people suffering from type C HE disclosed that antibiotic and disaccharide therapy are equally effective in controlling HE symptoms. A triple-arm randomized clinical trial was designed to compare the efficacy of different treatment combinations [HSD and lactulose (HSD + LACT), HSD and metronidazole (HSD + METRO), and solely HSD] in controlling the clinical signs in dogs suffering from cEHPSs. Dogs were not allowed to be receiving medical therapy at the time of inclusion. The allocated ‘initial’ treatment was continued for 4 weeks. Subsequently, all dogs received the combined treatment with HSD, LACT and METRO for an additional 2 weeks. Standardized questionnaires (assessing neurologic, gastrointestinal and urinary signs) were completed by the owners and a clinical score was calculated at diagnosis and at 4 and 6 weeks.

Thirty-six dogs were included, 12 were allocated to each group. Two dogs, 1 from HSD + LACT group and 1 from HSD + METRO groups were subsequently excluded due to concomitant diseases. Thirty-three dogs had their clinical scores available at the 4-weeks recheck and 27 dogs completed the trial until week 6 (10 HSD + LACT, 9 HSD + METRO, and 8 HSD). At diagnosis, the clinical scores were similar in all treatment groups (P = 0.89). Dogs in the “HSD + METRO” as well as the “HSD + LACT” group had a significant reduction in their clinical scores after the initial treatment compared to at diagnosis (P = 0.03 and P = 0.002, respectively). In contrast, no clinical improvement was observed in the HSD group (P = 0.401). At 6 weeks, a trend to improvement was noticed in the HSD group (P = 0.06), but no further improvement of the clinical score was detected in both other groups (P = 1).

Combination of HSD + LACT or HSD + METRO effectively reduces the clinical scores in cEHPSs dogs, while sole HSD therapy is not sufficient to control the same clinical signs.

Disclosures
No disclosures to report.

SCH-O-3

Hepatic lead and copper concentrations in dogs with chronic hepatitis

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Copper (Cu) influence on chronic hepatitis (CH) have been thoroughly studied in dogs, while a few information on other metals accumulation is currently available. Both Cu and lead (Pb) may cause hepatic injury if their hepatic metabolization is defective or due to oxidative stress mechanisms linked to their presence within hepatocytes, especially for Pb. The aim of the study was to evaluate liver Pb and Cu concentration in dogs with CH. Retrospective evaluation of the Teaching Hospital clinical database was performed searching for dogs with CH and hepatic copper ([Cu]) concentrations, on which lead concentrations ([Pb]) were measured. CH was defined using current ACVIM consensus. [Cu] and [Pb] were evaluated using square wave anodic stripping voltammetry (SWASV; limit of detection (LOD) 10ppm and 0 ppm, respectively). Dogs were divided into two groups based on hepatic [Cu]: Group A <400ppm and Group B ≥400 ppm. [Pb] and [Cu] were correlated using a Spearman correlation test. [Pb] and alanine aminotransferase (ALT) were compared between Group A and Group B using Welch’s t-test and Mann-Whitney U-test, respectively. Thirty-six dogs were screened for eligibility and the final population was composed by 29 dogs, since 7 dogs had [Pb] < LOD and they were censored (6 dogs of Group A and 1 dog of Group B, respectively). The median age was 9 years (range 1-15 years), equally divided between males and females, mainly Labrador Retrievers and Cocker Spaniels (n = 3 each), followed by mixed-breed (n = 9). Twenty-one
dogs (58%) were assigned to Group A, while the remaining 15 dogs were in Group B. [Pb] and [Cu] were strongly positively correlated ($P = 0.0002; r = 0.65$). Group A had a mean [Cu] and [Pb] of 236.6±98 and 44±22.3 ppm respectively, whereas Group B had a [Cu] and [Pb] of 1289±1944 and 103.1±59.8 ppm, respectively. Group B showed a significantly higher [Pb] than Group A ($P = 0.003$). ALT was not significantly different between groups (159 vs. 94 ppm groups A and B, respectively). Although further studies are needed to better understand the clinical role of hepatic [Pb], dogs with hepatic abnormal [Cu] may also have higher [Pb] than dogs with normal [Cu], which may be a concomitant storage defect or a direct consequence of Cu hepatic accumulation. However, since few data are available a concomitant oxidative damage caused by increased [Pb] cannot be excluded.

Disclosures

No disclosures to report.

POSTER RESEARCH COMMUNICATIONS

ESCG-P-1

Correlation between the middle width of the right pancreatic limb and serum trypsin-like immunoreactivity or pancreatic lipase immunoreactivity concentrations in cats with chronic gastrointestinal signs

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Based on anecdotal clinical experience, cats with chronic GI signs occasionally have a small pancreas during laparotomy, some of which turn out to have a low normal or a subnormal serum feline trypsin-like immunoreactivity (fTLI) concentration. The aim of this study was to evaluate the correlation of gross pancreatic size and serum fTLI concentration in cats with chronic GI signs. Ninety-nine cats with chronic GI signs underwent laparotomy for the purpose of collection of pancreatic, intestinal, and hepatic biopsy, as part of the diagnostic workup. Intraoperative photos were taken with a sterile stainless-steel ruler held beside the middle portion of the right pancreatic limb and the width was measured with an image analysis software. Serum fTLI and feline pancreatic lipase immunoreactivity (fPLI) concentrations were measured in surplus serum samples that had been obtained at the time of surgery. The relationships between pancreatic widths and serum fTLI and fPLI concentrations were assessed using Spearman’s rank correlation. The widths of the pancreata of cats in the subgroups detailed below were compared using Kruskal-Wallis tests. Statistical significance was set at $P < 0.05$.

Three cats had a serum fTLI concentration ≤ 8 μg/L, suggesting exocrine pancreatic insufficiency and no cats had a serum fTLI concentration between 8 and 12 μg/L, which is considered equivocal. The width of the middle portion of the right pancreatic limb (median: 1.2 cm, range: 0.6 – 2.2 cm) was weekly correlated with body weight ($r = 0.3, P = 0.005$), but not significantly different between cats with a body condition score that was suboptimal (1 – 3/9, 8/99 cats), optimal (4 – 5/9, 75/99 cats), or high (6 – 9/9, 16/99 cats) ($P = 0.90$). There was no significant correlation between the width of the middle portion of the right pancreatic limb and serum fTLI ($P = 0.74$) or fPLI concentrations ($P = 0.43$) in the 99 cats, nor in the subgroups of cats with a normal or abnormal pancreatic, intestinal, or hepatic biopsy results. Also, the width of the middle portion of the right pancreatic limb was not significantly different between cats with a serum fPLI concentration ≤ 3.5 μg/L (68/99 cats), a fPLI concentration 3.6 – 5.3 μg/L (10/99 cats), or a fPLI concentration ≥ 5.4 μg/L (21/99 cats) ($P = 0.92$). This study suggests that the gross pancreatic size might not significantly correlate with serum fTLI or fPLI concentrations in cats with chronic GI signs.

Disclosures

Norsworthy is the owner of Alamo Feline Health Center. Drs. Wu, Lidbury, Suchodolski, and Steiner are employed by the Gastrointestinal Laboratory at Texas A&M University, which offers laboratory testing, including fTLI and fPLI testing on a fee-for-service basis. Dr. Steiner serves as a paid consultant for Idexx Laboratories, who offers fPLI testing on a fee-for-service basis. Drs. Lidbury, Suchodolski, and Steiner serve as paid consultants and provide CE lectures on behalf of a variety of organizations and companies. None of these relationships should have any impact on the data presented here.

ESCG-P-2

Effects of cyclosporine treatment on supranormal feline serum pancreatic lipase immunoreactivity concentrations

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Chronic pancreatitis (CP) is a common disease in middle-aged to older cats. Treatment with corticosteroids has been suggested, but clinical data on efficacy is lacking. In a study in mice, cyclosporine treatment significantly reduced the severity of experimentally-induced autoimmune pancreatitis. Thus, cyclosporine is an interesting treatment option in feline CP.

The aim of this retrospective study was to evaluate the efficacy of cyclosporine on supranormal serum specific feline pancreatic lipase immunoreactivity (s-Spec fPL) concentrations in cats with suspected CP treated at Evidensia Specialist Animal Hospital Helsingborg, Sweden, during 2013-2019. Inclusion criteria were a history and clinical findings suggestive of CP, s-Spec fPL concentrations above 5.4 mg/L (reference 0-3.5 mg/L) on at least two occasions and treatment with cyclosporine for at least three weeks. Exclusion criteria were incomplete clinical records. Spec fPL was analyzed at Idexx Laboratories, Ludwigsburg, Germany. Nineteen cats aged 6.9-17.5 years (median 11.6) were included. The most common breed was Domestic Short Hair (n = 10). Sixteen cats were neutered males (84%) and three
neutered females. Chronic enteropathy was confirmed with histopathology in 6/19 cats. In nine additional cats, chronic enteropathy was suspected based on ultrasonography and/or hypocobalaminemia. Other comorbidities were common, including but not limited to, hepatobiliary disease (n = 15) and diabetes mellitus (n = 7). Ultrasonography of the pancreas was performed in 18 cats. 13/18 had an ultrasonographically abnormal pancreas. Pancreatic biopsies were not collected from any of the cats.

Median (range) s-Spec fPL concentration at baseline was 14.2 mg/L (6.1-43.3) and 6.7 mg/L (0.9-23.6) at follow-up. Cyclosporine treatment (5.0-7.9 mg/kg orally SID) was associated with a significant reduction in s-Spec fPL concentrations (P = 0.0008) at follow-up after 23-206 days (median 35) compared with baseline. In three cats, no reduction in s-Spec fPL concentrations occurred. In cats responding to cyclosporine, dose tapering (n = 7) or withdrawal (n = 4) was associated with a significant increase in s-Spec fPL concentrations from median 4.5 mg/L (1.5-8.8) to 9.3 mg/L (2.1-27.0) (P = 0.012). Adverse effects were reported for 12 cats. The most common was hyper-salivation and poor drug palatability (n = 6).

This study has several limitations, including unstandardized treatment length and dose, no control group and lack of pancreatic biopsies. Despite the limitations, our results suggest that cyclosporine treatment reduces supranormal s-Spec fPL concentrations in cats with CP and dose reduction can lead to increasing s-Spec fPL concentrations. Future studies are warranted to investigate the effect of cyclosporine on chronic pancreatic inflammation and clinical outcome of cats with confirmed CP.

Disclosures
No disclosures to report.

ESCG-P-3

Esophageal neoplasia in cats: Retrospective study in 19 patients
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Primary esophageal neoplasms are rarely reported in cats. Squamous Cell Carcinoma is the most common primary malignant tumor. The aim of this work is to evaluate the clinical, diagnostic and follow-up aspects of cats with esophageal neoplasia. All cats with esophageal neoplasia detected by endoscopy between January 2008 and December 2019 were included in this metacentric retrospective study. Esophageal neoplasia were found in 19 cats on 2462 (0.77%) upper digestive endoscopies performed. Domestic short hairs cats (DSH) were over-represented (17/19), while two cats were Maine Coon. The median age was 12 years old (6-18 years); 13 were males (11 neutered) and 6 sterilized females. The median BCS was 3 (2-5; range 1-9) and the median body weight 3.63 Kilos (1.83-4.3 kilos). Regurgitation was the main symptom in 18/19 cats (94.74%), related to weight loss in 13/19 (68.42%) and dysorexia in 6/19 (32%) cases. Survey radiographs were performed in 16 patients (84.21%), showing changes in 14 cases (73.68%), 9 cats (47.37%) had contrast radiography, but only 5 (26.32 %) showed an intraluminal filling defect. Endoscopy highlighted cardia proliferative lesions in 14 cats (73.68%), with complete lumenal occlusion in 13 cases (68.42%). Findings were (73.68%) consistent with a sessile base proliferative mass with friable, ulcerated reddish surface in all cases that afterwards turned out to be carcinoma; multiple nodular-like lesions in lymphoma cases (11.1%); single pale mass in leiomyosarcoma one; pink proliferative mass in adenocarcinoma case. Cytology, performed in 16 patients, found a match with histopathology in 14 cases (87.5%). Histopathology, performed on 18/19 cats (94.74%) revealed: 14 (73.68%) squamous cell carcinoma; 2 (11.1%) lymphoma; 1 (5.26%) adenocarcinoma; 1 (5.26%) leiomyosarcoma. 6 cats (31.58%) were immediately euthanized because of poor general conditions and prognosis; 1 cat died a natural death after 35 days. 1 patient with lymphoma received chemotherapy, showing best survival time (120 days). Others cats were euthanized with median survival time of 32 (10-60) days.

This study confirms that esophageal neoplasms are rare in cats and Squamous Cell Carcinoma is the most frequent form, with high mortality rate. It appears to be more present in DSH and older cats. The regurgitation is the most frequent symptom, progressively leading to poor general conditions and bad prognosis. Endoscopy provides direct display of the lesion, selective samples and accurate assessment of the lumenal patency. Moreover, endoscopic findings combined with cytology can provide useful advice on prognosis waiting for histological confirmation.

Disclosures
No disclosures to report.

ESCG-P-4

Gastro-duodenal ulceration (GDU) in cats: Retrospective study in 63 patients
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Gastroduodenal ulcer (GDU) is a rare disease in cats. GDUs are erosive changes in the gastric and duodenal mucosa that result in exposure of the submucosa or deeper layers. In literature, the most reported cause of GDU is neoplasia (mainly lymphoma) but studies are limited to a few cases. The aim of our work is to evaluate the clinical, diagnostic, histological and follow-up aspects of cats with GDU. In this multicenter retrospective study all cats with one or more ulcerative lesions, of at least 5 mm in diameter, detected by endoscopic examination were included. The information collected relates to signaling, anamnesis, clinical signs, radiographic, ultrasound, endoscopic and histological examinations and follow-up of at least 6 months as a minimum duration. GDUs were detected in 63 patients (5.14% of the 1224 total endoscopic examinations performed between January 2016 and January 2020). The median age was 9 years (0.6-16 years), 33 were females (32 sterilized) and 30 males (28 sterilized). Vomiting was the main symptom (sole symptom in 16 cats and associated with other symptoms in 42 cats). Hematemesis was present in 13 (20.6%)
cats. Ultrasound changes were present in 40 (63.4%) patients. The endoscopic examination shows single ulcerative lesions in 35 (55.5%) cats and multiple lesions in 28 (44.4%) cats. In 55 (87.3%) patients, ulcers were localized only in the stomach and in 8 (12.7%) cats only in the duodenum and in none of the patients were at both locations. In 20 (31.7%) cats, ulcerative lesions were exclusively in the antrapyloric area. The histological examination revealed benign lesions (lymphoplasmacytic, eosinophilic, neutrophilic inflammation) in 33 (52.38%) cases and malignant lesions (4 carcinoma and 26 lymphoma) in 30 (47.6%). Duodenal lesions were all inflammations except one lymphoma. In the six-month follow-up after diagnosis, 25 (39.6%) cats were dead, 23 (36.5%) were alive and in good condition while 15 (23.8%) were alive but with persistent symptoms.

The current work highlights that GDU are present only in a minimal percentage of patients (5.14%) with digestive symptoms. 52% of cats had benign GDU unlike what is reported in the literature in which lymphoma is reported as the most frequent cause of GDU ulcers. Excluding the neoplastic cause, the most frequent etiology is the idiopathic one followed by the eosinophilic inflammatory one. Furthermore, in our case history, duodenal ulcers were all benign except one lymphoma. All affected patients with benign ulceration were alive at six months.

Disclosures
No disclosures to report.

ESCG-P-5

Effect of stem cell therapy on serum albumin levels and its clinical effectiveness in dogs diagnosed with inflammatory bowel disease
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Hypoalbuminemia in dogs with inflammatory bowel disease (IBD) has been shown to be a poor prognostic factor. The CCECAI (canine chronic enteropathy clinical activity index) scoring system includes albumin concentration value and is the most accurate index in predicting the prognosis of patients with IBD. The treatment of these patients is challenging, and stem cell therapy is currently being investigated as a possible alternative. Our objective is to evaluate serum albumin concentration together with CCECAI in dogs with IBD after stem cell treatment. A total of 20 dogs diagnosed with IBD were included in the study, which was approved by the ethics committee. These animals had gastrointestinal symptoms for more than 3 weeks, did not respond to diet, antibiotics, or immunosuppressants, and the presence of intestinal inflammation was confirmed after the histologic exam of endoscopic gastrointestinal biopsies. A single infusion of allogeneic mesenchymal stem cells of adipose origin (MSCs) was carried out at doses of 2 x10⁶ cells per kilogram of weight. Serum albumin levels were determined prior to the administration of stem cells and one month, 3, 6 and 12 months after. The clinical evolution was evaluated using the CCECAI clinical activity index (normal values <3).

Safety and adverse effects during the stem cell infusion in dogs with inflammatory bowel disease
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The administration of mesenchymal stem cells (MSCs) is a therapy that is constantly being investigated, especially in the treatment of immunemediated diseases, such as inflammatory bowel disease (IBD), both in human and veterinary medicine. The route, dose and infusion rate are not yet established. The carcinogenic and immunogenic effects and their distribution after infusion have been studied, however, the immediate adverse effects after intravenous administration are not well described. In human medicine, adverse effects have been reported, consistent with type I hypersensitivity reactions (fever, rashes and pruritus) and headache in some patients. A high risk of pulmonary thrombosis has been observed in mice by rapidly administering high amounts of MSCs intravenously. In veterinary medicine, little studies report secondary effects during stem cells infusion. Apparently, they have been administered to treat atopic dermatitis, feline gingivostomatitis, IBD and other immunemediated pathologies, without any side effects. However, in a feline medicine study, after infusion of MSCs in cats with chronic kidney disease, vomiting, salivation, and increased respiratory rate were observed. Finally, only one dog had clinical signs (vomiting) during MSCs transplantation in an experimental study.
The objective of this work is to evaluate the safety of intravenous administration of allogeneic adipose MSCs in dogs with IBD, as well as to describe the resulting adverse effects.

A total of 20 dogs were included in the study approved by the ethics committee. These animals presented gastrointestinal symptoms longer than 3 weeks, did not respond to diet, antibiotics or immunosuppressants and the inflammatory process was confirmed in the biopsy obtained by digestive endoscopy, thus diagnosing IBD. All were administered intravenously MSCs at doses of 2x10^6 per kilogram of weight in a time of 30 minutes.

In most animals (15/20) the infusion of MSCs happened without complications, however, a small number of animals presented adverse effects (5/20). These events were vomiting (3/5), hyperthermia (2/5), bradycardia with hypotension (2/5), pruritus (2/5) and hypersalivation (1/5). Diphenhydramine (Antihistaminico Syva®) was administered to animals with adverse effects at a dose of 1 mg/kg intravenously and the infusion of MSCs was continued more slowly, thus disappearing the symptoms.

The results show that the administration of MSCs can be carried out safely. However, we must consider the side effects that may occur. Therefore, it would be necessary to carry out further studies to know exactly the specific dose, the infusion rate and all factors that affect the safety of administration of this therapy.

Disclosures
No disclosures to report.

ESCG-P-7

Detection of anti-erythrocyte antibodies in dogs with immunosuppressant-responsive enteropathy (IRE)

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Immunosuppressant-responsive enteropathy (IRE) is a chronic gastrointestinal inflammation with a significative immune system involvement. Several extra-intestinal manifestations are reported in human IBD, including immune-mediated cytopenias, while they are not documented in dogs. The aim of the study was to evaluate the erythrogram, as part of complete blood count (CBC), and the presence of anti-erythrocyte antibodies in dogs with IRE. IRE was diagnosed with the following criteria: chronic gastrointestinal signs, no improvement with diet trial, evidence of inflammatory infiltration on intestinal histology and subsequent improvement after immunosuppressant therapy. Canine Chronic Enteropathy Activity Index Score (CCECAI) was recorded for each dog, as well as WSAVA endoscopic and histopathological score. Each dog had a CBC evaluation prior the endoscopic procedure as part of the routine care. CBC was performed using Procyte® Hematology analyzer (IDEXX Laboratories) and blood smears were reviewed by a single clinical pathologist and the presence of nucleated RBC (nRBCs), anisocytosis, polychromasia, and Howell-Jolly bodies was recorded. Anti-erythrocyte antibodies (IgG) were evaluated on the same blood sample with flow cytometry (Cytomics FC 500 Beckman Coulter®). The presence of anti-erythrocyte antibodies was associated with CCECAI score, endoscopic and histological score of IRE dogs using Chi-square test. Seventeen dogs with IRE were enrolled. Eight out of 17 dogs (47%) had anemia which was normocytic normochromic (62%), followed by microcytic normochromic (25%) and macrocytic normochromic (13%). The main alterations in blood smears were: presence of nucleated RBC (57.1%), anisocytosis (42.9%), polychromasia (28.6%), and Howell-Jolly bodies (49.9%). Anti-erythrocyte antibodies were revealed (IgG>0.2%) in 70.6% of the study population and in 87.5% of anemic dogs, although they were not statistically associated with CCECAI, endoscopic and histopathological score.

The present study revealed a high frequency of positive cases for anti-erythrocyte antibodies in dogs with IRE. Moreover, about the half of the entire population study showed some hematologic features of RBC regeneration (e.g., polychromasia, NRBCs, anisocytosis) in addition to chronic inflammation hematologic findings (e.g., microcytia). Although prospective, larger-scale studies are needed, the presence of anti-erythrocyte antibodies and signs of erythroid regeneration may suggest a possible immune-mediated hemolysis that can induce anemia in dogs with IRE, together with the chronic inflammation.

Disclosures
No disclosures to report.

ESCG-P-8

Prognostic factors and long-term follow-up in Immunosuppressant Responsive Enteropathy (IRE): Prospective study in 165 dogs

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Canine immunosuppressant-responsive enteropathy (IRE) is defined as an idiopathic, multifactorial intestinal inflammation. The study aimed to evaluate negative prognostic factors to predict clinical response, relapse or mortality in IRE dogs.

In a multicentric prospective study on 165 dogs with IRE, canine enteropathy clinical activity index (CCECAI) score was evaluated at presentation and respectively 1, 3, 6, 12 and 18 months from diagnosis. Body condition score (BCS), serum total proteins (TP), albumin (ALB), cholesterol (COL) and CRP, endoscopic and histopathological WSAVA score were also evaluated at T0. The presence/absence of duodenal crypts distension (CD), intraepithelial lymphocytes (IEL), mucosal fibrosis (MF), ligation (LD) and the sum (SUM) of the histopathological lesions reported above were also recorded. Response to treatment was evaluated based on the comparison of CCECAI calculated at presentation and 1 month after it. Dogs with a CCECAI reduction ≥25% were classified as responders. Relapse
ABSTRACT

Chronic inflammatory enteropathies (CIE) are an important disease group in dogs, the pathogenesis of which involves dysregulated signaling mechanisms in innate immune responses. The receptor for advanced glycation end products (RAGE), an innate immune pattern recognition receptor, plays a role in chronic inflammatory responses. Abrogation of proinflammatory transmembrane RAGE signaling by ligand-soluble RAGE (sRAGE) binding might present a therapeutic avenue. Serum sRAGE levels are decreased in dogs with CIE, normalize with clinical remission, and correlate with the severity of histologic lesions. However, tissue RAGE expression has not been investigated in canine CIE. Aim of the study was to evaluate the gastrointestinal mucosal RAGE expression in dogs with CIE and its association with serum sRAGE concentrations and other disease markers.

Epithelial RAGE expression was evaluated in gastric, duodenal, ileal, and colonic biopsies from 15 dogs with CIE and 9 healthy control dogs. After fluorescence-labelling, RAGE expression was quantified using photon counting based on laser scanning microscopy. RAGE expression was compared between the two groups of dogs and was tested for an association with patient characteristics, clinical variables, histologic lesion severity, and biomarkers of extra-gastrointestinal diseases, systemic or gastrointestinal inflammation, function, or protein loss. Statistical significance was set at \( P < 0.05 \).

RAGE positivity was detected in all biopsies from healthy dogs and dogs with CIE. Gastric epithelial RAGE expression was significantly lower in dogs with CIE than in healthy dogs (\( P = 0.0066 \)). Compared to healthy controls, RAGE expression in dogs with CIE was higher in the duodenum and lower in the ileum, but both differences did not reach statistical significance. No differences were observed in the colon. A shift towards more apical epithelial RAGE expression was detected in the stomach, duodenum, and colon in dogs with CIE (all \( P < 0.05 \)). RAGE expression in the ileum and duodenum was inversely correlated with clinical disease activity (both \( P < 0.03 \)), in the duodenum it was associated with serum sRAGE (\( P = 0.0240 \)), albumin (\( P = 0.0163 \)), total calcium (\( P = 0.0047 \)), and C-reactive protein concentrations (\( P = 0.0074 \)), and in the ileum it was correlated with the severity of histologic lesions (\( P = 0.0403 \)).

This study showed a dysregulation of epithelial RAGE expression, along the gastrointestinal tract in canine CIE. These findings suggest that RAGE signaling plays a role in canine CIE, but RAGE over-expression was seen with less severe disease and was paralleled by higher anti-inflammatory decoy receptor sRAGE levels.

Disclosures

No disclosures to report.

ESCG-P-9

Dysregulation of gastrointestinal RAGE (receptor for advanced glycation end products) expression in dogs with chronic inflammatory enteropathy

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Chronic inflammatory enteropathies (CIE) are an important disease group in dogs, the pathogenesis of which involves dysregulated signaling mechanisms in innate immune responses. The receptor for advanced glycation end products (RAGE), an innate immune pattern recognition receptor, plays a role in chronic inflammatory responses. Abrogation of proinflammatory transmembrane RAGE signaling by ligand-soluble RAGE (sRAGE) binding might present a therapeutic avenue. Serum sRAGE levels are decreased in dogs with CIE, normalize with clinical remission, and correlate with the severity of histologic lesions. However, tissue RAGE expression has not been investigated in canine CIE. Aim of the study was to evaluate the gastrointestinal mucosal RAGE expression in dogs with CIE and its association with serum sRAGE concentrations and other disease markers.

Epithelial RAGE expression was evaluated in gastric, duodenal, ileal, and colonic biopsies from 15 dogs with CIE and 9 healthy control dogs. After fluorescence-labelling, RAGE expression was quantified using photon counting based on laser scanning microscopy. RAGE expression was compared between the two groups of dogs and was tested for an association with patient characteristics, clinical variables, histologic lesion severity, and biomarkers of extra-gastrointestinal diseases, systemic or gastrointestinal inflammation, function, or protein loss. Statistical significance was set at \( P < 0.05 \).

RAGE positivity was detected in all biopsies from healthy dogs and dogs with CIE. Gastric epithelial RAGE expression was significantly lower in dogs with CIE than in healthy dogs (\( P = 0.0066 \)). Compared to healthy controls, RAGE expression in dogs with CIE was higher in the duodenum and lower in the ileum, but both differences did not reach statistical significance. No differences were observed in the colon. A shift towards more apical epithelial RAGE expression was detected in the stomach, duodenum, and colon in dogs with CIE (all \( P < 0.05 \)). RAGE expression in the ileum and duodenum was inversely correlated with clinical disease activity (both \( P < 0.03 \)), in the duodenum it was associated with serum sRAGE (\( P = 0.0240 \)), albumin (\( P = 0.0163 \)), total calcium (\( P = 0.0047 \)), and C-reactive protein concentrations (\( P = 0.0074 \)), and in the ileum it was correlated with the severity of histologic lesions (\( P = 0.0403 \)).

This study showed a dysregulation of epithelial RAGE expression, along the gastrointestinal tract in canine CIE. These findings suggest that RAGE signaling plays a role in canine CIE, but RAGE over-expression was seen with less severe disease and was paralleled by higher anti-inflammatory decoy receptor sRAGE levels.

Disclosures

No disclosures to report.

ESCG-P-10

Immunohistochemical expression of β-catenin, Ki67, CD3 and CD18 in canine colorectal adenomas and carcinomas

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Inflammation is believed to influence the human colorectal carcinogenesis and may have impact upon prognosis and survival. High presence of tumor-infiltrating T-cells, evaluated by the CD3 marker is associated with a better outcome in humans with colorectal cancer. The mucosal immunophenotype in dogs with colorectal cancer is poorly described.
The aim of this study was to characterize and quantify mucosal histiocytes and T-cells, using immunohistochemistry (IHC) scoring of CD18 and CD3, respectively, in colorectal adenoma and adenocarcinoma of dogs. β-catenin and Ki67 were evaluated, as markers for tumor progression. The study was a retrospective case-control study. Tissue samples from dogs with colorectal adenoma (n = 18) and adenocarcinoma (n = 5) were collected retrospectively from archived samples. These samples were collected for clinical purposes, and yielding tissue samples were archived. Control samples were healthy colonic tissue collected from dogs euthanized of reasons not involving the gastrointestinal tract (n = 9).

IHC scoring of CD3, CD18 and β-catenin were compared between dogs with tumors and control dogs.

The tumor samples had significantly lower numbers of intraepithelial CD3 positive cells (Wilcoxon test, $P = 0.0006$), as well as significantly lower expressions of CD18 positive cells in the lamina propria, compared to control samples (Wilcoxon test, $P = 0.001$). The Ki67 positive cells showed a strong signal in adenomas and adenocarcinomas. There was no clear distinction with regards to expression levels of the markers for tumor progression (β-catenin, and Ki67) between adenoma and adenocarcinoma. Colonic samples from control dogs had uniform staining of β-catenin within the cytoplasm. When compared with normal colonic cells, the expression levels of cytoplasmic β-catenin were significantly higher in adenomas and adenocarcinomas (Wilcoxon test, $P = 0.0002$). None of the control samples showed positive staining of β-catenin in the nucleus of colonic cells. In contrast, adenocarcinoma and adenoma showed moderate or strong staining of the cell nucleus.

Colorectal adenomas were more common in dogs than adenocarcinomas in this study, β-catenin and Ki67 were not useful markers in distinguishing adenomas from adenocarcinomas. The lower presence of CD18- and CD3 positive cells in tumors compared to controls, indicates a reduced presence of T-cells, which may be of importance in the development of canine colorectal cancer.

Disclosures
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ESCG-P-11

Fecal bile acid profiles in cats with chronic enteropathy, intestinal neoplasia, and in healthy control cats.

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Feline intestinal neoplasia is challenging to distinguish from chronic enteropathy (CE), and reliable non-invasive biomarkers are needed. Altered fecal bile acid (FBA) profiles have been reported in humans and dogs with enteropathy or intestinal neoplasia and may have diagnostic potential in cats. We aimed to investigate FBA profiles as a biomarker to differentiate feline CE from intestinal neoplasia. Fecal samples were collected from healthy cats (n = 12), cats with chronic lymphocytic-plasmacytic enteropathy (n = 4), or intestinal neoplasia (n = 3; 1 with large-cell and 2 with small-cell lymphoma). Fecal unconjugated bile acids were measured using gas chromatography and mass spectrometry, and profiles included both concentrations and proportions of total-, primary-, and secondary FBAs, as well as five individual FBAs. Differences between groups were tested using Fisher’s exact and Kruskal-Wallis tests, and $P < 0.05$ was considered statistically significant.

Sex and breed distribution were similar between groups, but healthy cats were younger (median: 45 months; range: 14-133 months) compared to the CE group (109 months; 77-149 months) and the intestinal neoplasia group (87 months; 77-202 months) ($P = 0.014$). Although the concentrations of total FBAs ($P = 0.013$), deoxycholic acid ($P = 0.038$), and the proportion of ursodeoxycholic acid in the intestinal neoplasia group (0.05%; 0.00-0.09) compared to the healthy group (0.26%; 0.09-3.34), and the CE group (0.53%; 0.08-1.66) were statistically different, the post-hoc analyses failed to show significant differences comparing groups.

Though we were not able to distinguish CE from intestinal neoplasia using fecal bile acid profiles in our study, future studies with larger groups and age-matched controls should be performed.

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ESVC-P-1

Myxomatous mitral valve disease in Cavalier King Charles Spaniels: a clinical and genetic study

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Cavalier King Charles Spaniels (CKCSs) show a genetic predisposition to myxomatous mitral valve disease (MMVD) development, even at an early age. It is estimated that 50% of CKCSs before 6-7 years of age and almost 100% over 11 years are interested by MMVD, against 14% in other breeds. The aim of this study was to characterize the clinical and echocardiographic features of healthy and MMVD affected CKCSs, through the description of MV morphology, prolapse and annulus. A cohort of 90 healthy and affected by MMVD at different stages (B1, B2, C) CKCSs was analyzed and phenotypic, pedigree and echocardiographic parameters were recorded. Log$_{10}$ body weight indexed length (AMVL), width (AMVW) and area (AMVA) of the anterior mitral valve leaflet, prolapse, diameters of the mitral valve annulus in diastole (MVA$_d$) and systole (MVA$_s$) and sphericity index (SI) were
measured. In the whole sample AMVL was significatively longer in class B2 than class A ($P = 0.04$) and B1 ($P < 0.001$) and in class C than B1 ($P = 0.02$). AMVW and AMVA were higher in classes B2 and C than A and B1 ($P < 0.05$). LA/Ao ($P = 0.01$), MVA2 ($P < 0.01$), MVA4 ($P = 0.001$) and left ventricle longitudinal end-diastolic diameter ($P = 0.03$) were higher in males. MVA2 was significantly higher in neutered-females than in intact-females ($P = 0.02$). Subjects in class B1 were classified in age-related classes: under 3 y (group 1, 15.6%), between 3 and 6 y (group 2, 46.9%) and over 6 y (group 3, 37.5%). AMVW and AMVA were greater in group 3 than 1 (both $P = 0.02$). MVA2 and MVA4 were higher in group 3 compared to 2 ($P = 0.02, P = 0.01$). SI was lower in group 3 than 2 ($P < 0.01$). A subset of 34 CKCSs, non-relatives in direct line, was genotyped with Canine 230K SNP BeadChips to evaluate genomic regions of interest useful to distinguish between case and control groups of dogs. The first study was created according to age and ACVIM classification, the second to auscultation findings. These analyses highlighted, beside known regions on CFA 4-17 and 13-14, new regions of interest on several chromosomes (CFA 2-11-14-19-21-25). Although further investigations of the genes are needed, these results may contribute to disentangle the complexity of the etiopathogenic mechanism involved in early development and rapid progression of MMVD in CKCSs. Essential will be the follow up of the subjects in class B1 which will allow us to uniquely characterize the association between the genetics and the ventricular and valvular echocardiographic characteristics.

Disclosures

No disclosures to report.

ESVC-P-2

Factors affecting the urinary aldosterone-to-creatinine ratio in healthy dogs and dogs with naturally occurring myxomatous mitral valve disease

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Chronic renin-angiotensin-aldosterone system (RAAS) activation in course of heart diseases contributes to cardiac remodeling and congestive heart failure. The urinary aldosterone-to-creatinine ratio (UAldo:C) reflects RAAS activation in dogs and might be an important marker of disease progression. Data about this parameter in dogs with myxomatous mitral valve disease (MMVD) needs to be expanded. The aims of this study were to assess UAldo:C in healthy dogs and dogs with MMVD and determine if associations with certain clinical, echocardiographic and laboratory variables exist.

All dogs enrolled in this prospective study underwent complete physical examination, systemic pressure measurement, echocardiography, urinalysis, serum urea and creatinine analysis. Dogs with MMVD were classified according to ACVIM guidelines. Urinary aldosterone was measured on left-over urine samples with an enzyme-linked-immunosorbent-assay, previously validated in dogs.

One hundred fifty-one dogs were included, 49 healthy, 40 stage B1, 20 stage B2 and 42 stage C. At the enrolment, 11/20 B2 dogs were already receiving pimobendan. There were no significant differences in UAldo:C among healthy (1.75 IQR 0.83-4.02 μg/g), B1 (1.64 IQR 0.71-2.99 μg/g), B2 (1.93 IQR 1.02-4.13 μg/g) and C (2.03 IQR 1.16-4.85 μg/g) dogs. Urinary aldosterone-to-creatinine ratio was not significantly different between untreated B2 dogs (2.27 IQR 1.51-6.84 μg/g) and those receiving pimobendan (1.17 IQR 0.81-2.49 μg/g). Excluding those taking spironolactone ($n = 20$), C dogs treated with ace-inhibitors for 6 months or more had significantly higher UAldo:C ($n = 8$; 1.75 IQR 1.45-3.80 μg/g) than those treated for less than 6 months ($n = 14$; 1.09 IQR 0.78-2.68 μg/g). Intact females and Chihuahua, Cavalier King Charles Spaniel and Jack Russell had significantly higher UAldo:C than other sexes and breeds respectively in healthy and B1 dogs. A significant moderate negative correlation was found between UAldo:C and age in healthy ($ρ = -0.346$) and B1 ($ρ = -0.589$) dogs. In the entire study population, UAldo:C showed a significant, but weak, positive correlation with left atrium-to-aortic ratio ($ρ = 0.166$) and with urinary protein-to-creatinine ratio ($ρ = 0.286$).

Preclinical MMVD seems not to lead to a significant RAAS activation and chronic administration of pimobendan appears not to significantly interfere with neurohormonal activity. In stage C dogs, longer ace-inhibitors treatment seems to be associated with an increase in aldosterone secretion, suggesting the risk of the aldosterone breakthrough phenomenon. Moreover, UAldo:C showed high individual variability within both healthy and MMVD dogs and was affected by demographic factors (age, sex, breed). Individual serial monitoring of this parameter, instead of the use of a population-based reference value, should be considered.

Disclosures

No disclosures to report.

ESVC-P-3

Left atrial volume assessment and survival in 160 Cavalier King Charles spaniels with or without degenerative mitral valve disease (2017-2019)

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Degenerative mitral valve disease (DMVD) is the most common acquired canine heart disease, with a high predisposition of the Cavalier King Charles Spaniel (CKC) breed. Echocardiographic evaluation of canine DMVD includes measurement of left atrial (LA) size, which is one of the strongest prognostic factors for survival or disease worsening. The LA size is usually assessed using a linear measurement from a two-dimensional image, and then indexed to the aortic diameter (left atrium-to-aortic ratio, LA:Ao). As LA enlargement can happen in various directions, quantification of LA volumes (LAV) using the biplane
Utility of clinical and electrocardiographic findings in the prediction of the severity of pulmonic stenosis in dog

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Pulmonic stenosis (PS) is the most common congenital right heart disease in dog, and it is usually suspected by first opinion veterinarians. Echocardiography is needed to confirm the diagnosis and define the severity of the stenosis. Pulmonary balloon valvuloplasty (PBV) is the elective treatment for dogs with severe PS. The aim of this study was to evaluate the utility of clinical and electrocardiographic findings in the prediction of PS severity for the selection of dogs that could benefit from PBV.

This was a retrospective observational study. Medical records were reviewed for dogs with PS that had undergone echocardiography and ECG on the same day. The ECG tracings were reviewed and data regarding heart rate, P wave amplitude, QRS complex duration, mean electrical axis of the QRS complex (MEA) and presence of atrial or ventricular arrhythmias were gathered. Correlation between the severity of PS and MEA deviation was evaluated. The ROC analysis and the Youden index were used to assess the diagnostic accuracy of clinical and ECG parameters in the prediction of severe PS.

A total of 69 dogs were included, with 28 females and 41 males. The most frequent breeds were French Bulldog (n = 15), English Bulldog (n = 11) and Amstaff (n = 5). Median age was 1.6 years (0.3–13 years) and median body weight was 14 kg (1.5–58 kg). PS severity was assessed according to the Doppler-derived peak pulmonary gradient (PG) and was classified into mild (<50 mmHg; n = 7), moderate (51–80 mmHg; n = 14) and severe (>80 mmHg; n = 48). Four dogs had syncope and 8 had right-sided heart failure, all of these had severe PS [specificity (Sp) = 100%]. All dogs had a systolic murmur and a murmur grade ≥4/6 was predictive of severe PS [area under curve (AUC) = 0.72; P = 0.004; sensitivity (Se) = 94%; Sp = 52%]. Regarding ECG findings, 33 (72%) dogs with severe PS showed a right axis deviation of the MEA. A MEA cut-off >85° was highly predictive of severe PS (AUC = 0.82; P < 0.0001; Se = 86%; Sp = 74%). The entity of right MEA deviation was positively correlated with the PG (r = 0.63; P < 0.0001). A P wave amplitude >0.35 mV was predictive of severe PS (AUC = 0.67; P = 0.029; Se = 29%; Sp = 100%). Heart rate, QRS complex duration and presence of arrhythmias were not predictors of severe PS.

In conclusion, syncope, right-sided heart failure, murmur grade ≥4/6, MEA >85° and P wave amplitude >0.35 mV in a young dog are predictive of severe PS, thus necessitating an urgent echocardiographic evaluation because probably benefiting from PBV.

Disclosures

No disclosures to report.

ESVC-P-5

Assessment of global and regional right ventricular function in dogs with congenital pulmonic stenosis using echocardiography, speckle tracking imaging, and two-dimensional color tissue Doppler imaging: A prospective study of 105 cases (2013-2020)

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Pulmonic stenosis (PS) is one of the most common congenital canine heart diseases. To the best of our knowledge, no study has focused on right ventricular (RV) function evaluated by speckle tracking echocardiography (STE) and two-dimensional color tissue Doppler imaging (TDI) in dogs with PS. The aims of this prospective observational study were therefore, to investigate the global and regional systolic and dia-
Willebrand factor (vWF) in this condition. We aimed to characterize ATE in cats. There is limited information regarding the role of von

Left atrial (LA) thrombosis may result in aortic thromboembolism (ATE) in cats. There is limited information regarding the role of von

The study population consisted of 105 cases, 75 dogs with PS and 30 control dogs. As compared with controls, PS dogs showed significantly increased and decreased values of RV fractional area change (RFAC) \((P < 0.01)\) and tricuspid annular plane systolic excursion indexed to the aortic valve diameter \((P < 0.001)\), respectively. A markedly reduced longitudinal RVFW S1S was also found in PS dogs (median [interquartile range] = 14%[10-20] versus 32%[27-34]); \(P < 0.0001\), with greater systolic impairment at the base, as demonstrated by a significantly lower S1S base:S1S apex ratio \((0.87 [0.71-1.0] versus 1.0 [1.0-1.0]; \ P < 0.0001)\). A post-systolic peak S1S was identified in 61/69 dogs (88%) of the PS group and in only 5/30 control dogs (17%), with an early pre-stretch in 42/69 PS dogs (61%) and only one control dog (3%). Dogs in the PS group also presented a longer time-to-peak S1S \((P = 0.02)\) and an increased synchrony time index \((P < 0.001)\) in comparison to controls. Furthermore, a significantly reduced RFAC was found in PS dogs with congestive heart failure (CHF) as compared to those without \((P < 0.001)\). In addition, PS dogs with RV dilation had decreased RFAC \((P = 0.04)\) and S1S values \((P < 0.01)\), and higher time-to-peak S1S \((P = 0.04)\) and pre-stretch index \((P = 0.02)\) as compared with PS dogs without. Decreased systolic and early diastolic SR and segmental RV TDI velocities were also demonstrated in PS dogs in comparison to controls.

In conclusion, PS dogs show various systolic and diastolic RV alterations, more pronounced for basal segments and associated with intra-RV mechanical dyssynchrony particularly in dogs with RV dilatation.

**Disclosures**

No disclosures to report.

**ESVC-P-6**

Von Willebrand factor, endothelial injury and left atrial enlargement in cats with cardiomyopathy

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Left atrial (LA) thrombosis may result in aortic thromboembolism (ATE) in cats. There is limited information regarding the role of von Willebrand factor (vWF) in this condition. We aimed to characterize the expression of vWF, a marker of endothelial injury, in LA samples from the following cats:

(1) Control group: cats without structural cardiac changes.
(2) Pre-LAE group: cats with preclinical cardiomyopathy and a normal-sized LA.
(3) Pre+LAE group: cats with preclinical cardiomyopathy and LA enlargement (LAE).
(4) CHF group: cats with CHF attributable to cardiomyopathy and LAE.
(5) ATE group: cats with ATE attributable to cardiomyopathy and LAE.

Exclusion criteria were cats with systemic diseases that might cause cardiac structural changes. All control cats died of non-cardiac causes and their hearts were confirmed to be normal by histopathology. Affected cats were categorized into groups based on necropsy and histopathology ± cardiac imaging (echocardiography or T-FAST). Left-over LA samples from group 1, 4 and 5 were collected for multiplex RT-PCR and run in duplicate to quantify transcripts for VWF and RPS7 (reference gene). La/Ao and La minor (mm) measurements were recorded in a proportion of the cats. Three sections of full thickness LA myocardium from all 5 groups of cats were immunostained for vWF and integrin αIIb (platelet marker). Data were analysed using one-way ANOVA, Pearson’s correlation test, and Kruskal-Wallis test.

There were a total of 14, 5, 4, 14, and 21 cats in the Control, Pre-LAE, Pre+LAE, CHF, and ATE group, respectively, with a median age (range) of 2.5 (0.4-15.3), 7.5 (4.5-11), 6.5 (2.5-8.1), 7 (0.6-17.3), and 7 (1.8-18.5) years. The majority of cats were DSH. Male cats were overrepresented except for the Control group.

VWF transcript level was higher in the ATE (mean 0.66 [0.098], \(n = 13\); \(P = 0.006\) and CHF (0.66 [0.11], \(n = 9\); \(P = 0.006\)) cats compared to the controls (0.45 [0.18], \(n = 6\)). No difference was detected between the ATE and CHF cats. VWF transcripts moderately correlated with La/Ao (\(r = 0.511\) [CI 0.058-0.790], \(n = 18\); \(P = 0.030\)) and La minor (\(r = 0.551\) [CI 0.028-0.837], \(n = 14\); \(P = 0.041\)).

The fluorescence of immunostained vWF was significantly increased at the endocardium in the ATE (median 44.7 [IQR 34.9-54.6], \(n = 11\); \(P < 0.001\)), CHF (46.0 [36.6-56.8], \(n = 8\); \(P < 0.001\)), and Pre+LAE (42.7 [27.7-69.3], \(n = 4\); \(P = 0.04\)) cats compared to the controls (30.8 [28.1-34.2], \(n = 11\)).

Endothelial injury at the level of the endocardium revealed by increased vWF expression occurs as LA remodels in cats with cardiomyopathy.

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**ESVC-P-7**

Echocardiographic measurements in a large population of Italian healthy cats: The Osservatorio Veterinario Italiano Cardiopatie data

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Cardiomyopathies are the most frequently diagnosed cardiovascular disorders in cats and the most common of these is hypertrophic cardiomyopathy. Echocardiography is used to investigate cats with heart murmurs, but has become a common screening test for breeding purposes. The objective of this study was to evaluate normal echocardiographic measurements in a population of healthy cats from Italy. The Osservatorio Italiano Veterinario Cardiopatie (OVIC – http://www.osservatorioveterinariocardiopatie.com) is an Italian animal screening program, mainly for breeding purposes, of canine and feline cardiovascular diseases. Two thousand one-hundred thirty-eight echocardiographic examinations of cats from 2007 to 2019, were analyzed. Cats were included if they were at least one year old, if they had no history of systemic diseases, if pulmonary and cardiac auscultation was normal and if echocardiographic examination showed no congenital or acquired heart disease, or other cardiovascular abnormalities. Isolated hypertrophic or abnormal papillary muscles and presence of systolic anterior motion of the mitral valve were considered equivocal parameters and so met the exclusion criteria. Pregnant or lactating cats were excluded as were cats with incomplete data. Although sedation is acceptable, we considered data only from non-sedated cats.

Animals were scanned from below on echocardiographic table both from right and left recumbency according to previous published standards.

Descriptive analysis was performed. The ANOVA test was carried out to check the influence of breed on echocardiographic variables. Only breeds represented by >30 cats were included. We performed linear regression modeling with allometric transformation to assess the association between bodyweight and cardiac variables. A value of $P < 0.05$ was considered significant.

One thousand three hundred fifty-seven cats met the inclusion criteria. The results confirmed the positive relation between echocardiographic measurement and body weight while no significant correlation for gender or breed or age and any measurements were found. Our study provided normal reference measurements, allometric scale and body weight prediction intervals in a population of Italian healthy cats. Body weight should be considered when cats are evaluated for cardiomyopathy, especially if HCM is suspected. In our acknowledgements, this is the largest study of echocardiographic measurements in apparently healthy Italian cats.

**Disclosures**

No disclosures to report.

**ESVC-P-8**

**Analysis of PDK4 gene deletion in a population of Doberman Pinschers from Argentina**

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Dilated cardiomyopathy (DCM) in Doberman Pinschers (DP) is a polygenic disease inherited as an autosomal dominant trait with incomplete penetrance. In American DP, DCM development has been associated with a 16-bp deletion in the 5’ splice site of intron 10 of the pyruvate dehydrogenase kinase, isozyme 4 (PDK4) gene on chromosome 14. However, such association has not been observed in European DP. The aim of this study was to estimate the prevalence of PDK4 16bp INDEL variant and determine its association with DCM in a DP cohort from Argentina.

A total of 134 DP (58 male and 76 female) aged 4-15 years were assessed to evaluate the prevalence of the 16 bp deletion allele. For the association study, dogs were allocated into two groups: 1) DCM (n = 20, ≥ 4 years old, left ventricle diastolic diameter ≥ 49 mm and left ventricle systolic diameter ≥ 42 mm), and 2) control (n = 45, ≥ 7 years old, without clinical, electrical or morphological findings). The remaining 69 DP were not included because they did not comply with the inclusion criteria. According to the reported variant in the PDK4 gene (CFA14: g.20,829,667_20,829,682del; genome build CanFam 3.1), primers were designed to amplify a fragment that included the INDELS (forward primer, 5′-GTTTTGGTTATGGCTTACCAATT-3′ and reverse primer, 5′-ATGGACTCTCTCTCTCAAAT A-3′). Amplicons of 210-bp for the wild type (ins) and 194-bp for the variant (del) allele were obtained with polymerase chain reaction (PCR). The genotype of each animal was determined in a 1% agarose electrophoresis gel. Finally, the fragments obtained were sequenced with ABI 3500 Genetic Analyzer (Applied Biosystems). The prevalence of the variant allele and its association with DCM development were evaluated using a Fisher’s Exact Test with the odds ratio function of the R epitools package. $P < 0.05$ was considered significant.

Both alleles were observed in DCM and controls. However, no association was observed between the 16-bp deletion in PDK4 gene and DCM development ($P = 0.83$; OR = 1). Overall 16-bp deletion allele frequency was 0.15 (+/-0.11-0.20).

It is concluded, that the association between the 16 bp deletion in the PDK4 gene and DCM reported in American DP was not confirmed in the DP cohort from Argentina. This result is in agreement with that reported in a population of European DP. Moreover, the prevalence of the 16-bp deletion PDK4 variant was lower than that described in the American and European DP cohorts.

**Disclosures**

No disclosures to report.
ESVC-P-9

Clinical characteristics for differential diagnosis and prognosis of non-cardiogenic pulmonary edema in dogs with concurrent congestive heart failure: 45 cases (2018-2010)

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Precise identification of underlying causes and key factors leading to pulmonary edema is essential for proper treatment and prognosis. In patients with congestive heart failure, non-cardiogenic pulmonary edema (NCPE) can be easily misleading to a diagnosis of cardiogenic pulmonary edema (CPE) when occurring simultaneously. However, there have been very few reports describing the differential characteristics between NCPE and CPE. Here, we report a retrospective study to determine the clinical characteristics of NCPE in dogs with symptomatic myxomatous mitral valve disease (MMVD).

Emergently referred dogs with MMVD showed severe acute respiratory distress caused by either CPE or NCPE. The detailed groups are as follows: CPE (n = 49) and NCPE (n = 45; acute respiratory distress syndrome [n = 23], post-anesthetic events [n = 6], aspiration [n = 6], upper airway obstruction [n = 4], neoplasia [n = 4], acute pancreatitis [n = 1], multiple plasma transfusion [n = 1]). We have retrospectively compared clinical characteristics, including medical history, laboratory findings and diagnostic imaging, and survival outcome between the CPE and the NCPE groups.

Recurrent episodes of pulmonary edema in the medical history of the NCPE group (1.4±1.6) significantly outnumbered those of the CPE group (0.2±0.6) (P < 0.01). The differences in SPO2 (%) (93.1 ± 4.5 vs. 97.5 ± 2.0) and hematocrit (%) (39.4 ± 6.0 vs. 43.3 ± 3.7) were significantly less in the NCPE group (P < 0.01) than the CPE group. Furthermore, the NCPE group showed higher levels of blood work parameters related to inflammatory responses compared to the CPE group (P < 0.01): leukocytosis (WBCs, k/L; 19.6 ± 7.4 vs. 10.3 ± 2.1) and C-reactive protein (CRP, mg/dL; 1.9 ± 0.3 vs. 0.5 ± 0.3). In contrast, the NCPE group exhibited significantly lower peak velocity of E wave (cm/s; 130.8 ± 36.6 vs. 157 ± 21.2) and survival outcome (%: 33.3 vs. 83.7) than the CPE group (P < 0.01). The prevalent radiographic findings in the NCPE was unilateral asymmetrical opacities (68.9%), although bilateral diffuse pulmonary infiltration (75.5%) was more often observed in the CPE group (P < 0.01). Simple logistic regression to identify survival predictors demonstrated a significant correlation with the lower SPO2 level and the higher recurrent incidence of pulmonary edema (Cox-Snell’s R squared; 0.512 and 0.581, respectively) (P < 0.01). These two predictors were also found to exert a significant effect on survival outcome in multiple logistic regression (Cox-Snell’s R squared, 0.689; P < 0.05).

In summary, several clinical characteristics, in particular the SPO2 level and the history of recurrent pulmonary edema, showed highly differential significance and strong correlation with survival outcome. Our retrospective study could provide insight into improving diagnosis and prognosis of NCPE developed in dogs with concurrent congestive heart failure.

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ESVC-P-10

Selected hematological, biochemical and echocardiographic parameters as predictors of survival in canine patients with mitral valve disease and heart failure

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Data demonstrating the association of routine blood and echocardiographic parameters with survival in canine heart failure patients is still lacking. We aimed to investigate the association of selected blood and echocardiographic parameters with the survival of dogs with mitral valve disease (MVD) and heart failure.

In this retrospective study white blood cell count (WBC), absolute monocyte (MONO) and neutrophil (NEUT) counts, and their relative numbers (MONO%, NEUT%), urea, creatinine, potassium, chloride, left ventricular end-diastolic diameter (LVIDd) and left ventricular end-systolic diameter (LVIDs), both indexed per weight1/3 and mitral E and A wave (MvE, MvA), MvE/A ratio and left atrium to aorta ratio (LA/Ao), and tricuspid regurgitant pressure gradient were analyzed in dogs with MVD and heart failure (ACVIM class C2 and joined class ACVIM C1+D). The latest blood and the first and latest echocardiographic results were included. Additionally, we investigated the same parameters in hospitalized patients. Parameters were analyzed using Cox proportional-hazards models. Hazard ratios (HR), 95% confidence intervals (CI), and corresponding P values were calculated. The significance was set to 5%.

We included 165 dogs with MVD: 97 males and 68 females (96 in ACVIM C2 and 69 in ACVIM C1+D). The mean (standard deviation) age at the start of heart failure therapy was 10.97 ± 2.34 years, with no difference between classes. The most common breeds were mixed breed (18.2%) and Cavalier King Charles Spaniel (17.5%). 107 dogs (64.8%) died and 58 (35.2%) were censored. The median survival time of deceased dogs was 11.5 months (11 days to 4.3 years). Group ACVIM C1+D had higher NEUT (P = 0.023) and NEUT% (P < 0.001), lower potassium (P = 0.047), larger indexed LVIDd (P < 0.001), indexed LVIDs (P = 0.004) and LA/Ao (P < 0.001), higher MvE (P < 0.001) and MvE/A (P < 0.001) and lower MvA (P = 0.003). Hospitalized patients (59 dogs) had higher WBC (P < 0.001), NEUT (P < 0.001), NEUT% (P = 0.003), MONO (P < 0.001), urea (P < 0.001) and creatinine (P = 0.003) compared to non-hospitalized dogs. The following parameters (HR: 95% CI; P value) were negatively associated with
survival: age (1.171; 1.056, 1.299; 0.003), WBC (1.081; 1.017, 1.149; 0.013), and urea (1.047; 1.006, 1.089; 0.023). Chihuahuas had lower risk of death for 66.7% (0.333; 0.114, 0.972; 0.044). With the exception of LA/Ao (1.93; 1.058, 3.522; 0.032), other echocardiographic parameters showed no association with survival. Higher age and increasing WBC, urea, and LA/Ao are associated with decreased survival in dogs with MVD and heart failure.

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ESVC-P-11

Longitudinal Speckle-Tracking echocardiography of the left and right ventricular myocardium in trained and untrained Italian blood hound dogs
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Physical activity, as in human athletes, may influence the cardiovascular system of dogs. Longitudinal left (LV) and right ventricular (RV) deformation assessed by speckle tracking echocardiography (STE) in healthy dogs subjected to intensive exercise has hitherto not been investigated. This study aimed to investigate if LV and RV 2D echocardiographic and STE variables differed between a group of healthy trained and untrained dogs. Forty-four healthy Italian blood hound dogs were recruited. Twenty-two dogs were trained, and 22 untrained. Standard echo 2D variables and systolic longitudinal (SL) LV and RV strain and strain rate acquired from left apical four chamber view were measured over three cardiac cycle, and mean and standard deviation were calculated. Statistical tests included non-parametric groupwise tests. P < 0.05 was considered statistically significant. Heart rate, right ventricular fractional area change, left ventricular ejection fraction and shortening fraction were lower in trained compared to untrained dogs (P < 0.0006, P < 0.01, P < 0.05, P < 0.05 respectively). Left ventricular end systolic internal diameter normalized, right ventricular end systolic area and right atrial area, right ventricular end diastolic and end systolic volume indexed for body weight were higher in trained dogs compared to untrained dogs (P < 0.05, P < 0.05, P < 0.01 and P < 0.01, P < 0.05 respectively). In trained dogs, global SL RV strain was lower compared to global SL RV strain of untrained dogs (P < 0.01).

In conclusion, several left and right echocardiographic variables and longitudinal systolic RV deformation by STE differed between trained and untrained dogs. These results likely reflect functional and morphologic adaptations of the heart in response to exercise.

Disclosures
No disclosures to report.

ESVC-P-12

Assessment of longitudinal left ventricle deformation by 2-dimensional speckle tracking echocardiography obtained from different views in cats
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Two-dimensional speckle tracking echocardiography (STE) is a novel, angle-independent imaging technique useful to assess myocardial function by strain and strain rate (Sr) analysis in human and veterinary medicine. Commonly, the left apical four-chamber (Lap4Ch) view is used to assess left ventricular (LV) longitudinal deformation in dogs and cats. However, the right parasternal four-chamber view (RP4Ch) is often more easily obtained than the Lap4Ch view in cats. No studies exist comparing longitudinal strain and Sr values using STE from different views in cats. Therefore, we examined the agreement between RP4Ch and Lap4Ch for assessment of LV longitudinal strain and Sr in cats. We acquired 2D echocardiographic cineloops from RP4Ch and Lap4Ch views and analyzed LV longitudinal strain and Sr in 50 cats (31 healthy cats and 19 cats with different disease states) using Xstrain™ software. Peak systolic strain and SR values of endocardial and epicardial border were used for the analysis. All echocardiographic values were measured three times and averaged. The two echocardiographic views were compared using limits-of-agreement analyses and intra-observer measurement variability was assessed.

We could obtain longitudinal strain and strain rate from the RP4Ch view in all cats. Strain, but not SR, had good intra-observer measurement variability (<10% vs −20%). However, only endocardial longitudinal strain and Sr values obtained with the two views agreed (95% limits of agreement: −3.28, 2.58; −1.41, 1.36). Epicardial longitudinal strain and Sr values did not agree sufficiently to be used interchangeably (95% limits of agreement: −11.58, 9.19; −2.28, 1.74).

Our study suggests that RP4Ch view was feasible for assessment of the LV longitudinal deformation analysis by STE in cats, but only endocardial longitudinal strain and Sr values obtained from the two different views were interchangeable. Clinicians can use RP4Ch view for endocardial longitudinal deformation analysis when Lap4Ch view demonstrates sub-optimal in cats.

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ESVC-P-13

Prognostic significance of left cardiac remodeling in dogs with asymptomatic myxomatous mitral valve disease
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We could obtain longitudinal strain and strain rate from the RP4Ch view in all cats. Strain, but not SR, had good intra-observer measurement variability (<10% vs −20%). However, only endocardial longitudinal strain and Sr values obtained with the two views agreed (95% limits of agreement: −3.28, 2.58; −1.41, 1.36). Epicardial longitudinal strain and Sr values did not agree sufficiently to be used interchangeably (95% limits of agreement: −11.58, 9.19; −2.28, 1.74).

Our study suggests that RP4Ch view was feasible for assessment of the LV longitudinal deformation analysis by STE in cats, but only endocardial longitudinal strain and Sr values obtained from the two different views were interchangeable. Clinicians can use RP4Ch view for endocardial longitudinal deformation analysis when Lap4Ch view demonstrates sub-optimal in cats.

Disclosures
No disclosures to report.
In dogs with asymptomatic myxomatous mitral valve disease (MMVD), the distinction between the ACVIM stage B1 and B2 is mainly based on the echocardiographic evaluation of left cardiac remodeling. The recently updated ACVIM guidelines consider stage B2, dogs with both left atrium (LA) and left ventricle (LV) enlargement. Thus, dogs only presenting LA enlargement are classified as stage B1. The aim of this study was to evaluate the prognosis of dogs in stage B according to different degree of left cardiac remodeling.

This retrospective, multicenter observational study included dogs with asymptomatic MMVD imaged between 2011 and 2019. Dogs were reclassified into stage B1 and B2 according to the 2019 ACVIM guidelines, with LA enlargement defined as a left atrium-to-aorta ratio (LA/Ao) ≥1.6 and LV enlargement by a left ventricular end-diastolic diameter normalized (LVIDDn) ≥1.7. Long-term outcome was assessed by telephone interviews with the owners. Survival was analysed using Kaplan-Meier curves. ROC curve analysis and the Youden index were used to define the best cut-offs of LA/Ao and LVIDDn to predict cardiac mortality in the stage B2.

A total of 444 dogs with asymptomatic MMVD were included, 277 in ACVIM stage B1 and 167 in stage B2. Among stage B1, 203 dogs (73%) did not present cardiac remodeling and 74 (27%) had LA enlargement with normal LV size. In the study, 76 dogs died for cardiac-related causes (24 in stage B1 and 52 in stage B2). Dogs in stage B1 lived longer (median survival time 2344 days, 95%CI 1905-2783 days) than stage B2 (1341 days, 95%CI 984-1698 days). In the stage B1, the median survival time of dogs with LA enlargement [1882 days, 95%CI 1185-2579 days] was not significantly lower than those without cardiac remodeling [2344 days, 95%CI 1943-2744 days; P = 0.33]. In the stage B2, a LA/Ao > 1.91 (AUC = 0.65; P = 0.0035) and a LVIDDn > 1.90 (AUC = 0.61; P = 0.035) were predictors of cardiac mortality. The median survival time of dogs in stage B2 with both LA/Ao > 1.91 and LVIDDn > 1.90 was lower (848 days, 95%CI 406-1286 days) than those with LA/Ao < 1.91 and LVIDDn < 1.90 (1588 days, 95%CI 1167-2009 days; P = 0.0017).

In conclusion, the prognosis of dogs in stage B1 is not significantly influenced by LA enlargement. In the stage B2, the entity of left cardiac remodeling has prognostic significance and the proposed cut-offs of LA and LV enlargement could be useful for risk stratification of cardiac death and clinical decision making in this stage.

Disclosures
No disclosures to report.

Echocardiographic analysis of dogs before and after the surgical treatment of brachycephalic obstructive airway syndrome
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Brachycephalic Obstructive Airway Syndrome (BOAS) is characterized by numerous airways abnormalities that can affect the heart. Surgical treatment of BOAS improves clinical signs of respiratory distress; however, it has not yet been investigated whether surgery affects the morphology and function of the heart. This study aims to compare echocardiographic parameters of dogs before and six to twelve months after the surgical treatment of BOAS.

Complete echocardiographic examination of the left and the right heart (2-D, M-mode, spectral and color Doppler, tissue Doppler of right and left ventricular walls, the global strain of the left and the right ventricle, tricuspid and mitral annular velocities, vena cava at inspiration and expiration, right chambers linear and area dimensions) was performed according to guidelines in 18 client-owned dogs with BOAS (7 French bulldogs, 6 Boston Terriers, and 5 Pugs) scheduled for rhinoplasty and folded flap palatoplasty. Echocardiographic follow-up was performed 6 to 12 months (median 9 months) after surgical treatment and parameters were compared. Weight-dependent variables were indexed (one dimensional as variable/weight1/3, and two dimensional as variable/weight2/3). Normally distributed variables were compared with paired t-test and not normally distributed with Wilcoxon signed-rank test. Statistical significance was defined as P < 0.05.

There were 12 males and 6 females included in the study. The median age of dogs before surgery was 1.8 years (interquartile range: 0.9–3.7 years). The mean ± (standard deviation) weight of the dogs was 9.9 ± 2.4 kg before and 10.7 ± 2.6 kg after the surgery (P = 0.014). Dogs after surgery had larger left atrium to aortic ratio (P = 0.005; mean ± SD: 1.50 ± 0.12 vs 1.63 ± 0.13), left atrium in short (P = 0.012), and long axis (P = 0.012), left atrium in long axis (LA LAX: P = 0.002), indexed LA LAX (P = 0.018) and increased global right ventricular strain (P = 0.033; −19.3 ± 5.4% vs −23.1 ± 5.6%), and global left ventricular four-chamber strain (P = 0.02). Right ventricular inner diameter at the base, absolute and indexed (P < 0.0005), and indexed right ventricular area in systole (P = 0.038) were smaller after surgery. Vena cava collapsibility index (P = 0.049) was higher after surgery (41.6 ± 11.3% vs. 49.2 ± 7.9%). Tricuspid inflow E wave velocity (0.68 ± 0.13 m/s) did not differ from mitral (0.74 ± 0.16) before (P = 0.218) and after surgery (P = 0.484); while mitral A was higher than tricuspid before (P = 0.028), but not after surgery (P = 0.529).

Despite noticeable clinical improvement after surgery, echocardiographic changes were mild. High tricuspid inflow velocities suggest higher diastolic pressure in the right heart, which does not change with the surgery; however, the right ventricular function seems to improve.

Disclosures
No disclosures to report.
ESV CN-P-1

Plasma amino acid and taurine concentrations in cats with obesity before and after a period of controlled weight reduction


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Limited data are available regarding adequacy of essential nutrient intake during dietary caloric restriction using a therapeutic weight management diet. Therefore, the aim of this study was to determine changes in plasma amino acid concentrations in cats with obesity during a period of caloric restriction.

Eleven cats were included in this non-randomized observational cohort study. All remained systemically well with no significant abnormalities on physical examination and clinicopathological assessments. Each cat followed a tailored weight reduction programme, involving feeding a high protein, high fiber therapeutic weight management diet and increased physical activity through regular play sessions. Before and after weight reduction, blood was taken after a fast of ≥16h, for routine clinicopathological analyses. Surplus heparinized plasma was immediately frozen at -20°C for storage, and subsequently shipped on dry ice, as a single batch, to the laboratory where they were analysed.

Concentrations of 16 amino acids and taurine were measured using high-performance liquid chromatography with cation exchange column separation and post column ninhydrin-reactive colorimetric detection (Biochrom 30). Concentrations of cysteine and methionine were not measured due to known sensitivities with storage. The study protocol was approved by the University Research Ethics Committee, and all owners gave informed written consent. Analysis of covariance was used to determine the effect of weight reduction on plasma amino acid concentrations, whilst controlling for the effect of storage time.

Median (range) weight loss was 23% (12-29%) starting weight, over a median (range) weight loss period was 50 (35 to 60) kcal/kg0.711 ideal weight per day. Median (range) weight loss was 23% (12-29%) starting weight, over a median (range) weight loss period was 50 (35 to 60) kcal/kg0.711 ideal weight per day. Storage time.

ESV CN-P-2

Influence of three different diets on lipid and fructosamine concentrations in a population of healthy cats


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Hypercholesterolemia in cats with diabetes mellitus has been associated with lower remission rates. Both lean and obese cats with diabetes mellitus fed a high-protein/low carbohydrate diet had significantly elevated serum cholesterol concentrations. However, it is unknown whether a high-protein/low-carbohydrate diet causes increased cholesterol in healthy cats.

A randomized, crossover diet trial was performed in thirty-five healthy shelter cats. The aim of this study was to determine the influence of either a high-protein or a high-carbohydrate diet on serum concentrations of cholesterol, triglycerides and fructosamine in healthy cats. The fat content of the high-protein and washout diet were equal, but nearly double that of the high-carbohydrate diet. The washout diet had the highest fiber content followed by the high-protein and high-carbohydrate diet, respectively. Before enrolment into the study, cats were fed a commercial baseline diet. Following baseline health assessments, cats were randomized to one of the two diets for 4 weeks. After 4 weeks cats were fed a washout diet for 4 weeks before being transitioned to the cross-over diet. Each cat was transitioned onto each of the different diets over 7 days. Fasting serum cholesterol, triglycerides and fructosamine were determined after 4 weeks of each diet. Body condition score, body weight and environmental temperatures were evaluated serially throughout the study.
After 4 weeks, cats on the high-carbohydrate diet had significantly lower serum cholesterol concentrations ($P < 0.001$) compared to the baseline diet. While cats eating the high-protein and washout diets had significantly higher serum cholesterol concentrations compared to the baseline diet at the start of the study ($P < 0.001$). The increase in serum cholesterol from the high-protein diet was reduced significantly in cats with a body condition score $>5$ ($P = 0.007$). Similarly, cats on the high-protein diet also had significantly higher serum triglyceride concentrations ($P < 0.001$) compared to the baseline diet but the increase was higher in cats with a body condition score $<5$. The high-protein ($P < 0.001$) diet lowered serum fructosamine concentrations significantly compared to the baseline diet.

In conclusion, diets higher in protein, fat and fiber and lower in carbohydrates appear beneficial for short-term glucose control in healthy cats. Additionally, high protein/fiber diets influence the lipid profile in healthy cats.

Disclosures

No disclosures to report.

ESVCN-P-3

Study of blood pressure parameters in lean and obese client-owned dogs: Preliminary results


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Obesity in dogs is a growing nutritional disease with several presumed adverse health effects such as systemic hypertension. It has been suggested in some studies that body condition had only a minor effect on blood pressure and that hypertension was probably more related to age, concurrent diseases, exercise, size and breed of the dog. The aim of this study was to compare blood pressure in healthy lean and obese adult client-owned dogs of similar breed.

Fifteen lean (LD) (Body Condition Score (BCS) = 5 on a 9-point scale) and 28 obese (OD) (BCS ≥7/9) privately-owned adult Labradors and Golden Retrievers were recruited for this study, and declared healthy based on clinical examination, blood biochemistry and complete blood count. After a 10-minute acclimatization period, blood pressure was measured by oscillometry following ACVIM guidelines. Activity level was measured by accelerometry during 1 week. Data were analyzed with a Kruskal-Wallis test and a Mann Whitney U test. Results are expressed as mean (±SD) or median (first quartile – third quartile).

Dogs were: 23 females (17 neutered) and 20 males (14 neutered). The mean body weight for LD and OD respectively were 30.2±4.5kg with a BCS of 5, and 40.3±7.0kg with a mean BCS of 7.8±0.6. No significant differences between groups were found for age ($P = 0.13$) and activity ($P = 0.09$). Only heart rates were statistically significant (in bpm): LD 112 (105-135); OD 98 (86-110); $P = 0.017$. No difference was found for blood pressures that were, respectively in OD and LD (in mmHg): systolic: 161 (141-172), 142 (126-182), $P = 0.49$; diastolic: 89 (72-103), 70 (65-120), $P = 0.17$; and mean arterial: 115 (96-127), 96 (89-138), $P = 0.29$.

This lack of significance can partly be explained by the small sample size, but no correlation was found between obesity and blood pressure, like in previous studies, where it has been more related to age and associated disorders, breed, temperament and level of exercise. This study failed to show differences in blood pressure parameters between lean and obese adult client-owned dogs of a similar breed, without any concurrent disease.

Disclosures

I. Jeusette (Affinity Petcare) provided the dry food for the next part of the study.

ESVE-P-1

Evaluation of kidney function in diabetic dogs: biomarker analysis

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Diabetes mellitus (DM) is the most common disease of the endocrine pancreas in dogs. It’s a syndrome characterized by chronic hyperglycemia, glycosuria, polyphagia, polyuria/polydipsia and weight loss. Diabetic nephropathy (DN) is a possible complication of DM that, in humans, is the main cause of chronic kidney disease in western countries. DN includes microalbuminuria, proteinuria, systemic hypertension and impaired kidney function. There are only a few studies regarding canine DN and its clinical relevance is still unclear.

The aim of this study was to evaluate the renal function of dogs diagnosed with DM, as well as the occurrence of proteinuria, and to compare them with healthy dogs. We also tested if there was a correlation between these biomarkers and the time of diagnosis of DM and insulin dosage.

The study included 18 dogs diagnosed with DM and undergoing insulin therapy, and 17 healthy dogs, based on physical examination and clinical history. Dogs that presented with diabetic ketoacidosis, acute kidney disease, urinary tract infection, active urinary sediment, hyperadrenocorticism, or that were undergoing chronic treatment with glucocorticoids, were excluded. Blood and urine samples were collected, the latter by cystocentesis. Serum urea, creatinine, symmetric dimethylarginine (SDMA) concentrations and urinary protein:creatinine ratio (UPC ratio) were determined.

There were no significant differences in weight ($P = 0.65$) or age ($P = 0.81$) between the two groups. Even though serum urea concentrations weren’t significantly different ($P = 0.67$), diabetic dogs had lower serum concentrations of creatinine and SDMA ($P = 0.01$ and $P = 0.02$, respectively). 38.9% of the diabetic dogs had an UPC ratio higher than 0.5. This group had an odds ratio of 10.87 (95% IC, 1.71-127.08, $P = 0.06$) of developing proteinuria when compared to healthy dogs. There was no significant correlation between serum urea, creatinine or SDMA and UPC ratio and the time of diagnosis ($P = 0.85$, $P = 0.52$, $P = 0.1, P = 0.84$, respectively). We also didn’t
find an association between any of these variables and insulin dosage ($P = 0.19$, $P = 0.20$, $P = 0.91$, $P = 0.23$, respectively).

Even though the occurrence of clinical DN is unlikely in dogs, our results show the possible impact of DM on the kidney in this species. The lower levels of serum creatinine and SDMA seen in diabetic dogs when compared to healthy ones suggest that glomerular hyperfiltration is present, which may be related with hemodynamic changes in the kidney. Besides, these dogs showed a higher chance of developing proteinuria, which reinforces the importance of UPC ratio assessment when monitoring these patients.

**Disclosures:** This study was supported financially by the Centre for Interdisciplinary Research in Animal Health (CIISA), which is hosted by the Faculty of Veterinary Medicine of the University of Lisbon. MTVB is a member of the CIISA’s Clinical Research Lab, and an employee of the Faculty of Veterinary Medicine, and receives funding support for other unrelated research projects from CIISA. All testing was conducted at Laboratório de Análises Clínicas Prof. Dr. Braço Forte Júnior, which is also hosted by the Faculty of Veterinary Medicine of the University of Lisbon.

**ESVE-P-2**

Lack of training on proper use of insulin syringes leads pet-owners to significant deviations from target dose


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The success of insulin therapy greatly depends on pet owners’ skills in handling the device chosen for insulin administration. This study aimed to assess whether previous training might influence the ability of pet owners to properly handle U-100 insulin syringes. After Research Ethics Committee approval, fifty pet owners were asked to obtain 1 and 10 IU of insulin with no training from the researchers. Then, after have received instructions on how to properly handle insulin syringes by the researches, they were asked to repeat the initial procedure 4 times. Each dose was weighed on an analytical scale, and accuracy and precision were calculated. The proportions of clinically-important-deviation (CID: ≥ ± 20% off target) outcomes were compared between "before" and "after" training.

The averages of the first acquisitions of 1 and 10 IU of insulin were x15.1 and x3.75 times higher than targets ($P < 0.0001$; $P < 0.0001$), respectively. After have received training by a veterinary professional, pet owners showed significant improvement in their ability to acquire both 1 and 10 IU of insulin ($x1.07$ [$P < 0.00001$] and $x0.92$ [$P = 0.0016$] times off target). There was a significant reduction in the frequency of CID outcomes after training in 1 IU (98% before vs. 84% after, $P = 0.03$) and in 10 IU (76% before vs. 12% after, $P < 0.00001$).

These data suggest a great risk of inaccuracy in insulin administration by pet owners when not appropriately trained, especially when administrating small doses of insulin.

**Disclosures**

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**ESVE-P-3**

Glycemic control and owner preference in insulin delivery in diabetic dogs

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In human medicine, numerous studies have shown that insulin injection pen devices have several advantages over insulin syringes for subcutaneous insulin injection, including improved patient satisfaction and adherence, greater ease of use and superior dosing accuracy. A reusable insulin pen (VetPen™, MSD Animal Health) with insulin cartridges (Caninsulin®, MSD Animal Health) has been designed specifically for use in diabetic dogs and cats. This study aimed to assess owner preference and compare glycemic control following two different methods of subcutaneous injection (insulin pen and U40 insulin syringes) of porcine insulin zinc suspension (Caninsulin®) in a randomized, 2-period crossover study in client-owned dogs with naturally occurring diabetes mellitus (DM). Eighteen dogs with DM on insulin treatment and on the same prescription diet were enrolled in the study. Dogs were randomly assigned to receive insulin by syringe ($n = 11$) or pen ($n = 7$) for 2 months, followed by 2 months of the other injection method. The owner’s preference for the delivery method was assessed using a questionnaire. A total score was assigned for glycemic control: good (8-12), moderate (4-7), poor (1-3), based on scores using a 12-point scale (including clinical signs, blood glucose curve parameters and serum fructosamine) while serum fructosamine (SF) and glycated hemoglobin (HbA1c%) concentrations were analyzed. Preference of 50% of the owners for the pen and 50% for the syringe was not affected by the order that each device was used ($P = 0.620$). Median clinical score was 8, 8, and 8 at inclusion, after 2 months of pen and after 2 months of syringes, respectively ($P = 0.445$). Median SF (µmol/L) was 377.5, 457.9, and 388.6 at inclusion, after 2 months of pen and after 2 months of syringes,
respectively (P = 0.327). Median HbA1c(%) was 5.75, 6.2, and 5.65 at inclusion, after 2 months of pen and after 2 months of syringes, respectively (P = 0.290). While an equal number of owners expressed a preference for each device, a larger sample size would be required to show a difference. It was not possible to demonstrate differences in glycemic control between the two devices. In humans, adherence to insulin injections is strongly influenced by the selection of the injection device. Further studies are needed to help veterinarians match the owners of diabetic pets with the injection device best suited to their needs and capabilities.

Disclosures
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ESVE-P-4
Use of the continuous glucose monitoring system ‘Freestyle Libre’ in diabetic cats
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Continuous glucose monitoring systems facilitate monitoring of diabetic patients. Aims of this study were to assess the flash glucose monitoring system “Freestyle Libre” regarding its measurement accuracy and tolerability in cats.

Results from 66 sensors applied to 34 cats (33 diabetic, 1 hypoglycemic) were included. The behavior during the application, wearing and removing of the sensor and the skin site of application were assessed. Blood samples were regularly collected for comparative measurements using the hexokinase method.

Minimal signs of discomfort were noted, although the sensor was additionally fixed using individual skin stitches. 46 sensors, which stopped working in situ, had a median functional life of 8.3 (1.6–14) days. Only nine reached a functional life of 14 days as specified by the manufacturer for humans. Skin reactions on the glued surface occurred after removal of 23/66 sensors (mild erythema: n = 21; superficial dermatitis: n = 2) and were not detectable in the remaining 37/66 cases. Due to the upper limit of the measurement range of 27.8 mmol/l (500 mg/dl), the reading device displayed "hi" in 21 cats at least at one individual time point. In 17 of these cats this was repeatedly the case or for longer time periods and required additional measurements with the reference method. There was a high correlation with the results of the reference method (r = 0.90, n = 359), which was however lower in the hypoglycemic and normoglycaemic range of values.

In conclusion, the device proved to be practicable, less stressful for the animals and generated acceptable results. Although the upper limit of the measurement range is a limiting factor, the device promises to significantly facilitate the management of diabetic cats.

Disclosures
No disclosures to report.

ESVE-P-5
Reliability assessment of a novel feline glucosuria home screening test
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Diabetes mellitus (DM), frequent in feline endocrinology, may be laborious to diagnose due to its insidious clinical signs and stress-induced hyperglycemia observed in non-DM cats. It needs tedious survey (regular in-clinics or at home blood glucose curves) to detect uncontrolled DM or spontaneous remission. As a key clinical feature of DM is the presence of glucosuria, a non-invasive, at-home, easy-to-use test to detect glucosuria would be of interest to suspect or to monitor DM. The aim of this study was to evaluate the reliability of a novel at-home test using granules, turning blue with glucose, to be added on top of the cats litter to detect glucosuria.

To assess the feasibility of the test at home, 16 cats (10 healthy and 6 diabetic) were enrolled. 20g of granules were poured on the cat litter. Their color, when trapped in the urinary clumps, was noted by the owner according to a visual color scale (0 to 3+), twice a day for 14 days.

Reliability was assessed in a field study in 132 cats at risk of glucosuria (aged > 12 years, overweight, or receiving corticosteroids, n = 113) or diabetic (n = 19) recruited in private practices. Urine was obtained by cystocentesis and standard urinalysis were performed. 2 drops were poured parallelly on 4 granules and their color evaluated 3 minutes later, using the color scale. 0.3mL of each urine sample were stored at -20°C to determine glucosuria by spectrophotometry (ADVIA® 1800). A cat was considered glucosuric if glucosuria was ≥25mg/dL; a test was considered positive if the mean score of the 4 granules was ≥1+. The test sensitivity (Se), specificity (Sp), and positive and negative predictive values (PPV, NPV) were determined using the spectrophotometry as gold standard.

At home, the granules were easy-to-use for owners and well-tolerated by cats. 100% of the granules remained white for healthy cats (n = 260/260). In diabetic cats, 91.2% were ranked ≥1+ in well-controlled diabetic cats (n = 52/57) and 67.3% were ranked ≥2+ (n = 37/55) in cats with severe hyperglycemic episodes. Concerning the field study, color of the granules were in accordance with the results of the dipstick in 132/132 cats (26 of which glucosuric) and strongly correlated with glucosuria (r = 0.823, P < 0.0001), resulting in Se = 96.15%, Sp = 99.06%, PPV = 96.15%, NPV = 99.06%.

This study suggest that these granules would be useful in order to easily diagnose glucosuria at home and may be of interest in detection and management of feline diabetes mellitus.
Disclosures

The study was financially supported by Blücare Lab Inc. One of the authors (A. Diquélou) has a research contract for this study only, and the others (E. Khenifar, A. Gagnon and C. Gara-Boivin) have contracts with this lab for this study and other ones.

ESVE-P-6

Bacteriuria in dogs with spontaneous hyperadrenocorticism: A retrospective study of 89 cases (2009-2019)

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Hyperadrenocorticism (HAC) has been previously reported as a predisposing factor for urinary tract infection (UTI) or subclinical bacteriuria in dogs. However, recent studies on characteristics of bacterial isolates in HAC and on evolution of bacteriuria during HAC treatment are lacking.

The aims of this retrospective, observational study were to describe the frequency of bacteriuria in a cohort of dogs newly diagnosed with spontaneous HAC, investigate associations with clinicopathological variables, and detail the follow-up of dogs with bacteriuria. Dogs recruited had a definitive diagnosis of spontaneous HAC and a bacterial culture performed at the time of diagnosis (+/- one month) on urine collected by cystocentesis. Dogs were excluded if they were receiving antimicrobials or immunosuppressants during the last month, and if they suffered from another significant predisposing disease for UTI. Clinicopathological variables were compared between dogs with and without bacteriuria using Fischer’s and Mann-Whitney tests with P < 0.05 considered as significant.

Eighty-nine dogs were included, among which 24 (27%) had a Positive Urine Culture (PUC), representing 26 bacterial isolates. Four dogs (17%) with PUC had clinical signs of UTI. There was no significant association between age or gender (including neutering status) and bacteriuria. A Positive Leucocyte Esterase Test (PLET) on the dipstick (12/24, 50%), pyuria (11/23, 48%) and bacteriuria (14/23, 61%) on sediment examination were significantly associated with PUC (P < 0.001), whereas specific gravity, pH and proteinuria did not differ significantly between groups. Six dogs with PUC (25%) had no abnormality on both dipstick and sediment examination. Escherichia coli was the most frequent micro-organism isolated (15/26, 58%), followed by Enterococcus faecalis (4/26, 15%). Antimicrobial resistance was common, with 14/25 (56%) isolates showing multi-drug resistance (≥3 antimicrobial categories tested), and 4/25 (19%) showing extreme drug resistance (all but ≤2 antimicrobial categories tested). All 24 dogs with PUC were treated with targeted antimicrobial therapy. Sixteen dogs (67%) had a follow-up urine culture (median time 35 days, range [14-98]), with 7/16 (43%) showing persistence of bacteriuria, and 9/16 dogs cured, among which 3 showed reinfection later on (72 days, [61-202]).

The frequency of PUC in this population was 27%. PLET and active sediment (pyuria/bacteriuria) had good positive predictive value (respectively 80%, 79% and 93%) to detect PUC despite low sensitivity. There is no definitive consensus regarding treatment of asymptomatic bacteriuria in dogs with HAC, however the high frequency of antimicrobial resistance highlights the need for antibiotic susceptibility testing prior to medical treatment if intended.

Disclosures

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ESVE-P-7

The diagnostic performance of the heat-stable alkaline phosphatase in dogs with suspected hyperadrenocorticism

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Increased total alkaline phosphatase (tALP) activity is a common biochemical finding in dogs with hyperadrenocorticism (HAC). From the three isoenzymes detectable in serum, the corticosteroid-induced ALP assessed by the thermostability method (heat-stable ALP) has been used as a source of additional diagnostic information for HAC, although clear cutoffs and recent studies that corroborate its diagnostic value are lacking.

The aim of this study was to evaluate the diagnostic performance of the heat-stable ALP (HS-ALP) as an absolute value and as a percentage of tALP (%HS-ALP), and to compare it to that of tALP alone in dogs with suspected HAC.

The electronic database of the central laboratory in our institution was retrospectively searched for dogs with suspected HAC without acute non-adrenal illness that underwent a low dose dexamethasone screening test (LDDST) between April 2001 and January 2020. Dogs were divided into two groups: HAC group (confirmed by at least one positive diagnostic test and response to treatment/histopathology) and non-HAC group. As the diagnostic performance of the HS-ALP was unexpectedly low in these likely hypercortisolemic patients, the non-HAC group was complemented with patients suspicious of HAC with HS-ALP records and normal urine cortisol/creatinine ratio. tALP was assessed by the Cobas ALP2-assay on a Roche/Hitachi Cobas c502. Dogs were excluded when both tALP and HS-ALP records were missing.

The final study population consisted of 103 dogs (57%) with HAC and 78 (43%) without HAC. There was no difference in age (P = 0.53), weight (P = 0.15) and gender (P = 0.95) between the groups. Classical clinical signs except excessive panting (P = 0.18) were significantly more common in dogs with HAC. Median values of tALP and HS-ALP, but not those of %HS-ALP (70% [1-103] in the HAC group vs. 61.5% [0-106] in the non-HAC group, P = 0.17) were significantly higher in the HAC group (P < 0.001). The areas under the ROC-curves were 0.72 (0.63-0.8), 0.69 (0.6-0.78) and 0.56 (0.46-0.66), respectively. The cutoff values associated with the highest sensitivity and specificity
Feline hyperthyroidism is the most common endocrinopathy in cats. Several epidemiological studies suggest that hyperthyroidism is a common disease in countries such as UK (11.9%), Germany (11.4%), Portugal (9%), Poland (20.4%) and Ireland (21%). However, in Spain it has been historically considered a rare disease. A retrospective study in 2005 found a prevalence of 1.53% and 10% in a second prospective study in 2015 in 207 geriatric cats. The aims of this study were to assess the overall and regional prevalence of feline hyperthyroidism in Spain, to determine the age of hyperthyroid and non-hyperthyroid cats and to evaluate the percentage of animals with more than one measurement of total thyroxine (tT4) and the time between these. The study was performed retrospectively, including serum blood samples submitted to a reference laboratory during a 3-year period (January 2016-December 2018). Prevalence in this population referred to the total number of hyperthyroid cats divided by the total number of individual cats tested. Serum tT4 concentrations were determined in all cats by use of a chemiluminescent competitive immunoassay (Immulite®) validated for use in dogs. A cat was considered hyperthyroid when the tT4 concentration was greater than 4.7 μg/dL (reference range 0.8-4.7 μg/dL). A total of 27,893 client-owned cats from different regions of Spain were included in the study. The overall prevalence of feline hyperthyroidism was 6.35%. The prevalence was variable according to the area, with a lower prevalence in Castilla Leon (3.17%) and a higher prevalence in Balearic Islands (9%). Age data was available from 6,470 cats. The mean age of the hyperthyroid cats was 14 years (range 2-25) and 11.7 years (range 1-27) in non-hyperthyroid group. Total thyroxine measurement was repeated in 8.5% of the cats. Average of repeated measurements in the hyperthyroid group was 4 months compared to 8.6 months in the non-hyperthyroid cats. The number of cats in which tT4 was measured increased from 2016 to 2018 (from 7652 to 10345 cats tested per year) which might suggest a more thorough follow-up or greater effort in challenging cases. The overall prevalence of feline hyperthyroidism was 6.35% in a population of 27,893 cats in Spain, but these results were variable according to the area. To the authors’ knowledge this has been the largest prevalence study performed in Spain about feline hyperthyroidism.

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The main limitation of this study was the lack of a highly objective method (e.g. scintigraphy or histopathology) to classify the 2 groups of dogs. Thus, some dogs could have been misclassified. This study suggests that TSHst using 75 μg/dog of rhTSH is accurate in distinguishing hypothyroidism from NTI in a population of dogs in which hypothyroidism is suspected.

Disclosures

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ESVE-P-10

Use of desoxycorticosterone pivalate by veterinary surgeons: A Western European survey


A desoxycorticosterone pivalate (DOCP) product was approved by the European Medicines Agency in 2015. Lower-than-label doses and extended treatment intervals have been reported by several authors. The frequency of use of DOCP as first-line mineralocorticoid supplementation, initial doses and treatment intervals have not been evaluated among Western European veterinary surgeons (WEVS). This study aimed to characterize the use of DOCP by WEVS in dogs diagnosed with Addison’s disease.

An online survey translated into four different languages (Portuguese, Spanish, French and Italian) was developed using an electronic platform. Respondents were recruited through social network veterinary groups and mailing lists. Questions focused on initial treatment regimen, DOCP starting dosage, clinical and electrolytic monitoring schedule in dogs diagnosed with Addison’s disease. Responses from participants who had diagnosed canine Addison’s disease over the previous 12 months were included.

Overall, 167 responses from six European countries were included (Portugal [n = 65], Spain [n = 55], Italy [n = 34], France [n = 8], Belgium [n = 4] and Luxembourg [n = 1]). Among respondents, 83% had already used DOCP and 78% indicated they preferred its use over fludrocortisone acetate as first-line treatment for mineralocorticoid supplementation. Among 138 respondents who had used DOCP, 61% indicated using 2.2mg/kg as initial dosage while 15% stated using lower dosages. The remaining 34% indicated monitoring electrolytes twice a month (67% at day 10 and day 25 and 22% at day 10 and day 28-30) and 11% once a month (6% at day 10, 3% at day 25 and 2% at day 28-30) until stabilization. Out of 89 WEVS that specified a preferable therapeutic adjustment, 51% indicated changing administration interval rather than dosage while 49% stated changing dosage rather than frequency. Following the initial administration, 9% of respondents indicated administering subsequent DOCP injections only in case of clinical relapse. In dogs with stable electrolytes concentrations, 34% of respondents reported reassessing dogs monthly, 44% quarterly, 17% twice yearly and 5% yearly.

In Western Europe, DOCP is the preferred treatment for mineralocorticoid supplementation. The large majority of WEVS follow the manufacturer’s recommendations for initial dosage and short-term monitoring schedule. Subsequent preferred therapeutic adjustments regarding change in dosage or dosing intervals vary among WEVS, most likely reflecting the absence of strict guidelines. Of particular concern, a significant proportion of WEVS only administer DOCP in case of clinical relapse following the initial administration, which likely increases the risk of addisonian crisis.

Disclosures

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ESVE-P-11

Evaluation of basal cortisol testing in dogs with signs consistent with hypoadrenocorticism

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Basal cortisol concentration <55 nmol/L is well described as a screening test for hypoadrenocorticism and commonly performed in suspected cases. Canine hypoadrenocorticism can manifest with a variety of vague and nonspecific clinicopathological features, mimicking conditions such as renal, gastrointestinal or neurological disease.

The aim of this study was to evaluate basal cortisol testing in dogs with clinical signs and clinicopathological abnormalities consistent with hypoadrenocorticism presenting to a referral institution.

Medical records for all dogs having had a basal cortisol performed between May 2013 and November 2018 were reviewed for signalment, basal cortisol or ACTH stimulation results, and final diagnosis. Dogs were excluded if testing was performed as a screening for hyperadrenocorticism.

A total of 1182 cases were included. Six hundred and forty-four dogs were male (54.5%) and 538 were female (45.5%). Labrador Retriever was the most common breed (15.1%). Most common clinical signs on presentation were gastrointestinal signs, such as vomiting (37.2%), diarrhea (32.2%), lethargy (25.0%), weight loss (11.8%) and hyporexia (9.2%). Other frequent presenting complaints included collapse (6.8%), regurgitation (6.1%), polydipsia (4.4%), polyuria (4.1%) and abdominal pain (4.1%).
Basal cortisol ranged between <13.8 to 988 mmol/L (median 90 mmol/L). A result <55 mmol/L was obtained in 327 cases (27.7%). Basal cortisol was retested in 136 patients and 82/136 were <55 mmol/L; ACTH stimulated cortisol was tested in 225 dogs. Hypoadrenocorticism was the final diagnosis in 17 dogs (1.4%). Multivariate logistic regression analysis was performed on the 327 dogs with an initial basal cortisol <55 mmol/L to explore routine blood variables and the most common presenting signs associated with hypoadrenocorticism. The following changes were associated with hypoadrenocorticism within this group: increased potassium ($P = 0.003$), decreased cholesterol ($P < 0.001$), increased globulin ($P = 0.003$) and increased urea ($P = 0.029$). Overall, the most common diagnosis was chronic primary inflammatory enteropathy (18.2%), followed by pancreatitis (4.5%) and kidney disease (3.5%). A final definitive diagnosis was not obtained in 16.5% of patients.

In this study, basal cortisol screening for hypoadrenocorticism was frequently assessed in a population of dogs due to its wide variety of clinicopathological abnormalities and it was the final diagnosis in only 17 of 1182 dogs (1.4%) tested for clinical suspicion presenting to a referral institution. No presenting clinical signs were specifically signif-

### Disclosures

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### ESVIM-P-1

**Respiratory and digestive abnormalities in a population of dogs with chronic idiopathic lymphoplasmacytic rhinitis**

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Chronic idiopathic lymphoplasmacytic rhinitis (CILPR) is a common inflammatory disorder of the nasal cavity in dogs due to unknown etiology. The definitive diagnosis is made by exclusion of other causes of nasal disease and specific therapeutic protocols are lacking. In human medicine, a relationship between CILPR and gastrointestinal symptoms has been postulated, and a remission of respiratory signs after clinical trials with oral proton-pump inhibitors, prokinetics and/or diet has been observed. The aims of the present study were to describe history, clinical presentation, endoscopic and histopathologic concurrent respiratory and digestive abnormalities; and to evaluate the eventual improvement of respiratory signs after treatments for gastrointestinal signs. The following information from 25 dogs with CILPR was recorded and studied: respiratory/digestive signs, airway/digestive endoscopic abnormalities, histologic evaluation of respiratory and gastrointestinal tract biopsy specimens, clinical response to different treatment strategies. Overall, a high proportion of dogs (88%) showed endoscopic gastrointestinal lesions, while thirteen dogs (52%) had concurrent gastrointestinal signs. Most esophageal and duodenal endoscopic abnormalities were classified as moderate/severe. Most gastric endoscopic abnormalities were classified as mild. Respiratory and gastrointestinal histologic evaluation identified mostly chronic inflammation. All dogs that received only treatments for gastrointestinal signs (30.4%) showed remission or marked improvement of respiratory signs at two-month follow up. A significant association between age and respiratory symptoms was found. Nasal clinical signs of some dogs treated exclusively with gastrointestinal approach notably improved or disappeared. Further studies are need to explore the possibility of a cause-effect relationship between the two processes.

### Disclosures

No disclosures to report.

### ESVIM-P-3

**Influence of concurrent lower respiratory tract disease on point-of-care lung ultrasound in small-breed dogs with mitral valve disease**

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Small-breed dogs commonly suffer with concurrent heart and respiratory disease. In previous studies, various respiratory etiologies can produce false-positive results with point-of-care lung ultrasound (POC-LUS) for cardiogenic pulmonary edema (CPE). Therefore, we hypothesized that small-breed dogs with lower respiratory tract disease (LRTD) have increased numbers of B-lines and are prone to misdiagnosis. Eighty-four small-breed dogs with preclinical stage B mitral valve disease (MVD) were included. POC-LUS was obtained by a single clinician using the Vet BLUE protocol. The number of B-lines was recorded at each scan site. The presence/absence of LRTD was assessed by clinicians blinded to the POC-LUS results. LRTD was present in 72.6% of MVD dogs. When a previously used criterion for CPE diagnosis ($\geq 2$ sites with $\geq 3$ B-lines/site) was applied, false-positive results were observed in 14.3% of dogs with preclinical MVD. Total B-line score was significantly higher in dogs with LRTD compared with dogs without LRTD ($4$ vs. $0$, $P = 0.0009$); however, the proportion of false-positive results was not statistically different between dogs with and without LRTD ($18.0\%$ vs. $4.3\%$, $P = 0.17$). Multivariable logistic regression showed that with presence of abnormalities other than B-line on POC-LUS (eg, thickened pleura or consolidation) could predict false-positive results (OR = 14.6, $P = 0.006$) after adjusting for the effects of LRTD and echocardiographic hemodynamic parameters.

In conclusion, small-breed dogs with concurrent MVD and LRTD could have increased B-lines before CPE development. Adhering to
previously reported criteria for CPE diagnosis and carefully evaluating abnormalities other than B-line on LUS may help to prevent misdiagnosis in small-breed dogs.

Disclosures
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ESVIM-P-4

Signalment, clinical presentation and diagnostic imaging findings in 14 dogs and 3 cats with lobar emphysema

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Lobar emphysema is an uncommon condition described in infant humans and dogs and cats. It is caused by bronchial collapse during expiration, leading to air trapping and subsequent hyperinflation of the affected lung lobe. The mass effect associated with the hyperinflated lobe can lead to severe clinical signs.

Congenital forms of the disease are most frequently associated with bronchial cartilage defects in both human and veterinary medicine. However, acquired forms caused by neoplasia or anomalous pulmonary vessels have been reported.

The purpose of this study was to review the clinical presentation and imaging findings in a series of dogs and cats diagnosed with lobar emphysema.

Seventeen cases of lobar emphysema (14 dogs, 3 cats) were retrospectively recruited from referral veterinary hospitals. Diagnosis was based on diagnostic imaging, surgery and histopathology when available. Signalment, presenting signs, clinicopathological findings and surgical reports were also reviewed. All images were reviewed by a board-certified radiologist.

Small breed dogs were overrepresented (median - 4.7kg) and there was a bimodal age distribution amongst the group of dogs (median - 15 months, local peaks - 1 year and 12 years). The most common presenting signs included dyspnea, coughing, dysphagia and vomiting. Clinicopathological findings were non-specific.

The most common imaging findings included decreased opacity/attenuation and bronchial collapse of the affected lung lobe, atelectasis of the adjacent lung lobes and mass effect. Computed tomography was superior in identifying the affected lung lobe. The right middle lung lobe was most frequently affected (13 of 17) followed by the right cranial lobe (4 of 17). Multiple lobes were involved in several patients (4 dogs, 1 cat). Acquired forms of lobar emphysema were identified in 3 cases including two cases of pulmonary carcinoma (1 cat, 1 dog) and one case of diaphragmatic hernia (1 cat).

Ten patients underwent surgery (9 lung lobectomy, 1 diaphragmatic hernia repair) with 8 surviving to discharge. Histopathology confirmed congenital lobar emphysema in 8 cases following lung lobectomy whilst pulmonary carcinoma was diagnosed in one case.

This case series suggests that computed tomography provides superior information for the diagnosis of lobar emphysema. In keeping with previous reports, the right middle lung lobe is most frequently affected in veterinary patients. In older patients presenting with lobar emphysema, acquired causes of bronchial compression should be suspected.

Disclosures
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ESVIM-P-5

Suitability of commercial human rheumatoid factor rapid tests for detection of rheumatoid factors in dog serum

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The diagnosis of rheumatoid arthritis can be supported by detection of serum rheumatoid factors (RF), autoantibodies directed against IgG immunoglobulins. Rapid tests are commercially available, time-saving, and would be useful in veterinary practice. However, it is unclear if RF tests developed for human RF detection are suitable to detect canine RF.

In total, 7 and 3 commercially available rapid tests based on Waaler Rose hemagglutination and latex agglutination principles, respectively, were examined (further information available on request). We used surplus material from samples obtained for diagnostic purposes, in total 12 dog sera tested positive (n = 6) or negative (n = 6) with the routine method used in the author’s lab (Waaler Rose principle, sensitivity 86%, specificity 97.9%). According to the manufacturer’s recommendations, sera were mixed with reagent, and slides were examined macroscopically for presence of agglutination after 2-3 min of undisturbed incubation or incubation on a rotator. When agglutination occurred, sera were diluted 1:10 to exclude false positives which may be caused by heterophilic antibodies.

We verified that a human serum tested positive with the routine method was positive with all rapid tests. Noticeable, this serum was clearly positive applying latex agglutination tests which use particles coated with species-specific IgG to detect agglutination. In contrast, in tests using Waaler Rose principle, the human serum showed clear agglutination only after a prolonged incubation time of 5-8 min. In dog sera, however, none of the rapid tests detected RF. Because canine RF has low affinity for human IgG, this result was foreseeable when using rapid latex agglutination tests which all use human IgG coated latex particles. In contrast, it could have been expected that rapid tests based on non-species-specific Waaler Rose principle based on sheep erythrocytes sensitized with rabbit IgG may be suitable for canine RF detection. However, also the use of the latter tests was not successful.

The investigated rapid tests are not suitable for the detection of RF in dog sera, thus specialized laboratory testing for canine RF is recommended.
Disclosures

Some of the evaluated tests were provided for free from the companies. Our lab offers testing for rheumatoid factors, but uses none of the tests mentioned in the abstract.

ESVIM-P-7

Multiple abdominal granuloma caused by Scedosporium spp in a dog

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Infections caused by Scedosporium spp. are occasionally described in dogs causing rhinitis, keratitis, osteomyelitis, discospondylitis, and rarely disseminated infections. Granulomatous lesions have been reported in urinary bladder and ureter, and nasal cavity. This case describes multiple abdominal pyogranulomatous lesion secondary to Scedosporium spp. infection.

A 2-year-old female spayed mixed-breed dog presented with a 3-week history of vomiting, lethargy and weight loss. Previous history included abdominal evisceration secondary to postsurgical dehiscence 5 days after being spayed, 12 months prior to presentation. Physical examination revealed an abdominal mass, discomfort on palpation and hyperthermia (39.2°C). Systolic blood pressure was 80 mmHg. Hematology showed a non-regenerative normocytic normochromic anemia (PCV 30.7; range, 37.3 – 61.7 percent), moderate neutrophilia 22.64 (range, 2.95 – 11.64 x 10⁹/L), hypalbuminemia 21 (range, 23-40 g/L), hyperglobulinemia 60 (range 25 – 45 g/L) and increased ALP 534 (range, 23-212 U/L). Coagulation profile, urinalysis and culture and thoracic radiographs were unremarkable. Abdominal ultrasound showed an ill-defined mass with irregular margins involving the stomach, spleen, liver and pancreas. The liver parenchyma was heterogeneous, there was portal hypertension and ascites. Computed tomography showed a soft tissue peritoneal mass involving the previously mentioned organs compressing the portal vein with multiple acquired portosystemic shunts and generalized abdominal lymphadenomegaly. Fine-needle aspirations from the liver and spleen and peritoneal ascites were consistent with pyogranulomatous inflammation and pure transudate, respectively. Exploratory laparotomy was performed; complete resection of the mass was not possible. Splenectomy and omental biopsies were taken. Histological examination revealed pyogranulomatous splenitis, peritonitis and omentitis with intralymphatic fungal organisms. Tissue culture grew Scedosporium spp. and Staphylococcus epidermidis.

The patient was treated with itraconazole (5 mg/kg PO q24h), marbofloxacin (2mg/kg PO q24h) and S-adenosylmethionine (10 mg/kg PO q24h). The dog made a full recovery after surgery with transient improvement of clinical signs. However, was euthanized 2 months after diagnosis due to clinical deterioration.

Scedosporium spp is an opportunistic pathogen and infections are reported in dogs, cats and humans with a very poor outcome. Most of the dogs reported in the literature were immunocompromised. The dog we report here was not immunosuppressed. There are few reports of granulomatous lesions caused by Scedosporium spp. in dogs and one involving liver and abdominal cavity caused by Pseudallescheria boydii, actually classified as a distinct specie in the Pseudallescheria/Scedosporium complex. This is the first multiple abdominal granuloma caused by Scedosporium spp reported in dogs.

We hypothesize that this multiple organ involvement was secondary to the previous postsurgical abdominal evisceration.

Disclosures

No disclosures to report.

ESVIM-P-9

Indications and outcomes of feeding tubes in cats : 56 cases (2015-2018)

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Appetite disturbance is very common in cats. Prolonged anorexia can be a life-threatening condition in this species, which can develop hepatic lipidosis.

This study aimed to report the clinicopathological findings and outcome of anorectic cats with enteral feeding tube placement during hospitalization in a tertiary care referral center.

Medical records of 56 cases (representing 53 cats) managed with a feeding tube between January 2015 and July 2018 were retrospectively reviewed. Thirty-four cases were spayed females and 22 were castrated males. Mean age was 9 years (range: 1-18). The reason for tube placement was complete anorexia (39 cases, 70%) or partial anorexia (17 cases, 30%). Mean duration of anorexia before tube placement was 10 days (range: 2–61 days). Mirtazapine was unsuccessfully attempted before feeding tube placement in 21/56 (38%) cases. The most common clinical signs associated with anorexia were lethargy (86%, 48/56), vomiting (57%, 32/56) and icterus (30%, 17/56). Main biological abnormalities encountered before tube placement were increased ALT in 33/50 (66%), hyperbilirubinemia in 20/31 (66%) and hypokalemia in 24/48 (50%) cases. Most commonly associated medical conditions were digestive: hepatic (12/56), pancreatic (8/56), gastrointestinal (9/56) or mixed (3/56). Forty-six (82%) cases had a naso-esophageal feeding tube and 10 (18%) an esophagostomy feeding tube. Eight of 10 cases (80%) with esophagostomy feeding tubes were previously managed with naso-esophageal tubes without spontaneous refeeding during the first seven days of enteral nutrition. Those 8 cases had neutrophilic cholangitis (3/8), hepatic lipidosis (2/8), infectious rhinitis (2/8) and digestive neoplasia (1/8). The two remaining cases had a neutrophilic cholangitis and a triaditis, respectively. Complications were reported.
in 2/56 (3.5%) cases (dislodgement of the esophagostomy tube in one case, aspiration pneumonia due to malposition of the tube in one case). The type of tube chosen did not seem to be associated with any clinicopathological and diagnostic finding or the duration of anorexia. Forty-seven (84%) cases were discharged from hospital while 9 (16%) cases died or were euthanized during hospitalization. Spontaneous refeeding occurred in 29/47 cases during hospitalization. The mean time between feeding tube placement and removal for naso-esophageal and esophagostomy tubes was 5 (range: 1-17) and 33 days (range: 5-61), respectively.

This retrospective study shows that medical conditions associated with feeding tube placement varied widely, though hepatic diseases were frequent. Most cats were discharged and recovered. A larger prospective case-controlled study is needed to identify putative historical and clinicopathological prognostic factors.

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ESVIM-P-10

Central venous catheter associated thrombosis in dogs
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Thrombosis in the jugular vein related to central venous catheters (CVC) is a complication empirically observed in canine patients treated in intensive care units. This study aimed to determine the incidence of catheter-induced thrombosis in canine intensive care patients and, thereby, the efficacy of a routine prophylactic heparin treatment. In addition, it should be assessed whether initial changes of different hemostasis parameters can be used to predict an increased risk for thrombosis formation.

Canine in-patients of a small animal clinic receiving a central venous catheter in the jugular vein for medical reasons from March 2017 to December 2019 were included in the study. All animals received standard anticoagulant treatment with low dose unfractionated heparin (UFH), i.e. 150 IU [75 IU in surgical patients]/kg BW TID subcutaneously. Before the catheter was inserted (day 0) and on days 1, 3, 5, etc. the respective vein was assessed by color Doppler sonography and blood samples were collected. Hemostasis tests performed included routine coagulation tests, rotational thromboelastometry, thrombin generation assay (TGA), D-dimer, antithrombin and the heparin activity using a chromogenic anti-factor Xa test. After removal of the CVC, catheter tips were examined electron microscopically.; 32 dogs entered the study including 12 dogs receiving the reduced heparin dose. Based on ultrasonographic findings of the external jugular vein, 8 dogs (all of them receiving 150 IU UFH/kg BW TID) showed thrombus formation (in none of the cases completely occluding the vein) and 6 dogs fibrin precipitates within or attached to the CVC. These findings did not correlate well with the electron microscopic assessment of the catheter tips (unfortunately these studies are not complete at this time). Heparin plasma activities showed a wide variation with median values (minimum-maximum) of 0.36 (0.00–0.72) IU/ml and 0.11 (0.02–0.32) IU/ml in dogs treated with 150 and 75 IU UFH/kg BW TID, respectively, with no significant difference between dogs with and without sonographically detectable thrombi. Fibrinogen concentrations were higher in dogs with thrombi, whereas the residual hemostasis parameters including low antithrombin activities (65.5, 42.8–104 %) were not significantly different between these patient groups.

Results demonstrate that catheter associated thrombotic changes detected by ultrasonography do not correlate with results of electron microscopy of the catheter tip. The used anticoagulatory treatment is not completely effective to prevent catheter-induced thromboses in dogs, whose development may be supported by the wide variation of UFH plasma activities and low antithrombin activities in intensive care patients.

Disclosures
No disclosures to report.

ESVIM-P-11

Importance of bone marrow examination in reaching the final diagnosis in a referral population of dogs with non-regenerative anemia: 23 cases (2015-2020)
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There is very little information or criteria available in the veterinary literature, allowing clinicians to plan diagnostic work up, including bone marrow sampling, in dogs with severe non-regenerative anemia. The aim of this study was to determine the importance of bone marrow examination in reaching the final diagnosis in dogs with non-regenerative anemia.

Medical records from a referral hospital were searched retrospectively from 2015 to 2020 for all dogs presenting with a more than 5 days history of documented non-regenerative anemia defined as PCV < 20% and reticulocyte count < 60.000/mcL. To be included, a complete history, physical examination findings, routine and specialized laboratory testing, diagnostic imaging and sampling of documented abnormalities necessary to reach the final diagnosis, had to be available as well.

A total of 23 client owned dogs fulfilled the inclusion criteria. Median age of dogs was 6.5 (range, 9 months to 13 years). There were 4 sexually intact and 7 spayed females, 3 intact and 9 neutered males. Median PCV and reticulocyte count on presentation were 16.7 (range, 6.1 – 20 percent) and 16.9 (range, 3.1 – 58.8 x 10⁹/L), respectively. Lethargy, anorexia, pale mucous membranes and hemic heart murmur were most common physical examination findings. Chronic kidney
disease as a suspected cause of non-regenerative anemia was documented in 3/23 dogs, precursor-targeted-immune-mediated hemolytic anemia (PT-IMHA) in 7/23, and one each of stage 5b hepatosplenic lymphoma, metastatic insulinoma with secondary iron deficiency anemia, submandibular round cell neoplasia with suspected myeloid leukemia and one dog with severe gastrointestinal bleeding. Bone marrow sampling was performed in 14/23 dogs and was imperative for obtaining the final diagnosis in 9/14 dogs of which four were diagnosed with primary or metastatic bone marrow neoplastic disease and five with myelofibrosis; 6/9 of these dogs presented with either pancytopenia or bicipotopenia. Coombs test was performed in 6/9 of these dogs and was negative in four.

In remaining 5/14 dogs, BM examination revealed PT-IMHA; all five dogs had positive Coombs test results. Myelofibrosis was diagnosed in total of 8/14 dogs in this cohort and was associated with PT-IMHA and bone marrow neoplasia (primary or secondary) in 3 and 2/8 dogs, respectively.

Results of this study revealed that BM examination is valuable in obtaining the final diagnosis in most dogs with non-regenerative anemia, but might be particularly vital in dogs with negative Coombs test results and depression of two or more blood cell lines.

**Disclosures**

No disclosures to report.

**ESVIM-P-12**

**Thrombocytosis in iron deficient dogs and cats**

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Iron deficiency is a well-known cause of anemia in dogs and cats, and is often found as a consequence of chronic gastrointestinal bleeding. In humans, iron deficiency anemia is associated with reactive thrombocytosis and, in patients with inflammatory bowel disease (IBD), an elevated platelet count can be used as a marker of active disease. There is also accumulating evidence to support concurrent platelet activation and an increased thromboembolic risk. The aim of this study was to investigate whether there is an association between iron parameters and platelet count in dogs and cats.

A search of clinical records from 2010-2020 was done to identify patients in whom a serum iron panel had been submitted. Serum iron, % saturation, total iron binding capacity (TIBC), concurrent hematocrit and platelet counts were documented and assessed for correlation. Automated hematologic parameters were verified by microscopic smear evaluation in all cases.

A total of 111 patients (94 dogs and 17 cats) with serum iron panel results and concurrent platelet counts were identified, all of whom had confirmed anemia, microcytosis or chronic blood loss. In 67 patients (54 dogs and 13 cats) iron deficiency (iron < 20 µmol/L) was documented, of these, 12 patients (10 dogs and 2 cats) had thrombocytosis (platelet > 500 *10^9/L). No significant correlation was established between serum iron parameters and platelet count, nor between the magnitude of iron deficiency and degree of anemia in either dogs or cats.

This study did not identify any association between platelet count and serum iron parameters in dogs or cats. These preliminary results suggest that iron deficiency may not immediately stimulate megakaryopoiesis in dogs and cats to the same degree as in humans. Accordingly, until further information is available, thrombocytosis should not be used by practitioners as a surrogate marker to raise the suspicion of iron deficiency.

**Disclosures**

No disclosures to report.

**ESVIM-P-14**

Clinical and laboratory findings and their association with AA-amyloidosis in shelter cats: A retrospective study

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Amyloidosis is a group of diseases characterized by tissue deposition of amyloid fibrils. In animals AA-amyloidosis is the most common form and chronic inflammation is deemed crucial to promote fibril deposition. In cats, AA-amyloidosis has been mainly described in the familial form in Abyssinian and Siamese breeds, and rarely in domestic short-hairs. Recently, a high prevalence of AA-amyloidosis has been reported in shelter cats but the underlying reason is unknown. The aim of this study is to explore the association between clinical and laboratory findings and AA-amyloidosis in cats of a shelter with high disease prevalence.

Cats from one shelter were included if necropsies were performed within 6 hours from death. Kidney, spleen and liver samples were collected and a diagnosis of AA-amyloidosis was given if any of the 3 organs had amyloid fibrils. An association between clinical and laboratory findings and AA-amyloidosis was investigated using available data at onset of clinical illness and before death. Variables retrieved from medical records were duration of stay in the shelter and of illness, white blood cells count (WBC), hematocrit, erythrocyte mean corpuscular volume (MCV), serum creatinine, bilirubin, albumin and globulin concentrations, urine protein-to-creatinine ratio (UPC). Additionally, an illness severity score derived from abnormal laboratory findings was assigned to cats. Comparisons were performed between cats with and without AA-amyloidosis.

Twenty domestic shorthair cats were included: 12 with AA-amyloidosis and 8 with other diseases (3 chronic kidney disease,
2 lymphoma, 2 infectious diseases, 1 discospondylitis). At onset of clinical illness, cats with AA-amyloidosis vs. those without AA-amyloidosis had significantly lower albumin [median 2.3 g/dL (interquartile range 2.1-2.4) vs. 2.6 g/dL (2.5-2.9), P = 0.031] and higher illness severity score [0.60 (0.50-0.69) vs. 0.40 (0.38-0.50), P = 0.025]. Before death, cats with AA-amyloidosis vs. those without AA-amyloidosis had significantly higher WBC [18400/μL (15900-23000) vs. 9550/μL (4800-12100), P = 0.005] and lower albumin [2.3 g/dL (2.1-2.4) vs. 2.9 g/dL (2.4-3.0), P = 0.039]. Differences between the 2 groups were not observed for duration of stay and of illness, hematocrit, MCV, creatinine, bilirubin, globulin and UPC, at either time point. Age and gender did not differ.

In conclusion, AA-amyloidosis in shelter cats is associated to lower serum albumin concentrations throughout clinical illness. Hypoalbuminemia does not seem caused by proteinuria. The higher illness score at onset of clinical signs might suggest that disease severity has a permissive role in the pathogenesis of AA-amyloidosis in shelter cats. Further studies are needed to confirm these preliminary findings.

Disclosures
No disclosures to report.

ESVIM-P-15

Alendronate treatment in cats with idiopathic hypercalcemia: A retrospective control study of 20 cases
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Alendronate has been advocated for long-term management of idiopathic hypercalcemia of cats (IHC). To date, only three case reports and one prospective uncontrolled study have documented the usefulness of alendronate in IHC. The aims of this study were to investigate whether treatment with alendronate is associated with a decrease of ionized calcium (iCa) in comparison with other (or no) treatment.

Cats with IHC were recruited. IHC was defined by persistently elevated ionized calcium. Cats treated with alendronate were 2 (median not reached; 150 days; 2.1-2.4) and occurrence of a 15%-decrease in iCa in comparison to iCaT0 (iCa < iCaT0 - 15% iCaT0 : iCaT0-15%). Kaplan-Meier method with logrank testing was used to compare time to endpoints between groups. Variables were presented as medians [25th quartile ; 75th quartile] and compared with Mann-Whitney test. Differences were considered significant when P < 0.05.

Twenty cats were included. The two groups were comparable regarding epidemiologic and biological data. Three cats in group 2 received other treatments: prednisolone (2) or furosemide (1). In 6/11 cats (55%), alendronate dose had to be increased from 10 to 15 (1) or 20 mg (5) weekly. Median iCa variation from iCaT0 at 6 months of follow up (+/- 60 days) was -18% [-21 ; 3] in group 1 and -1% [-6 ; 3] in group 2 (P = 0.35). Median percentage of days spent with normocalcemia over total duration of follow-up was 66% [6 ; 18] in group 1 and 17% [17 ; 40] in group 2 (P = 0.106). Median time to iCa15% was significantly longer in group 1 (119 days) than in group 2 (median not reached; P = 0.02). Median times to iCa<1.40 were not significantly different between group 1 (80 days) and group 2 (150 days; P = 0.81). Severe hypophosphatemia was observed in one treated cat; alendronate was stopped. No other sign of toxicity was observed.

These results suggest that treatment with alendronate in IHC seems to be associated with a shorter time to a 15%-decrease of iCa from baseline, as compared with other (or no) treatment. Alendronate might be more indicated than other or no treatments for IHC.

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ESVNU-P-1

A prospective evaluation of contrast-induced nephropathy (CIN) in dogs
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Administration of intravenous iodinated contrast (IVIC) in humans has been causally associated with the development of acute kidney injury, known as contrast-induced nephropathy (CIN). Serum creatinine has been shown to increase 3-5 days after IVIC and kidney injury could range from subclinical forms to severe kidney failure. Scattered information exists in dogs in vitro as well as in laboratory studies. In a recent retrospective study, an increase in serum creatinine after IVIC was observed in dogs, however, a causal association was not demonstrated. A population of dogs undergoing computed tomography examination was prospectively evaluated for evidence of CIN after IVIC administration. Biochemical parameters (serum creatinine, blood urea nitrogen, total protein, albumin, chloride, phosphorus, potassium, calcium, sodium and symmetric dimethylarginine) and urinalysis (specific gravity, dipstick, sediment, protein/creatinine ratio, alkaline phosphatase/creatinine ratio, γ-glutamyl transferase/creatinine ratio) were evaluated at the time of IVIC administration (T0) and after 3-7 days (T1). Twenty-three dogs of different age, breed and sex were enrolled. Three dogs showed increased symmetric dimethylarginine and hyperphosphatemia at T1, whereas 6 dogs showed isosthenuria, cilindruria and proteinuria. An increased in serum creatinine >25% and γ-glutamyl transferase/creatinine ratio >50% from baseline was found in 2 and 4 dogs, respectively. None of these dogs had a pre-existing kidney disease. A significant difference between T0 and T1 for serum
Examination of serum hepcidin concentration in dogs with kidney disease

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Hepcidin is the key regulator hormone of the iron homeostasis. According to human studies, the serum hepcidin concentration in patients with kidney disease is frequently elevated, and the consequently evolved iron sequestration contributes the non-regenerative anemia and even may lead to erythropoietin resistance.

Our study aimed to measure serum hepcidin concentration in dogs with kidney disease; the hypothesis was that serum hepcidin in these sick dogs is elevated compared to healthy ones.

The study population included 21 dogs (7 with acute kidney injury [AKI] and 14 with chronic kidney disease [CKD]) from patients presented in the Small Animal Hospital Nephrology Service or Intensive Care Unit of the University of Veterinary Medicine - Budapest. Routine hematology, biochemistry (including C-reactive protein, iron, total iron-binding capacity) and urinalysis were performed by all patients. Left-over serum samples were used to measure hepcidin with liquid-chromatography tandem mass spectrometry method (LC/MS-MS). Results from our previous study evaluating serum hepcidin in 86 healthy dogs were used as control.

All dogs with AKI (7/7) and 50% of the dogs with CKD (7/14) had hepcidin concentration above the reference range, with mean hepcidin of 63.45 ng/mL (40.1-110.1) in AKI group and 38.45 ng/mL (17.7-66.9) in CKD group compared to the healthy population 16.6 ng/mL (2.3-41.1). The difference was significant in all dogs vs healthy (P < 0.001), in AKI vs healthy (P = 0.015), AKI vs CKD (P = 0.031), but not between the CKD and healthy groups (P = 0.067).

Serum hepcidin significantly correlated with C-reactive protein levels in the kidney disease population (P = 0.037, rho = 0.6142), but not with hematocrit, serum iron and iron-binding capacity.

This study showed that correspondingly to human studies, elevated serum hepcidin concentrations were frequently detected in dogs with kidney disease.

3D bladder ultrasound for estimation of urine volume in dogs vs. traditional 2D ultrasound methods

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Urinary bladder volume (UBV) and residual volume can provide important clinical information for hospitalized dogs and dogs with micturition disorders. UBV can be measured directly via urethral catheterization or indirectly via 2D ultrasound formulations. However, these techniques impose risk such as those associated with sedation, moderate restraint needed, catheter-associated urinary tract infections and/or need for appropriate operator skill and equipment. 3D ultrasound for point-of-care volumetric assessments of the urinary bladder is the method of choice for monitoring UBV in people but is not routinely performed in dogs.

The aim of this study was to validate the application of 3D ultrasound at small, medium, and large urinary volumes in dogs, compare measurements of 3D bladder estimation obtained by a novice to traditional 2D measurements by a board-certified veterinary radiologist, and compare time required for examination by 3D ultrasound to traditional 2D, B-mode ultrasound calculations.

In this prospective, experimental study, 10 laboratory-bred Beagle dogs were utilized for estimation of UBV. Bladders were infused with a calculated amount of sterile saline to represent small, medium, and large volumes. Each UBV was estimated and calculated by a boarded radiologist using 2D ultrasound followed by a 3D ultrasound device by a novice. Measured UBVs were compared to the instilled UBV for each method, and the two methods were compared to each other. Time from start to end of examination was recorded for both methods.

Use of 2D ultrasound overestimated infused UBV with a mean [SD] difference of 4.2 ml +/- 13.1 ml. The 3D ultrasound underestimated infused UBV with a mean difference [SD] of -9.8 ml +/- 9.8 ml. 3D ultrasound took less time to measure UBV with a mean of 80 seconds per measurement compared to 165 seconds per measurement for 2D.

The tested 3D ultrasound device is a safe, efficient, and clinically effective tool for measuring UBV in dogs. The device decreases need for operator skill or board certification, reduces time for bladder estimation and provides a quick estimate of bladder volume in real time.
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Use of 3D ultrasound for investigation of urinary retention in hospitalized dogs
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Urinary residual volume (URV) is the volume of urine remaining in the bladder immediately after the completion of micturition and is a clinically important measurement for assessing bladder function. URV >1 ml/kg can lead to serious clinical consequences such as detrusor atony and urinary tract infections. Multiple factors, such as anesthesia and surgery have been implicated to cause postoperative urinary retention (POUR) in people, which has led to routine bladder monitoring. Clinical application of a 3D ultrasound device has been used for point-of-care volumetric assessments and is routinely used for fast, ‘bedside’ estimation of urinary bladder volumes.

The aim of this study is to investigate the degree of urinary retention in hospitalized dogs using a 3D ultrasound device, and to describe various factors that could be contributing to urinary retention.

In this prospective, observational study, a total of 25 hospitalized dogs were enrolled. All dogs were hospitalized for more than 24 hours, weighed more than 5 kg, and had no concurrent urinary or neurologic disease that would affect their ability to ambulate or voluntarily urinate. Pre- and post-void bladder volumes were measured within 12 hours of presentation, and subsequent measurements were obtained during the length of their hospitalization at the same time daily. Use of a 3D ultrasound device was used to measure pre- and post-void urinary volumes.

URV was increased and urine retention was observed in all hospitalized dogs. Urine retention was observed in all dogs regardless of the length of hospitalization and the majority of dogs experienced the greatest degree of urinary retention on the second day. Of the 25 dogs enrolled, 18 dogs had an anesthetic event during their hospitalization. Of the 18 dogs who underwent anesthesia, 16/18 (88%) had a degree of urinary retention above the normal reference range (0.4 ml/kg) with an average URV of 4.34 ml/kg.

Urinary residual volume is an important clinical measurement and can be used as a direct parameter for monitoring bladder function and urine retention in dogs. Use of a safe and efficient ‘cage-side’ 3D ultrasound device to measure daily urinary bladder volume in hospitalized dogs could help in early identification of patients that are retaining urine, and ultimately prevent the effects of urinary retention. All dogs that undergo an anesthetic event during their hospitalization should be monitored for complete urine voiding and increased residual volume.

Disclosures

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ESVNU-P-5

Elevated blood creatinine: A biomarker of renal function—associates with multiple metabolic perturbations in dogs
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Chronic kidney disease (CKD) is a common, progressive disease in dogs. While the kidneys have multiple important metabolic functions, the occurrence of metabolic disturbances in canine CKD has not been extensively studied. Here we utilize a canine NMR metabolomics platform to identify metabolic changes in blood samples exhibiting elevated blood creatinine, a hallmark of CKD.

Clinical samples analyzed by the 1H NMR-based metabolomics platform were used as the base population. Twenty-three samples with creatinine over 125 μmol/l were included in the case group and 873 samples with creatinine within the analysis reference interval were included in the control group.

Biomarker association with elevated creatinine concentration was evaluated utilizing three different statistical approaches: Wilcoxon rank-sum test and logistic regression analysis (P-values FDR-corrected), and classification using random forest. A created heatmap visualized these changes. Means of the groups and their 95% CI were compared to reference intervals and histograms were plotted to further visualize the observed changes.

Each of the used statistical methods identified similar biomarkers associated with elevated creatinine concentration. The levels of 9 biomarkers—citrate, tyrosine, branched-chain amino acids, valine, leucine, albumin, acetate, linoleic acid % and the ratio of phenylalanine to tyrosine—were significantly different between cases and controls in the Wilcoxon rank-sum test, as well as the amino acid alanine. The Wilcoxon rank-sum test and logistic regression analysis (P < 0.05). The ten biomarkers with the highest variable importance in the random forest model were the same that reached significance in the Wilcoxon rank-sum test, as well as the amino acid alanine.

This study identified multiple metabolic changes associated with elevated blood creatinine, including prospective diagnostic markers and therapeutic targets. The NMR metabolomics platform is a promising tool for improving diagnostics and management of canine CKD. Further research is needed to verify the association of these changes to the patient’s clinical state.

Disclosures

The study was funded by PetBIOMICS Ltd. CO is an employee, KV a previous employee, and HL is an owner and the Chair of the Board of PetBIOMICS Ltd. AMM is the CEO, and NH a member of board of Movet Ltd. SS is an owner and CEO of Kuopio Animal Health Center Ltd. LJ is an owner and chairman of board of Punaturkki Ltd.

ESVNU-P-6

Effect of hypoxia on mineralocorticoid expression and activation in primary cultures of feline renal cortical fibroblasts and proximal tubular epithelial cells

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Chronic kidney disease (CKD) is the most common cause of mortality in ageing cats. Feline CKD is pathologically characterized by tubulointerstitial fibrosis and inflammation. Renal ischemia/hypoxia is proposed as an initiating and/or progression factor in feline CKD; hypoxia induces pro-fibrotic gene expression in feline renal cortical fibroblasts (FCFs) and changes consistent with epithelial-to-mesenchymal transition in feline proximal tubular epithelial cells (FPTECs) in vitro. Aldosterone is emerging as a contributor to renal fibrosis and inflammation and mineralocorticoid receptor (MR) blockade is beneficial in rodent models of renal ischemia/reperfusion injury. Data from in vitro studies investigating the effect of hypoxia on MR activation are limited and conflicting and appear to be cell specific. The aim of this study was to assess the effect of hypoxia on MR expression and activation (indicated by serum glucocorticoid-regulated kinase-1 [sgk1] expression) in FCFs and FPTECs.

Primary cultures of FCFs and FPTECs were isolated post-mortem from cats with (FCFs, n = 3) and without (FPTECs, n = 3) CKD. Cells were exposed to 24- or 72-hours hypoxia (1% oxygen) or normoxia (18% oxygen). One to three repeats were performed per isolate for each treatment and timepoint. MR and sgk1 mRNA expression (relative to reference gene [RPS7] expression) was assessed by reverse transcription-qPCR. Paired t-tests were used to compare gene expression between normoxic and hypoxic conditions; 24-hours hypoxia had no effect on MR and sgk1 expression compared to control conditions in either cell type. MR expression tended to decrease following 72-hours hypoxia in FCFs (mean ±standard deviation) fold change in gene expression 0.68 ±0.079, P = 0.0573) and decreased in FPTECs (0.49 ±0.097, P = 0.0352), whereas sgk-1 expression was decreased at this timepoint in FCFs (0.50 ±0.023, P = 0.0019) but not FPTECs (0.72 ±0.094, P = 0.0907). Importantly, responses in gene expression appeared to vary between isolates (i.e. individual cats) after 24-hours hypoxia whereas a consistent decrease was evident following 72-hours hypoxia.

These preliminary findings suggest that MR expression and activation decreases following prolonged hypoxia in FCFs and FPTECs. The effect of this downregulation on protein expression and response to renal injury requires further investigation.

Disclosures

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ESVNU-P-7

Palatability and tolerance evaluations of a new formulation of a supplement dedicated to maintain the balance of renal function in dogs and cats (Pronefra)

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Pronefra oral suspension (Virbac) is a supplement dedicated to maintain the balance of renal function of dogs and cats. It contains phosphate binders, chitosan to bind uremic toxins and a marine oligopeptide to help maintain a balanced blood pressure.

A new formulation of Pronefra with these main ingredients in olive and corprah oils, has been developed. The palatability and tolerance of this formulation were tested on healthy cats and dogs.

Palatability: In dogs, the supplement was added once with food of 38 dogs and the number of dogs taking the product and food was evaluated. For cats, the suspension was mixed with food of 83 cats daily for 7 days and the mean ration consumed with the supplement was compared to the usual ration (without the suspension).

Tolerance: The tolerance was evaluated for 28 days in healthy dogs and cats receiving either 1 time the recommended dose (0.25 and 0.2 ml/kg, BID – 8 cats and 8 dogs, respectively) or 5 times the recommended dose (8 cats and 8 dogs). Four cats, receiving no supplement, were used as control. The animals’ general health, food consumption and stools consistency were assessed daily. A complete clinical examination and body weight measurement were performed once a week. Blood samples were taken before the start and at the end of the study for standard hematology and blood biochemistry (plus SDMA).

Palatability results: all dogs (100%) took the product, 84% consumed more than 95% of the product and 92% of dogs totally consumed their kibbles. For cats, the food with product was accepted by 94% of cats with no impact on the mean food consumption on Day 7. The owners gave a mean acceptability score of 7.7/10 (median of 8/10) and 72% judged the acceptability was good enough to give it for 30 days.

Tolerance results: No product-related clinical signs were observed. The supplement did not affect body weight, food consumption or blood parameters. Therefore, Pronefra oral suspension is considered as very palatable and well tolerated by cats and dogs.

Disclosures

All authors are Virbac employees.
Use of 3D bladder ultrasound for characterization of urinary incontinence in male dogs
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Urinary incontinence in male dogs can be further subdivided into dogs with higher urinary bladder volume and urinary retention with USMI and the remaining 3 were diagnosed with ectopic ureters (EU). Dogs with evidence of overflow incontinence had a significantly higher post-void urine residual volume (URV) than those with USMI or EU (P = 0.04). Urinary incontinence in male dogs can be further sub-divided into dogs with urinary retention due to USMI or EU. Use of a safe and efficient 3D ultrasound device to measure post-void URV is a useful diagnostic tool at time of initial evaluation. A URV of >1 ml/kg can be utilized to begin treatment for detrusor atony prior to considering USMI as a cause of urinary incontinence in a male dog.

Disclosures
No disclosures to report.

Comparison between non-injected computed tomography and ultrasonography for detection of ureteral stones in the cat: a prospective study
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Computed tomographic scan (CT) is now considered as the gold standard in human medicine for renal colic. This prospective study aims to compare ultrasound (US) and CT for detection of ureteral stones in cats.

Fifty-one cats with a ureteral obstruction were included. An ultrasound followed by a non-injected CT were performed. The number of stones and their location (proximal, middle and distal) were recorded in both modalities and their numbers were compared with a Student test. Pelvic distension was measured on US only. Based on the US results, 3 groups were created: without stone, stone not detected, stone detected. Pyelic dilatation between the three groups was compared with a Wilcoxon test.

There are significantly more stones detected with the CT compared to US (126 versus 90; P < 0.05). More stones were detected in the proximal and distal regions (P < 0.05) with CT. Pyelic dilatation is significantly different between the 3 groups (1.2 mm [SD 1.5 mm], 3.4 mm [SD 2.2 mm] and 8.3 mm [SD 5.9 mm] in the "no stone", "stone not detected" and "stone detected" groups respectively).

CT seems to be more informative than ultrasound for detection of ureteral stones in cats, as in human medicine. Proximal and distal stones seem to be the most difficult to diagnose with US. Distension of the pelvis is more pronounced when a ureteral stone is detected. However, a ureteral stone should not be ruled out if there is no or a small distension of the pelvis.

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administration) and 28-day administration phase. A complete clinical examination was performed once a week. Food consumption and stool consistency were assessed daily. Blood samples were taken on Day -5 (during the acclimation phase) and on Day 28 for standard hematology and blood biochemistry.

Palatability results: 82/89 cats (92%) accepted to eat the food with the product and the mean food consumption did not change by day 7 for these cats. The mean acceptability score given by the 89 owners was 7.2/10 (median of 8/10) and 73% of owners judged the palatability was good enough to give the product over 30 days. Seventy-four percent (74%) of owners judged there was either no impact or a positive impact of the suspension on the cat’s eating behavior.

Tolerance results: no product-related clinical signs were observed and all cats remained healthy throughout the study. The supplement did not affect body weight, food consumption or blood parameters. In conclusion, this product developed to help maintain a healthy urinary tract in cats is therefore considered as very palatable and well tolerated by cats.

Disclosures
All authors are Virbac employees.

Usefulness of Serum Amyloid A in diagnosing pyelonephritis in cats
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Veterinary literature is scarce about pyelonephritis in cats, despite its probable underestimated prevalence. More specifically, diagnostic features are not well defined. They rely on the association of both evocative clinical and paraclinical modifications (e.g. fever, painful abdominal palpation, azotemia, bacteriuria), as well as ultrasonographic abnormalities (e.g. renal pelvic dilation). However, these signs are often non-specific. Definitive diagnosis is based on pyelocentesis or biopsy for bacterial culture, which remains technically challenging. Accurate diagnosis is crucial, considering both therapeutic and prognostic implications.

The aim of this retrospective study was to evaluate the utility of measuring serum amyloid A (SAA), a major positive acute phase response protein, as a marker of pyelonephritis in cats.

Medical records were reviewed and animals were classified in 2 groups. Group 1 included cats with confirmed (1a) or presumed (1b) pyelonephritis (1a: cats with positive bacterial culture on urine collected via pyelocentesis. 1b: cats fulfilling 3 out of 4 criteria among azotemia, hyperthermia, bacteriuria and pelvic dilation on ultrasound). Group 2 included cats with chronic kidney disease (CKD) in which pyelonephritis was either excluded (2a: negative urine culture via pyelocentesis) or considered unlikely (2b). Cats with incomplete medical data were excluded, as well as Abyssinian cats. Statistical analysis was performed using Mann-Whitney test, and differences were considered significant when P < 0.05. Variables are presented as medians [25th percentile; 75th percentile].

Forty cats (46 observations) were included in the study. Median age at presentation was 9 years old [6.3 ; 11.8]. Median SAA concentrations (reference interval: 0 – 12 mg/L) in group 1 (n = 13) and 2 (n = 33) were 127 mg/L [51.3 ; 216.8] and 5.6 mg/L [2.6 ; 9.7], respectively. Median SAA in group 1 was significantly higher than in group 2 (P = 0.0005). Group 2 included seven cats with ureteral obstruction, six with subclinical bacteriuria, and the remainder were presented for worsening of clinical condition or for regular recheck (CKD or subcutaneous ureteral bypass). Seven cats in group 2 had increased SAA. Among them, median SAA was 59.4 mg/L [25.2 ; 70]. In two of them, a patent extrarenal disease was identified. Two cats in group 1b showed SAA concentration within reference range.

These results suggest that cats with pyelonephritis are very likely to have higher SAA concentrations as compared with cats with CKD. SAA might be a valuable diagnostic tool for feline pyelonephritis.

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No disclosures to report.

Urine protein to creatinine ratio (UPC) in puppies and young dogs
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Some of hematological variables and biochemical analytes have specific reference intervals for puppies. Nevertheless, the literature concerning urine parameters in puppies is scarce. The purpose of this study was to determine the urine protein to creatinine ratio (UPC) in puppies and young dogs aged 2-12 months, compare the results with results of adult dogs and evaluate them according to the general reference interval for dogs.

In total, 176 voided urine samples of clinically healthy puppies, young dogs and adult dogs were enrolled. Five groups according to the age were created: puppies aged 2-3 months (n = 25), puppies aged 3-4 months (n = 22), puppies aged 4-6 months (n = 36), young dogs aged 6-12 months (n = 26), and adult dogs older than 1 year (67). To calculate the UPC, urine protein concentration was measured by use of benzethonium chloride; creatinine concentration was measured with Jaffe method; both in an automated analyzer (Abbott Architect c4000, Abbott Diagnostics). The UPC was classified in accordance with IRIS guidelines. Therefore, dogs with a UPC<0.2 were classified as nonproteinuric, dogs with a UPC ratio from 0.2 to 0.5 had borderline proteinuria, and dogs with a UPC ratio >0.5 had proteinuria.

In puppies aged 2-3 months, the mean UPC (± standard deviation) was 0.77±0.42 (range 0.24-2.25); in puppies aged 3-4 months, it was 0.57±0.45 (range 0.18-2.31), in puppies aged 4-6 months it was 0.23 ±0.14 (range 0.05-0.57), in young dogs aged 6-12 months it was 0.10±0.14 (range 0.05-0.57), in young dogs aged 6-12 months it was 0.10±0.14 (range 0.05-0.57).
Comparison of two quantitative methods for urine protein measurement used for calculation of urine protein to creatinine ratio (UPC)

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Urine protein to creatinine ratio (UPC) is considered as a gold standard for quantification of proteinuria in dogs and cats. For this ratio, urine creatinine concentration is mostly measured via the Jaffé method with picric acid. Nevertheless, various methods are reported for assessment of urine protein concentration. The aim of this study was to compare two commonly used quantitative methods for urine protein measurement.

Voided urine samples obtained from 67 dogs were used for this study. In all samples, creatinine concentration was determined by Jaffé method with automated biochemical analyzer Konelab 20i (Thermo Fisher Scientific). In paired samples, protein concentration was measured via turbidimetric method with benzethonium chloride (automated biochemical analyzer Abbott Architect c4000, Abbott Diagnostics) and using photometric method with pyrogallol red (automated biochemical analyzer Konelab 20i). Both methods were calibrated using Total protein Urine/Liquor calibrator with concentration 1300 mg/L. Urine protein to creatinine concentration was calculated for both methods. The UPC was classified in accordance with International Renal Interest Society (IRIS) guidelines. Therefore, samples with UPC < 0.2 were classified as nonproteinuric, samples with a UPC ratio from 0.2 to 0.5 as borderline proteinuria, and samples with a UPC ratio > 0.5 as with proteinuria. To compare results, Wilcoxon signed-rank test was used.

Median UPC calculated from results obtained by method with benzethonium chloride was 0.18 (range 0.02-1.19). In case of pyrogallol red method, median UPC was 0.09 (range 0.04-0.78). Method with benzethonium chloride gave us significantly higher results (p<0.001). According to the IRIS guidelines, in samples evaluated by method with benzethonium chloride 11 cases were classified as proteinuric, 19 had borderline proteinuria, and 37 were nonproteinuric. In samples assessed by pyrogallol red method, only one case was classified as proteinuric, 9 samples had borderline proteinuria, and 57 samples were nonproteinuric.

This study shows that urine protein measurement using different quantitative methods leads to significantly different results of UPC and it may have clinical consequences when general limits recommended by IRIS are adopted.

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Erythrocyte and platelet changes in dogs managed with hemodialysis

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In human medicine, different changes in some hematological parameters such as packed cell volume (PCV), hemoglobin concentration (HGB), red blood cell (RBC) (hemolysis, broken cells, lower hematocrit), platelet activation and thrombocytopenia have been reported in patients managed with hemodialysis (HD). These alterations could be due both to the severe uremia and to HD treatment (blood cell physical and chemical stress). The aim of the study was to evaluate erythrocyte and platelet changes in uremic dogs managed with HD. Seven dogs with Acute Kidney Injury, AKI or Acute on Chronic Kidney Disease, AKI/CKD were enrolled: three dogs were in stage IV and four in stage V, and managed with 25 HD sessions overall. Each dog at every pre and post HD treatment was evaluated with a complete blood count. The data collected were assayed with D’Agostino test for normality. In order to compare the values of RBC, HCT, HGB, red cells distribution width (RDW) mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) between pre- and post-HD Wilcoxon matched pairs test or paired t-test were used. Chi squared test was used to evaluate the difference on platelet estimation between pre- and post-HD treatment. Four males and 3 females of different breed, age and body weight were included. There were no statistically significant differences between pre and post HD treatment about RBC, HCT, HGB, red cells distribution width (RDW) mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW) between pre- and post-HD Wilcoxon matched pairs test or paired t-test were used. Chi squared test was used to evaluate the difference on platelet estimation between pre- and post-HD treatment. Four males and 3 females of different breed, age and body weight were included. There were no statistically significant differences between pre and post HD treatment about RBC, HCT, HGB and RDW. On the contrary, MCV pre-HD 61.9 fl (range 58.6-63.6) and post-HD 64.2 fl (range 59.3-65.4) and MCH values pre-HD 22.7 pg (range 21.7-23.2) and post-HD 23.3 pg (21.7-23.4) were significantly increased post-HD (P < 0.0001 and P = 0.002, respectively). The PLT pre-HD 181 K/mL (range 98-407.5) and post-HD 110 K/mL (range 58-422)
and PCT pre-HD 0.19% (range 0.13-0.35) and post-HD 0.15% (range 0.07-0.27) were significantly decreased post-HD (P < 0.0001). Chi squared test was not significant difference in platelet estimation between pre- and post-HD. Canine HD seems to induce changes in few RBC and PLT parameters. The increase of MCV and MCH were striking post-HD probably linked to the persistency of RBC in the fluid tonicity used in HD. The reduction of PLT count and the overall mass post-HD was significant probably due to the activation of coagulation process. These information could be useful to the clinician in order to adjust the fluid tonicity and the anticoagulant protocol used in HD dogs.

Disclosures
No disclosures to report.

ESVNU-P-17

Retrospective study of cystine lithiasis in dogs in France
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Cystine lithiasis are likely to form in case of cystinuria, which is secondary to a lack of reabsorption by the proximal convoluted tubule. North American studies show low prevalence among lithiasis in dogs (0.3-0.8%), unlike European publications (3.0 to 5.6%). This disease is still poorly described.

The aims of this retrospective, observational study were 1/ to describe the lithiasis characteristics and breed predispositions of dogs with cystine urolithiasis recruited from a French veterinary analysis laboratory over two years and 2/ to report epidemiological, clinical, parasitological and prognostic aspects of dogs diagnosed with cystine urolithiasis at our hospital. Dogs were included if stone analysis by infrared spectroscopy confirmed cystine composition (100%). Results are presented as percentage or odd ratio (OR) subjected to a 95% confidence interval, or as median subjected to interquartile range (IQR).

In the first population, 104 dogs were included. Cystine lithiasis represented 8.9% [7.7-10.1] of all analyzed stones (n = 2054). Stones were in the urethra in 68% [61.75] and in the bladder in 77% [71-83] of cases. Median number of stones was 7 (IQR: 12) and median size was 5mm (IQR: 6.25). 99.4% [98.4-100] of dogs were males and 90% [85-94] were entire. In dogs with lithiasis, English Bulldogs (OR: 55.62 [27.41-112.8]), American Staffordshire Terriers (OR: 38.22 [20.33-71.84]), French Bulldogs (OR: 14.84 [8.26-26.67]), Staffordshire Bull Terriers (OR: 13.46 [6.48-27.94]), Dachshunds (OR: 3.43 [1.72-6.82]) and Chihuahuas (OR: 2.41 [1.43-4.05]) were predisposed to have cystine lithiasis. 22% [16-28%] of dogs had recurrent stones. At our hospital, 25 dogs were included over twenty years. Overall diagnostic frequency was 0.021% [0.013-0.029]. All dogs were entire males. Sub-obstruction of urethra was present in 71% [53-89] of cases. Uroliths were radio-opaque in 74% [54-93]. Cystine crystals were observed on sediment examination in 35% [15-54] of cases. In 88% [74-100] of cases, a low purine food was prescribed; otherwise homemade diet or wet industrial diet was advised. Tiopronine, D-penicillamine, antibiotics and potassium citrate were respectively used in 60% [41-79], 28% [10-46], 24% [15-34] and 16% [2-37] of cases. D-penicillamine and potassium citrate were respectively interrupted in 1 out of 4 and 2 out of 8 cases for which follow-up was available. 40% [21-59] of dogs recurred once or more, up to 5 times for 1 case. Recurrence and treatment interruption were not associated. This study highlights the significant part of cystine among stones in French dogs and the unreported radio-opacity of the stones.

Disclosures
Blavier A and Nelaton C work for Royal Canin. Bogey-Lambert A and Sulter A work for Vet’Analys which is in partnership with Royal Canin.

ESVNU-P-18

Urinalysis alterations in dogs affected with urinary tract infection: A retrospective case/control study
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Urinary culture and antimicrobial susceptibility test are the gold standard in order to select a correct treatment in urinary tract infections (UTI). However, a complete urinalysis is the first line investigation in dogs with urinary symptoms and results can help the clinician in the diagnostic workup.

The aim of this study was to compare signalment and urinary parameters obtained from dogs with positive urine culture (“UTI-group”) to those with sterile culture (“nUTI-group”) in a population in which UTI was considered among the differential diagnosis.

Two-hundred-eighty-two culture and urinalysis results, from urine sampled by cystocentesis in 214 dogs, between 2013 and 2019, were included in this retrospective study. Statistical analysis was performed by chi-square, Wilcoxon or Kruskal-Wallis test using JMP 14 (SAS Inc., Cary, USA).

One-hundred-nine urine samples from 85 dogs were positive and 173 samples from 129 dogs were negative to culture. Single isolates were 92.7% and Escherichia coli was the main pathogen (50.5%).

Dogs in UTI-group were significantly (P < 0.01) older (9.8±4.2 years) compared to nUTI-group (7.6±4.6). No significant difference regarding breed and sex were found.

Urine appearance was predominantly yellow in both groups, but pale yellow (11% vs 5%) was overrepresented in UTI-group. Turbid aspect was predominant in UTI-group (32% vs 9%), but 35% of UTI-group samples was clear.

UTI-group had lower (P = 0.03) urine specific gravity (USG); pH was similar between groups.
Positivity to blood and hemoglobin was higher in UTI-group (P < 0.01) but negative results (44% and 60% respectively) were present in UTI-group and positive (28% and 17% respectively) in nUTI-group. Nitur test was positive in 13% of UTI-group and 0% in nUTI-group. Urinary red blood cells were not significantly different between groups. White blood cells (>5/hpf) were present in 72% of UTI-group and in 15% of nUTI-group showing a significant difference (P < 0.05).

Bacteria were detected in 75% of urinary sediments of UTI-group and apparently evident in 5% of nUTI-group. In the 27 dogs of UTI-group in which bacteria were not evident, USG ranged from 1002 to 1048 and resulted below 1014 in 11 cases.

Proteinuria staged according to IRIS guidelines was significantly different (P < 0.01) between groups: proteinuric and borderline proteinuric were respectively 44% and 28% in UTI-group, while in nUTI-group were 30% and 14%.

Although the set of found alterations can lead to a suspicion of infection, urinalysis is not diagnostic of UTI; based on these results, the diagnosis could be missed in at least 25% of patients.

Disclosures
No disclosures to report.

ESVNU-P-19

Cystoscopic-assisted urinary bladder lavage in male cats with recurrent urethral obstructions: Treatment and outcome in 9 cases
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Urethral obstruction (UO) is a common and potentially life-threatening complication in male cats with feline lower urinary tract disease (FLUTD). Consensus regarding the most effective medical treatment to prevent recurrence of UO is lacking. Recurrence of UO can lead to repeated hospitalization, increase risks of urethral injury secondary to catheterization or need for surgical intervention (perineal urethrostomy, PU).

The aim of this work is to report the use of cystoscopic-assisted urinary bladder lavage and the outcome in 9 male cats with recurrent UO.

Exclusion criteria were age less than 12 months, less than 2 episodes of UO, presence of any underlying disease different from FLUTD or existing PU.

All nine cats were DSH castrated males with a mean age of 5.3 years (2-12 years).

All nine cats showed recurrent pollakiuria and stranguria and 3/9 cats macroscopic hematuria. Mean duration of clinical signs was 15.6 months (1-48 months).

All cats were anesthetized and the urinary bladder was distended with warm saline infused through a preoperatively placed rigid polypropylene open-ended, 3.5 F, tomcat catheter. A mini-laparotomy incision on the midline, about half of the way between the pubis and the umbilicus, was performed to expose and secure the cranial region of the bladder to the abdominal wall.

A 2.4 mm rigid cystoscope was placed, within its 3.5 mm cannula, through a small incision made into the ventral wall of the urinary bladder.

Amorphous debris, mucous plugs, sand, blood clots and small size uroliths (<2mm) were flushed from the urinary bladder under high pressure saline solution infused through the catheter and removed with suction attached to the ingress/egress portal of the cannula. The scope was often withdrawn from its cannula to remove material trapped within the lumen.

All cats were discharged uneventfully with medical and dietary therapy depending on clinical condition, stress factors and urinalysis. Mean follow-up was 9.6 months (3-24 months). One cat had recurrence of UO and perineal urethrostomy was performed.

In this case series 8/9 cats (88.8%) showed long term remission of FLUTD, without recurrence of UO. Cystoscopic-assisted urinary bladder lavage might represent a more effective technique than decompressive cystocentesis or urethral catheterization in treating UO in male cats. Increased endoscopic visualization and high pressure saline flow allow more accurate removal of mucous plugs, clots and small uroliths reducing the risks for repeated catheterizations or need for PU.

Disclosures
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ESVNU-P-20

Increase in canine cystine urolithiasis in Norway
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Cystine is a dibasic amino acid, a dimer consisting of two molecules of the non-essential amino acid cysteine. Cystinuria occurs when there is insufficient reabsorption of dibasic amino acids (cystine, ornithine, lysine and arginine) in the renal proximal tubules. Compared to the other dibasic amino acids, cystine has low solubility in acidic urine and may form cystine crystals and uroliths. Mutations in two genes (SLC3A1 and SLC7A9) involved in the reabsorption of these amino acids in the proximal tubules have been identified in various breeds in addition to an androgen-dependent type of cystinuria which seems less breed dependent.

Due to a suspected increase in dogs with cystinuria in Norway, the aim of the present study was to investigate possible changes in the proportion of cystine uroliths among all analyses of canine uroliths performed from January 2010 until May 2019 in a reference laboratory.

A gradual increase in cystine uroliths was noted, constituting 12% (10/81) of the total number of uroliths analyzed in 2010, 11% (7/66) in 2011, 9% (7/80) in 2012, 7% (6/87) in 2013, 12% (10/84) in 2014, and 10% (8/80) in 2015.
There were 8 mixed-breed dogs, 1 unknown breed and 50 pure-breed dogs of 28 different breeds. Sixteen dogs (27%) were toy breed dogs (0-5 kg), 22 (37%) small breed dogs (5-10 kg), 9 (16%) medium breed dogs (10-25 kg) and 11 (19%) large breed dogs (25-45 kg). More than 80% of the dogs had PU/PD and more than 60% were lethargic and showed signs of inappetence. Vomiting was reported in 38% of the dogs.

Urinary specific gravity ranged from 1.000-1.060 (mean 1.018 and median 1.015). Aminoaciduria was examined and confirmed in 49/59 dogs. Proteinuria was detected by dipstick in 76%, hypokalemia was detected in 37% and hypophosphatemia in 21% of the dogs. Twenty-nine percent had serum creatinine levels above the reference interval. Blood gas analysis was performed in 35/59 dogs, of which 43% had acidosis and 33% had bicarbonate concentrations below the reference interval.

The majority of the dogs (90%) had ingested jerky treats. No other potential causes for aFS were detected.

Acquired Fanconi syndrome has previously not been described in Norwegian dogs. While clinicopathological findings are in line with existing literature, the present study included a larger proportion of medium and large breed dogs than previously reported.

Disclosures
No disclosures to report.

ESVNU-P-21

Outbreak of acquired Fanconi syndrome in dogs in Norway
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Fanconi syndrome (FS) is defined as a generalized inherited or acquired proximal renal tubulopathy, characterized by inadequate reabsorption of substances such as glucose, amino acids, bicarbonate, potassium, calcium, sodium, chloride, phosphate, magnesium, ketones and lactate. Metabolic acidosis and progressive renal failure may develop.

Acquired Fanconi syndrome (aFS) has been reported in association with several different causes including various infections, diseases, drugs and intoxications. In addition, aFS has been associated with ingestion of pet jerky treats of Chinese origin. Dogs diagnosed with aFS have been reported in North America, Australia, Asia and Europe. In the late autumn 2017, an increase in cases diagnosed with aFS was noted in Norway and confirmed by analysis of patient records from 2010-2017. Therefore, a national register for aFS in dogs was established.

The aim of the present retrospective study was to describe signalment and clinicopathological findings for 59 dogs with normoglycemic glucosuria registered between October 2015 and February 2019. The study sample consisted of 23 (39%) females and 36 (61%) males between 1 and 13 years of age. Mean and median age was 6.6 and 7 years, respectively.
and esomeprazole on validated in vitro human (LAD2) and canine (C2) neoplastic MC structure and cytokine expression.

The LAD2 line, which best models degranulation and cytokine release of neoplastic MCs, was evaluated along with the canine C2 line. Light and transmission electron microscopy along with immunofluorescence techniques were used to assess MC structure and cytokines (IL-3, 4, 6, 10, and 12, CXCL8, TNF-α, and IFN-γ) following vehicle-control, H2RA, or PPI treatment.

Concentration and time-dependent structural changes were observed in MCs following drug treatment, with more pronounced effects seen with esomeprazole. Granule morphology was dramatically altered, with some cells demonstrating loss of macronuclei and increased cytoplasmic vacuolization. A significant decrease in CXCL8 was seen only with esomeprazole (P < 0.01; ANOVA with Holm-Sidak).

Acid suppressants altered in vitro MC structure, but only esomeprazole reduced pro-inflammatory cytokine production. This work indicates that acid suppressants may directly impact neoplastic mast cells, and that selection of the optimal class of acid suppressant for use in dogs with MCTs, especially those with non-resectable tumors, requires further study in vivo.

Disclosures
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ESVONC-P-2

Do feline solid and cystic pancreas tumors influence different pancreatic lipases?

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In feline pancreatic tumors, solid and cystic growth has been described in detail. Additional inflammation was frequently seen in feline pancreatic neoplasms. The aim of the study was the evaluation of feline pancreatic lipase immunoreactivity (fPLI) and 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin)ester (DGGR) lipase in cats with solid and cystic pancreatic tumors. Corresponding tissue and serum from 21 cats with primary pancreatic tumors, routinely submitted between 2014 and 2019, were examined. The animals were 5–20 years old (median 11) and predominantly Domestic Shorthair (n = 14). FPLI (>3.5 μg/l) (n = 21) and DGGR lipase (>26 U/l) (n = 13) were measured in serum. Pancreatic tumors were macroscopically either cystic (n = 7) or solid (n = 14) and up to 16.0 x 7.0 x 7.0 cm in size. Histologically, all solid neoplasms were malignant, whereas cystic tumors were benign (n = 4) or malignant (n = 3). Mild (4 cystic, 7 solid), moderate (3 cystic, 4 solid) or severe (2 solid) lymphoplasmacellular (n = 7) or mixed (n = 13) pancreatitis was present. One cat showed no pancreatitis. FPLI values were mostly elevated (cystic 7/7, solid 11/14; 1.7–40.0 μg/l, median 14.3 μg/l), regardless of dignity, degree or character of additional pancreatitis. DGGR lipase (9.3–287.4 U/l, median 39.8 U/l) was increased in 9/13 cats. None showed solitary increased DGGR lipase. In conclusion, feline solid/cystic pancreatic tumors can cause elevation of fPLI and DGGR concentrations. For differentiation from pancreatitis, pathohistological examination is required. Increased blood values were probably due to epithelial cell transformation, because no correlation to character or degree of the pancreatitis was obvious.

Disclosures
No disclosures to report.

ESVONC-P-3

Tumors of the retrobulbar space in cats: 31 cases

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The objective of this retrospective case series is to describe the clinicopathological features of feline neoplasia involving the retrobulbar space.

Medical records of 31 cats diagnosed between 2007 and 2019 with neoplasia involving the retrobulbar space were reviewed. Signalment, reason for referral, physical and ophthalmological examination, imaging and pathological findings were recorded. The most common breed was a domestic short hair (21). Median age was 11 years (range: 2-18). Twenty-one cats were referred for ocular-related problems; 10 cats presented for non-ocular clinical signs only, most frequently respiratory signs (nasal discharge, sneezing and dyspnea), facial swelling/mass or anorexia.

The most common ocular findings included exophthalmos (16), serous ocular discharge (13), decreased retropulsion (13), third eyelid prolapse (12), periorbital swelling (6), anterior and/or posterior uveitis with associated decrease in vision (2). Cats with exophthalmos exhibited exposure keratitis and corneal ulcerations (6) with one cat presenting with a corneal perforation.

All cats underwent advanced imaging of the head including 3 MRI and 28 CT exams. In 4/31 cats, primary retrobulbar tumors were documented and in the remaining cases tumors with secondary retrobulbar extension were identified.

The diagnosis was achieved via cytology, histopathology or both in 11, 14 and 6 cases, respectively. Tumor types included lymphoma (19), carcinoma (7), sarcoma (3), extramedullary plasma cell tumor (1) and undifferentiated neoplasia (1).

In this study, secondary neoplastic local involvement of the retrobulbar space was most common, with intranasal neoplasia being the most frequent cause. Lymphoma was identified as the most frequent neoplasia involving the retrobulbar space in cats.
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ESVONC-P-4
Are severe adverse events commonly observed in dogs during cancer chemotherapy? A retrospective study on 155 dogs
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Severe adverse events (AE) might be induced by maximum tolerated dose chemotherapy and often require protocol modification, or even chemotherapy arrest which has an impact on the prognosis of cancer bearing pets and on owners’ acceptance. The aim of this retrospective study was to assess the rate and risk factors of severe AE secondary to chemotherapy in dogs. Medical records from dogs receiving chemotherapy between January 2013 and December 2018 were retrospectively reviewed. A causality link between chemotherapy and clinical and/or biological signs was established, mainly based on the chronology and compatibility with known AE. The severe AE were graded according to VCOG-CTCAE grading system. Multiple correspondence analysis and Fisher’s exact Chi-2 tests were performed. 155 dogs were included in the study. AE were reported at least once in 123/155 dogs (79.4%) and severe AE were observed in 70/155 dogs (45.2%). Among these dogs, 43/70 (58.9%) had gastro-intestinal and 30/70 (42.9%) had myelotoxic events. Severe AE led to delay and/or dose reduction in 46/70 dogs (65.7%), to molecule modification in 21/70 dogs (30%), to chemotherapy arrest in 12/70 dogs (17.1%) and to euthanasia or death in 9/70 dogs (12.9%). Multiple Correspondence Analysis showed relationship between the presence of severe AE, hematopoietic tumors, and L-COP chemotherapy for instance, but also an influence of the body condition score. Conversely, no association between AE and in charge clinician, age, gender or body weight was observed. These associations were further investigated. Significant relationship between occurrence of severe AE and tumor type (P < 0.005) or multi-agents chemotherapy protocol (P < 0.005) were observed. Contrary to previous studies, severe AE following chemotherapy and leading to modification of the chemotherapy regimen was relatively common in dogs.

Disclosures
No disclosures to report.

ESVONC-P-5
Retrospective comparative analysis of some clinical and clinicopathological features of canine lymphoma from Italy and Thailand
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Non-Hodgkin’s lymphoma is one of the most common hematopoietic tumor in dogs and represents 7-24% of all canine tumors. Middle-aged dogs and some purebreds (i.e. Boxer, Bull mastiff, etc.) are more affected. Few studies regarding the distributions of lymphoma subtypes in different countries have been published. The aim of this retrospective study was to compare some clinical data and the cyto-morphological aspects of canine lymphoma cases collected in two different countries, Italy (Pisa) and Thailand (Bangkok). This study included 192 dogs with lymphoma (cytologically and/or histologically diagnosed) collected at the Veterinary Teaching Hospital of Pisa (VTHP) between January 2010 and May 2017, and 436 dogs collected at the VTH of Bangkok (VTHB) between January 2015 and November 2017. The data analysed included breed, size (small, medium, and large), sex, age, and lymphoma classification (anatomo-clinical, tumor grade, immunophenotype). Differences for age have been evaluated by the Mann-Whitney test, while other parameters have been investigated with the Chi-squared or Fisher’s exact tests (P-value of <0.05 was statistically significant). Breeds affected by lymphoma were compared with the whole canine population presented at the same period in the two facilities. Döbermann and Rottweiler for VTHP and Golden Retriever for VTHB were significantly overrepresented. VTHB-dogs were considerably older (median 9 vs. 8 years) and were mostly small sized breeds compared to the population at VTHP. More than 90% of lymphomas were classified as high-grade in both groups. Multicentric lymphoma (83%) was significantly more frequent in the VTHP. Extra-nodal (34%) and cutaneous lymphomas (26%) were significantly more frequent in the VTHB. B-cell lymphomas (71%) were significantly more frequent in VTHP and T-cell lymphomas (34%) in VTHB. Striking differences were found in the signalment data and the higher frequency of cutaneous lymphomas in VTHB dogs should be pointed out. Moreover, such findings probably influenced the immunophenotype results, since almost all cutaneous forms were T-cell lymphomas. Different breed and size distribution, lifestyle and environmental factors could influence the two study populations, as shown by our results.

Disclosures
No disclosures to report.

ESVONC-P-6
Toceranib phosphate in the management of insulinoma in dogs
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Pancreatic tumors are uncommon in dogs, being those that arise from the islets of Langerhans (insulinoma: β cell insulin secreting tumors or
β cell carcinomas) the most representative. Insulinomas commonly metastasize to regional lymph nodes and liver (50% at presentation). Clinical signs are mainly due to hypoglycemia secondary to the increase of insulin secretion. Classical treatment is based on surgery and control of clinical signs due to that, so far, no specific medical treatment has shown clear efficacy. Toceranib phosphate is a tyrosine kinase inhibitor (TKI) that may inhibit angiogenesis and others kinase receptors involved in the development of the neoplasm and whose use in the management of neuroendocrine neoplasms has been consolidated in the last years. Dogs with insulinoma, treated with surgery and toceranib or toceranib as monotherapy, were included in this retrospective study. Diagnostic was performed by glucose and insulin serum levels, cytology and/or biopsy. Clinical stage was based on imaging techniques (mainly CT). All dogs received 2.5 mg/kg of toceranib in Monday-Wednesday-Friday schedule. Response to therapy was based on glycemic control and imaging monitoring. Descriptive data analysis and Kaplan-Meier survival function was calculated with IBM SPSS V22 software. Twenty dogs were included, 8 males (40%) and 12 females (60%). West Highland Withe Terrier was the most represented breed (6/20; 30%). Median age was 10.05 years (+/- 1.99). Ten (10/20; 50%) were treated with surgery and adjuvant toceranib and 10 (10/20; 50%) with toceranib as monotherapy. Median survival time was 577 days for the group managed with surgery and toceranib, and 984.80 days for the group which received toceranib as monotherapy, however no significative differences were observed between both groups (P = 0.85). The objective of this study was to evaluate retrospectively the efficacy of toceranib in the management of canine insulinoma, with or without surgery. Survival time was superior to previously report for both groups (381 days for surgery and toceranib, and 74 days for medical therapy; Tobin et al. 1999). In our study no differences in survival time were found in dogs that underwent surgery. However, the role of the surgery in canine Insulinomas should be clarified in prospective studies with a larger number of cases.

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ESVONC-P-8

Comparison between oral chlorambucil and dose-intense chemotherapy for the treatment of feline transmural low-grade alimentary T-cell lymphoma

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While several studies have been published on feline alimentary low-grade T-cell lymphoma of the mucosa, limited data are available for its transmural counterpart (tLGAL).

This retrospective study aimed at comparing clinical benefit (CB), response rate (RR) at 3 and 6 months, time to progression (TTP) and survival time (ST) of cats with tLGAL receiving chlorambucil-prednisolone (Chl-P) or dose-intense chemotherapy. tLGAL was defined as a neoplastic infiltrate composed of small T-cells extending markedly into the submucosa and muscularis propria.

Cats with newly-diagnosed, histologically and immunohistochemically-confirmed tLGAL that underwent complete staging work-up, had abdominal ultrasound performed at admission, received treatment, and had an adequate follow-up were included. Cats were classified into 2 groups: Chl-P and dose-intense chemotherapy. To determine treatment efficacy, the following variables were compared between groups: previous administration of corticosteroids, hematology, serum albumin, LDH, cobalamin, FIV/FELV status, presence of epitheliotropism, serosa infiltration, extra-intestinal involvement. RR was evaluated according to RECIST criteria; adverse treatment events (AEs) were recorded. Data were compared with non-parametric statistics or crosstabs (Fisher exact test). P < 0.05 was considered significant.

Fifteen cats were included. At admission vomit and/or diarrhea (median 60 days; range 2-700) were frequent. Three cats underwent enterectomy due to sub-occlusion. In 8 cats, LGAL extended to the serosa. Nine (60%) cats received Chl-P and 6 (40%) received dose-intense chemotherapy (5 CHOP, 1 lomustine). Enterectomy (P = 0.047) and previous steroid treatment (P = 0.047) were more common in the dose-intense chemotherapy group; otherwise, groups were well-balanced for all other variables. Overall, 12 (80%) cats obtained CB within a median of 22 days (range, 7-60) documented by the resolution or improvement of symptoms. Fourteen cats that were alive at 3 months were re-scanned: there were 2 complete and 9 partial remissions (RR 73%). At 6 months, 13 cats that were still alive were re-scanned: there were 6 complete and 5 partial remissions. Median TTP was 395 days (range, 58-997). At the end of the study, 8 (53%) cats were alive, 7 (47%) had died of lymphoma; median ST was 374 days (range, 58-1700). TTP was significantly longer for cats receiving Chl-P than dose-intensive chemotherapy (867 vs 200 days, respectively) (P = 0.028), whereas there was no significant difference between groups in CB, RR, ST and AEs.

Results of this study suggest that Chl-P and CHOP-based may be equally effective in tLGAL. Further prospective studies are warranted to confirm these findings given our small sample size and the retrospective nature of the data.

Disclosures
No disclosures to report.

ESVONC-P-9

The effect of age and body weight on the incidence of neutropenia in dogs receiving chemotherapy

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Old age and low body weight of cancer patients may cause veterinarians to reduce the dose of chemotherapy. However, reducing the dose of chemotherapy leads to a decrease in its effectiveness. In the literature, data on the effects of age and body weight on neutropenia as side effect of chemotherapy in dogs are controversial. The aim of our retrospective study was to determine whether old age (≥10 years) or low body weight (<11 kg) cause a more frequent occurrence of neutropenia. Statistical analysis was carried out using Pearson's Chi-squared test.

The medical database from 04.2003 to 08.2018 was analyzed for dogs receiving chemotherapy. Overall, 971 chemotherapy sessions were analyzed in 295 dogs. In dogs ≥10 years, 415 chemotherapy administrations resulted in 122 (29.4%) episodes of neutropenia. In patients <10 years, 556 chemotherapy administrations resulted in 204 (36.7%) episodes of neutropenia, a significant difference ($P = 0.021$). Younger dogs had more often neutropenia before chemotherapy compared to those ≥10 years old.

The weight of dogs receiving chemotherapy ranged from 1.8 kg to 55.6 kg. In patients <11 kg, 198 chemotherapy sessions and in patients ≥11 kg 773 chemotherapy sessions were analyzed. The doxorubicin administrations were excluded since the dose calculation of 1 mg/kg in patients <11 kg was used. 164 chemotherapy administrations in dogs <11 kg resulted in 66 (40.2%) episodes of neutropenia. In patients ≥11 kg, 600 chemotherapy administrations resulted in 190 (31.7%) episodes of neutropenia. The difference was statistically significant ($P = 0.049$).

This study suggests, that high age (≥10 years) does not increase the risk of chemotherapy induced neutropenia, but in contrast young dogs (<10 years) have a significantly increased risk of neutropenia. As expected, small dogs with a low body weight (<11 kg) have a significantly increased risk of neutropenia caused by other chemotherapeutics than doxorubicine.

Disclosures

No disclosures to report.

ESVONC-P-10

Collection, Culture, and Characterization of Canine Urothelial Carcinoma Organoids: Reverse Translational Clinical Research in the Veterinary Patient

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Urothelial carcinoma (UC) is the most common type of bladder cancer in both dogs and humans. UC is incurable with minimal treatment success due to tumor heterogeneity and frequency of distant metastases at the time of diagnosis. Dogs function as physiologically relevant models for UC in humans due to similarities in genetic predispositions, environmental risk factors, clinical presentation, responsiveness to common chemotherapeutics, and tumor molecular and behavioral phenotypes. Recent optimization of adult stem cell-derived organoid cultures in various species has shown an increasing value to reverse translational clinical research and personalized medicine.

This preliminary study aimed to culture and characterize UC organoids from urine collected from a canine clinical patient and characterize UC organoids based on shared histology and molecular markers of UC. Further, we aimed at developing assays for drug screening of chemotherapy to be used for precision-medicine purposes both in veterinary and human patients suffering from UC.

Free-catch urine was collected from one dog at time of UC diagnosis. Sample was centrifuged and supernatant was removed. Pellet was washed with phosphate-buffered saline (PBS), then incubated in complete chelating solution (CCS) with EDTA and plated in Matrigel for establishment of organoid culture within one week. Sub-samples of differentiated UC organoids were taken for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for metabolic activity assessment after incubation with chemotherapeutic agents. Remaining UC organoids were characterized with H&E, RNA in-situ hybridization (RNA-ISH), and immunohistochemistry (IHC) staining techniques.

Differentiated organoids showed structural similarity to UC tumor epithelium on H&E staining. RNA-ISH showed high expression of Keratin-7 (KT-7, a marker specific for urothelial epithelium) in UC organoids. Ki-67 (epithelial proliferation marker), vimentin (marker upregulated in metastatic UC) and CD44 (presumptive urothelial stem cell marker) were overexpressed in canine UC organoids, consistent with in vivo canine UC tissue and human muscle invasive bladder cancer tissue and organoids. Results from MTT assay on maintained, differentiated canine UC cultures demonstrated reduced metabolic activity of UC organoids after incubation with cisplatin for 24-48 hours. These preliminary results indicate that urine-derived canine UC organoids share histological and molecular similarities to UC tissue in vivo. In addition, we show proof-of-concept for a precision-medicine test using cisplatin on canine UC organoids. Collectively, these results show the potential value of the organoid technology for characterization of UC phenotype and treatment responsiveness as an emerging tool for personalized medicine applications in veterinary and human medicine.

Disclosures

No disclosures to report.

ISCAID-P-1

Chronic diarrhea as a main clinical sign of canine leishmaniosis: 22 cases

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Urothelial carcinoma (UC) is the most common type of bladder cancer in both dogs and humans. UC is incurable with minimal treatment success due to tumor heterogeneity and frequency of distant metastases at the time of diagnosis. Dogs function as physiologically relevant models for UC in humans due to similarities in genetic predispositions, environmental risk factors, clinical presentation, responsiveness to common chemotherapeutics, and tumor molecular and behavioral phenotypes. Recent optimization of adult stem cell-derived organoid cultures in various species has shown an increasing value to reverse translational clinical research and personalized medicine.

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Differentiated organoids showed structural similarity to UC tumor epithelium on H&E staining. RNA-ISH showed high expression of Keratin-7 (KT-7, a marker specific for urothelial epithelium) in UC organoids. Ki-67 (epithelial proliferation marker), vimentin (marker upregulated in metastatic UC) and CD44 (presumptive urothelial stem cell marker) were overexpressed in canine UC organoids, consistent with in vivo canine UC tissue and human muscle invasive bladder cancer tissue and organoids. Results from MTT assay on maintained, differentiated canine UC cultures demonstrated reduced metabolic activity of UC organoids after incubation with cisplatin for 24-48 hours. These preliminary results indicate that urine-derived canine UC organoids share histological and molecular similarities to UC tissue in vivo. In addition, we show proof-of-concept for a precision-medicine test using cisplatin on canine UC organoids. Collectively, these results show the potential value of the organoid technology for characterization of UC phenotype and treatment responsiveness as an emerging tool for personalized medicine applications in veterinary and human medicine.

Disclosures

No disclosures to report.
Chronic diarrhea, originating from either small or large intestine, is a clinical sign associated with canine leishmaniosis, varying from 3–8% to as high as 30% of prevalence. However, in the majority of the cases, its occurrence has been mostly associated with chronic kidney or liver disease. Furthermore, Leishmania organisms can also cause inflammation of the digestive tract in an isolated manner causing only chronic diarrhea, although it has been poorly documented in dogs.

The aim of this retrospective observational study was to describe dogs with mainly gastrointestinal clinical signs associated with a diagnosis of leishmaniosis by serology and/or identification of the agent by microscopy or PCR at four referral hospitals from endemic areas between 2006 and 2019. All selected cases had a complete medical record including CBC, biochemistry, urinalyses, and diagnostic tests for leishmaniosis. Exclusion criteria were evidence of renal or hepatic disease and previous gastrointestinal disease diagnosed.

Twenty-two dogs (4FN, 1MN, 5FE, 12ME; median age 4y) were included. Small bowel diarrhea was present in 6/22 (27%), large bowel diarrhea in 9/22 (41%), mixed diarrhea in 7/22 (32%). Vomiting, weight loss and, hyporexia were found in 4/22 (18%), 10/22 (45%) and 3/22 (14%), respectively. Ten dogs (45%) showed anemia that was more frequently non-regenerative 9/10 (90%). Hypoalbuminemia and hyperglobulinemia were a common finding (45%) (median serum albumin 2.1 g/dL) and (63%) (median serum globulin 5.4 g/dL) respectively. Abdominal ultrasound was performed in 18/22 dogs, which mostly in cold and humid climates (Northwest of Spain). Generally, Prevalence studies in Europe have described the presence of Babesia spp. in dogs divided by the total number of individual test result but negative specific individual test. Prevalence in this population referred to the total number of positive Babesia spp. dogs divided by the total number of individual dogs tested. A total of 481 samples were included, the overall prevalence was 5.1% (68% to B. Vogeli, 28% to B. canis, and one case (4%) of B. vulpes). Coinfections were not detected with other Babesia species. Prevalence studies in Europe have described the presence of B. canis mostly in cold and humid climates (Northwest of Spain). Generally, B. canis is transmitted by D. reticulatus which is frequently associated with these environments. However, B. canis has been also detected in I. hexagonus and R. sanguineus, that are easily found in the Mediterranean area. This fact may be the explanation of the current unexpected high prevalence of B. canis in our study. In conclusion, prevalence of Babesia spp. in Catalonia area was estimated 5.1%, including B. canis and B. vogeli and a single case of B. vulpes. It is important to consider Babesiosis in the differential diagnosis in dogs with suggestive clinical sings in Northeaster of Spain.

Disclosures
Raquel Santiago residency program has been sponsored by Idexx Laboratories

ISCAID-P-3

A study of 78 new Angiostrongylus cantonensis infections in Australian dogs
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Canine neuroangiostronglasis is caused by migration of Angiostrongylus cantonensis (rat lungworm) larvae through the central nervous system. In the present study, 78 dog owners from four referral veterinary hospitals in Sydney, Australia were recruited and a total of 947 A. cantonensis antigens were detected by ELISA. In all, 33 (43%) dogs had antibodies to the A. cantonensis antigens. The seroprevalence was highest in Sydney dogs with 15 (47%) seropositive dogs. This study highlights the importance of routine serological testing for A. cantonensis antibodies in Australia and underscores the need for public education on the importance of avoiding exposure to A. cantonensis-infested soil and leaf litter.
nervous system. It is an emerging infectious disease in New South Wales, Australia and endemic in coastal east Queensland. The aim of this study is to further define the epidemiology and clinical features of canine neuroangiostrongliasis in a large cohort of dogs (the largest cohort studied to date) and to determine which tests was more likely to confirm a presumptive diagnosis in dogs with eosinophilic pleocytosis in cerebrospinal fluid (CSF).

A total of 78 dogs were presumptively diagnosed with neuroangiostrongliasis (2010 to 2019). 58% of dogs were less than 6 months old. The gender breakdown was 51 males (18 neutered; 33 entire) and 26 females (13 spayed; 13 entire), with gender unrecorded for two patients. The striking preponderance of male dogs presumably reflects males being more exploratory and thus more likely to consume molluscs. There has been a progressive increase in the number of cases diagnosed each year over the study period, with 17 cases in 2019. Most cases were diagnosed in autumn or winter. The disease was rarely seen in summer.

CSF records were available from 71 dogs: cell counts ranged from 2 to 146,150 cells/μL (median 4,470). The percentage of eosinophils varied from 15% to 98% (median 83.5%). CSF was obtained from the cisterna magna in 65 dogs, from the lumbar cistern alone in six dogs and from both sites in 3 dogs. Nucleated cell counts in lumbar CSF was substantially higher than in the corresponding cisternal CSF, suggesting inflammation was more severe caudally. 54 leftover CSF samples were available for both ELISA testing (for antibodies) and qPCR (for larval DNA), 43 dogs (80%) were both ELISA and qPCR positive, 4 (7%) were ELISA positive but qPCR negative, while 7 (13%) were qPCR positive but ELISA negative. Of the qPCR positive cases, CT values ranged from 24 to 38.

Although the presentation of neuroangiostrongliasis is usually syndromic with hyperaesthesia and caudal spinal involvement, some cases are atypical with encephalitic signs including blindness. Preventative therapy consisting of monthly moxidectin should be considered to prevent dogs acquiring this infection. We are currently determining if qPCR can diagnose this disease using blood or urine as diagnostic specimens.

The Angio Detect™ rapid point-of-care immunochromatography test for A. vasorum was run on six canine CSF specimens from dogs with presumptive rat lungworm disease. All samples tested negative.

Disclosures
No disclosures to report.

ISCAID-P-4

Pharmacokinetic profile of oral dosing of mefloquine to cats, as a potential treatment for FIP
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Feline infectious peritonitis (FIP) is a fatal disease in cats induced by coronavirus. Treatment options are limited. In searching for antiviral agents against feline coronavirus, mefloquine, a human anti-malarial drug has been demonstrated to reduce the viral load of FIPV in vitro. The aim of this study was to investigate the pharmacokinetic profile of mefloquine when administered orally, twice weekly for two weeks. The second objective was to identify the changes in hematological and biochemical analytes and physiological responses during the dosing period.

On ethics approval, mefloquine was administered orally (62.5 mg per cat) to seven clinically normal, mature cats (3 males: 4 females) on day 0, 4, 7 and 10, ideally administered with food. Serial blood samples were collected at 0, 1, 2, 4, 8, 12, 24, 48, 96, 168 and 240 hour after the first dose. Blood samples at 96, 168 and 240 hour were taken prior to dosing. Hematology and biochemistry were performed at 0, 168 and 336 hours. Plasma samples were quantified for mefloquine concentrations by high performance liquid chromatography (HPLC).

Pharmacokinetic (PK) analysis of mefloquine plasma concentrations was undertaken using a non-compartmental analysis for 4 cats over the first 96 hours. A single oral dose of mefloquine resulted in a Cmax of 2.71 ug/mL at 15 hour (Tmax) while the plasma concentration reached 4.06 ug/mL at 240 hour after second dose of mefloquine was given with food. The elimination half-life of mefloquine over the first 96 hours is 224 hour (s.d. 51.6). Two cats vomited and were excluded from PK analysis over 96 hours. Another cat’s PK profile was excluded as mefloquine concentrations were much lower than the others and skewed the data.

Hematology results were unremarkable in all cats at all time. Biochemical analytes were also unremarkable other than a significant increase in serum symmetric dimethylarginine (SDMA) concentrations at 168 and 336 hour, compared to t = 0 hour in all cats using a repeated measures one-way ANOVA (P < 0.002) and Tukey’s multiple comparisons test (P < 0.05). All cats seemed clinically well and had normal appetites at the end of mefloquine dosing.

Disclosures
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Serological and molecular study of Borrelia infection in dogs from different areas in Spain
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Several Borrelia spp. are transmitted by ticks and cause relapsing fever in humans and domestic animals. The disease in humans manifests
with recurrent episodes of fever while fever, lethargy, anorexia, anemia and thrombocytopenia are encountered in dogs infected by relapsing fever spirochetes. Two species of relapsing fever *Borrelia* causing disease in dogs have been described in the Mediterranean basin: *Borrelia hispanica* infection has been sporadically documented in southern Spain and *Borrelia persica* infection in Israel and Iran. Although these species have been reported, little information is available about the real magnitude of this infection in Mediterranean basin.

The aim of this study was to investigate the prevalence of relapsing fever *Borrelia* in blood samples from dogs living in Spain. For this reason, quantitative detection of reactive antibodies against *B. persica* antigen was performed by ELISA. Moreover, the presence of *Borrelia* DNA in blood samples was also carried out by real time PCR. Residual samples from 289 dogs from different areas of Spain were investigated: Mallorca (n = 95), Cádiz (n = 99), Córdoba (n = 42) and Asturias (n = 53).

The seroprevalence of *Borrelia* spp. in Spain was 41.9%. The results of the serological study differed depending on the geographical location ([P < 0.001](#)). The highest percentage of seropositivity was in the Island of Mallorca (61.1%) followed by the southern locations, Cádiz (41.4%) and Córdoba (28.6%), while Asturias, the most northern location, presented the lowest result (18.9%). Furthermore, seropositivity to *B. persica* antigen was also associated with young dogs (under the age of 1 year; [P < 0.001](#)), hunting dogs ([P = 0.003](#)), presence of ectoparasites ([P = 0.001](#)) and presence of clinical signs ([P = 0.024](#)). The borreliae-specific PCRs performed were all negative.

This study shows the geographical differences in seroreactivity with *B. persica* antigen in Spain, with higher prevalence in southern locations when compared with northern locations. Moreover, dogs exposed to ectoparasites, living in rural areas and presenting clinical signs were more prone to have *Borrelia* spp. exposure. The highest seroprevalence found for *B. persica* antigen might indicate a high exposure of a similar relapsing fever *Borrelia* spp. or other Lyme borreliosis causing species in dogs in Spain.

Disclosures

No disclosures to report.

ISCAID-P-6

A new in-clinic titer test detects antibodies to canine distemper, adenovirus type-2, and parvovirus in 10 minutes with high accuracy

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VETSCAN Rapid Canine Titer is a novel lateral flow assay (LFA) for simultaneous detection of antibodies to canine distemper virus (CDV), canine adenovirus type-2 (CAV-2) and canine parvovirus (CPV) from vaccination or exposure. A positive result should correlate to protective antibody titers measured by serum neutralization (SN) (≥1:24 for CDV or ≥1:16 for CAV-2) or hemagglutination inhibition (HI) (≥1:80 for CPV) assay. A negative result should indicate antibody titers below the protective titer cut-off. This study aims to evaluate the diagnostic performance of the LFA compared to SN or HI titers determined by a reference laboratory. Sensitivity was estimated by testing sera from ≤20-weeks-old, healthy client-owned dogs (n = 662) vaccinated ≥1 month to ≤3 years prior for CDV, CAV-2, and CPV. Specificity was estimated by testing sera from client-owned dogs with titers below the cut-off (n = 39-114) and specific pathogen free (SPF) dogs (n = 150). Using SN or HI titers as the reference, LFA showed good performance (CDV: sensitivity 90.9%, specificity 87.1%, accuracy 89.7%; CAV-2: sensitivity 99.0%, specificity 83.1%, accuracy 95.3%; CPV: sensitivity 100.0%, specificity 84.8%, accuracy 96.3%). Using only SPF canine sera, the specificity was 94.0 – 98.0% for all analytes. Positive predictive values for CDV, CAV-2 and CPV were 93.6%, 95.1% and 95.3%, respectively, and negative predictive values were 82.1%, 96.3% and 100.0%, respectively. LFA agreement by Kappa statistic with reference method for CDV, CAV-2 and CPV were 0.768, 0.862 and 0.894, respectively. These data conclude that VETSCAN Rapid Canine Titer may be beneficial in helping veterinarians make informed pet-side vaccination decisions based on antibody titers.

Disclosures

This work was funded by Zoetis, Inc. All the authors are employed by Zoetis, and VETSCAN Rapid Canine Titer is a product of the company with a business and/or financial interest.

ISCAID-P-7

Detection of pathogens implicated in feline upper respiratory infections in cats without respiratory signs hospitalized in a veterinary teaching hospital

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Most agents implicated in feline upper respiratory infections (FURI) are highly transmissible and can be harbored by healthy and convalescent carriers. Feline calicivirus (FCV) can also persist in the environment for prolonged periods. Nosocomial infection through indirect contact with healthy cat carriers or transmission via hospital members and facilities are therefore often feared, especially considering that some hospitalized cats can be immunocompromised and that cats seen for elective surgical procedure are sometimes not vaccinated. This risk might also be increased in teaching hospitals due to high densities of animals and humans and potential suboptimal adherence to biosecurity protocols. However, to our knowledge, the carriage of pathogens implicated in FURI has not been evaluated in cats hospitalized in a teaching hospital.

This study aimed to evaluate the detection rate of pathogens implicated in FURI: feline calicivirus (FCV), feline herpesvirus type 1 (FHV1), influenza virus type A (FluA), *Chlamydophila felis* (Cf), *Bordetella bronchiseptica* (Bb), and *Mycoplasma felis* (Mf) in cats without...
signs of upper respiratory tract disease hospitalized at a veterinary teaching hospital.

Conjunctival and oropharyngeal swabs were prospectively sampled from 101 cats admitted between January and October 2019, without upper respiratory tract disease. Samples were evaluated for FURI agents’ detection using multiplex PCR. All positive results were verified by simplex PCR. Descriptive statistics were used.

Detection rate by multiplex testing was 24.7% for Mf, 12.9% for FCV, 4% for FHV1, 2% for Bb, 0.99% for Cf and 0% for FluA. Detection was confirmed in most cases by simplex PCR, leading to a detection rate of 20.8% (Mf), 8.9% (FCV) and 0.99% (FHV1, Bb and Cf). 41 cats tested positive for at least one respiratory agent by multiplex testing, with respectively 54, 43 and 2% of them tested positive for 1, 2 or 3 agents.

Nucleic acid from various FURI pathogens was isolated in almost of half of the cases, even though the detection rate for FCV and FHV1 was lower than previously reported in mult-cat household. Whether this detection is associated with persistent carriage and further disease development and whether cats were contaminated prior or during their hospital stay remains to be elucidated. Results of the present study highlight teaching hospital visit as a risk factor for FURI pathogens transmission.

Disclosures

Disclosures to report.

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ISCAID-P-8

Negative or low levels of antibodies in dogs with overt clinical disease associated with leishmaniasis; 12 cases

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In canine leishmaniasis (CanL), antibody titers are high when clinical signs are evident and there is a direct relationship between clinical score and serology. Only dogs with papular dermatitis or uveitis due to localized leishmaniasis are seronegative or have low serology, however, there is the suspect of a modest number of dogs with low or negative serology despite overt clinical signs of leishmaniasis other than the two above presentations.

For this purpose, dogs diagnosed with leishmaniasis with low or negative serology were reviewed. A total of 12 cases were finally included. Five dogs presented for chronic diarrhea (4 small and 1 large intestine). One of these had ascites due to protein-losing enteropathy and one was pancytopenic. Two dogs presented for lethargy, weight loss and systemic lymphadenomegaly and one of these also had multifocal alopecia and blepharitis. One dog had diffuse exfoliative dermatitis and ulcerations. Two dogs were referred for pyrexia of unknown origin (PUO). One of these also had thrombocytopenia and moderate non-regenerative anemia. Another dog presented for pancytopenia and one for suspected non-regenerative IMHA. Both dogs and those with PUO were on immunosuppressive dose of steroids at time of presentation. Serology was low in 5 cases and negative in 7; CanL was diagnosed based on a combination of the following: PCR (blood = 3, lymph nodes = 2, bone marrow = 1), bone marrow biopsy (n = 5), cytology of cutaneous lesions (2), lymph nodes (1), spleen (1) and liver (1). Two dogs with chronic diarrhea were diagnosed on histopathology with granulomatous ileitis and enterocolitis with abundant Leishmania amastigotes, respectively. All dogs were treated with a combination of allopurinol and meglumine antimoniate (11) or miltefosine (1) and the majority of them (9) had a good response. Serology titers remained low or negative in all cases with a favorable outcome.

If the antibody titer is low or negative, CanL is considered unlikely. However, as shown by the present study, there is a population of dogs with leishmaniasis that behaves differently. These dogs perhaps build a predominant Th1 immune response that probably is not completely effective against the infection, allowing amastigotes to disseminate. Four dogs were on immunosuppressive doses of steroids possibly causing a reduced humoral immune response.

In conclusion, this is the first report describing cases of CanL other than papular dermatitis or uveitis that, despite overt clinical signs, presented low or negative serology. This is important to consider when evaluating dogs with suspected leishmaniasis.

Disclosures

No disclosures to report.

ISCAID-P-9

Development and validation of a species-independent whole proteome tick-borne encephalitis virus antibody detection assay


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Tick-borne encephalitis virus (TBEV) is a single-stranded, positive-sense RNA virus of the Flaviviridae family and the causative agent of tick-borne encephalitis in dogs. TBEV rarely leads to clinical illness, but then is likely to have a fatal outcome. Clinical manifestation ranges between individuals and can be asymptomatic to febrile illness in the first phase and results in severe neurological complications like meningitis, meningoencephalitis or meningoencephalomyelitis in the second phase. An early detection of animals on risk would be preferable. To compare the protective role of anti-TBEV antibody responses in different hosts and between individuals, we developed a luciferase immunoprecipitation system (LIPS) antibody detection assay. Antibody-reactions from overall 399 dog samples from unselected inpatients of a university animal hospital located in Lower Saxony (Germany) were screened, resulting in a seroprevalence of 1 %. ELISA detected 4 positive and 4 questionable results. Validation of these samples by LIPS showed 5 positive results, confirming the 4 ELISA-positive samples and 1 of the questionable samples. Results obtained by ELISA and LIPS assay showed a correlation of 97.5 and 100 %, indicating a good sensitivity of the LIPS assay. Expression of all antigen fusion proteins was confirmed and appropriate assay performance was verified with intra- and inter-assay coefficients of variation of 21 % and 17 %, respectively. In conclusion, initial results indicate the LIPS assay to be a useful tool for detection of TBEV antibodies.

Disclosures
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ISCAID-P-10

Therapeutic approach to glomerulonephritis secondary to canine leishmaniosis in Portugal: a questionnaire-based survey
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Canine leishmaniosis (CanL) is endemic in southern Europe. Despite the LeishVet and other existing guidelines, there is an important inconsistency about the medical management of glomerulonephritis in dogs with CanL, particularly in advanced stages. This study aims to investigate the main therapeutic approach of veterinarians in Portugal, regarding stage IV CanL, with emphasis on glomerulonephritis management.

An online questionnaire was developed, including 24 to 65 questions, depending on the answering pathway of each respondent. The questionnaire mainly focused on the medical approach of clinical cases strategically elaborated to reflect five theoretical scenarios of the LeishVet staging classification. After internal validation, it was uploaded using an electronic platform and diffused online, over 8 weeks, via Portuguese social network veterinary groups. For this study, only answers concerning LeishVet stage IV were selected.

Eighty-six answers were obtained. Faced with a theoretical scenario of a dog with stage IV CanL, showing severe azotaemia (creatinine of 3.5 mg/dl [<1.4]) and proteinuria (urinary protein/creatinine ratio of 6.2 [0.5]), 72.1% of the respondents admitted prescribing the association of allopurinol with meglumine-antimoniate or miltefosine. From these, 67.7% would use allopurinol with miltefosine, while 32.3% would prefer allopurinol with meglumine-antimoniate. Single-therapy with allopurinol was considered by 3.5% of the respondents and 2.3% would only prescribe supportive therapy, without any other compound. Non-scientific evidence-based protocols were considered by 16.2% of the respondents, while the remaining 5.8 % elected euthanasia.

Concerning proteinuria management, 93.8% admitted treating it. From these, 97.4% would switch to a renal diet. While 78.9% preferred angiotensine-converting enzyme inhibitors (ACEIs), 13.2% prioritized angiotensin-receptor-blockers. A few respondents still mentioned calcium channel blockers (5.3%) and anti-thrombotic therapy (2.6%). The use of immunosuppressants was considered by 44.2% of the respondents, who tended to prioritize prednisolone (94.7%) or mofetil mycophenolate (5.3%).

This study highlights that the association of allopurinol and miltefosine is the current preferred protocol for the medical management of stage IV CanL in Portugal. This is probably due to the assumed lower nephrotoxic effect of miltefosine when compared with meglumine-antimoniate. This study also shows that ACEIs are still the first therapeutic choice for dogs with CanL and concurrent severe proteinuria. Almost half of the respondents admitted using prednisolone when CanL associated glomerulonephritis is suspected, probably due to an immune-mediated etiology. These findings reinforce the urgent need of guidelines reassessment for dogs with suspected immune-mediated glomerulonephritis secondary to CanL. Further studies are needed to extrapolate these conclusions to other European countries.

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ISCAID-P-11

A review of automated hand sanitizer dispensers in a teaching hospital
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Alcohol-based hand sanitizers are routinely used in healthcare establishments worldwide. Two milliliters of 85% ethanol gel is required to fulfill the FDA requirement of reducing bacterial contamination by at least 2 log10. Sanitizers used by the teaching hospital are metered to dispense 1.2mL, therefore 2 aliquots of minimum 1ml would fulfill these requirements. There is a risk that automated dispensers may not achieve these volumes, for example through large variation in the amount dispensed or operational issues.
This study aimed to assess if the dispensed volume fulfills FDA requirements and if the implementation of a role in maintaining the sanitizers improved dispenser efficacy.

Samples were collected from 15 automated dispensers set to dispense 1.2 mL per aliquot. Two aliquots of gel were collected, the weight and volume of gel dispensed for each aliquot were calculated, and any malfunctions with the dispenser recorded. Samples were collected daily on six consecutive days (time point 1). This was repeated immediately following the assignment of a role to monitor and service the sanitizers (time point 2), and again eight months post implementation of the role (time point 3).

Of the 270 aliquots (135 samples) collected, 54 (20%) and 216 (80%) were <1 mL and >1 mL, respectively. The mean volume dispensed in a single aliquot (1.092 mL, 95% confidence interval = 1.055 - 1.128 mL) was significantly different to the target (1.2 mL) (P = 1.05x10^-8). The volumes of sanitizer dispensed and the number of aliquots <1 mL did not change significantly between the three time points (P = 0.829 and P = 0.327, respectively). A significantly higher number of malfunctions were reported when aliquots of <1 mL were dispensed compared to when aliquots ≥1 mL were dispensed (P = 4.71x10^-7).

This study suggests that there is a high risk of inadequate hand sanitation when using the automated dispensers, as twenty percent of samples (paired-aliquots) fell below FDA requirements. This may increase the risk of nosocomial infections in patients and zoonotic disease transfer to staff. Using sanitizers automated to dispense larger volumes of sanitizer and encouraging self-reporting may reduce these risks more than implementing a dispenser servicing role.

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SCH-P-1

Hepatocyte ploidy in cats with and without hepatocellular carcinoma
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Hepatocellular carcinoma (HCC) is one of the most common causes of cancer related death in humans, yet it is an uncommon neoplasm in the domestic cat. The difference in prevalence and etiology between these two species is unexplained. Recent work in mice has shown that hepatocyte polyplody (>2N) is protective against the development of hepatocellular carcinoma. Further, human hepatocarcinogenesis is associated with a reduction in hepatocyte ploidy. Hepatocyte ploidy in the domestic cat has not been previously evaluated. Our research objective is to establish a baseline hepatocellular chromosome number in normal cats and cats with HCC. We hypothesize that hepatocyte ploidy is significantly different between these groups. Samples were selected from archival cases at two veterinary schools. Seven confirmed feline HCC cases and seven age and sex matched normal control cases were selected following review by a veterinary anatomical pathologists. Using a nuclear stain that stoichiometrically binds to DNA, fluorescence intensity was measured to determine hepatocellular ploidy. Neoplastic, peri-tumoral, and normal hepatocytes were analyzed. There was a significant difference between normal to peri-tumoral, normal to neoplastic, and neoplastic to peri-tumoral hepatocytes. In addition, there is a significant difference between the number of 2N vs. 4N cells in normal and peri-tumoral and neoplastic cells, and peri-tumoral to neoplastic. There is no statistical difference in the number of multinucleated cells between other groups. Normal liver has a greater number of 4N cells than neoplastic samples. This difference may contribute to development or be a cellular response to carcinogenesis.

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SCH-P-2

Serum 25-hydroxyvitamin D in dogs with gallbladder mucocele
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Gallbladder hypokinesia is believed to have a large contributory role to the development of gallbladder mucocele (GBM) in dogs. Ursodeoxycholic acid is a common medical intervention in dogs with GBM that are subclinical; however, there are no clinical trials that demonstrate effectiveness. Studies in humans have identified vitamin D deficiency as a cause for gallbladder hypokinesia and oral vitamin D supplementation resolves gallbladder stasis. These studies suggest that vitamin D could have potential causal and therapeutic roles in dogs with GBM. Therefore, this study had two objectives 1) to compare serum 25(OH)D concentrations between controls, clinical GBM, and subclinical GBM and 2) to determine if serum 25(OH)D concentrations are different based on ultrasonographic GBM type. A prospective, multi-center study was performed. Dogs with an ultrasonographic diagnosis of GBM were eligible for inclusion. Control dogs were deemed healthy based on history, physical examination, diagnostic test results, and an unremarkable ultrasonogram. Static images from ultrasonograms were reviewed by two boarded radiologists and a consensus GBM type (i.e., 1 through 5) was recorded. Dogs with GBM were classified as clinical or subclinical based on whether they exhibited biliary tract clinical signs in the 7 days preceding presentation. Serum 25(OH)D concentrations were measured with a commercially available chemiluminescence immunoassay. A Kruskal-Wallis test with a post-
hoch multiple comparison procedure was used for multiple comparisons. A P-value of <0.05 was considered significant.

Sixty dogs with GBM (clinical n = 16; subclinical, n = 44) and 20 controls were included in this study. Healthy controls had significantly greater serum 25(OH)D concentrations (median, IQR: 63.4, 53.6-86.0 ng/ml) than subclinical GBM dogs (51.2, 28.9-71.4 ng/ml, P = 0.024) and clinical GBM dogs (37.6, 35.6-63.4 ng/ml, P = 0.027). There was no difference in 25(OH)D concentration between subclinical and clinical GBM dogs (P = 1.00). Serum 25(OH)D concentrations were not significantly different based on GBM type (P = 0.056).

These data indicate that dogs with GBM have reduced serum vitamin D concentrations. In people, hypovitaminosis D results in gallbladder hypokinesia and these data might indicate a similar pathology in dogs. Additional studies are needed to assess if hypovitaminosis D in GBM dogs is a cause or effect of their biliary disease and investigate if vitamin D supplementation could be used as a preventative or treatment for GBM.

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SCH-P-3

Use of NanoString technology to evaluate gene expression patterns in dogs with neutrophilic cholangitis
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Canine neutrophilic cholangitis is a liver disease which has been identified with increased frequency over the last ten years. To date, the pathophysiological and immunological mechanisms underpinning this liver disease remain poorly characterized. The aim of this pilot study was to explore pathophysiological mechanisms by comparing the differential gene expression in liver tissue from dogs with cholangitis and healthy controls.

Dogs with neutrophilic cholangitis (n = 20) and healthy controls (n = 12) were identified by review of histopathology reports and formalin-fixed, paraffin-embedded (FFPE) liver tissue blocks were retrieved from the pathology archive of the University College Dublin Veterinary Hospital. Following deparaffinisation using Depaaffinisation Solution (Qiagen, Hilden, Germany), RNA was isolated using RNeasy FFPE kit (Qiagen). A custom codeset was designed to include 42 genes encompassing the pathways of fibrosis, apoptosis, cholestasis, oxidative injury and immune response, as well as six reference genes. The RNA concentration of all samples was adjusted to 20 ng/µl in preparation for analysis using the Nanostring nCounter Analysis System (Nanostring Technologies, Seattle, WA). The normalized gene expression was determined for each gene within each sample using nSolver 2.6 software (Nanostring Technologies).

In total, 18 (42.8%) of the 42 genes investigated were significantly over-expressed in the dogs with neutrophilic cholangitis compared to the control dogs. Genes with significantly increased expression included: BSEP, CTGF, CXCL8, CYBB, IL-10, IL-12, IL-33, JAK2, JAK3, KLRG1, STAT3, TGF-β1, TLR1, TLR2, TLR4, TLR6, TNFSF10, and TNF-α, (P < 0.002).

The up-regulation of genes associated with neutrophil chemotaxis (CXCL8), the innate immune response (TLR 1,2,4 and 6), JAK-STAT pathway (JAK2, JAK3, KLRG1, STAT3) and fibrosis (CTGF, IL-33, STAT3) offer insight into the aetiopathogenesis of this poorly characterized condition. The up-regulation of a number of the TLRs indicates that bacterial infection or bacterial proteins are likely to be a primary driving force in the pathological progression of this disease. This suggests that early intervention is warranted to reduce the inflammatory response and subsequent fibrotic processes that occur over the course of the disease.

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SCH-P-4

The lidocaine/monoethylglycylxylidide liver function test to assess shunt closure in dogs with attenuated congenital extrahepatic portosystemic shunts
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Commonly used liver function tests do not always normalize after successful attenuation of congenital extrahepatic portosystemic shunts (EHPSS) in dogs. In human medicine, the hepatic metabolism of lidocaine is used as a dynamic liver function test. The advantages of the use of lidocaine are its short elimination half-life and its high hepatic extraction ratio, that is blood flow dependent. This study aimed to assess whether the dynamic lidocaine/monoethylglycylxylidide (MEGX) liver function test would be a useful test to determine postoperative EHPSS closure.

Dogs with EHPSS were prospectively enrolled. The lidocaine/MEGX test was performed at diagnosis, and at 1, 3, and 6 months postoperatively. At each time point, 1 mg/kg lidocaine was injected intravenously and blood samples were taken before (T0) and 15 minutes after injection (T15). Plasma concentrations of lidocaine and its metabolite MEGX were determined using a validated LC-MS/MS method. Three months postoperatively, a transspenic portal scintigraphy was performed to determine EHPSS closure.

At T15, dogs with a closed EHPSS (n = 16) had significantly higher median MEGX concentrations at postoperative time points compared to diagnosis (P < 0.001), whereas no significant differences were
noticed for dogs with persistent shunting (n = 5). Sensitivity and specificity to determine shunt closure were 96.2% and 82.8%, respectively. The lidocaine/MEGX test is a promising, rapid and non-invasive dynamic liver function test that seems helpful to determine EHPSS closure in dogs. Dogs with a normal fasted ammonia concentration but high MEGX concentration at T15 after surgical attenuation are very likely to have a closed EHPSS, and therefore additional medical imaging to determine shunt closure is not of added value in the vast majority of cases. In dogs with persistent shunting, advanced medical imaging remains important to differentiate between persistent EHPSS and multiple acquired portosystemic shunts. Further studies in a larger cohort of dogs are warranted to consolidate these promising findings.

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**SCH-P-6**

**Bile acid and bilirubin measurement in canine peritoneal fluid samples with and without biliary tract rupture**

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Diagnosis of biliary tract rupture can be challenging in dogs. Abdominal ultrasound, cytology and serum bilirubin do not detect all cases and evidence supporting the use of fluid bilirubin concentration is lacking in the literature.

The aim of this study was to evaluate bile acid and bilirubin concentrations in peritoneal effusions of dogs and determine whether there were any differences in the concentration of these analytes between dogs with biliary tract rupture and other causes of ascites.

Thirty-seven dogs with peritoneal effusion were included in this prospective observational study and divided based on the presence or absence of biliary tract rupture, determined by ultrasonographic and surgical findings. Bile acids and bilirubin were measured in serum and peritoneal effusion of all cases, as well as fluid-to-serum bile acid and bilirubin ratio, fluid cell counts, protein measurement and cytologic evaluation.

Four dogs were diagnosed with gallbladder rupture (4/37, 11%). The most frequent other conditions were septic peritonitis (8/37, 22%), neoplastic conditions (7/37, 16%) and chronic gastrointestinal pathologies (4/37, 11%). Bile acid and bilirubin concentrations in serum and peritoneal fluid were significantly higher in the group with biliary tract rupture, as was the fluid-to-serum bile acid ratio (P < 0.05). Fluid-to-serum bilirubin ratio was not significantly different between groups. Receiver operating characteristic curve analysis identified the abdominal fluid bile acid concentration to be 100% sensitive and specific for the diagnosis of biliary tract rupture with a cut off above 769 μmol/l.

Based on this limited number of cases, determination of bile acids concentration in canine peritoneal effusion may be a highly sensitive and specific test for the diagnosis of biliary tract rupture. Furthermore, fluid-to-serum bilirubin ratio, which is commonly used, was found not to be significantly different between groups.

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**SCH-P-7**

**Culture and maintenance of well-differentiated canine hepatic organoids and urinary bladder organoids**


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Recent advances in 3D culture technology allow adult mammalian stem cells to exhibit their remarkable self-organizing properties in vitro. The resulting organoids reflect key structural and functional properties of organs and can therefore be used to model canine pathologies. Additionally, patient-derived organoids hold promise to predict drug response/toxicity in a personalized fashion and in combination with gene editing technology open up new avenues for regenerative medicine. Adult hepatic stem cells have previously been utilized to culture canine hepatic organoids termed “hepatoids”. However, previously described culture media maintain hepatoids in a cystic, pre-differentiated state that do not fully replicate in vivo biological functions. Our group has previously established culture and maintenance conditions for healthy and diseased (small and large) intestinal organoids in dogs. In this study, we aimed to refine culture conditions for healthy hepatic organoids to induce differentiation into budding organoids, which more closely reproduce the adult physiology of canine hepatocytes. Furthermore, we investigated whether the same culture media could be used to grow and maintain canine organoids from healthy urinary bladder tissues with the objective to expand the portfolio of available canine organoids for translational biomedical research.

Canine hepatic organoids were isolated from wedge biopsies of 3 healthy dogs. Culture media were adapted from our previously published protocols in enteroids without addition of recombinant human IGF-1, HGF or FGF-2. Our results show that we are able to achieve differentiation of cystic organoids into budding hepatoids. Organoid survival was confirmed over three passages and successful repeat cultivation after multiple cycles of freezing (-80°C)/thawing. Bladder biopsies from 2 healthy dogs were processed for stem cell isolation as previously reported by our group. Organoids were cultured in the same medium as described above and differentiated into budding organoids within 7-10 days of culture. After differentiation, bladder organoids displayed hollow structures or solid spheres, as previously described for mouse and human bladder organoids.

Findings from this study demonstrate that our stem cell culture medium developed for the culture of canine enteroids/colonoids can be used to establish in vitro organoid cell lines from multiple tissues,
including hepatic and bladder epithelial cells. This is the first report of successful organoid culture from healthy bladder tissues. Importantly, and as opposed to previous description from the literature, our medium induces differentiation of hepatic cystic organoids into budding, differentiated organoids. Next steps are to phenotypically and functionally characterize these canine hepatoids and bladder organoids for further biomedical applications.

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