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16.10-16.25

ESVC-O-8

Takano

# RESEARCH COMMUNICATIONS OF THE 28th ECVIM-CA CONGRESS

# Rotterdam, The Netherlands, 6<sup>th</sup> to 8<sup>th</sup> September 2018

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## **SCH - Society of Comparative Hepatology**

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## ESVIM - European Society of Veterinary Internal Medicine

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12.35-12.50	ISCAID-O-2	Beatty	Novel hepatitis B-like hepadnavirus identified in a feline immunodeficiency virus-infected domestic cat
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14.40-14.55	ISCAID-O-4	Francey	Reduction in incidence of canine leptospirosis in Switzerland correlates with the introduction of a new quadrivalent anti-leptospiral vaccine
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14.55-15.10	ESCG-O-3	Berman	Hypoalbuminaemia as a possible biomarker of gastro-intestinal lymphoma in cats
15.10-15.25	ESCG-O-4	Werner	Treatment of Dogs with Acute Uncomplicated Diarrhea with Amoxicillin Clavulanate: A Prospective, Placebo-controlled, Randomized, Blinded Treatment Trial
15.25-15.40	ESCG-O-5	Burchell	Faecal microbial transplantation in a canine model of haemorrhagic diarrhoea syndron
15.40-15.55	ESCG-O-6	Ziese	Longitudinal Assessment of Clostridium perfringens encoding NetF toxin in Dogs with Acute Haemorrhagic Diarrhoea Syndrome
16.30-16.45	ESCG-O-7	Whittemore	Gastrointestinal changes in healthy dogs receiving sustained clopidogrel, prednisone, or combination therapy
16.45-17.00	ESCG-O-8	Whittemore	Antibiotic-associated gastrointestinal signs in healthy dogs administered antibiotics with or without synbiotics
17.00-17.15	ESCG-O-9	Jergens	Glucocorticoid effects on microbial community structure in canine inflammatory bowel disease
17.15-17.30	ESCG-O-10	Cabrera Garc	iaDecreased serum soluble rage concentrations correlate with the severity of histologic lesions in dogs with chronic inflammatory enteropathies
17.30-17.45	ESCG-O-11	Febo	Evaluation of plasma endocannabinoids in dogs with primary chronic enteropathies
17.45-18.00	ESCG-O-12	Salavati Schmitz	Retrospective analysis of dogs with inflammatory protein-losing enteropathy reveals that second-line immunosuppression is only necessary in 1/3 of cases and treatment requirements or outcome cannot be predicted by routine clinicopathological data

## ESVE - European Society of Veterinary Endocrinology

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14.40-14.55	ESVE-O-2	Fabres	Evaluation of telmisartan administration as a suppression test for primary
			hyperaldosteronism diagnosis in cats



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15.25-15.40	ESVE-O-10	Prasinou	The erythrocyte membrane lipidome profile in healthy dogs and changes in dogs with diabetes mellitus		
15.40-15.55	ESVE-O-11	Blomqvist	Presence of T- and B-lymphocytes in the canine pituitary gland		
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16.45-17.00	ESVE-O-5	Ramsey	Haptoglobin and pre-trilostane cortisol as monitoring tools for the treatment of canine hyperadrenocorticism		
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ESVCP - European Society of Veterinary Clinical Pathology					

## ESVCP - European Society of Veterinary Clinical Pathology

## Saturday 8 September 2018

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11.20-11.35	ESVCP-O-4	Tangalidi	Evaluation of the paraoxonase-1 concentration in dogs with mitral valve disease
11.35-11.50	ESVCP-O-5	Prieto	Biological Variation of Symmetric Dimethylarginine and Other Biochemical Analytes in Healthy Cats
11.50-12.05	ESVCP-O-6	De Witte	Corrected calcium as a predictor of hypocalcaemia in hypoalbuminaemic dogs
12.05-12.20	ESVCP-O-7	Норе	Evaluation of the clinical utility of a point-of-care serum amyloid A (SAA) assay for the detection of inflammatory disease in dogs
12.20-12.35	ESVCP-O-8	Davison	Targeted serum metabolomic and lipidomic profiling in Miniature Schnauzers – a pilot study
12.35-12.50	ESVCP-O-9	Klainbart	Acute organophosphate and carbamate intoxication in 102 dogs - a retrospective study



## **VBPS - Veterinary Blood Pressure Society**

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11.35-11.50	VBPS-O-2	Archontakis	Comparison of Doppler ultrasonography and oscillometry with or without proprietary optimizations for non-invasive blood pressure measurement in conscious cats
11.50-12.05	VBPS-O-3	Archontakis	Comparison of two Doppler ultrasonography devices for measurement of

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Saturday 8 September 2018

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		laboratory test results

## **ESVC** - European Society of Veterinary Cardiology

ESVC-P-1	Carnabuci	Left shift of the ventricular mean electrical axis in healthy Doberman Pinschers
ESVC-P-2	Chang	Effects of a structured exercise program in sedentary dogs with asymptomatic myxomatous mitral valve disease: quality of life, and radiographic and echocardiographic parameters
ESVC-P-3	Parmentola	Indeterminate mean electrical axis of the QRS complex in the dog
ESVC-P-4	O'Shaughnessy	Clinical narrative analysis of clinical findings associated with measurement of N-terminal pro-B-type natriuretic peptide in dogs and cats
ESVC-P-5	Duarte	Adverse Effects of amlodipine on the treatment of heart failure in dogs with myxomatous mitral valve disease: preliminary results
ESVC-P-6	Roels	Assessment of pulmonary hypertension in dogs with angiostrongylosis before and after treatment
ESVC-P-7	Michalek	Serum uric acid concentrations in dogs with chronic heart failure and atrial fibrillation
ESVC-P-8	Michalek	Oxidative stress in cats with asymptomatic and symptomatic hypertrophic cardiomyopathy
ESVC-P-9	Lo	Is there an association between environmental factors and cardiovascular disease in dogs?
ESVC-P-10	Caivano	Longitudinal right ventricle strain and strain rate by 2-dimensional speckle tracking echocardiography in dogs with pulmonary hypertension
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## **ESVONC** - European Society of Veterinary Oncology

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ESVONC-P-2	Cueni	Methadone potentiates the effect of doxorubicin on a canine transitional carcinoma cell line



		Technical y International Control	
ESVONC-P-3	Ignatenko	Clinical manifestations and response to the therapy of extragenital intranasal form canine transmissible venereal sarcoma(CTVT): retrospective study 11 dogs in Ukraine	
ESVONC-P-4	Granziera	The role of electrochemotherapy in management of tumours of the head in dogs	
ESVONC-P-5	Cervone	Feasibility, safety and diagnostic yield of ultrasound-guided fine needle aspiration of cardiac masses in dogs	
ESVIM - European Soc	ciety of Veterinary	Internal Medicine	
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ESVIM-P-2	Roels	A questionnaire-based survey of owner-reported environment and care of West Highland white Terrier with or without idiopathic pulmonary fibrosis	
ESVIM-P-3	Fastrès	Investigation of serum Kebs von den lungen 6 (kl-6) concentration as a predisposing factor and in the diagnosis of canine idiopathic pulmonary fibrosis in the West Highland white Terrier	
ESVIM-P-4	Nivy	Trends in serum cobalamin, folate and total iron binding capacity concentrations in pregnant bitches and their association with hematological parameters and neonatal survival	
ESVIM-P-5	Dominguez Ruiz	Inter-clinician reliability of the respiratory physical examination in dogs and cats with abnormal breathing patterns	
ESVIM-P-6	Galiazzo	Endoscopic bronchial anatomy in the dog	
ESVIM-P-7	Kehl	A novel CMAH gene variant leading to blood type b in ragdolls	
ESVIM-P-8	Pierini	Evaluation of diet's effects and ability of the $Hemoccult^{\otimes}$ assay for the detection of faecal occult blood in healthy dogs	
ESVIM-P-9	Johnson	Eosinophilic lung disease in 86 dogs (2006-2016)	
ESVIM-P-10	Viitanen	Systemic and local immunoglobulin concentrations in Irish wolfhounds with recurrent bacterial pneumonia	
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ESVIM-P-14	Grotheer	Evaluation of long-term therapy in cats with feline asthma and chronic bronchitis	
ESVIM-P-15	Määttä	Matrix-metalloproteinase-7 activity in serum of West Highland White Terriers with idiopathic pulmonary fibrosis	
ESVIM-P-16	Johnson	Bronchoalveolar lavage lymphocytosis in dogs (2006-2016)	
ESVIM-P-17	Bazelle	Assessment of adverse effects of mycophenolate mofetil for treatment of immune mediated diseases in dogs	
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ESVIM-P-19	Cocci	Hyperplasia of the suprarytenoid pharyngeal fold in brachycephalic and nonbrachycephalic toy dogs: clinical presentation, treatment and outcome in 10 cases	
ISCAID - International Society for Companion Animal Infectious Diseases			
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Clinical and laboratory findings of sixty naturally Babesia gibsoni infected dogs

ISCAID-P-2

Su



ISCAID-P-3	Aboim	Blood Cultures and the detection of multidrug-resistant bacteria in companion animals		
ISCAID-P-4	Duque	Use of neutrophil gelatinase-associated lipocalin (NGAL) in the early diagnosis of renal damage in canine leishmaniasis		
ISCAID-P-5	Xenoulis	Seroepidemiology of feline leukemia virus (felv) and feline immunodeficiency virus (fiv) in cats in Greece		
ISCAID-P-6	Carreton	Clinical Picture of Cats Seropositive for Dirofilaria immitis in a Hyperendemic Area: Is this Feline Disease Still Being Misdiagnosed?		
ESVNU - European So	ciety of Veterinar	y Nephrology and Urology		
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ESVNU-P-2	Lauenstein-Bosse	e Evaluation of serum transforming growth factor beta 1 in cats with chronic kidney disease		
ESVNU-P-3	Moberg	Overweight and obesity did not predispose to subclinical bacteriuria in a mixed population of 152 middle aged and elderly cats – a prospective cross-sectional study		
ESVNU-P-4	Sørensen	In-house culture and susceptibility reduce antibiotic over-prescription in dogs with suspected cystitis – a randomized controlled trial		
ESVNU-P-5	Oscarson	Bacterial culture results from urine and ejaculates in healthy intact male dogs		
ESVNU-P-6	Riesgo	Retrospective study (2004-2017) of 137 uroliths diagnosed at the Complutense Veterinary Teaching Hospital		
ESVNU-P-7	Dokuzeylül	Clinical efficacy of the Marbofloxacin usage in dogs and cats diagnosed lower urinary tract disorders		
ESVNU-P-8	Sargent	Moderate dietary phosphate restriction and fibroblast growth factor 23 in healthy older cats		
ESVNU-P-9	Monari	Pattern of renal tubular damage and dysfunction in dogs with intrinsic acute kidney injury due to leptospirosis and other causes		
ESVNU-P-10	Scarpa	Big endothelin-1 in cats with CKD: preliminary evaluation		
ESVNU-P-11	Carreton	Evaluation of renal function in dogs infected by Dirofilaria immitis in relation to microfilaremia, parasite burden and pulmonary pressure		
ESVNU-P-12	Defauw	Evaluation of acute kidney injury in dogs with Babesia rossi infections		
ESVNU-P-13	Burchell	Salt and sugar in your larder make your kidneys work harder		
ESCG - European Society of Comparative Gastroenterology				
ESCG-P-1	Cerquetella	Proteomics of canine feces from healthy Boxer dogs: a pilot study		
ESCG-P-2	Hayashi	Elevated blood urea nitrogen and thrombocytopenia during treatment as prognostic factors for canine acute pancreatitis		
ESCG-P-3	Ohta	Expression of leucine rich alpha-2-glycoprotein (LRG) in the duodenal mucosa of dogs with chronic enteropathy		
ESCG-P-4	Loze	Differential diagnosis of ileocolic masses associated with intestinal obstruction in cats: A retrospective study of 8 cases (2015-2017)		
ESCG-P-5	Marolf	Comparison of computed tomographic angiography and ultrasonography in the diagnosis of acute canine pancreatitis		
ESCG-P-6	Allenspach	Canine jejunum and enteroids derived from healthy dogs are useful models to evaluate the gastrointestinal side effects of NSAIDs		
ESCG-P-7	Marsilio	Characterization of the fecal microbiome of cats with chronic enteropathy		



ESCG-P-8	Lavoué	Serine proteases activity in intestinal tissue of dogs with inflammatory bowel disease (IBD) - a new insight into the pathogenesis of IBD?
ESCG-P-9	Bertolani	Hypercobalaminemia in 144 samples from cats and dogs
ESVE - European Socie	ety of Veterinary I	Endocrinology
ESVE-P-1	Oliveira Leal	The use of ACTH-depot for ACTH stimulation test in dogs with hyperadrenocorticism under trilostane therapy: changes to the conventional protocol
ESVE-P-2	Le Boedec	Predictive model to estimate ionized calcium from routine serum biochemical profiles in cats
ESVE-P-3	Burchell	Episodic nocturnal hypoglycaemia in healthy New Zealand working dogs
ESVE-P-4	Wehner	Efficacy of orally administered anti-thyroid drugs for the treatment of hyperthyroid cats
ESVE-P-5	Lamoureux	latrogenic hypoadrenocorticism following treatment with trilostane for hyperadrenocorticism in dogs: a description of 10 cases
ESVE-P-6	Stammeleer	Evaluation of free T4 (fT4) measurement after equilibrium dialysis (FT4ED) and a chemiluminescent enzyme immunoassay (FT4CEIA) in hyperthyroid cats before and after treatment with radioiodine (131I)
ESVE-P-7	García del Real Torralva	Prevalence of hypokalemia in feline diabetes
ESVE-P-8	Pérez Alenza	Evolution of trilostane dosage in dogs with pituitary dependent hyperadrenocorticism during the first year of treatment
ESVE-P-9	García San José	Survival of dogs with naturally occurring hyperadrenocorticism
ESVE-P-10	Alonso Miguel	Efficacy and safety of toceranib phospate in dogs diagnosed with beta cell neoplasia
ESVE-P-11	Burlacu	Neural network based model for glycemic dynamics predictions in feline diabetes mellitus
ESVE-P-12	Carotenuto	Use of tandem mass spectrometry (LC-MS/MS) for the measurement of thyroid hormones in dogs with spontaneous hypothyroidism
ESVE-P-13	Malerba	Accuracy of a flash glucose monitoring system in dogs with diabetic ketoacidosis
ESVE-P-14	Rapastella	Predictors of response to pituitary radiotherapy in dogs with and without pituitary dependent hyperadrenocorticism
ESVE-P-15	Gónzalez Sanz	Telmisartan versus benazepril on the management of systemic hypertension in dogs with hyperadrenocorticism
ESVCP - European Soc	ciety of Veterinary	Clinical Pathology
ESVCP-P-1	Nivy	Efficacy of specific antivenin and fresh frozen plasma in reversing Daboia palaestinae venom-induced hemostatic derangement - an in vitro study
ESVCP-P-2	Gerke	Insulin-like growth factor-1 and 26S proteasome as possible biomarker for metabolic states in dogs suffering from chronic diseases
ESVCP-P-3	Marazzi	Preliminary investigation of haematological and biochemical parameters as prognostic markers in dogs with severe inflammation
ESVCP-P-4	Cantos Barreda	Changes in anti-leishmania antibody levels in serum and saliva after three months of treatment in dogs with clinical leishmaniosis
ESVCP-P-5	Breu	Prevalence of braf variant V450E in urine, smears, and biopsies of canine transitional cell carcinoma
ESVCP-P-6	Nielsen	Global measurement of coagulation in plasma from healthy cats – a potential tool for detection of thrombosis
ESVCP-P-7	Miglio	Breed Specific hemato - biochemical reference intervals for the adult hunting dogs using a blood donor data base

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ESVCP-P-8	Rubio	$ \label{thm:conditions}  Effects of different storage conditions on markers of oxidative stress in canine serum samples $
ESVCP-P-9	Franco-Martínez	The salivary proteome in dogs with canine parvovirosis
ESVCP-P-10	Gomez Fernandez-Blanco	Beta-hydroxybutyrate and adiponectin in overweight healthy dogs
ESVCP-P-11	Franco-Martínez	Validation of a new point-of-care immunoassay for serum cortisol measurement in canine serum samples
ESVCP-P-12	Vizi	Evaluation of a point-of-care veterinary hematocrit meter in 57 dogs
ESVCP-P-13	Lidbury	Analytical validation of a point-of-care assay for the measurement of serum C-reactive protein concentrations in dogs

## **ESVCN - European Society of Veterinary Comparative Nutrition**

ESVCN-P-1 Koizumi Studies on a new body condition scoring by morphometric methodin dogs (2nd report)

DOI: 10.1111/jvim.15372

# Journal of Veterinary Internal Medicine AC



## ABSTRACTS

## ESCG-O-1

Are clinical and paraclinical data helpful in discriminating feline lowgrade alimentary lymphoma from inflammatory bowel disease? A prospective study of 36 cases

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Feline low-grade alimentary lymphoma (LGAL) is the most frequent digestive neoplasia in cats, characterised by the infiltration of neoplastic small T-lymphocytes, typically in the small intestine. With rising prevalence, the disease is of growing concern to clinicians. Due to similarities with inflammatory bowel disease (IBD), establishing a diagnosis is challenging and may require extensive histology, immunohistochemistry and clonality analyses. However, these diagnostic tests are not broadly available. The aim of this study was therefore to determine whether usual clinical and/or paraclinical parameters may differentiate LGAL from IBD.

This prospective study was conducted in the same referral centre between July 2016 and February 2018. Owners gave their consent for investigations. Cats were included if they had a final diagnosis of LGAL or IBD, based on histological, immunohistochemical and clonality analyses of full-thickness intestinal biopsies. All cats underwent a standardized diagnostic workup including history and physical examination, biochemical analyses, complete blood count, cobalamin dosage, urinalysis and abdominal ultrasonography. Values were expressed as percentage and median. Statistical analyses were performed using Wilcoxon Mann-Whitney and Fisher's exact tests.

Eighteen cats were included in each group. Domestic-Shorthair was the most frequent breed represented (83% in LGAL: 72% in IBD). Median age and weight were similar between groups (13.3 vs 11.5 years; 3.9 vs 3.7 kg in LGAL and IBD respectively). Neutered males were over-represented in LGAL compared to IBD (83% vs 39%; p=0.015). Median duration of clinical signs was higher in cats with LGAL than in cats with IBD (365 vs 80 days; p<0.01). Diarrhoea was significantly more frequent in LGAL group (72% vs 28%; p=0.02). Hypocobalaminemia (< 200 pg/mL) was strongly associated with LGAL (73% vs 27%; p=0.04). Median serum albumin concentration was comparable in both groups (29 vs 30 g/L in LGAL and IBD respectively). Major ultrasonographic findings included a diffuse thickening of the intestinal muscularis layer in both groups. Slight abdominal

effusion and ieiunal lymphadenomegaly (defined by thickness superior to 6 mm) were more frequently noted in LGAL (44% vs 6%; p=0.02 and 61% vs 24%; p=0.04, respectively).

To our knowledge, this is the first prospective study comparing cats diagnosed with LGAL or IBD. Our results indicate that paraclinical data such as hypocobalaminemia, abdominal effusion and jejunal lymph node thickening seem to be associated with LGAL and should prompt the clinician to investigate for this emerging disease.

#### Disclosures

No disclosures to report.

## ESCG-O-2

Histopathological and molecular characterisation of feline low-grade alimentary lymphoma and inflammatory bowel disease: a prospective analysis of 36 cases

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In cats, differentiation between inflammatory bowel disease (IBD) and low-grade alimentary lymphoma (LGAL) is definitely a challenge for clinicians. Final diagnosis is a major issue as treatment and prognosis are different. To date, LGAL characterisation is based upon combined morphological and immunohistochemical analyses. Moreover, clonality assessment can be helpful for questionable cases. We therefore performed an extensive histopathological and molecular characterization of cats diagnosed with LGAL or IBD to identify differential features between both entities.

This prospective study was conducted, with owner compliance, in the same referral centre between July 2016 and February 2018. Cats were included if they had a final diagnosis of LGAL or IBD. Haematoxylin and Eosin staining and immunohistochemistry using CD3, BLA36, MAC387 and MIB-1 (Ki67) antibodies were carried out on fullthickness intestinal biopsies. Markers expressions in lamina propria and intra-epithelial lymphocytes (IEL) were assessed separately and quantified using a semi-quantitative scale. Blinded reading was performed by two board-certified veterinary pathologists and one pathologist specialised in human digestive low-grade proliferative disorders. All samples were subjected to clonality testing, based on PCR targeting of the CDR3 region of T-cell receptor gamma for T-cells (TCRy).

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Eighteen cats diagnosed with LGAL localised in the duodenum (n=3), jejunum (n=12), and ileum (n=3) and 18 cats with IBD localised in the duodenum (n=1), jejunum (n=16) and ileum (n=1) were included. Villous atrophy (p=0.015), lamina propria high cellular density (p<0.01), and lymphocytic cryptitis (p<0.01) were significantly more frequent in LGAL group. An apical-to-basal gradient of cellularity was strongly associated with LGAL (29% vs 0%; p=0.02). In contrast to BLA36, CD3 expression levels in lamina propria and IEL were significantly increased in LGAL cases (p<0.01), reflecting the monomorphic feature of tumor cell population. No difference in MAC387 expression was detected. Median values of Ki67 in lamina propria and IEL were significantly higher in the LGAL group (p<0.01). Clonality assessment revealed monoclonal TCRg rearrangement in 82% of the LGAL cases and in 56% of the IBD cases.

To our knowledge, this is the first prospective histological and immunohistochemical characterisation relying on separate analysis of mucosal compartments in cats diagnosed with LGAL or IBD. Based on these results, CD3, BLA36 and Ki67 measurements could be of interest to differentiate those similar clinical entities. Clonality testing should be interpreted with caution and in the light of histopathological data.

#### Disclosures

No disclosures to report.

#### ESCG-O-3

## Hypoalbuminaemia as a possible biomarker of gastro-intestinal lymphoma in cats

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Both gastrointestinal (GI) lymphoma and inflammatory bowel disease (IBD) are common and debilitating disorders of cats with similar clinical findings and often both will show intestinal thickening on abdominal ultrasound. The diagnosis and differentiation between GI lymphoma and IBD requires the histopathological evaluation of biopsy samples taken from the GI tract, obtained either via laparotomy (fullthickness surgical biopsies) or mucosal biopsies (obtained endoscopically). The latter, however, can still be difficult to conclusively differentiate IBD from GI lymphoma.

The purpose of this study was to compare the degree of hypoalbuminaemia in cats with GI lymphoma and IBD with the hypothesis that cats with GI lymphoma would have a lower serum albumin concentration in comparison to those with IBD.

The records of 38 client owned cats that had been diagnosed with either GI lymphoma or IBD on histopathology from endoscopically derived mucosal biopsies were retrospectively evaluated.

Of the 38 cats, 25 were diagnosed with GI lymphoma and 13 with IBD. In the GI lymphoma group, ages ranged from 3-19 years with a median 10.1; whereas in the IBD group, ages ranged from 1-13 years with a median of 10.5. There was no statistically difference between the groups. In the GI lymphoma group, serum albumin concentration ranged from 1.5-2.5 g/dl with a median of 2 g/dl; whereas in the IBD group, serum albumin concentrations ranged from 2.5-3.5 g/dl with a median of 2.75 g/dl. There was a statistically difference between the groups (p < 0.05).

The study concluded that cats with GI lymphoma have a lower serum albumin concentration than cats with IBD and therefore serum albumin concentrations can be used as biomarker to aid in differentiating GI lymphoma from IBD in cats.

#### Disclosures

No disclosures to report.

## ESCG-O-4

## Treatment of Dogs with Acute Uncomplicated Diarrhea with Amoxicillin Clavulanate: A Prospective, Placebo-controlled, Randomized, Blinded Treatment Trial

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Although antibiotics can promote antimicrobial resistance and adverse drug reactions, they are commonly used in dogs with uncomplicated acute diarrhea (AD). No studies have been performed proving a beneficial effect of antimicrobials in AD. Thus, the aim of this study was to evaluate a potential benefit of amoxicillin clavulanate in dogs with AD and the effect on the proportion of resistant fecal E. coli.

Sixteen dogs with AD were randomly divided into a treatment (amoxicillin clavulanate 12.5-25mg/kg PO q12h for 7 days) or placebo group (lactose powder q12h for 7 days). All dogs received the same standardised symptomatic therapy and diet. Severity of clinical signs based on a disease activity index was compared. Furthermore, semiquantitative measurement of amoxicillin-resistant E. coli in fecal samples (day 0, 6, and 30) was performed.

Every dog improved to a clinical activity score ≤ 3 (insignificant disease) within 1-6 days (median 2 days) after presentation. No significant difference between treatment groups concerning disease activity scores or any individual parameter (activity, appetite, stool constistency, defecation frequency) was observed (p≥0.466). The proportion of amoxicillin-resistant fecal E.coli increased significantly (p=0.031) from 0.0 on day 0 to 0.9 on day 6 only in the antibiotic group. On day 30 microbial resistance against E. coli was significantly higher (0.2 versus 0.0) in the antibiotic compared to the placebo group (p=0.001).

Dogs with AD recover within few days with symptomatic treatment and diet only. No clinical relevant benefit of treatment with amoxicillin clavulanate could be observed in this study.

## Disclosures

No disclosures to report.

## ESCG-O-5

## Faecal microbial transplantation in a canine model of haemorrhagic diarrhoea syndrome

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In people, faecal microbial transplantation (FMT) cures recurrent Clostridium difficile-associated colitis possibly through the recovery



of lost microbiota diversity. Similar FMT donor-recipient microbiota interactions in recipient sick dogs have not been reported. The authors hypothesized that a single FMT would temporarily increase microbiota diversity and aimed to determine if FMT can substantially alter the microbiota diversity for a month. The hypothesis was tested in a canine haemorrhagic diarrhoea syndrome (HDS) model, which is associated with gut dysbiosis. Eight dogs with clinical signs of HDS (1:1 ratio) were randomized to receive FMT (recipient) or saline (control) via colonoscopy. The Pielou alpha bacterial diversity indexes (PABDI) were compared (through sequencing of the bacterial 16S ribosomal RNA gene) in faecal samples from recipients, controls and matched donors obtained at admission (before FMT), discharge, and at 30-day recheck. We also compared a previously validated HDS clinical score between recipients and controls. The recipient PABDI differed substantially between the time of admission and discharge (P 0.02) and was similar to the donors' PABDI at discharge (P 0.39). However, the recipients' PABDI did not differ between the time of admission and recheck (P 0.77). In contrast, the PABDI of the donors and controls did not change at any point during the study. The HDS clinical score did not differ between recipients and controls (P 0.14). In conclusion, FMT transiently altered the recipients' bacterial diversity. Further studies are required to determine the number of repeated FMT procedures that will lead to a long-term increase in bacterial diversity.

#### Disclosures

No disclosures to report.

## ESCG-O-6

## Longitudinal Assessment of Clostridium perfringens encoding NetF toxin in Dogs with Acute Haemorrhagic Diarrhoea Syndrome

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Recent studies suggest a significant association between C. perfringens encoding NetF toxin (netF) and acute haemorrhagic diarrhoea syndrome (AHDS) in dogs, but nothing is known about the persistence of netF over time in those patients. The study aim was to evaluate the presence of netF and concurrent clinical signs in dogs with AHDS.

Twenty-five dogs with AHDS without signs of sepsis were treated symptomatically without antibiotics. Faecal samples were tested for netF by PCR on day 0, 3, 7, 14, 21, and 90. Clinical signs were assessed daily for the first 8 days using the canine haemorrhagic diarrhoea severity index (CHDSI). Statistical analysis (significance p<0.05) was performed with Chi-square test for number of dogs positive for netF at each time point and Friedman test for comparison of CHDSI over time.

On day 0, 13/23 dogs (57%) were positive for netF. Percentage of netF-positive dogs was significantly lower on all days compared to day 0 (p<0.001), with 5/23 dogs (22%) being positive on day 3, 2/25 (8%) on day 7, 0/19 (0%) on day 14, 1/19 (5%) on day 21, and 0/19 (0%) on day 90. On day 0, mean CHDSI was 13.1 (standard deviation 3.2), indicating severe AHDS. Mean CHDSI decreased significantly on day 3 (p=0.015) compared to day 0 and stayed below 5.0 after day 3 indicating rapid recovery.

In conclusion, a rapid decrease of netF and fast clinical recovery were observed in dogs with aseptic AHDS without antibiotic

#### Disclosures

No disclosures to report.

## ESCG-O-7

## Gastrointestinal changes in healthy dogs receiving sustained clopidogrel, prednisone, or combination therapy

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Dogs with immune-mediated hemolytic anemia are often administered glucocorticoids and clopidogrel. Although both medications can cause gastrointestinal bleeding in people, gastrointestinal effects of combination therapy in dogs are unknown. The aim of this study was to compare gastrointestinal changes among dogs administered clopidogrel, prednisone, and combination therapy.

A double-blinded, placebo-controlled trial was performed using 24 healthy adult dogs (median age 3 years, range 2-7)> Dogs were randomized to 4 groups: placebo, clopidogrel 2-3mg/kg/d, prednisone 2mg/kg/d, or combined prednisone/clopidogrel PO for 28 days. Attitude, food intake, vomiting, and fecal score were determined daily. Digitally-captured endoscopy was performed at baseline and days 14 and 28. Mucosal hemorrhages, punctate erosions (PE), invasive erosions (IE), and ulcers in the gastric body, cardia, incisura, and antrum were numerated by 2 blinded investigators (JCW, JT). A value of 201 was assigned for >200 lesions in a region. Clinicallysignificant bleeding was defined as ≥15 hemorrhages, ≥5 PE, ≥1 IE, or ≥1 ulcer. Results were compared among groups using mixed model, split-plot repeated measures ANOVAs and generalized estimating equation proportional odds models. P<0.05 was considered significant.

Clinical signs did not differ among treatment groups or over time. Clinically-significant bleeding, PE, and IE differed by group, week, and group\*week (PE only). Post-hoc analysis revealed significantly increased lesion scores over time in the prednisone [day 14, PE/IE 82 (7-369); day 28, PE/IE 47 (12-402)] and prednisone/clopidogrel [day 14, PE/IE 60 (4-402); day 28, PE/IE 44 (0-128)] groups versus placebo [day 14, PE/IE 2 (0-270); day 28, PE/IE 2 (0-5)] and clopidogrel [day 14, PE/IE 5 (1-45); day 28, PE/IE 0 (0-14)] groups. Ulcers only occurred in the prednisone (4/6) and prednisone/clopidogrel (3/6) groups. Dogs receiving prednisone and prednisone/clopidogrel were 12.2 and 8.2 times more likely, respectively, to develop clinically-significant bleeding than dogs receiving placebo.

Gastrointestinal bleeding occurs commonly in healthy dogs administered prednisone or prednisone/clopidogrel therapy, but not clopidogrel monotherapy. Though lesions were severe in many cases, they



were not accompanied by changes in attitude, food intake, vomiting, or fecal score.

#### Disclosures

No disclosures to report.

## ESCG-O-8

## Antibiotic-associated gastrointestinal signs in healthy dogs administered antibiotics with or without synbiotics

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Antibiotic-associated gastrointestinal signs (AAGS) occur in up to 60% of dogs receiving metronidazole. In people, combination antibiotic therapy increases the risk of AAGS, while synbiotic administration decreases AAGS occurrence. The objective of this study was to compare AAGS in dogs administered antibiotics followed by placebos or synbiotics.

A randomized, double-blinded, placebo-controlled cross-over with an 8-week washout was performed using 24 healthy research dogs. After a 1-week baseline period, dogs received enrofloxacin (10mg/kg qd) and metronidazole (12.5mg/kg BID) with food for 3 weeks. One hour after each dose of antibiotics, dogs were administered placebos or synbiotics (Proviable®-Forte with Proviable®-SB). Mean food intake; days of hyporexia (food intake<50%), vomiting, or diarrhea (fecal score ≥6); and mean fecal score (1-7) per week were compared between groups using mixed model, repeated measure, cross-over ANOVAs. P<0.05 was considered significant.

Two dogs were excluded for high baseline fecal scoring (1 per group); 1 dog was withdrawn in each treatment period due to the severity of her AAGS. Hyporexia, vomiting, and diarrhea occurred in 42%, 83%, and 100%, respectively, of antibiotic-naïve dogs receiving the placebo. Food intake differed by treatment (F=7.0, P=0.02) and period (F=18.8, P<0.01), with less derangement in food intake in dogs receiving synbiotics. Although days of hyporexia did not differ between treatment groups (P=0.06), it was less common in period 2 (F=4.4, P=0.04). Days of vomiting (F=4.7, P<0.01) and fecal score (F=17.9, P<0.01) differed by week of treatment but not by treatment received. Fecal scores were significantly lower during period 2 (F=9.9, P<0.01).

Enrofloxacin/metronidazole administration resulted in an unexpectedly high incidence of AAGS in healthy dogs. Administration of synbiotics 1 hour after antibiotics mitigated food intake derangements. Period effects suggest that clinical effects of synbiotics persisted more than 9 weeks after discontinuation, decreasing AAGS in dogs that subsequently receive antibiotics alone.

## Disclosures

Disclosures to report.

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## ESCG-O-9

## Glucocorticoid effects on microbial community structure in canine inflammatory bowel disease

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The pathogenesis of canine inflammatory bowel disease (IBD) likely involves complex interaction between the mucosal immune system and the intestinal microbiota. While one study compared the intestinal microbiota of dogs with IBD and food-responsive diarrhea before and after treatment, there are no studies reporting changes in microbial community structure following glucocorticoid therapy. The objective of this study was to evaluate the effects of oral prednisone on the spatial distribution of mucosal bacteria in IBD dogs.

Eight dogs diagnosed with moderate-to-severe IBD (CIBDAI score > 6) were treated with immunosuppressive doses of glucocorticoids, (oral prednisone 1-2 mg/kg PO every 24 hours for 21 days, then tapered gradually to 0.5 mg/kg at 8 weeks as a typical protocol), for induction of clinical remission. The mucosal microbiota from endoscopic intestinal biopsies of IBD dogs and controls (n=15 dogs) was evaluated by fluorescence in situ hybridization (FISH) targeting the 16S rRNA genes of total bacteria, group-specific organisms, and individual bacterial species shown to be relevant in canine/human IBD. Epithelial tight junction protein (TJP) expression was studied using immunohistochemistry to investigate the effect of drug therapy on intestinal barrier integrity.

All IBD dogs showed a reduction in GI signs following 8 weeks of prednisone therapy compared with baseline CIBDAI scores (p<0.05). The mucosa-associated microbiota in the intestines of healthy and diseased dogs was most abundant in free and adherent mucus. In IBD dogs at diagnosis, the total number of EUB-338 positive bacteria, Faecalibacterium spp., and Streptococcus spp. was decreased (p<0.05) compared to healthy dogs. The spatial distribution of mucosal bacteria was significantly different (p<0.05) in IBD dogs following prednisone therapy, with higher numbers of total bacteria, Bifidobacterium spp., Faecalibacterium spp., and Streptococcus spp. detected predominantly within adherent mucus compared to other mucosal compartments. Relative to other bacterial groups, the number of mucosal Enterobacteriaceae was decreased (p<0.05) in prednisone-treated IBD dogs. The expression of TJPs occludin and zonulin was increased (p<0.05) in IBD versus healthy dogs; however, only occludin was increased (p<0.05) in IBD dogs following prednisone therapy.

In conclusion, the spatial distribution of mucosal bacteria differs between IBD dogs and healthy dogs and in response to glucocorticoid administration. Oral prednisone therapy was associated with positive clinical outcome and changes in microbial community structure but not TJP expression. Analysis of intestinal microbiota using FISH provides valuable insights on the abundance and spatial distribution of mucosal bacteria which interact most closely with the intestinal epithelium.

## **Disclosures**

No disclosures to report.



## ESCG-O-10

Decreased serum soluble rage concentrations correlate with the severity of histologic lesions in dogs with chronic inflammatory enteropathies

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Chronic inflammatory enteropathies (CIE) are an important group of diseases in dogs, and the innate immunity plays a key role in CIE pathogenesis. The receptor of advanced glycation end products (RAGE), which belongs to the innate immune system, is a pattern-recognition receptor recognizing pathogen-associated and endogenous molecular structures released with inflammation or tissue damage. In a pilot study, systemic concentrations of the decoy receptor sRAGE (soluble RAGE) were decreased in dogs with CIE and normalized only in those dogs with clinical remission after induction therapy. However, the association between serum sRAGE, disease severity, and other inflammatory biomarkers has not been extensively studied in canine CIE. This study aimed to evaluate serum sRAGE concentrations in a large group of dogs with CIE.

Serum and fecal samples from 102 dogs diagnosed with CIE were used for this study. Serum sRAGE concentrations were measured by ELISA and were compared among different groups of dogs using nonparametric group comparisons. A potential relationship of serum sRAGE levels with clinical disease activity (CCECAI scoring system), serum and fecal inflammatory marker concentrations, and histologic lesion severity (4-point semi-quantitative grading system) was assessed using a Spearman rank-sum correlation coefficient. Statistical significance was set at P<0.05.

Serum sRAGE concentrations ranged from 52-3,260 ng/L (median: 287 ng/L) in all CIE dogs, with 65 dogs (64%) having a serum sRAGE concentration ≤340 ng/L (cut-off previously best separating CIE dogs from healthy controls). Serum sRAGE levels were not associated with the severity of clinical signs (CCECAI scores), serum CRP, serum and fecal calprotectin, and fecal \$100A12 concentrations (all P>0.05). Serum sRAGE and S100A12 concentrations tended to be inversely correlated, but statistical significance for this association was not reached (P=0.095). The overall histologic score was not associated with serum sRAGE levels (P=0.157), but microscopic lesions in the duodenum were more severe in dogs with serum sRAGE levels ≤340 ng/L (P=0.013). Serum sRAGE was significantly and inversely correlated with the severity of lymphoplasmacytic infiltration in the gastric antrum (P=0.038) and duodenum (P=0.017), and with crypt dilation (P=0.027) and the neutrophilic infiltrate in the duodenum (P=0.044).

This study confirms that the majority of CIE dogs have decreased serum sRAGE concentrations, suggesting that the sRAGE/RAGE axis plays a role in canine CIE, and that sRAGE likely functions as a nonspecific decoy receptor. Further research is warranted to assess the gastrointestinal (and particularly the duodenal) mucosal expression of RAGE in dogs with CIE and in healthy controls.

#### Disclosures

Disclosures to report.

Dr. Steiner and Dr. Suchodolski are directors of the Gastrointestinal Laboratory at Texas A&M University where CRP, calprotectin, and S100A12 testing is offered on a fee-for-service basis.

## **ESCG-O-11**

Evaluation of plasma endocannabinoids in dogs with primary chronic enteropathies

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Diagnosis of canine chronic enteropathies (CE) is challenging and requires a thorough clinical, laboratory and histological evaluation. Nevertheless, disorders related to CE are further retrospectively classified by their responsiveness to sequential therapeutic trials. Convincing evidence suggests that the endocannabinoid (eCB) system is expressed in the gut and that eCBs can modulate major gastrointestinal functions. The purposes of the study were to estimate plasma levels of 2-arachidonoylglycerol (2-AG), arachidonoylethanolamide (anandamide, AEA), palmitoylethanolamide (PEA), and oleoylethanolamide (OEA) in healthy dogs, to ascertain their potential as diagnostic markers of canine CEs. Dogs with CE were divided into 4 groups: Food Responsive (FRE) and Antibiotic Responsive Enteropathy (ARE), Idiopathic Inflammatory Bowel Disease (IBD) and Protein Losing Enteropathy (PLE). Plasmatic 2-AG, AEA, PEA and OEA levels were determined by liquid chromatography/mass spectrometry (LC-MS). All data were expressed in pmol/ml as medians (interquartile range), and were compared using non-parametric tests. The diagnostic accuracy was assessed by a ROC-curve. P-values <0.05 were considered significant.

In healthy dogs (CONTROL, n=30) plasma levels of 2-AG, AEA, PEA and OEA were: 4.1 (2.80-6.40), 1.7 (1.50-2.20), 24.5 (17.50-31.60) and 52.1 (39.50-74.60), respectively.

Dogs with CE (n=33) showed significantly higher (P<0.01) 2-AG [10.7] (3.87-29.77)] and PEA [40.5 (30.05-49.27)], compared to the CON-TROL, while no statistical differences were found for AEA and OEA.In particular, in the comparison among the CONTROL, FRE (n=10), ARE (n=9), IBD (n=9) and PLE (n=5) groups, FRE showed higher levels of PEA [48.9 (40.5-57.3), P<0.05] and OEA [93.0 (65.3-122.1), P<0.05], ARE had higher levels of 2-AG [11.2 (6.4-24.9), P<0.01], the IBD group showed increased 2-AG [15.4 (7.5-130.3), P<0.01] and PEA [41.2 (38.2-50.2), P<0.05], while PLE dogs had increased concentrations of 2-AG [38.4 (11.2-92.1), P<0.01] and decreased amounts of OEA [26.2 (18.2-38.0), P<0.05] and PEA [11.9 (9.9-23.7), P<0.05]. The overall accuracy of 2-AG to exclude (-LR 0.18, Sensitivity 94.4%, Specificity 70% at ≤3.8) or predict (+LR 7.78, Sensitivity 77.78%, Specificity 90% at >6.4) ARE or IBD was very high (AUC 0.90). Additionally, plasmatic OEA showed a good accuracy (AUC 0.77) to exclude (-LR 0.18, Sensitivity 90.0%, Specificity 55.56% at ≤53.3) or predict (+LR 4.5, Sensitivity 50.0%, Specificity 88.89% at >90.7) FRE. Potential limitations were the sample size and the lack of eCBs analysis in intestinal biopsies. Yet, it can be concluded that 2-AG and OEA



may be a promising diagnostic tool for differentiating FRE from ARE and IBD in dogs with chronic gastrointestinal signs at presentation.

## Disclosures

No disclosures to report.

## ESCG-O-12

Retrospective analysis of dogs with inflammatory protein-losing enteropathy reveals that second-line immunosuppression is only necessary in 1/3 of cases and treatment requirements or outcome cannot be predicted by routine clinicopathological data

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It is commonly accepted that dogs suffering from Inflammatory Bowel Disease (IBD) with protein-losing enteropathy (PLE) need more aggressive immunosuppressive treatment than dogs with IBD but no PLE. At our hospital, treatment of PLE-IBD follows a stepwise algorithm to assess response to diet, antibiotics & prednisolone first, with second-line immunosuppressants (SLI) only added if initial response is not satisfactory. It was hypothesised that results from this approach will challenge the need for SLI in many cases. Files of dogs diagnosed with PLE from 2015 to 2017 (n=68) were retrospectively analysed. Exclusion criteria were lack of initial serum albumin (SA) value (n=5), lack of histopathological diagnosis (n=9), diagnosis not compatible with IBD (intestinal lymphoma n=3, intestinal adenocarcinoma n=2, intussusception n=3, granulomatous colitis n=2, nutritional deficit n=1), significant intestinal bleeding contributing to protein loss (n=1), and significant concurrent disease (PLN n=1, endocrinopathies n=2, EPI n=1). In the remaining 38 dogs (15 FN, 14 MN, 6 ME, 3 FE; mean age of 7 y, sd 2.5), initial mean SA was 19.8g/l (sd 4.5) with the lowest recorded SA after diagnosis 17.7g/l mean (sd 4.7). 17/38 dogs had concurrent hypoglobulinaemia, 24/37 hypocholesterolaemia, 36/38 total and 5/31 ionised hypocalcaemia, 18/38 total hypomagnesaemia. Serum cobalamin was low in 21/36 dogs (58%). According to the treatment algorithm, 2 dogs (5%) were treated with diet alone, 5 dogs (13%) with diet + antibiotics, 17 dogs (44%) with diet, antibiotics and prednisolone. 14 dogs (36%) subsequently needed SLI, which in 10/14 cases was cyclosporine and 4 dogs receiving another SLI (cyclophosphamide, mycophenolate, chlorambucil). Overall time until SA rose above 20g/l was median 11 days (range 0-136 days) and median follow-up time was 79 days (range 4-463 days). 8 dogs were deceased by the end of the study (21%). There was no difference between dogs with and without SLI regarding clinicopathological data (t-tests, Kruskal Wallis tests). No correlation between these variables, treatment requirements, time to normalisation of SA or outcome could be detected. In conclusion, contrary to previous literature, SLI were not needed in the majority of dogs with PLE-IBD (24/38 = 63%), which suggests a step-wise treatment approach often used in IBD without PLE can be appropriate in these cases and might lower costs for drugs substantially. In addition, none of the clinicopathological parameters routinely assessed in these cases correlated with prognosis or outcome, illustrating the lack of useful markers to predict the clinical course of PLE-IBD in dogs.

#### Disclosures

No disclosures to report.

## ESVCN-O-1

#### Arachidonic acid stimulates secretion of IL6 in feline primary adipose tissue culture

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This study aimed to determine the effects of body condition, fat depot, troglitazone, and fatty acids on secretion of adiponectin, interleukin-6 (IL6), and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) from adipose tissue of healthy cats. Subcutaneous and visceral adipose tissue samples were collected from 18 healthy intact female cats, and body condition score (BCS, Range 3-7/9) was determined. Concentrations of adiponectin were measured in mature adipocytes cultures and concentrations of IL6 and TNF $\alpha$  were measured in stromovascular cells (SVC) cultures following treatment with control medium, troglitazone at 10µM, eicosapentaenoic acid (EPA), arachidonic acid (AA), or palmitic acid, at 25μM, 50μM, or 100μM. SVC of visceral origin secreted higher concentrations of IL6 than corresponding cells of subcutaneous origin (P=0.003). AA treatment at 25μM, 50μM, and 100μM increased IL6 secretion in subcutaneous (P=0.045, P=0.002, and P<0.001, respectively) and visceral (P=0.034, P=0.001, and P<0.001, respectively) derived SVC. EPA treatment increased TNFα secretion in subcutaneous derived SVC at 25μM, 50μM, and 100μM (P=0.002, P=0.001, and P=0.015, respectively) and in visceral derived SVC at 50μM (P<0.001). No significant effect on medium adiponectin concentration was observed following troglitazone treatment (P=0.4) or fatty acids treatments at 25μM (P=0.2), 50μM (P=0.8), or 100μM (P=0.7). BCS did not have significant effects on medium concentrations of adiponectin (P=0.4), IL6 (P=0.1), or TNF $\alpha$  (P=0.8). This study demonstrated higher basal secretion of IL6 from visceral compared to subcutaneous adipose tissue, a stimulatory effect of AA on secretion of IL6 and a stimulatory effect of EPA on TNF $\alpha$  from feline adipose tissue.

#### **Disclosures**

No disclosures to report.

## ESVC-O-1

## Echocardiographically obtained left-atrial-to-aortic ratio in healthy dogs: re-evaluation of historic reference intervals

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Echocardiographic estimates of left atrial (LA) size (indexed to the aorta, LA:Ao) continue to be a cornerstone of determining cardiac disease severity in dogs and cats. However, reference limits were established from only 36 healthy dogs, and only at a single point in the cardiac cycle. Therefore, we re-examined the reference limits of LA:Ao in a 230 healthy adult dogs of various breeds, at 3 points in the cardiac cycle.

Three left atrial measurements were obtained from echocardiographs at end-systole (just prior to mitral valve opening) in the right parasternal long-axis view, and at end-systole (at aortic valve closure, LA<sub>max</sub>), onset of atrial systole (p-wave, LA<sub>p</sub>) and end-diastole (R-wave, LA<sub>min</sub>) in the right parasternal short axis view. Three diastolic left ventricular (LV) dimensions were obtained in the short axis view. Measurements were averaged and LA:Ao calculated. The LA<sub>max</sub> and LV measurements were indexed to bodyweight-adjusted aortic measurements as previously described (wLA, wLV). Dogs were considered to have no LA enlargement if wLA and/or wLV were within previously established reference intervals. Reference intervals were calculated nonparametrically.

225 dogs were included in the analysis. Upper reference limits for LA: Ao<sub>max</sub>, obtained in both views, were 1.73 (90% CI: 1.67-1.76 for short axis) and 2.11 (90%CI: 2.07-2.17 for long axis), marginally greater than originally proposed. 28/225 dogs had LA:Ao<sub>max</sub> greater than the short-axis limit of 1.6; 16/225 dogs had LA:Ao<sub>max</sub> greater than the long axis limit of 2.05. Upper reference limits for LA:Ao<sub>min</sub> and LA:Ao<sub>n</sub> were 1.53 (90%CI: 1.48-1.65) and 1.71 (90%CI:1.62-1.83), respectively.

Our study provides more robust reference limits for LA:Ao, obtained from 2D echocardiographic images, which are marginally larger than originally proposed.

## Disclosures

No disclosures to report.

## ESVC-O-2

Comparison of ECG-gated multi-detector computed tomography angiography and two-dimensional echocardiographic methods in the assessment of left atrial size using left atrial-to-aortic root short-axis ratio in dogs

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Left atrial size (LAS) is an important prognostic indicator in canine cardiac diseases. A widely used, unidimensional, echocardiographic method to estimate LAS is the left atrial to-aortic-root ratio (LA:Ao) using a short axis plane at the level of the root of the aorta. However, observer-dependant variability of this ratio has been reported, and measurements may vary considerably even among highly trained cardiologists. In humans, ECG-gated cross-sectional imaging modalities, such as multi-detector computed tomography angiography (MCTA) are used as reference methods for measuring LAS. MCTA limits cutplane variability and should therefore provide a more accurate and reproducible evaluation of left atrial morphology and borders compared to echocardiography in dogs.

We hypothesized that there is a poor correlation between LA:Ao calculated by echocardiography (LA:Ao, s) and by ECG-gated MCTA (LA: Ao<sub>MCTA</sub>) when aligned to the same right parasternal short axis view. To investigate this hypothesis, we compared two-dimensional echocardiographic and 64-slice ECG-gated MCTA measurements of LA:Ao (LA:Ao<sub>us</sub> and LA:Ao<sub>MCTA</sub> respectively) at end-diastole and end-systole in dogs. Measurements of LA:Ao were performed using the Hansson

method. Levels of agreement between these modalities and intra- and inter-observer variability were calculated.

This single centre, prospective, cross-sectional study was approved by the local Veterinary Ethical Review Committee. Dogs of various breeds undergoing anaesthetised thoracic MCTA for various diagnostic purposes were enrolled from a previous study on left atrial volume. Eligible dogs underwent a physical examination including blood pressure measurement prior to anaesthesia and cardiac auscultation to ensure that no heart murmur was present. Echocardiography was performed immediately following ECG-gated MCTA. Measurements of LA:Ao were obtained at end-diastole and endsystole, identified as the frame at the onset of the QRS complex and the frame immediately after aortic valve closure respectively. Agreement between LA:Aous and LA:AoMCTA was evaluated using Bland-Altman analysis and Pearson correlation. Coefficients of variation (CVs) were calculated to quantify intra- and inter-observer variability.

The correlation between LA:Ao $_{us}$  and LA:Ao $_{MCTA}$  was moderate at end-systole (r=0.52, p=0.018), whereas there was no correlation at end-diastole (p= 0.99). Echocardiography underestimated LA:Ao compared to MCTA at end-systole with a bias of -0.22 (95% limits of agreement: -0.68 to 0.24). Intra- and inter-observer variability for LA:  $Ao_{us}$  and LA: $Ao_{MCTA}$  at end-systole and end-diastole were adequate (CVs<15%).

In conclusion, our data demonstrate that the end-systole measurement of LA:Aous is a superior ratio-metric estimate of LA:Aous than the end-diastole measurement, but the ratio is underestimated compared to LA:Ao<sub>MCTA</sub>.

## **Disclosures**

No disclosures to report.

## ESVC-O-3

Assessment of left atrial volume in dogs: comparisons of twodimensional and real-time three-dimensional echocardiography with ECG-gated multi-detector computed tomography angiography

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Left atrial size (LAS) aids clinical decision-making in left-sided heart disease. Left atrial to-aortic-root ratio on short axis view is commonly used to assess LAS. This unidirectional measurement might not represent changes in other dimensions. Therefore, measurement of LA volume (LAV) is recommended for evaluation of LAS. Two-dimensional methods such as biplane area length method (ALM) and biplane method of disks (MOD) can estimate LAV, assuming the LA to be a fixed shape. Alternatively, real-time three-dimensional echocardiography (RT-3DE) measures LAV without geometric assumptions. In veterinary medicine none of these have been compared with volumetric gold standard like multi-detector computed tomography angiography (MCTA), which correlates well with cardiac magnetic resonance imaging.

We hypothesised that RT-3DE estimation of LAV is more accurate than ALM and MOD and correlates more strongly, using ECG-gated MCTA as gold standard.

To investigate this hypothesis, we compared maximum and minimum LAV (LAV<sub>max</sub> and LAV<sub>min</sub> respectively), identified as the frame immediately prior to mitral valve opening and immediatly after mitral valve closure respectively. ALM, MOD and RT-3DE were compared with 64-slice ECG-gated MCTA in 20 client-owned dogs (9.1-44.5kg; 24.9±9.3). Levels of agreement and intra- and inter-observer variability were calculated.

This single centre, prospective, cross-sectional study was approved by the local Veterinary Ethical Review Committee. Dogs of various breeds undergoing anaesthetised thoracic MCTA for various diagnostic purposes were enrolled. Eligible dogs underwent a physical examination including blood pressure measurement prior to anaesthesia and cardiac auscultation to ensure that no heart murmur was present. Echocardiography immediately followed ECG-gated MCTA.

The agreement between ALM, MOD, RT-3DE and MCTA were evaluated using Bland-Altman analysis to express bias and 95% limits of agreement (95%LoA). Correlations were calculated along with coefficients of variation (CVs) to quantify intra- and inter-observer variability.

ALM (r=0.79 and 0.72), MOD(r=0.81 and 0.70) and RT-3DE (r=0.94 and 0.82) correlated with MCTA for LAV<sub>max</sub> and LAV<sub>min</sub> respectively (all p<0.05). Biases for LAV $_{max}$  (-0.96mL, (95%LoA: [-5.6-3.7]) and LAV<sub>min</sub> (-0.67mL, (95%LoA: [-5.4-4.1]) were minimal with 3D-RTE, reflecting a systematic underestimation. Conversely, MOD (LAV<sub>max</sub>bias=3.19mL, 95% LoA: [-5.7-12.1]; LAV<sub>min</sub>=1.96mL, 95% LoA: [-4.6-8.5]) and ALM (LAV<sub>max</sub>bias=4.05, 95%LoA: [-5.7-13.8]; LAV<sub>min</sub>=2.80mL, 95%LoA: [-3.9-9.5]) systematically overestimated LAV. Intra- and inter-observer variability for LAV<sub>max</sub> and LAV<sub>min</sub> were adequate using ALM, MOD, RT-3DE and MCTA (CVs<15%).

In conclusion, assessment of LAV with RT-3DE is a simple, non-invasive, accurate and feasible method for measuring LAS that has superior accuracy to ALM and MOD in dogs without cardiac disease.

#### Disclosures

No disclosures to report.

#### ESVC-O-4

## Post mortem assessment of left ventricular mass by micro-computed tomography in feline cardiomyopathic hearts

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Micro-computed tomography (micro-CT) is a post mortem imaging modality that provides high-resolution three-dimensional imaging, which could be a useful tool in imaging cardiomyopathies. Left ventricular mass (LVM) is an important variable in cardiomyopathy classification and this prospective cross-sectional study aimed to assess LVM by micro-CT in cats undergoing routine necropsy.

Hearts were excised, formalin-fixed and immersed in potassium triiodide (I2KI) for 48 hours. Whole heart micro-CT scans were reconstructed into an isotropic volume allowing virtual dissections of the feline hearts in any plane, (mean cohort voxel size 24 microns). LVM

was determined by micro-CT using 2 different methods and each compared with actual LVM (aLVM). aLVM was determined by weighing the left ventricle after dissecting the great vessels, atria and right ventricle free wall.

The method with better agreement with aLVM was then used for mass determination in cats with hypertrophic cardiomyopathy. Method 1 (LVM-M1) was based on short-axis images of the heart, manually tracing endocardial and epicardial borders in multiple slices from base to apex. Method 2 (LVM-M2) used automated software to calculate ventricular surface area and volume from 3D reconstructions of the whole left ventricle. LVM was obtained in both methods by multiplying total volume by myocardial density. Data are reported as mean (95%CI).

aLVM from 7 cardiomyopathic hearts (4 HCM, 2 RCM, 1 DCM) was greater (8.4 g (95%CI 6.0-10.8)) than LVM-M1 (6.8 g (95%CI 4.6-8.9), p=.001) and LVM-M2 (6.9 g (95%Cl 4.7-9.1), p=.001). LVM-M2 was greater than LVM-M1 (p=0.003). Both methods underestimated LVM with mean bias of 1.6 g (95% LA 0.04-3.2) for LVM-M1 and 1.5 g (95% LA -0.04-3.0) for LVM-M2. LVM-M2 was greater in HCM (n=10) than normal cats (n=4) (6.5 g [4.6-9.8] vs 3.5 g [3.3-4.1], p=0.005). The regression equation to predict aLVM from LVM-M2 was 0.87 (aLVM)-0.40.

Micro-CT can be used to determine LVM, although underestimated aLVM by approximately 2% in our study. This may be attributable to tissue preparation technique or mass determination calculation factors.

#### **Disclosures**

Disclosures to report.

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## ESVC-O-5

## ECG-gated computed tomography angiography of pulmonic stenosis in 31 dogs

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Pulmonic stenosis (PS) is a common congenital cardiac disease in dogs. Accurate sizing of the pulmonic annulus, routinely performed with intraoperative angiography, is essential for successful balloon valvuloplasty. Most dogs have valvular PS (with or without annular hypoplasia), but some Bulldogs can have subvalvular PS that is associated with an aberrant coronary artery (CA). If an aberrant CA is suspected, balloon valvuloplasty might be contraindicated. ECG-gated computed tomography angiography (CTA) can be a complementary minimallyinvasive way in early planning of interventional treatment. The aim of this prospective observational study was therefore to report CTA finding prior to balloon valvuloplasty.

A total of 31 dogs (of which 3 Australian, 3 English, and 4 French Bulldogs) diagnosed with PS aged 3 to 114 months and weighing 2.1 to



ventricular pacing. When the AP was localized, radiofrequency energy was delivered by a conventional generator and the procedure was considered successful if no recurrence was noted 45 minutes postablation.

A total of 83 APs were identified (92.1% single; 7.9% multiple), of which 96.4% were right-sided and 3.6% left-sided. In 68.7% of the cases, conduction along the AP was unidirectional and retrograde, while bidirectional in 31,3%. Retrograde decremental properties were noted in 6% of the cases. The APs mediated orthodromic AVRT in 92.1% of the cases, and permanent junctional reciprocating tachycardia in 6.5%. In one case, AVRT was not inducible. In 97.4% of dogs, RFCA was attempted with a 100% short-term success rate. Within 18 months, recurrence of tachycardia occurred in 6.6% of cases, followed by a second successful ablation. No mortality was observed and major complications (pacemaker implantation) were identified in 2.6% of dogs.

This study showed that, not only AP distribution and characteristics in dogs are similar to what has been previously reported but also that the success and complication rates of RFCA appear similar to humans. RFCA should be considered the first line therapy in dogs, as it can be performed with a high success rate and low incidence of complications.

#### Disclosures

No disclosures to report.

## ESVC-O-7

Breed does not affect the association between murmur intensity and disease severity in dogs with pulmonic or subaortic stenosis

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Previous studies have demonstrated that murmur intensity is associated with stenosis severity in dogs with pulmonic and aortic stenosis. However, anecdotally, some breeds provide challenges to auscultation, while others are easily auscultated. Cardiologists have suggested that this could affect the interpretation of murmur intensity in estimating disease severity - dogs that are difficult to auscult would be expected to have more severe disease than their murmur intensity suggests, while dogs that are easily auscultated would be expected to have less severe disease than their murmur intensity suggests, especially at the ends of the murmur scale. We retrospectively examined murmur intensity in 1065 dogs with pulmonic or aortic stenosis, presenting to 7 cardiology centers, and evaluated the effect of breed on ability to predict disease severity.

Breeds represented by 10 or more individuals were used to examine breed effect: 131 dogs, representing 5 breeds, had soft murmurs; 123 dogs, representing 7 breeds had moderate murmurs; 179 dogs, representing 9 breeds, had loud murmurs and 284 dogs, representing 11 breeds, had palpable murmurs. We found no association between

27.5 kg were enrolled: all dogs were planned to undergo possible balloon valvuloplasty under the same anaesthetic directly after CTA. The contrast medium injection protocol was individually tailored with total injection time equaling the sum of diagnostic scan delay and scan duration. First, 1 ml/kg undiluted contrast medium (350 mgl/ml) was administered for two thirds of the total injection time, directly followed by 2 ml/kg of 1:1 diluted contrast medium for one third of the total injection time. The CT scan was triggered manually under a single breath hold when the contrast bolus reached the descending aorta. In all dogs, the sagittal plane of the images was optimized for visuali-

zation of the pulmonic annulus and compared to the aortic annulus in the transverse plain. Ten dogs (32%) were considered to have annular hypoplasia (aortic annulus: pulmonic annulus > 1.2). The CAs could be assessed in all dogs, and 6 dogs had an aberrant CA detected (4 dogs with a single right coronary ostium with prepulmonic left CA, 3 Bulldogs and 1 Chihuahua; 2 dogs with a single left coronary ostium with prepulmonic right CA, 1 Bulldog and 1 German Shorthair Pointer). A total of 27 dogs (all except 4 of the dogs with an aberrant CA) subsequently underwent balloon valvuloplasty, and in all these dogs the suspected PS morphology could be confirmed with fluoroscopy.

In conclusion, CTA is a is a complementary guick and minimallyinvasive diagnostic method that may provide additional relevant information on sizing of the pulmonic ostium and assessment of CA anatomy prior to balloon valvuloplasty. Non-Bulldogs can also have an aberrant CA as part of the pathophysiology of PS.

#### Disclosures

No disclosures to report.

#### ESVC-O-6

## Radiofrequency catheter ablation of accessory pathways in the dog: the Italian experience (2008-2016)

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Accessory pathways (APs) are extra-nodal muscular bundles that connect atrial and ventricular myocardium. In dogs, APs are mostly rightsided, with non-decremental conduction, and mediate atrioventricular reciprocating tachycardias (AVRT). Radiofrequency catheter ablation (RFCA) is the first line therapy in people and veterinary medicine data suggest that it can be safe and successful in dogs. The objective of this study is to confirm the anatomical distribution of APs and their electrophysiologic characteristics in a large canine population and to evaluate long-term success and complication rates of RFCA.

Records of 76 dogs were retrospectively reviewed. All dogs presented clinical signs or electrocardiographic evidence of ventricular preexcitation (VPE) and/or AVRT. After physical examination, 12-lead ECG, thoracic radiography, and echocardiogram, electrophysiological mapping was performed. Presence and location of APs, conduction pattern, refractory period of AP and AV node, type of inducible supraventricular arrhythmia, and cycle length of inducible AVRT were considered. The presence of APs was confirmed by the presence of VPE during sinus rhythm or atrial pacing and/or the presence of rapid ventriculo-atrial activation during supraventricular tachycardia or



breed and disease severity at any level of murmur intensity. Power analyses suggested that we should have detected at least a moderate effect if one existed. Nine dogs with soft murmurs had more-thanmild stenoses, and were of various breeds, not usually associated with auscultation difficulty.

Our data suggest that, despite perceived difficulties in auscultating certain breeds, such issues do not affect clinicians' ability to estimate disease severity.

#### Disclosures

No disclosures to report.

## ESVC-O-8

## Breed characteristics in cardiac reverse remodeling and left ventricular systolic function after mitral valve repair in dogs

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Mitral valve repair (MVR) can be considered as a treatment option for severe myxomatous mitral valve disease in dogs, and significant cardiac reverse remodeling (CRR) after a repair has been reported. Although predisposed breeds and the breed-specific progression of this disease have been investigated, the breed characteristics and the outcome of cardiac reverse remodeling for MVR remain unclear. We hypothesized that there is a difference in breed characteristics in CRR for MVR, and some insights for suitable surgical timing can be obtained.

The consecutive medical records of 117 dogs that underwent MVR between November 2016 and July 2017 at JASMINE Veterinary Cardiovascular Medical Center were reviewed. Breed; age; sex; body weight; heart failure classification; presence of coughing; vertebral heart sum (VHS); pre- and postoperative echocardiographic parameters including left atrial and ventricular sizes, fractional shortening (FS), and severity of regurgitation; and number of cardiac medications used were obtained. Postoperative examination was performed 1 month after surgery. The dogs were divided into three groups-Cavalier King Charles Spaniels (CKCSs), Chihuahuas, and all dogs-and parameters were compared amongst the groups.

A total of 99 dogs were included in the analysis, after the exclusion of 10 dogs that died before the 1-month postoperative review, 6 dogs with missing data, and 2 dogs that underwent secondary surgery. The overall survival rate 1 month after surgery was 91.5%. In multiple comparison analysis, the CKCSs were significantly younger, had higher body weight, preoperative VHS, postoperative normalized left ventricular end-diastolic and end-systolic diameters, and had lower postoperative FS compared amongst the groups. Although the pre- and postoperative number of cardiac medications in the CKCSs tended to be high, no significant difference was observed amongst the groups. Although the heart failure classification did not differ amongst the groups, the CKCSs were significantly younger than the dogs in the other groups. Therefore, the progression of mitral regurgitation is considered to have occurred at an earlier age in the CKCSs and this finding is consistent with previous reports. Stronger postoperative irreversible cardiac dilation and systolic dysfunction observed in the CKCSs amongst the groups, with a similar reduction of regurgitation, could be explained by severe myocardial remodeling, and these findings might be hard to detect preoperatively because of regurgitation. These results suggest that MVR should be recommended earlier for CKCSs compared with other breeds to minimize LV systolic dysfunction and dilation after surgery.

#### **Disclosures**

No disclosures to report.

## ESVC-O-9

#### Iron deficiency assessment in dogs with chronic heart failure

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In humans suffering from chronic heart failure (CHF) iron deficiency is a common comorbidity and intravenous iron therapy significantly improves exercise capacity and quality of life. Consequently, the European Society of Cardiology Guidelines (2016) recommend intravenous iron therapy in symptomatic patients with CHF and iron deficiency defined as serum ferritin <100 µg/L, or ferritin between 100–299 μg/L and transferrin saturation <20%. In dogs it is unknown if iron deficiency is a comparable comorbidity requiring supplementation. Therefore, the aim of this study was to evaluate the iron status in dogs at various stages of chronic heart failure.

In this multicenter, prospective case-control study, in 2017 75 dogs with Myxomatous Mitral Valve Disease (MMVD) and Dilated Cardiomyopathy (DCM) Chief Class B2 to C3 were included and compared to 25 healthy dogs. The disease stage was based on clinical, radiologic and echocardiographic examinations. Cardiovascular integrity of the control group was based

on anamnesis, clinical examination and echocardiography. Laboratory examinations included biochemical analysis and complete blood count including RET-HE; iron status was assessed based on serum iron, ferritin, TSAT and total iron binding capacity (TIBC). Non-parametric tests were used for statistical analysis. With increasing severity of heart failure serum iron (p=0.002) and TSAT significantly decreased (p=0.026); medium and range of TSAT were 35% (21-63%, n=25) in healthy controls, 36% (5-85%, n=39) in class B2, 33% (19-67%, n=17) in C1, 33% (10-38%, n=15) in C2, and 28% (11-39%, n=4) in C3. Specifically, dogs in C2 had significantly lower TSAT compared to healthy controls (p = 0.007) and dogs in B2 (p = 0.002). No differences were observed in TIBC (p=0.098), ferritin (p=0.81) and RET-HE (p=0.5).

Decreasing serum iron concentration and TSAT support the hypothesis that iron deficiency may be present in dogs with heart failure. Normal ferritin does not rule out iron deficiency; TSAT is considered a more reliable diagnostic marker in humans suffering from CHF, because of inflammatory mediators affecting ferritin. RET-HE, a newer parameter considered sensitive to assess iron status in humans and dogs, was not affected by the degree of heart failure in our dogs.

In conclusion, this study provides some evidence for iron deficiency as comorbidity in dogs with advancing heart failure. However, it is



difficult to assess if also in dogs this affects quality of life. Therefore, routine screening for iron deficiency in heart failure dogs and iron supplementation may not be recommended at this time.

#### Disclosures

Disclosures to report.

-Angela Betschart is a former employee of Vifor Pharma -Susanna Burckhardt is an employee of Vifor Pharma -The study was partially funded by an unrestricted research grant from Vifor Pharma - Monique Wenger is married to Tony Glaus.

## ESVC-O-10

## Effects of oral trazodone on echocardiographic and hemodynamic measurements in healthy cats

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Stress and transportation for veterinary visits can impact comprehensive evaluations and limit diagnostic testing of cats. Oral anxiolytic drugs are available by prescription; however, all have disadvantages limiting their usefulness. Recently, oral trazodone administered to cats prior to a veterinary visit decreased anxiety greater than placebo and was well tolerated. While trazodone is a promising drug for reducing veterinary care related anxiety in cats, its effects on echocardiographic and hemodynamic variables are unknown. The purpose of this study was to evaluate the effects of oral trazodone on echocardiographic variables, heart rate, and blood pressure in healthy cats.

Client-owned domestic cats between 3 and 8 years of age were recruited. Cats were considered healthy on the basis of physical examination, complete blood count, blood chemistry profile, urinalysis, total thyroid level, non-invasive blood pressure measurement by Doppler method, and a screening transthoracic echocardiogram with simultaneous electrocardiogram. Cats were discharged from the hospital after screening evaluation and returned for a separate visit within one week. A physical examination, baseline echocardiogram, and blood pressure measurement were performed prior to trazodone administration. Echocardiographic variables assessed included left ventricular internal diameter in systole and diastole, interventricular and left ventricular free wall thickness in systole and diastole, left atrial size, left auricular function, and left ventricular diastolic function assessed by spectral Doppler transmitral and pulmonary venous flow velocities and tissue Doppler profiles. Subsequently, 50 mg of trazodone was administered orally to each cat. After 90 to 120 minutes, physical examination, echocardiography, and blood pressure measurements were repeated. Mean echocardiographic and hemodynamic variables were calculated for pre-trazodone and post-trazodone exams for each cat. Pre-trazodone and post-trazodone variables were coded as separate groups and data were analyzed by repeated measures ANOVA with significance set at P < 0.05.

Twelve healthy cats were evaluated. Mean time for repeated evaluation after trazodone was 96.3 minutes and mean trazodone dose was 10.7 mg/kg (minimum 7.9 mg/kg; maximum 16.6 mg/kg). Trazodone was safely administered and a sedative effect was observed in all cats. There were no significant differences in any echocardiographic variables after trazodone administration. Mean blood pressure was

significantly decreased after trazodone (pre-trazodone: 131 mmHg; post-trazodone: 107 mmHg; P = 0.0005) with no significant change in heart rate.

The results of this study suggest that trazodone is well tolerated and may aid in obtaining transthoracic echocardiograms. Trazodone significantly lowers systemic blood pressure without affecting heart rate or echocardiographic variables in healthy cats.

#### Disclosures

No disclosures to report.

## ESVC-O-11

Prevalence of hypersomatotropism in non-diabetic cats with left ventricular hypertrophy - a silent and curable phenocopy for hypertrophic cardiomyopathy

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Hypersomatotropism (HS) causes reversible left ventricular hypertrophy (LVH) in humans and cats. The estimated prevalence of HS among UK diabetic cats is 26-32%, however recently HS has also been identified in non-diabetic cats. Given the high prevalence of feline hypertrophic cardiomyopathy (HCM) at 14.7% which, similar to HS increases with age and given that to date only two HCM associated mutations have been identified; we hypothesise that HS may be an important phenocopy of HCM in the non-diabetic feline population. If identified, treatment of HS can cure the cardiomyopathy.

The aim of this study was to establish the prevalence of HS in a population of UK non-diabetic cats with LVH and therefore presumptively diagnosed with HCM.

Sixty six cats previously diagnosed with HCM at two referral hospitals were included retrospectively. Inclusion criteria were a diagnosis of HCM by a cardiology diplomate based on a left ventricular wall thickness in diastole > 6mm by echocardiography. Hypertension (BP>150 mmHg), hyperthyroidism (serum TT4) and other causes of LVH such as aortic stenosis were excluded. Cats were considered non-diabetic based on normal blood glucose and/or fructosamine concentration or absence of glycosuria. Serum IGF-1 concentration was measured in duplicate on residual samples frozen at -80°C for a maximum of 5 years using a validated radioimmunoassay; HS was defined as a mean IGF-1 concentration of > 1000ng/ml (positive predictive value for HS 95%).

IGF-1 concentrations ranged from 25-1179ng/ml with a mean+/-SD of 539.2 +/- 230.4. Four cats had serum IGF-1 concentrations consistent with HS (1179, 1131, 1106 and 1051ng/ml). Of these, two cats presented with LVH, dilated atria with spontaneous contrast, pleural effusion and complex arrhythmias. Both cats had evidence of reduced motion and thinning of one segment of the left ventricular free wall. A third cat presented with LVH, an enlarged left atrium with spontaneous contrast and pulmonary oedema and the final cat with a murmur LVH and a normal sized left atrium.

A prevalence of HS of 6% was established in a cohort of UK nondiabetic cats initially diagnosed with HCM. It is estimated that there are over 1 million cats in the UK with HCM; these results suggest that over 60,000 of them in fact suffer from HS-induced cardiomyopathy,



due to excess growth hormone and IGF-1. This has significant clinical impact since medical and surgical management of HS can lead to resolution of LVH and restoration of health.

#### Disclosures

Disclosures to report.

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#### ESVC-O-12

## New genetic variants of MYH6 gene were associated with sporadic hypertrophic cardiomyopathy in cats

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Hypertrophic cardiomyopathy (HCM) is the most common cardiomyopathy in cats and humans. It is a heterogeneous genetic heart disease characterized by left ventricular hypertrophy in the absence of another disease that could explain the wall thickening. It is considered a familial disease with autosomal dominant transmission. In humans, more than 1500 causative mutations, mainly from 11 sarcomeric genes have been associated with HCM. In cats, only two causal mutations affecting the myosin protein binding C (MYBPC3) gene have been described (A31P in Maine Coons and R820W in Ragdolls). The aim of the study was: a) to identify possible causative mutations that could be associated with HCM in different breeds by next generation sequencing and b) to explore the relationship between genotype and phenotype.

Nine cats with HCM (5.4±4.1 years, 77.8% males; 4Persian, 3Domestic shorthair, 1Sphynx and 1Chartreux) and 6 controls (3.8±1.8 years, 33,3% males, 2Persian, 1Domestic shorthair, 2Maine Coon and 1Norwegian Forest) were included. HCM was defined as LVH ≥6mm, when other causes of LVH were excluded. DNA was extracted from peripheral blood sample (left-over from routine haematology sample). A customized panel of 18 genes associated with HCM (ACTC1, DES, FLNC, GLA, LAMP2, MYBPC3, MYH7, MYH6, MYL2, MYL3, PLN, PRKAG2, PTPN11, TNNC1, TNNI3, TNNT2, TPM1, TTR) was sequenced by next generation sequencing. The variants were classified as pathogenic when they were no present in control population and were in conserved domains.

In the affected cats, 7 new causal variants, and 1 previously described (FLNC p.T440M rs785588805 - dbSNP-), not present in the controls, were identified. 5/8 variants were missense (MYH6: p.A1398T, p. A1438T; DES: p.A430P; FLNC: p.T440M; TNNT2: p.F126C) and 3/8 affected splicing (MYH6: c.1411-4G>A; MYBPC3 c.1882+4A>C; FLNC: c.2187+4A>C). All affected cats were carriers at least of one variant, 7 of them were carriers of one variant in MYH6.

Signalment, clinical findings at diagnosis (asymptomatic, congestive heart failure, cardiac murmur) and the echocardiographic phenotypes of affected cats such as, basilar septal bulge (1/9), obstructive HCM (4/9) heterogeneous hypertrophy (5/9) and false tendons (5/9) were independent of variants detected.

In conclusion, this study describes heterogeneous novel variants in new genes associated with HCM in cats. The main gen involved in the

affected cats was MYH6. There were no correlation between breeds and the variants described.

#### Disclosures

Disclosures to report.

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## ESVC-O-13

## Discovery of novel biomarkers for cardiomyopathies in cats with congestive heart failure

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Cardiomyopathies (CM) are the most common heart diseases in cats. The disease presentations are variable; prediction of disease course is difficult, particularly due to a lack of knowledge of underlying pathogenesis. Congestive heart failure (CHF) and arterial thromboembolism are common late consequences associated with feline cardiomyopathies. In this study, by using proteomic techniques we aimed to identify novel serum biomarkers for feline primary cardiomyopathies with congestive heart failure. The findings may aid understanding disease pathogenesis and assist in disease diagnosis, prognosis and management.

The study population comprised 15 cats diagnosed with CHF and CM, 5 cats with asymptomatic CM and 15 healthy cats. Serum proteomic profiles of these different cat groups were obtained by using liquid chromatography-tandem mass spectrometry techniques. The Wilcoxon test and Bonferroni correction were used for statistical analysis on differential protein expressions. In total 28 serum proteins were found differentially expressed between CHF and healthy control cats. Seventeen proteins were upregulated in CHF, mainly involved in lipoprotein metabolism, inflammation and coagulation pathways. Apolipoproteins, acute phase protein ceruloplasmin, prothrombin, platelet factor 4 and serine protease inhibitors were significantly higher in CHF cats compared with healthy controls (P<0.05). Ten proteins were downregulated in CHF. Tetranectin, a protein regulating fibrinolysis and proteolysis was significantly lower in CHF cats compared with healthy controls (P<0.05). In human medicine this biomarker has recently been associated with coronary artery disease. Subsequent bioinformatics analysis (Gene Ontology) predicted 21 best candidate biomarkers, which were primarily associated with extracellular matrix organization and metabolism.

These results suggest systemic inflammation, coagulation abnormalities and extracellular matrix remodelling occur in feline cardiomyopathies particularly with CHF. Identification of these biomarkers provides new insights for feline cardiomyopathy research.

#### **Disclosures**

Disclosures to report.

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## ESVC-O-14

Effect of breed and signalment on N-terminal Prohormone of Brain Natriuretic Peptide concentrations measured by ELISA in healthy cats and comparison to a point of care test

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Causes of plasma variations in the cardiac biomarker B-type natriuretic peptide (NT-proBNP) have not been fully evaluated in healthy cats. The objectives were to investigate effect of breed and other signalment variables on NT-proBNP concentrations in healthy cats using quantitative ELISA and to compare with a point of care test (POC). 100 healthy cats; 33 Birman, 35 Norwegian Forest and 32 Domestic Shorthair cats were included. Absence of heart disease and other significant diseases was ensured by physical examination, measurement of systolic blood pressure, echocardiography, hematology and blood biochemistry. NT-proBNP concentrations were analysed by ELISA at a reference laboratory. The POC test was analysed by visual inspection and an automated method, considered "positive" if ≥100 pmol/l. Data was analysed using non-parametric tests and regression analyses. NTproBNP concentration for all cats was 12 pmol/I (IQR 12-31) and there was no significant effect of body weight or age on the NTproBNP concentration. Median concentration of NT-proBNP was twice as high in male (25 pmol/l, IQR 12-49) compared to in female cats (12 pmol/l, IQR 12-25.5) P = 0.0072. Three cats had ≥100 pmol/l with the ELISA, two of them were positive using visual inspection of the POC test. One cat with titre 155 pmol/l and 4 with lower titres < 100 were positive when the POC test was automatically analysed. Sex, but not breed, body weight or age, influenced NT-proBNP concentrations in healthy cats. Visual inspection of the POC test showed higher agreement with ELISA compared to using automated analysis.

## Disclosures

No disclosures to report.

## ESVC-O-15

Evaluation of NT-proBNP-levels in sport dogs after immediate physical activity and after an intense two months training session

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Cardiac biomarkers like N-terminal pro-B-type natriuretic peptide (NTproBNP) are used as a diagnostic tool for the clinical assessment of heart diseases in dogs, but this parameter is influenced by extracardiac factors. In humans, different types of physical activity, duration of training and pre-training physical condition have all been shown to affect NTproBNP. The aim of this study was to compare NTproBNP levels of two dog breeds before and after different physical activity and training.

In this prospective cohort study, seven untrained German shepherd dogs (GSD) entering the military service and seven Scandinavian hounds (SH) used for competitive racing were examined. The latter underwent a three week training pause before entering into the study. All dogs had an, echocardiographic examination at baseline. NTproBNP was measured at baseline and after exercise. GSD were exercised for 10 minutes in protection training activities, i.e. high intensity interval training, while the SH were running continuously for 10 minutes with a biker attached. After two months of scheduled training NTproBNP levels were reassessed under the same conditions. Nonparametric tests were used for statistical analysis, and data are reported as median [range].

At baseline, normalized LV diameter in diastole (LVDDN) (1.60 [1.49-1.76] versus 1.43 [1.29-1.69], p = 0.014) and NTproBNP (410 [211-2685] versus 111 [18-611] pmol/l, p = 0.010) were higher in SH than in GSH. Both groups showed a similar significant increase of NTproBNP (ΔBNP) after physical exercise (ΔBNP SH 85 [-7-272], p = 0.032, GSH 60 [20-147], p = 0.004). The increase between groups was not different. After two months of training, NTproBNP levels at rest were similar compared to baseline, but still higher in SH than in GSD (p = 0.017). After exercise  $\triangle$ BNP was higher in SH, but not significantly (292 [62-1394], p 0.453), whereas it was lower, but not significantly, in GSD ( $\Delta$ BNP -73 [-244-162], p = 0.212). The exercised induced change between the groups was significant (p = 0.007).

In conclusion NTproBNP levels were higher in SH compared to GSD, training and exercise affected NTproBNP in dogs, but this depended on intensity of training and type of exercise. Echocardiographically SH had larger hearts than GSD and if higher NTproBNP reflects higher wall tension, eccentric hypertrophy in these athletic dogs may be a sign of inappropriate compensatory hypertrophy. Alternatively, other factors like excitability and excitation associated with manipulation or upcoming exercise may cause mild transient increase in wall tension.

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## ESVC-O-16

Interest of a cardiac troponin I using a bedside analyzer for clinical orientation in dogs with mitral insufficiency and/or dyspnea

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Diagnostic investigation of an heart murmur or dyspnea in dogs requires diagnostic imaging procedures, thoracic radiographic examination (TRE) or cardiac ultrasound (CUS). Those procedures represent a high cumulative cost. In asymptomatic dogs with mitral valve disease, dogs with cardiac enlargement require CUS and sometimes treatments, whereas dogs without cardiac enlargement do not. Dogs with dyspnea of cardiac origins will benefit from CUS whereas in dogs with "non-cardiac" dyspnea, TRE will be more informative. The use of cardiac biomarkers for patient stratification is a proposed option for accelerating diagnostic process and limiting diagnostic procedures costs. Cardiac troponin I (cTnI) is a biomarker of myocardial damage and can be used for diagnostic and prognostic purposes. The main limitation of cTnI in veterinary cardiology is the lack of bedside analyzer and the high lower detection limit of some analyzer. The aim of this study was to evaluate the interest of a highly sensitive cTnl bedside



analyzer to categorize patient in a prospectively recruited population of dogs with mitral insufficiency and/or dyspnea.

A population of 86 dogs in which CUS and TRE were performed, were prospectively recruited in an observational cross-sectional study. A measurement of cTnl was performed using a highly sensitive bedside analyzer (MiniVidas Biomerieux). An additional population of 21 dogs without cardio-pulmonary symptoms and with normal CUS was prospectively included as a control group.

Fifty-eight dogs presented with mitral insufficiency (MI), corresponding to ACVIM classification B1 (n= 29), B2 (n=10), C (n=14) and D (n=5). Over the same period, 28 dogs with non-cardiac dyspnea were recruited, corresponding mostly to inflammatory/infectious disease (n=13) or pulmonary neoplasia (n=8).

Cardiac Troponine I values were significantly lower in dogs with ACVIM B1 MI and control group, compared to ACVIM B2 and symptomatic (C-D) dogs with MI (p<0,01). Dogs with non-cardiac dyspnea presented with significantly lower cTnl compared to symptomatic (C-D) dogs with MI (p<0,01). In dogs with asymptomatic MI, a cut-off value of 58 ng/L identifies dogs with cardiac enlargement with a sensibility and specificity of 100% and 79%, respectively. In dogs with dyspnea, a cut-off value of 53 ng/L identifies dogs with cardiac disease with a sensibility and specificity of 89% and 60%, respectively. This study suggests that cTni Could be proposed as a screening test in dogs with MI or dyspnea, and particularly as an exclusion test when a

#### Disclosures

Disclosures to report.

highly sensitive bedside analyzer is used.

Analyzer and furnitures for cTnl measurement were provided by BioMerieux.

## ESVC-O-17

## Effect of pimobendan on left atrial function in dogs with stage B2 myxomatous mitral valve disease

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Left atrial (LA) function is an important determinant of left ventricular (LV) filling and plays a key role in maintaining an optimal cardiac performance. LA size is negatively associated with the prognosis of dogs with mitral valve disease (MVD). Pimobendan is a phosphodiesterase III inhibitor with positive inotropic and vasodilator effects and it has been reported to decreases the LA pressure and the overall heart size in dogs with MVD. Based on the EPIC study, dogs with stage B2 MVD have been shown to benefit from administration of pimobendan. This study sought to investigate the effects of Pimobendan on LA function in dogs with stage B2 MVD.

Twenty dogs with stage B2 MVD were retrospectively included. Left atrial function was assessed prior to and 1-6 months after starting pimobendan. For each dog, 2-dimensional echocardiographs from right parasternal long axis 4 chamber view optimised for the LA was recorded to assess LA diameter and volume (Simpson's method) for each phase of the LA function cycle: at end-systole, one frame before opening of the mitral valve; immediately before the P wave of the

ECG and at end-diastole, one frame before closure of the mitral valve. Using those variables we assessed complete, passive and active LA function as previously described. Pulsed wave tissue Doppler imaging (PW-TDI) of the left ventricular longitudinal myocardial velocity associated with atrial contraction (A'), both at the level of the interventricular septum and the LV free wall, were also used as an indicator of LA function.

Of the all variables assessed, TDI A' of the septum and TDI A' of the free LA wall were significantly increased after pimobendan administration (p <0.01 and p= 0.04 respectively), whereas LA complete EF was significantly decreased after treatment (p=0.03). None of the other variables assessing the LA function or LA size, were significantly different between pre and post pimobendan administration. The velocity of mitral valve regurgitation was significantly increased (p= 0.01) post institution of pimobendan treatment.

Estimates of LA function by TDI A' in dogs with MVD improves after treatment with pimobendan suggesting an effect of this drug on LA function although it is difficult to separate its effect from improvements in left ventricle function. The decrease in LA complete EF may reflect reduced mitral regurgitant fraction. Despite reduction in LV size, we did not document decreased LA size in the short time-course of this study.

#### **Disclosures**

No disclosures to report.

## ESVC-O-18

The misuse (overdose) of pimobendan may potentially induce myocardial fatigue in dogs with myxomatous mitral valve disease

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Off-label use of pimobendan may be considered for management of acute pulmonary edema or refractory congestive heart failure (CHF). However, the risk of continuous pimobendan overdose has not yet been well investigated in dogs with stable CHF. This observational study is to evaluate the potential effect of overdosing of pimobendan on cardiotoxicity with home-based chronic therapy in myxomatous mitral valve disease (MMVD).

In referred dogs with MMVD, eight client-owned dogs have been treated with a regimen of overdose of pimobendan (case patients; 0.9~2.7mg/kg daily for 60~240 days). Among patients treated with a recommended dosage of pimobendan (0.4~0.6mg/kg daily for 60~345 days), controls (n=12) were selected based on matching variables such as signalment/medical history, physical examination findings, and severity of CHF. For the initial analysis of homogeneity between case and control groups, comprehensive blood test profile (CBC, serum biochemistry, NT-proBNP, SDMA), electrocardiographic, radiographic, and echocardiographic data were collected. Serum cardiac troponin I (cTnI) and QTc interval values (formula of Van de Water) were compared between groups. In addition, these two endpoints were analyzed to assess the relationship to the dosage and the duration of administration of pimobendan in the case patients.



The patients group with the overdose use of pimobendan showed a significantly higher level of cTnl (1.45±0.60 ng/mL) and a longer QTc interval (158.50±8.50 msec) than controls (cTnl, 0.23±0.08 ng/mL; QTc, 137.80±8.40 msec; P<0.01). Regression analysis adjusted by the duration of pimobendan administration in the case patients revealed that both cTnl level and QTc interval were highly correlated to the dosage (P<0.01: Adjusted R<sup>2</sup>=0.849 and 0.784, respectively).

The significantly elevated cTnI level and prolonged QTc interval and their strong correlations with the dosage and the duration of improper overdose of pimobendan in the case patient group can indicate myocardial damage indicative of cardiac fatigue.

#### Disclosures

No disclosures to report.

## ESVC-O-19

## Heart-specific and fibrosis related microRNAs in whole blood samples of dogs with cardiomyopathy

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Fibrosis and extracellular matrix remodeling has a causative role in the pathophysiology of cardiac diseases of different origin. At cellular level, fibrotic processes are prior to clinical manifestation of symptoms. Currently there is no biomarker, which is able to detect heartspecific fibrosis and remodeling in the peripheral blood sample despite it's advantages in cardiac diagnostics and monitoring of therapy. Previously, using microarray method we identified heart-specific gene expression profile representing fibrotic and inflammatory processes in the peripheral whole blood of tachypacing-induced cardiomyopathy model dogs and we successfully selected a heart-specific fibrosis related mRNA-panel which gene expression levels in blood samples clearly differentiated clinical cases from healthy dogs. MicroRNAs are key regulators of gene expression and highly stable molecules thus ideal biomarker candidates.

Based on our previous results and using the same samples, now we aimed to investigate the cardiac and whole blood expression levels of selected microRNAs directly involved in cardiac fibrosis and heart failure.

The selected panel consisted of 7 different microRNAs which have key regulatory role in fibrosis, remodeling and impaired contractility. MiR-208a, miR-208b, miR-499 are located in genes coding different isoforms of myosin heavy chain and are related to impaired contractility. MiR-133a, miR-21 and miR-29 have key role in the regulation of fibrosis and miR-1 is an important regulator of cardiac hypertrophy. Whole blood and left ventricular samples of tachypaced dogs (n=13), healthy controls (n=7) and whole blood samples (surplus material) of canine clinical patients (n=10) with different severity and etiology of heart failure caused by cardiomyopathy were collected in RNA stabilizing solution. RNA integrity was confirmed by capillary electrophoresis (RIN>7). Expressions of selected microRNAs were measured by gRT-PCR (TagMan-chemistry) and normalized to U6 snRNA by ddCt method. Data evaluation was made by descriptive statistics and Mann Whitney U-test.

In the tachypacing-induced model miR-1, miR-208a, miR-208b and miR-499 were up-regulated, whilst miR-21 was down-regulated in both heart and blood samples. MiR-29 was up-regulated in heart samples, while down-regulated in blood samples whilst miR-133a showed the opposite tendency. In blood samples of clinical patients the same expression pattern could be observed and the results were significant (p<0,05) except miR-21. Based on the selected microRNAs, clinical cases could be clearly differentiated from healthy dogs using their microRNA expression profile in blood samples. Moreover clinically asymptomatic cases with tachycardiomyopathy could be identified. Our results suggest that peripheral blood may have a potential to indicate early heart-specific fibrosis in dogs.

#### Disclosures

No disclosures to report.

## ESVC-O-20

## Heart rhythm characterization by 24-hour Holter monitoring during sudden cardiac death in dogs - A case series

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Malignant arrhythmias are poorly defined as the cause of sudden cardiac death (SCD) in dogs. Although SCD has been described in dilated cardiomyopathy, arrhythmogenic cardiomyopathy, hypertrophic cardiomyopathy, and myocarditis, as well as in inherited and congenital arrhythmic disorders (German Shepherd, Rhodesian Ridgeback and Springer Spaniels, respectively), only few contextual 24-hour Holter monitoring data is available in the veterinary literature. The aim of this case series was to describe the terminal arrhythmia recorded by 24-hour Holter monitoring in dogs in the moment they experienced SCD.

The database of a Holter referral center was retrospectively searched for client-owned dogs that experienced SCD during 24-hour Holter monitoring between January 2006 and July 2017. Fifteen dogs of different breeds were included in the study. The Holter analysis included the dominant rhythm during the recording, the prevalent arrhythmia in the period preceding death, and the terminal arrhythmia causing SCD. Clinical data and echocardiographic diagnosis were also considered.

The mean age was 8.7  $\pm$  3.9 years (median 9 years) and mean body weight was 30.8  $\pm$  17.2 kg (median 30.0). In 60% of dogs (9/15), the cause of SCD consisted of ventricular premature complexes (VPCs), accelerated idioventricular rhythm (AIVR), or ventricular tachycardia (VT) that degenerated into ventricular fibrillation (VF). In two dogs (13%) complete atrioventricular block (AVB) preceded VF. In 3 dogs (20%), asystole was the cause of cardiac arrest. Finally, in 1 dog (7%), low penetrance atrial fibrillation was followed by ventricular arrest and electromechanical dissociation. Ten dogs were affected by a variety of structural heart diseases (5 dilated cardiomyopathy; 3 arrhythmogenic right ventricular cardiomyopathy; 1 myxomatous mitral valve



disease; 1 myocarditis), while echocardiographic examination detected no structural abnormality in the remaining 5 dogs. Sotalol was the antiarrhythmic therapy at the time of recording in 2 dogs.

In agreement with the data previously reported in humans, the present study showed that VPCs, AIVR, VT, complete AVB degenerating into VF and asystole are the most common causes of SCD in dogs. Additionally, the fact that 2/3 of the study population had underlying structural heart disease suggests that the presence of an anatomical substrate, when associated with a modulator (autonomic nervous system) and a trigger, increases the risk of sustained ventricular arrhythmias and degeneration into VF. To the authors' knowledge this is the first report detailing causative arrhythmia of SCD in an extended series of dogs.

#### Disclosures

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## ESVC-O-21

## R-peak time in normal dogs

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R-peak time (RPT) is an ECG parameter that represents the time from the QRS complex onset (Q or R wave) to the apex or peak of the R or R' wave, if present. It represents the time for excitation to spread from the endocardial to the epicardial surface. In human medicine, right ventricular RPT is measured in lead V1 (upper normal limit 35 ms), while left ventricular RPT is measured in lead V6 (upper normal limit 45 ms). The aim of this study was to define the duration of RPT in normal dogs with different thoracic conformation.

Records of thirty privately-owned healthy dogs were retrospectively reviewed. All dogs underwent physical examination, thoracic radiography, 12-lead electrocardiogram using the lead system described by Santilli in 2016, and echocardiogram. The dogs were allocated in three groups according to the thoracic index (dorso-ventral thorax diameter x 100/latero-lateral thorax diameter): 90-100 brachymorphic, 50-60 dolichomorphic, and 60-90 mesomorphic. For each ECG, three measurements were performed. Normality was tested using the Shapiro-Wilcoxon W-test. To evaluate differences between morphotypes and leads, nonparametric analysis of variance was performed by Kruskas Wallys test, as data were not normally distributed. In the brachymorphic morphotype, RPT median duration and range were: V1: 10 ms (7-16), V2: 17 ms (10-19), V3: 18 ms (11-24), V4: 19.5 ms (11-24), V5: 21 ms (14-24), V6: 21 ms (12-25). In the mesomorphic morphotype, RPT median duration and range were: V1: 13 ms (9-19), V2: 21 ms (12-26), V3: 21.5 ms (18-25), V4: 22 ms (17-28), V5: 24 ms (18-30), V6: 24.5 ms (17-31). In the dolichomorphic morphotype, RPT median duration and range were: V1: 15.5 ms (7-21), V2: 27 ms (19-35), V3: 28 ms (20-34), V4: 28 ms (20-36), V5: 29 ms (24-37), V6: 29.5 ms (25-37). In all precordial leads, RPT was statistically different between morphotypes (P<0.0001). In lead V1, RPT was statistically shorter (P<0.00001) compared to V2 through V6 in all morphotypes.

These results demonstrate a difference in the RPT duration in different morphotypes and a difference between V1 and the other

precordial leads, which can be explained by the fact that V1 reads the thinner-walled right ventricle and V2 through V6 read the thickerwalled left ventricle. These data can be used as reference for normal RPT in the dog. Further studies are needed to evaluate the use of RPT in cardiac diseases.

## **Disclosures**

No disclosures to report.

#### ESVE-O-1

Radioiodine treatment of feline hyperthyroidism: measuring 24-hour thyroid 131-I uptake helps predict treatment failure or development of iatrogenic hypothyroidism

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Successful treatment of hyperthyroidism in humans requires effective uptake of the <sup>131</sup>I by the thyroid gland to deliver its radioablative effects. We sought to analyze the effects of <sup>131</sup>I uptake on subsequent outcome in hyperthyroid cats.

We prospectively included 937 cats referred to the Animal Endocrine Clinic for <sup>131</sup>I treatment. Following quantitative thyroid scintigraphy, we calculated thyroid volume and percent thyroidal uptake of pertechnetate for each cat. We administered a radioiodine dose based on an algorithm that considers the serum T4 and T3 concentrations, percent uptake of pertechnetate, and calculated thyroid volume. Twenty-four hours later, we then determined the percent <sup>131</sup>I thyroid uptake by measuring radioactive counts directly on the skin surface over the thyroid tumor. After correction for background (thigh count), the thyroid radioactivity (in counts per minute) was compared with the activity measured from a standard containing pprox350  $\mu$ Ci  $^{131}$ I. To evaluate the effect of  $^{131}$ I uptake on final outcome, cats were divided into 4 quartiles, based on thyroid uptake values.

Recheck examinations were performed at 4-7 months (median, 6 months). Four outcomes were determined based on serum concentrations of T4 and TSH concentrations: euthyroid (normal T4, normal TSH), overt hypothyroid (low T4, high TSH), subclinically hypothyroid (low-normal T4, high TSH), and persistently hyperthyroid (high T4, suppressed TSH).

Hyperthyroid cats had  $^{131}$ I thyroid uptake values ranging from 4.3-49.2% (median, 21.5%). Cats in the lowest uptake quartile (<17%) had more than twice the incidence of persistent hyperthyroidism than cats in the other quartiles (8.6% vs. 3.4%, P < 0.001). In contrast, the cats in the highest uptake quartile (>27%) had the higher incidence of iatrogenic hypothyroidism (29.1% vs. 12-21%, P <0.001). Cats in all 4 uptake quartiles were treated with similar doses of radioiodine (median dose, 1.9 mCi).

In conclusion, the 24-hour thyroid uptake value affects successful outcome of hyperthyroid cats treated with radioiodine. Cats with low <sup>131</sup>I thyroid uptake values have ~10% chance of treatment failure, whereas cats with the highest uptakes are predisposed to developing iatrogenic hypothyroidism.

## **Disclosures**

No disclosures to report.



## ESVE-O-2

Evaluation of telmisartan administration as a suppression test for primary hyperaldosteronism diagnosis in cats

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Diagnosis of feline primary hyperaldosteronism (PHA) remains challenging especially in cases with concurrent azotaemia. In human medicine, the losartan suppression test is used as a safe and accurate test for hyperaldosteronism diagnosis.

The aim of the study was to determine if oral telmisartan administration suppress aldosterone secretion in healthy cats and cats with PHA.

Following ethical committee approval, 10 healthy student-owned cats received telmisartan administration (2mg/kg per os). Serum aldosterone concentration (PAC), potassium concentration and systolic blood pressure were measured before (T0), 1h (T1) and 1.5h (T1.5) after oral administration of telmisartan. Six clientowned cats with PHA were also tested. A percentage of the relative variation of PAC was calculated at T1 ((T1-T0)/T0) and at T1.5 (T1.5-T0)/T0). Results were expressed as median [range] and variables were compared by a wilcoxon signed-rank test or by a mann-whitney test.

Baseline PAC was significantly higher (P < 0.01) in PHA cats (1866 pmol/L [range, 265 - 2419]) than in healthy cats (274 pmol/L [65-1364]. In healthy cats, telmisartan administration resulted in a significant decrease of PAC at T1 and T1.5 compared with T0 (P < 0.01) with a relative variation of -40% [5%- -64%] and -47% [-15%- -60%] respectively. No significant decrease of PAC was identified in PHA cats. Systolic blood pressure significantly decreased but no significant variation of potassium concentration was identified.

Our results suggest that oral telmisartan administration suppress aldosterone concentration in healthy cats but not in PHA cats. Further studies are needed to evaluate this suppression test in cats with secondary hyperaldosteronism.

#### Disclosures

Disclosures to report.

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## ESVE-O-3

## Pheochromocytoma in dogs undergoing adrenalectomy: an exploratory investigation of 24 cases

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Pheochromocytoma is relatively common in dogs and carries a guarded prognosis. Outcome of adrenalectomy is the most important predictor of survival, although younger age and absence of vascular invasion may also be associated with a favourable prognosis. Current histological criteria do not predict a malignant behaviour in dogs, similar to humans. In the latter, the characterization of tumors has been refined by the "Pheochromocytoma of the Adrenal gland Scaled Score" (PASS), which includes 12 microscopic features, and by immunohistochemical analysis. The aim of the study was to investigate PASS and several immunohistochemical markers used in human medicine in dogs with pheochromocytoma that underwent adrenalectomy. Pathology records of dogs with pheochromocytomas were reviewed to identify those treated surgically and tumors were collected. Sections were stained with haematoxylin and eosin to apply the PASS and were single-labelled for chromogranin A, proliferation marker Ki-67, cyclooxygenase-2 (COX-2), tumor suppressor gene product p53, proto-oncogene products BCL-2 and c-erbB-2, vascular endothelial growth factor (VEGF), protein S100. The results of PASS and of immunohistochemistry were compared for surgical outcome, age and frequency of capsular and vascular invasion caused by the tumor. In addition, PASS was assessed in relation to immunohistochemical findings. Twenty-four dogs with pheochromocytomas were included. PASS did not differ based on surgical outcome, age, vascular and capsular invasion. Immunohistochemistry was also not different for any variable. Tumors showing BCL-2 expression in >50% cells had lower PASS than those with lower expression (7 $\pm 2$  vs. 9 $\pm 2$ ; P=0.011). Tumors positive for S100 had higher PASS than those negative ( $10\pm2$ vs.  $7\pm2$ ; P=0.001). Associations between immunohistochemical markers were not identified. In conclusion, in dogs with pheochromocytoma, survival, age, vascular and capsular invasion are not linked to the results of PASS and of the selected immunohistochemical markers. The lower PASS in dogs with elevated BCL-2 expression suggests that pheochromocytomas with high anti-apoptotic rate have few morphologic changes. The higher PASS in S100-positive tumors may indicate that pheochromocytomas developing morphologic changes acquire S100 expression. Overall, the use of PASS and of the present immunohistochemical markers is of limited clinical value in dogs with pheochromocytoma.

#### Disclosures

No disclosures to report.

## ESVE-O-4

Temporal changes in thyroid status in euthyroid dogs with positive thyroglobulin autoantibodies

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Thyroid autoantibodies are used as markers of autoimmune thyroiditis in humans and dogs. In humans, their presence is associated with a 2.5-5.0% per year progression from subclinical thyroiditis to hypothyroidism. Several survey studies have shown an increased prevalence of positive thyroglobulin autoantibody (TgAA) in dogs with thyroid

test results indicative of primary hypothyroidism versus euthyroid dogs. The implications of a positive TgAA result with otherwise normal concentrations of thyroid hormones and TSH (subclinical thyroiditis) on the subsequent progression to hypothyroidism in dogs are less clear. The objective of this study was to define the outcome of thyroid status in dogs with subclinical thyroiditis over a multi-year interval. Purebred dogs with subclinical thyroiditis (>25% specific TgAA binding but with normal concentrations of T4. FT4 and TSH) were identified from the Orthopedic Foundation for Animals (OFA) Thyroid Registry database. Owners were invited to arrange submission of a follow-up serum sample with their veterinarian along with inclusion of a medical history form including subsequent treatments. Serum samples were obtained from 114 dogs with subclinical thyroiditis that met inclusion criteria. The study population included 62 female and 52 male dogs from 30 breeds with English Setters, Boxers, Rhodesian Ridgebacks and Dalmatians most frequent. The average age at study completion was 6.3 years (SD = 2.2 years, range 2-13). The mean follow-up time was 3.9 years from initial testing (SD = 2.0 years, range 1-9). At the time of retesting, 33% of the dogs had progressed to hypothyroidism, or were treated with thyroxine. Fifty-one percent maintained positive or equivocal TgAA results while remaining euthyroid. In 16% of dogs, TgAA results had normalized with dogs remaining euthyroid. Initiation of thyroxine supplementation following a TgAA-positive result was usually, but not always, based on suggestive clinical signs and decreased thyroid hormones at subsequent testing. These results suggest that the majority of dogs with elevated thyroglobulin autoantibodies either exhibit persistent autoimmune thyroiditis with continued risk of hypothyroidism or progress to hypothyroidism.

#### Disclosures

Disclosures to report.

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## ESVE-O-5

#### Haptoglobin and pre-trilostane cortisol as monitoring tools for the treatment of canine hyperadrenocorticism

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Haptoglobin is increased in untreated cases of canine hyperadrenocorticism (HAC) and reduces following trilostane treatment. We hypothesised that haptoglobin measurements may assist therapeutic monitoring of HAC.

The first part of this study compared haptoglobin concentrations in two samples obtained 1 hour apart with known pre-trilostane cortisol concentrations. The second part compared haptoglobin and pretrilostane cortisol concentrations in the monitoring of HAC. In both studies, cortisol was measured using a chemiluminescent assay (Siemens Immulite) and as part of normal therapeutic monitoring protocols at our institutions.

1) Haptoglobin was measured using an in-house enzymatic method adapted for the ABX Pentra in 20 pairs of residual stored samples obtained 1 hour apart before the administration of trilostane. The coefficient of variation between the haptoglobin measurements was low (median=2.3%, range 0-7.9%), even when there were clinically significant differences between the pre-trilostane cortisol concentrations (median=12.3%, range 0-61.2%, 8 pairs of samples greater than 20%)., 2) Cases of HAC that had received a consistent dose of trilostane for more than two weeks were blood sampled and clinical scored using an owner questionnaire, from which cases were then categorised as being unwell, controlled, or under-controlled. Unwell dogs were excluded from further analysis, regardless of the control of their HAC. Haptoglobin was measured as part of our normal monitoring using a commercially available immunoturbidimetric assay (Avacta Laboratories, Wetherby). There were 44 samples, obtained from 19 dogs. One dog was noted as being particularly anxious before 2 sampling points and its pre-trilostane cortisol concentrations were excluded from all subsequent analysis. Mann Whitney tests demonstrated that there were significant differences between both the pretrilostane cortisol (p=0.036) and haptoglobin (p=0.012) of dogs classified as being controlled and those that were uncontrolled. There was a significant correlation between all the haptoglobin concentrations and the owners score (r=0.52, p<0.001) whereas pre-trilostane cortisol concentrations were not significantly correlated with the score (r=0.27, p=0.13). ROC analysis suggested that a previously established reference range of haptoglobin (0.1-3.0g/l) could serve as a useful target range for therapeutic monitoring. Similar analysis for pre-trilostane cortisol was consistent with the current therapeutic range (40-138nmol/I).

Haptoglobin should be further investigated as an ancillary method for monitoring trilostane therapy. As the reference range for haptoglobin extends to nearly 0, it will not detect over-treatment and so other methods (such as pre-trilostane cortisol) are still needed. One advantage of haptoglobin appears to be that it is unaffected by short-term changes in cortisol.

#### Disclosures

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Dechra Pharmaceuticals directly supported this study and also provided indirect support to the first author. Glasgow University provides haptoglobin and pre-trilostane cortisol measurements on a commercial basis. Siemens, ANIPoc, Woodleys, Boehringer and many other companies have supported work at one or both institutions but are not directly involved in the funding of this project.

## ESVE-O-6

## Effect of monthly injections of GLP-1 analogue exenatide extended release on β-cell function in newly diagnosed diabetic cats

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Exenatide (GLP-1 analogue) has proven beneficial in diabetic people. Clinical experience in cats is limited and effect on β-cell function (BCF) in diabetic cats is unclear.



Diabetic cats (diagnosed <6 months) were recruited for a 6-month, double-blinded, randomised, placebo-controlled clinical trial assessing the effect of once monthly exenatide extended release (EER; Bydureon, AstraZeneca) injections on glycaemic control and BCF. Cats were excluded if screening identified relevant co-morbid conditions. Cats were treated with protamine zinc insulin (ProZinc, Boehringer Ingelheim), fed a low-carbohydrate diet and randomised to EER (0.13mg/ kg SQ g 27-32 days) or placebo (saline). Diabetic control and BCF were assessed using fructosamine, and insulin peak response (IPR) and total insulin secretion (TIS) calculated from glucagon stimulation tests (insulin at baseline, 15 and 60 mins post 20ug/kg IV glucagon), respectively. Enrolment body condition score (BCS) and fructosamine were compared between groups (t-test). Linear mixed effects modelling assessed the effect of treatment group, trial timepoint and enrolment BCS (> or <5/9) on glycaemic control and BCF (significance p<0.05).

Eleven cats received EER, 13 received placebo. Groups were similar at enrolment, apart from higher BCS in placebo-group (mean (±SD): 4.4 ( $\pm 1.4$ ) vs. 5.7 ( $\pm 1.5$ ); p=0.045). There was no significant effect of treatment group or enrolment BCS on IPR/TIS and fructosamine, but IPR and TIS significantly increased (p=0.002), and fructosamine decreased (p=0.0002), over successive trial timepoints.

EER did not affect BCF or glycaemic control, however, inadvertent randomisation of lean cats to EER means the use of EER in obese cats could not be assessed

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Disclosures to report.

Katarina Hazuchova's PhD, which the clinical trial has been a part of, is funded by Boehringer Ingelheim. Diabetic Remission Clinic, where the study took place, receives support from Purina.

## ESVE-O-7

## A visual aid to judgement in the diagnosis of canine hyperadrenocorticism and the development of a diagnosis score

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Diagnosing canine hyperadrenocorticism (HAC) can be complicated. Dynamic adrenal function tests have low specificity and/or less than perfect sensitivity. Anecdotally, HAC can be incorrectly diagnosed or excluded when key clinical or laboratory features are overlooked or disregarded.

The aim of this study was to construct an educational visual aid to assist clinicians in balancing and appropriately weighting clinical and laboratory observations to support judgement in the diagnosis of HAC.

The first aid (Version 1) comprised a 6-box grid arranged in 2 columns of 3 including a list of relevant observations. One column recorded observations known to support a diagnosis of HAC (e.g. thin skin, polyuria, polydipsia, positive adrenal function test) and the other those that would support non-adrenal-illness (NAI; e.g. significant weight loss, absence of stress leukogram, reference interval alkaline phosphatase activity, negative low-dose-dexamethasone suppression test (LDDST)). The rows divided observations conveying mild, moderate or strong support for the respective diagnosis. The second aid (Version 2) included author-opinion-based numeric weightings for specific observations allowing calculation of a score indicating whether HAC was likely, unlikely or equivocal. Initial cut-offs were derived from a small development dataset (n=19).

Performance of the summary score was assessed using an anonymised validation dataset of 119 suspect HAC cases from UCD Veterinary Hospital classified as HAC (n=58) or NAI (n=61). A diagnosis of HAC was supported by a documented improvement with treatment (n=42), postmortem examination (n=3) and independent expert review of the medical records (n=13). A diagnosis of NAI was supported by an alternative diagnosis (n=55), spontaneous resolution of clinical signs (n=3) and absence of progression or onset of additional clinical signs over a followup period > 1 year (n=3). The sensitivity of the score ranged from 53 to 66%, depending on whether equivocal results were treated as positive or negative for HAC. Specificity was 93%. Area under ROCC was 0.926 indicating that cut-off adjustment could improve diagnostic sensitivity. Four false negative scores were complex cases where HAC was diagnosed in the face of a negative LDDST adversely affecting sensitivity. In conclusion, an educational visual judgement aid was developed to support HAC diagnosis in first opinion practice. When modified to generate a numeric score, the tool had good specificity but less good sensitivity, likely reflecting the authors' concerns for over- rather than

under-diagnosis. Future improvements to cut-off values statistically-derived weightings could improve diagnostic accuracy and HAC diagnostic judgement in primary care practice.

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#### ESVE-O-8

Functional impact of single nucleotide polymorphisms within the putative promoter of feline ACP1 gene in lean Domestic Shorthair cats with diabetes mellitus

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A genome-wide association study of diabetes mellitus (DM) in Domestic Shorthair cats identified single nucleotide polymorphisms (SNPs) at the end of chromosome A3. The ACP1 gene, encoding an enzyme involved in insulin signalling, is located close to these SNPs and was selected as a candidate diabetes susceptibility gene.

A DM-associated haplotype (c.-227A, c.-378G, c.-420G, c.-452G; p=0.007) was identified within the putative promoter of ACP1 in a case-control association study (residual blood samples used for PCR). A dual-luciferase reporter assay was used to investigate the potential impact of these SNPs on gene expression. DNA sequences carrying two variant promoter haplotypes (variant 1 - DM-associated haplotype, variant 2 - non-diabetic haplotype) were amplified by PCR, and cloned separately into the pGL4 vector containing Firefly luciferase [FLuc]. The pRL\_CMV vector, containing Renilla luciferase [RLuc] was used as a control vector for co-transfection of CHO cells, to enable

normalisation of FLuc activity. Positive (pGL3) and negative (promoterless pGL4) controls were included. There was no difference in the normalised luminescence comparing the recombinant plasmids (FLuc: RLuc: mean (SE); variant 1: 5.6 (0.42); variant 2: 5.13 (0.27); p=0.7 [Mann-Whitney-U]).

The DM-associated ACP1 promoter haplotype did not influence gene expression in CHO cells, compared with variant 2, which was more prevalent in non-diabetic cats. This might be due to a lack of any functional impact of these polymorphisms or the result of the experimental system used. Further experiments are required to assess the functional consequences of ACP1 promoter genetic variability in cells expressing the insulin receptor.

#### Disclosures

Disclosures to report.

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#### FSVF-O-9

## Thyroidectomy in dogs with thyroid tumors: survival analysis in 99 cases (1994-2013)

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Few studies assessed outcome predictors in dogs with thyroid tumors undergoing thyroidectomy. Median survival ranged between 500-1100 days. Larger tumors, local invasiveness and excision of both thyroid lobes negatively affected outcome, whereas adjuvant chemotherapy did not improve survival. Metastasis was not associated with a poor outcome. The aims of the present study were to calculate survival and identify prognostic factors in a large series of dogs with thyroid tumors treated with thyroidectomy.

Medical records of dogs with thyroid tumors that underwent thyroidectomy between 1994 and 2013 were reviewed. From each dog the following variables were collected at admission to determine their influence on overall survival, defined as the time from surgery to death: age, gender, breed, T4 and TSH concentration, unilateral or bilateral lobectomy, type of surgery (thyroidectomy combined or not with muscular and jugular vein resection), institution where surgery was performed, tumor major diameter, histological type, presence of thrombosis and metastasis, administration of adjuvant chemotherapy. Median survival was calculated with Kaplan-Meier product-limit. Outcome predictors were studied using Cox proportional hazard models. The final model was built with stepwise deletion guided by the Akaike information criterion.

Ninety-nine dogs were included; median age was 10 years (range: 5-14), 47% were males and 53% females, 29% were crossbreed and 71% pure-breed. Except one adenoma, all tumors were carcinoma. Median survival was 693 days (range: 27-3018), with 10% living <154 days="" and="" 25="">902 days. From univariate analysis older age, presence of thrombosis and presence of metastasis were associated with shorter survival. In the multivariable model, only metastasis was significantly associated with survival. Dogs with metastasis had a hazard ratio (HR) of 5.97 (confidence interval [CI] 95%: 1.97-18.1) compared to those without (p=0.002). Metastasis were identified in 8 (8.1%) cases and 4 of them lived >500 days. Dogs with thrombosis tended to be at increased risk of death (HR: 2.24; CI95%: 0.92-5.48; p=0.077).

Dogs with thyroid tumors undergoing thyroidectomy have long life expectations. Metastasis may carry a poor outcome although extended survival is observed in some of them. Differently from previous studies, size of the tumor, local invasiveness and bilateral thyroidectomy did not affect outcome.

#### Disclosures

No disclosures to report.

## ESVE-O-10

## The erythrocyte membrane lipidome profile in healthy dogs and changes in dogs with diabetes mellitus

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Analysis of erythrocyte membrane lipidome represents a powerful diagnostic tool for assessing the quantity and quality of fatty acids and for the follow-up of the membrane fatty acid remodeling that is associated with different physiological and pathological conditions. Several studies have shown modification of the fatty acids but also of the phospholipid content of cell membranes in human diabetic patients and in animal model of diabetes, however, a systematic study of the membrane fatty acids of dogs to evaluate membrane homeostasis has not yet been established. The aims of the present study were to evaluate 1) the erythrocytes membrane lipidomic profile in healthy dogs (HD, n=24) and 2) changes in membrane lipidome of dogs with newly diagnosed diabetes mellitus (DM, n=6).

Erythrocyte membranes were isolated from EDTA-treated blood samples from dogs and a cluster of 10 saturated [SFA (palmitic; stearic)], mono-unsaturated [MUFA (palmitoleic; oleic; vaccenic)] and polyunsaturated [PUFA (linoleic; dihomo-gamma-linolenic; arachidonic; EPA; DHA)] fatty acids was determined by Gas-Chromatography. Relevant lipid parameters (SFA/MUFA, SFA/PUFA, ω6/ω3, PUFA balance, unsaturation and peroxidation indexes) were calculated.

Healthy dogs, aged from 2 to 98 months (median 38.5) 10 were males (1/10 neutered) and 14 females (4/14 sterilized), while DM dogs, aged from 96 to 158 months (median 120) 2 were males and 4 females (2/4 sterilized).

Among the fatty acids, the ω3 (median 1.75%) showed the wider variability in HD (minimum 0.5-maximum 6.8). No significant differences were observed regarding the age. The EPA levels in intact females were significant lower (P<0.01) compared to sterilized subjects and intact males. Palmitic and arachidonic acids showed less SD variability (<12% and <6%, respectively) in medium body size dogs (10-20 Kg) compared to small (<10 kg="" and="" large="">20 Kg) dogs (27-40% and 27-14%, respectively).



In comparison to the HD, DM dogs showed increased concentrations of MUFAs (P<0.05), specifically, palmitoleic (P<0.0001) and oleic (P<0.05) acids showed higher levels, while no differences were observed for vaccenic acid. The EPA concentrations in DM dogs were higher (P<0.01) as compared to the HD.

Potential study limitations were the sample size and the lack of data in geriatric healthy dogs.

The present data suggest a variability of ω3 expression in erythrocytes membranes of healthy dogs, probably due to the individual dietary variations. Furthermore, these preliminary results suggest the involvement of the SFA-MUFA pathway in canine diabetes mellitus, involving higher palmitic-palmitoleic and palmitic-oleic transformations and an accelerated delta-9 desaturase enzymatic activity.

#### Disclosures

No disclosures to report.

## ESVE-O-11

## Presence of T- and B-lymphocytes in the canine pituitary gland

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Hypophysitis is a well described group of diseases in humans, and a few cases have been reported in dogs resembling lymphocytic hypophysitis in humans. Although, there is a common assumption that lymphocytes are not normally present in the pituitary glands of dogs, there is a paucity of studies to confirm this. Lymphocytes have been found in pituitary glands of both humans and horses, without clinical suspicion of hypophysitis.

The aim of the present study was to investigate the occurrence of lymphocytes in pituitary glands of dogs without clinical suspicion of pituitary disease.

Pituitary tissue was collected at routine necropsy from 20 dogs, without clinical suspicion of pituitary disease, in this cross-sectional study performed at the Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala. Pituitary tissue was fixed in 10% formalin. The pituitary glands were sagittally incised before embedding in paraffin. Sections (4 µm) were stained with haematoxylin and eosin (H&E). Sequential sections were subjected to immunohistochemistry using primary antibodies specific for the T-cell marker CD3 and the B-cell marker CD79a. An indirect avidin-biotin-peroxidase procedure using diaminobenzidine as chromogen was used for detection of antigen-antibody binding. The number of cells per area unit (CPA) was determined in pars tuberalis, pars distalis, pars intermedia, infundibulum and lobus nervosus.

Two dogs were excluded because of presence of metastatic malignant lymphoma and pituitary adenoma. In the pituitary glands of the remaining 18 dogs, occasional scattered CD3+ cells were found on immunohistochemistry. The CD3+ CPA differed significantly between parts of the pituitary gland (p < 0.001). The highest CD3+ CPA was found in the pars tuberalis (median CPA 41.3 cells/mm<sup>2</sup>, range 8.9 to 94.7 cells/mm<sup>2</sup>). The lowest CD3+ CPA was found in the infundibulum (median CPA 2.0 cells/mm<sup>2</sup>, range 0 to 10.7 cells/mm<sup>2</sup>). Only 6 of the 18 dogs (33%) had detectable CD79a+ cells in their pituitary glands, and when present they were in small numbers (median total cell number 0 cells/section, range 0 to 14 cells/section).

This study shows that it is common to find CD3+ cells in the pituitary gland at necropsy of dogs with no clinical suspicion of pituitary disease, and that there is a regional difference in cell density with the highest CD3+-cell density in the pars tuberalis. In contrast, CD79a+ cells were generally absent or present in only small numbers.

#### Disclosures

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## ESVE-O-12

## Comparison of continuous glucose monitoring profiles and glycemic variability during day and night in healthy and diabetic cats

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Glycemic variability represents swings in blood glucose levels including episodes of hypo- and hyperglycemia throughout the day and on different days. Glucose fluctuations were shown to be one of the risk factors for diabetes-related complications in humans. So far, knowledge about glycemic variability in cats is scarce. Continuous glucose monitoring systems (CGMS) have been introduced into veterinary medicine some years ago and are useful tools to evaluate the glycemic situation. They measure interstitial glucose continuously during day and night for up to seven days. The aim of this study was to evaluate the continuous glucose monitoring profiles of healthy and diabetic cats and to compare glycemic variability during day and night.

Six healthy and eleven diabetic cats were included. CGMS iPro2® (Medtronic) was placed in the dorsolateral neck area. For calibration glucose measurements were obtained every 8-12 hours with a validated portable blood glucose meter (AlphaTRAK2®, Abbott). Glucose measurements by the iPro2® were achieved every five minutes. Day and night were defined as the time (approx. 12 hours) between the insulin injections and/or feeding. The mean glucose concentration, standard deviation (SD) and coefficient of variation (CV) were determined to assess glycemic variability. Additionally, the minimum and maximum glucose concentration were identified. Data were analysed by nonparametric tests (P<0.05). Values are reported as median and

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

In diabetic cats, minimum glucose concentration during the day (5.4, 3.4-15.4 mmol/l) was significantly lower (P=0.032) than during the night (7.7, 4.1-17.1 mmol/l). There were no significant differences between day and night with regard to mean and maximum glucose concentration, SD and CV.

Healthy cats had lower mean, minimum, maximum glucose concentration and SD during the day (P=0.0011, P=0.0019, P=0.0012, P=0.0029) and night (P=0.0006, P=0.0002, P=0.0048, P=0.044) than diabetic cats, while CV did not differ.

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

#### Disclosures

No disclosures to report.

## ESVE-O-13

Validation of human immunoturbidimetric assays for the measurement of glycated hemoglobin (HbA1c) and serum fructosamine in dogs and comparison of the two variables for the assessment of glycemic control in dogs with diabetes mellitus

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The aims of this study were the validation of human assays for serum fructosamine (SF) and glycated hemoglobin (HbA1c) in dogs and the comparison of the ability of the HbA1c and SF in reflecting the glycemic control in dogs with diabetes mellitus. SF (nitrotetrazole blue method, Fructosamine 17350H, Sentinel Diagnostics) and blood HbA1c (HbA1c, OSR6192 Beckman-Coulter) were analyzed on an automated chemistry analyzer (Beckman-Coulter AU 480). Linearity, precision and accuracy were determined; a reference interval for HbA1c and SF was established from 40 healthy dogs, using the percentile method. Performances of HbA1c and SF in assessing the glycemic control were evaluated; correlation between the two variables and a clinical score was studied in 200 re-evaluations of 47 diabetic dogs treated with insulin q12h. The clinical score used to classify diabetic dogs in good (GGC), moderate (MGC) or poor (PGC) glycemic control was set on the basis of stability of body weight, presence of poliuria/polydipsia, median glucose of the blood glucose curves (BGCs), blood glucose nadir and overall evaluation of BGC. The average intra- and interassay coefficient of variation (CV) for HbA1c were 1.5% and 10.9%, respectively; the average intra- and interassay CV for SF were 4.1% and 2.5%, respectively. Excellent accuracy was obtained for both assays (r>0.99). The reference interval for HbA1c was 1.6-4.5% and for SF was 222-382 μmol/L. In diabetic dogs, HbA1c and SF were significantly correlated (r=0.48) and they were also correlated with the clinical score (r=-0.33; r=-0.39, respectively). ROC curves analysis of SF and HbA1c to distinguish GGC from

MGC/PGC dogs showed an AUC of 0.69 and 0.66, respectively. Values of HbA1c<5.5% and SF<400 μmol/L had Sp=79%, Se=41%, and Sp=71%, Se=61%, in discriminating dogs with GGC from MGC/PGC dogs, respectively. ROC curves analysis of SF and HbA1c to distinguish PGC from GGC/MGC dogs showed an AUC of 0.75 and 0.69, respectively. Values of HbA1c>6.8% and SF>500 μmol/L had Sp=79%, Se=52%, and Sp=85%, Se=45%, respectively, in discriminating dogs with PGC from GGC/MGC dogs. Using the clinical score as the reference method, SF and HbA1c identified correctly the glycemic control (GGC/MGC/PGC) in 50% and 44% of cases, respectively. In conclusion, SF and HbA1c have similar performances in classifying the glicemic control. Neither of the two parameters should be used as the sole indicator of glycemic control, and must always be evaluated in association with history, physical findings and BGC results.

#### **Disclosures**

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#### ESVE-O-14

#### Spontaneous primary hypothyroidism in adult cats: More common that we think?

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Spontaneous primary hypothyroidism appears to be an extremely rare clinical disorder in adult cats, with only 4 reported cases over the last 25 years. Given the paucity of data regarding adult-onset feline hypothyroidism, we sought to describe the history, clinical features (including presence or absence of goiter), diagnostic testing, treatment, and long-term outcome of 7 adult cats with spontaneous primary hypothyroidism.

This prospective case series included 7 adult cats with spontaneous hypothyroidism referred for evaluation over a 3.5-year period (from March 2014 to September 2017), and then followed until March 2018. In each case, we collected data on cats' signalment, clinical signs, results of physical examination, routine laboratory and thyroid hormone testing, and thyroid imaging (thyroid scintigraphy or ultrasound). We subsequently treated cats with levothyroxine and evaluated their response to treatment.

Cats ranged from 3.5-11 years, with no breed predilection; 6/7 cats were male. Only 2/7 cats were initially tested because of signs of hypothyroidism (hair-coat changes, lethargy, obesity); others were tested for routine thyroid monitoring or palpable thyroid nodules. Four were azotemic (serum creatinine, 2.2-3.4 mg/dL). Six of the cats had low serum thyroxine (T4) and free T4 (fT4) concentrations, whereas all 7 cats had high thyroid-stimulating hormone (TSH) concentrations. In 6/7 cats, thyroid scintigraphy revealed bilateral goiter with intense radionuclide uptake; imaging showed no visible thyroid tissue in the other. After levothyroxine treatment, serum concentrations of T4 and fT4 increased and TSH fell; high serum creatinine normalized in azotemic cats; and repeat imaging showed reduction in goiter size.



In conclusion, primary hypothyroidism develops in adult cats, with a higher prevalence than previously assumed. Most cats appear to develop a goitrous form of hypothyroidism associated with thyroid hyperplasia, whereas thyroid atrophy appears to be less common. With levothyroxine replacement, clinical and laboratory abnormalities (e.g., azotemic) improve or resolve. Although the condition is rare, we suspect that these documented cases represents only "the tip of the iceberg" of cats affected with this syndrome, because of the mild clinical signs displayed by these cats, and the lack of awareness of this condition by first opinion clinicians. Heightened awareness that adult hypothyroidism can develop in cats, together with increased screening

for the disorder, will ultimately determine if this condition is indeed

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rare or more common than currently thought.

## **ESVE-O-15**

Short- and long-term follow-up of kidney function using creatinine, SDMA and GFR in hyperthyroid cats treated with radioiodine

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Sensitivity of serum creatinine (sCr) for detection of early chronic kidney disease is poor and its performance is even worse in hyperthyroidism due to poor muscle mass and hyperfiltration. Serum symmetric dimethylarginine (sSDMA) is a more sensitive renal biomarker in dogs and cats. A recent study showed relatively low sSDMA sensitivity (33.3%) in revealing masked azotemia in hyperthyroid cats. The objective of this study was to provide a short- and long-term follow-up of sSDMA and glomerular filtration rate (GFR) in hyperthyroid cats treated with radioiodine.

Ten non-azotemic hyperthyroid cats were prospectively included and sCr, sSDMA (IDEXX SDMA<sup>TM</sup> Test) and GFR (plasma exogenous creatinine clearance) were measured before (T0), after 1 month (T1) and within 14-24 months (mean: 16 months) (T2) after radioiodine treatment. Nine cats finished the study, 1 cat died of unrelated cause before T2. Serum creatinine significantly increased from T0 to T1 (P<0.001) and further increased from T1 to T2 (P<0.05), but all cats remained non-azotemic (sCr <203 µmol/L) during the study. Significant decrease of GFR was seen between T0 and T1 (P<0.001) but not between T1 and T2 (P=0.28). Serum SDMA did not significantly differ between times.

Serum SDMA was increased (>14 μg/dL) before treatment in 1 cat. This cat had normal GFR at all times and sSDMA normalised after treatment. At T1, 8 cats had normal GFR with normal sSDMA, GFR was borderline low (<1.9 ml/min/kg) with increased sSDMA in 1 cat and GFR was low (<1.4 ml/min/kg) with normal sSDMA also in 1 cat. At T2, GFR was borderline low in 2 cats with increased sSDMA in 1 of them and GFR was low in 3 cats with increased sSDMA in 1 of them. Interestingly, all 3 cats with borderline low or low GFR and normal sSDMA were hypothyroid. Remaining 6 cats were euthyroid.

Borderline low or low (abnormal) GFR was seen in 20% of cats 1 month after treatment of hyperthyroidism and in 50% of cats during subsequent follow-up. Serum SDMA correctly identified about half of the patients with abnormal GFR. Remaining cats with abnormal GFR but normal sSDMA were hypothyroid which might have contributed to GFR decline.

In this small population of cats, sSDMA did not identify cats with low GFR prior to treatment but was a better biomarker than sCr at identifying cats with lower GFR after treatment.

#### **Disclosures**

Disclosures to report.

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## ESVIM-O-1

#### Effect of antimicrobial drug on lung microbiota in healthy dogs

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In human and dogs, baseline differences in lung microbiota (LM) have been associated with important clinical features in chronic lung diseases (CLD) and there is growing evidence that an altered LM contributes to disease pathogenesis. The common use of antibiotic drugs throughout the management of CLD likely represents a major confounding factor in the study of the LM. However, the effect of antibiotic treatment on the LM in healthy individuals has not been specifically investigated yet.

The aim of the present study was to assess the short and mediumterm effect of an oral treatment with a large spectrum antimicrobial drug on the LM in healthy dogs.

Six healthy experimental beagle dogs were included. Amoxycillin/clavulanic acid (AC) was administered at a dose of 20 mg/kg twice daily for 10 days. In each dog, bronchoalveolar lavage fluid (BALF) was collected at 3 different timepoints: before administration of AC (J0) and immediately (J10) as well as 16 days (J26) after interruption of AC.

In each BALF, total and differentiated cell counts were obtained and metagenetic analyses were performed on the V1-V3 hypervariable region of 16S rDNA after total bacterial DNA extraction and sequencing on a MiSeq Illumina sequencer. Taxonomical assignation and microbiota community analysis were done with MOTHUR V1.35 with an OTU clustering distance of 0.03. Statistical comparisons between events for microbiota community and BALF cell counts were made using Friedman test and post-hoc t-tests with Bonferroni correction. Statistical differences in bacterial population relative abundance between timepoints were assessed using a mixed linear model with FDR correction for multiple comparisons.

Administration of AC did not induce significant changes in BALF cellular counts and had no effect on the richness, evenness and alpha diversity. Bacteroidetes and Proteobacteria abundance increased from JO to J10 (mean: 12.0 to 24.1, and 12.3 to 26.2%, respectively; p<0.001) and decreased at J26 (13.1 and 9.3%; p<0.001). Firmicutes abundance decreased from J0 to J10 (45.6 to 18.3%; p<0.001) and increased at J26 (28.8%; p<0.001). Actinobacteria abundance increased at J26, compared with J0 and J10 (43.7 compared with 23.2 and 23.5% respectively; p<0.001). We failed to show significant differences between timepoints at the level of genera and species.



In conclusion, in healthy dog, oral administration of a commonly used large spectrum antimicrobial drug induces significant changes in microbial population abundance at the phyla level. Most but not all of these changes normalize within 2 weeks after discontinuation of the drug.

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No disclosures to report.

## ESVIM-O-2

## Comparison of the pulmonary deposition of nebulized 99mTc-DTPA through three different inhalation devices in healthy dogs

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In human medicine, the administration of inhaled drugs for chronic respiratory diseases has been used for a long time, with proven efficacy and low systemic side effects. In veterinary medicine, this treatment modality has gained popularity during the last years, although there is still little information about the distribution and the effect of these drugs in the lower airways of dogs and cats.

The aim of this study was to compare the pulmonary deposition of a nebulized radiopharmaceutical agent through three different inhalation devices in healthy dogs in order to detect possible devicedependent differences in drug deposition.

Ten healthy beagles were included in this prospective cross-over study. All dogs inhaled a dose of nebulized <sup>99m</sup>technetium-diethylenetriamine-pentaacetic acid (<sup>99m</sup>Tc-DTPA) through the following devices: Aerodawg® with a custom-made tightly fitting nose-muzzle mask, Aerochamber® for children with the same custom-made nose-muzzle mask and Aerodawg® with the original corresponding nose mask. Wash out period between treatments was at least 1 week. The inhalator bowl was filled with 1.9-2.5 GBq <sup>99m</sup>Tc-DTPA and the inhalation time was five minutes. Immediately afterwards dogs were scanned with planar scintigraphy in right lateral, left lateral and sternal recumbency. The deposition of <sup>99m</sup>Tc-DTPA in the head region (upper airways, mouth), the lungs, the stomach, as well as the whole-body distribution were recorded and quantified by manual or isocontour region of interest (ROI) analysis. Deposition calculated as percentage of delivered dose in the ROI was compared.

Median percentage uptake in the lungs using Aerodawg® with a nosemuzzle mask, Aerochamber® with a nose-muzzle mask and Aerodawg® with a nose mask was 9.8% (range 2.4 – 16.8%), 12.5% (range 2.4 – 18.3%), and 9.3% (range 5.3-22.3%), respectively. Combined median uptake in the head region and stomach was 82,9% (range 62.6 – 96.4%), 81.3% (range 71.5 – 93.4%), and 78.5% (range 63.2 – 97.1%), respectively. Although the Aerochamber® with a nose-muzzle mask group had the highest median uptake in the lungs, differences were statistically not significant. The inhalation procedure was well tolerated with all devices.

This study suggests that oro-nasal inhalation and nasal inhalation devices provide similar deposition of nebulized <sup>99m</sup>Tc-DTPA in the lungs and that spacers intended for use in small children (Aerochamber®) adapted with custom-made nose-muzzle masks

perform equally to more expensive chambers specifically designed for dogs (Aerodawg<sup>®</sup>).

#### Disclosures

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#### ESVIM-O-3

Long-term survival and complications in end-stage tracheal collapse dogs treated with intraluminal self-expanding Wallstents  $^{\rm TM}$ 

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Intraluminal stenting is used to treat end-stage tracheal collapse (ESTC) in dogs refractory to medical treatment. The study's objective was to evaluate a new technique for estimating the size of stainless-steel intraluminal Wallstents<sup>TM</sup> used for tracheal stenting, and to report short- and long-term survival and complications.

Dogs presented for dyspnoea due to ESTC (confirmed by endoscopy) treated by intraluminal stenting under fluoroscopic guidance between 2006 and 2016 were included in this retrospective study. Stent size was selected based on a lateral neck-thoracic radiograph performed under general anaesthesia with spontaneous breathing. Stents were chosen to be ideally 1-2 mm larger than the laryngeal diameter and to cover the trachea from 1 cm caudal of the cricoid to 1 cm cranial of the carina. Dogs were re-examined (clinical signs; radiographs; endoscopy if possible) preferably 6 weeks post-stenting. Long-term outcome was assessed via owner questionnaire.

Thirty-six dogs (median age 7.3, range 2-12 years) were included. Thirty-one dogs survived to discharge; 2 were lost to follow-up. Median survival time post-stenting was 1176 days (range 0-2338). Twenty-nine dogs initially showed partial (16/29) or complete (13/29) resolution of clinical signs post-stenting. Control radiographs (n=23) revealed stent fracture in 1 dog; none had stent migration. Stent length was significantly shorter during follow-up compared to post-deployment (p=0.02). Endoscopy (n=18) revealed tracheobronchitis (n=15) and granulation tissue formation (n=13). Most owners were satisfied with clinical improvement and their pets' quality of life.

The presented estimation technique is a straightforward method achieving satisfying results, good long-term outcomes and overall client satisfaction.

#### Disclosures

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## ESVIM-O-4

# Association between abnormal respiratory clinical signs and respiratory localization in dogs and cats

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Using the pattern of abnormal respiration to localize respiratory disease origin has been under-investigated in dogs and cats.

The aim of this cross-sectional study was to identify associations between respiratory clinical signs and disease localization in dogs and cats.

Dogs and cats with abnormal breathing patterns presenting to Fregis, University of Missouri, or University of Pennsylvania veterinary hospitals were recruited over a one-year period. Animals were included if case investigation allowed for respiratory disease localization and definitive diagnosis. Thoracic radiographs and minimal bloodwork were mandatory for inclusion. Associations between respiratory signs and disease localization were evaluated via two-level mixed-effects logistic regression, sensitivity, specificity, and likelihood ratio calculation.

One-hundred and eighteen dogs and 46 cats were included. Decreased nasal airflow accurately localized the disease to nasal cavities. Although stertor was most specific for pharyngeal diseases, it was also associated with nasal diseases in both species as well as laryngeal diseases in dogs. Although stridor was most specific for laryngeal diseases, it was also associated with nasal and extra-thoracic tracheal diseases. Inspiratory efforts were associated with extrathoracic diseases. Goose honking and wheezes were more specific but less sensitive than tracheal sensitivity for intra-thoracic tracheal diseases. Expiratory efforts, expiratory snap and coughing were specific for bronchial localization, especially in cats, while crackles and increased respiratory rate referred to pulmonary diseases in both species. Combination of attenuated lung/cardiac sounds and paradoxical breathing was specific for pleural diseases.

Patterns of abnormal respiration can localize origin of respiratory disease, useful to tailor subsequent diagnostic evaluations.

## Disclosures

No disclosures to report.

## ESVIM-O-5

Association between tomodensitometry, rhinoscopy, and histopathology characteristics and treatment response in canine idiopathic rhinitis

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Treatment of canine idiopathic rhinitis may be frustrating, as the underlying causes are not identified and treatment response is often limited.

The aim of this retrospective observational study was to look for tomodensitometric (CT), rhinoscopic, and histopathologic parameters that would be associated with treatment response and risk of relapse in canine idiopathic rhinitis.

Dogs with a final diagnosis of idiopathic rhinitis after an appropriate diagnostic work-up, including CT, MRI and/or rhinoscopy, and histopathology, were screened over a 4-year period. Follow-up information regarding treatment response and relapse was gathered from owners and local veterinarians. Associations with treatment response or risk of relapse were screened across CT, rhinoscopic, and histopathologic findings via logistic regressions and calculation of predicted probabilities and Spearman's rank correlations.

Fifty dogs were included in the study. Most dogs were treated with corticosteroids (38 dogs) and antimicrobials (23 dogs), with a

satisfactory overall treatment response: 7 failures (14%), 19 partial remissions (38%), and 24 complete remissions (48%). Irregular nasopharyngeal mucosal surface on rhinoscopy and presence of mucous exudate on histopathology were associated with a worse overall treatment response (p=0.02 and p=0.04, respectively). Twenty-eight dogs (56%) relapsed after treatment was discontinued. There was a high negative correlation between treatment response and risk of relapse (Spearman rho=-0.74, p<0.001). Furthermore, risk of relapse was significantly associated with intensity of the exudate on histopathology (p=0.02).

Relapse is common among dogs with idiopathic rhinitis and may be anticipated if treatment response is incomplete and if exudate is observed histologically on nasal biopsies.

#### Disclosures

No disclosures to report.

#### ESVIM-O-6

#### Pulmonary complications in canine acute pancreatitis: a pilot study

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In human beings, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) in acute pancreatitis (AP) represents important complications with a high mortality rate (30-40%). To our knowledge, there are no clinical veterinary studies on this topic. The aim of the present study is to evaluate pulmonary complications during canine AP and their association with the outcome. AP diagnosis was made if there were compatible clinical signs and laboratory parameters, abnormal SNAP cPL test and a positive abdominal ultrasound within 48 hours from the admission. Thoracic radiography was performed for each patient using a digital radiological equipment and subgraded base on the pulmonary pattern (normal, interstitial or alveolar). At the admission, arterial blood samples, obtained from the dorsal pedal artery, at room air (FiO<sub>2</sub>=21%), were analyzed (ABL 700 series, Radiometer, Denmark). ALI/ARDS were diagnosed using the current veterinary consensus: (1) acute onset (<72 hours) of respiratory distress (RD) (tachypnea and laboured breathing at rest), (2) known risk factors, (3) evidence of pulmonary capillary leak without cardiac disease and (4) evidence of inefficient gas exchange. Dogs were divided into 2 groups according to outcome at 15 days from their admission: survivors and non-survivors. Normal distribution was assessed using D'Agostino-Pearson test. Welch's t-test was used to compare PaCO<sub>2</sub>, P[A-a]O<sub>2</sub> gradient with the outcome; meanwhile, pH, PaO<sub>2</sub>, PaO<sub>2</sub>/ FiO<sub>2</sub> were compared to the outcome using Mann-Whitney U-test. Exact tests were used to compare the presence of radiographic abnormalities, RD and ALI/ARDS to the outcome. Odds ratio (OR) was calculated. Twenty-three client-owned dogs with owners' consent, admitted to the Veterinary Teaching Hospital, were prospectively enrolled. Ten dogs (43%) died during the study period. Two out of 10 dogs were euthanized due to poor prognosis or to progressive disease. Ten out of 23 dogs showed RD which was associated with poor outcome (p=0.0001; OR 108 95% CI 7-1225). Nineteen dogs (83%) showed radiographic alterations (10 alveolar pattern and 9 interstitial pattern) and they were associated to death (p=0.04). Non-survivors



showed a lower PaCO<sub>2</sub> levels than survivors (p=0.009). P[A-a]O<sub>2</sub> gradient, pH, PaO2 and PaO2/FiO2 were similar between groups. ALI was diagnosed in seven dogs (30%) and no dogs had ARDS. The presence of ALI was associated with poor outcome (p=0.0005). In dogs with AP, pulmonary complications seemed to be frequent and associated with risk of death. Moreover, ALI/ARDS may be a severe pulmonary complication affecting the prognosis, as well as in human medicine.

#### Disclosures

No disclosures to report.

## ESVIM-O-7

## Effectiveness of aspirin versus clopidogrel in dogs with immune mediated haemolytic anaemia evaluated by serial thromboelastography and platelet mapping

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Immune mediated haemolytic anaemia (IMHA) is associated with a high risk of thromboembolism. Most dogs with IMHA are hypercoagulable, as measured by thromboelastography (TEG). Platelet mapping (PM) has been used to assess platelet function in human patients treated with aspirin or clopidogrel.

The aims of this study were to a) compare the efficacy of aspirin versus clopidogrel in inhibition of platelet activation in dogs with primary IMHA (pIMHA) and b) determine if TEG and/or PM are reliable to monitor treatment response.

This prospective double blinded study included 18 client-owned dogs with pIMHA randomized to receive aspirin (loading dose 10mg/kg, then 1mg/kg PO SID, n= 10) or clopidogrel (loading dose 10 mg/kg, then 2 mg/kg PO SID, n=8) in addition to standard therapy. TEG, haematocrit (HCT), platelet count (PLT), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, antithrombin (AT) activity and D-dimers were measured before, and 1 and 4 days after commencing treatment. PM was performed on day 1 and 4. Non-responders were defined as <50% inhibition of thromboxane A<sub>2</sub>-receptor activity (TXA<sub>2</sub>-RA) stimulated by arachidonic acid (AA) in the aspirin group and <50% inhibition of ADP-receptor activity (ADP-RA) in the clopidogrel group, on day 4. For statistical analysis an Anderson-Darling test was used to determine normality with variables not meeting assumptions loge transformed. A restricted maximum likelihood model was run for each measurement with fixed effects of treatment, day and their interaction and the random effect of patient. Significance was set at p<0.05.

Significant differences identified between the two groups at all time points included higher TEG G (clot strength) in the clopidogrel group (p=0.021) and lower PM MA<sub>AA</sub> and PM G<sub>AA</sub> (MA and G generated by TxA<sub>2</sub>-RA) in the aspirin group (p=0.009 and 0.016, respectively). Mean platelet TxA<sub>2</sub>-RA and platelet ADP-RA were not significantly different between groups. The overall mean % inhibition of TxA<sub>2</sub>-RA was 25% (aspirin 33%, clopidogrel 15%), and of ADP-RA was 82% (aspirin 83%, clopidogrel 80%). On day four 6/9 dogs (66%) in the aspirin group and 2/8 dogs (25%) in the clopidogrel group were non-responders (p=0.086).

Overall, there was no significant difference in efficacy between aspirin and clopidogrel and TEG was not reliable for monitoring treatment response in dogs with pIMHA. PM is reliable to detect nonresponders, monitor response to treatment and help adjust treatment in individual dogs.

#### **Disclosures**

Disclosures to report.

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## ESVIM-O-8

## Platelet function in healthy dogs receiving sustained clopidogrel, prednisone, or combination therapy

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Clopidogrel is commonly administered to dogs receiving glucocorticoids for immune-mediated hemolytic anemia, but the impact of sustained therapy on platelet reactivity is currently unknown. The aim of this study was to compare platelet reactivity among dogs receiving sustained clopidogrel, prednisone, and combination therapy.

A double-blinded, placebo-controlled trial was performed using 24 healthy dogs that were randomized to 1 of 4 treatment groups: placebo, prednisone 2 mg/kg/d, clopidogrel 2-3 mg/kg/d, or combined prednisone/clopidogrel therapy PO for 28 days. Complete blood counts, manual platelet counts, PFA-100® closure times (collagen/ADP), and area under the curve (AUC) for Multiplate® wholeblood aggregometry (ADPtest) were determined at baseline, day 14, and day 28. Platelet reactivity was categorized as controlled if closure times increased ≥30% compared to baseline or AUC was ≤46U; control based on AUC was subcategorized into adequate (19-46U) and excess (<19U) control. Closure times, AUC, platelet reactivity control, and degree of control were compared among groups using mixed model, split-plot repeated measures ANOVAs, generalized estimating equation proportional odds models, and Fisher's exact tests as appropriate. P<0.05 was considered significant.

All dogs had normal hematocrits and platelet counts at all timepoints. Significant (P<0.01) group, week, and group\*week interactions were present for closure times and AUC, due to significant differences for the clopidogrel and prednisone/clopidogrel groups compared to placebo and prednisone groups. Based on closure times on days 14 and 28, significantly more dogs had adequate control in the clopidogrel (5/6) and prednisone/clopidogrel (5/6) groups versus the placebo (1/6) and prednisone (0/6) groups. On days 14 and 28, all dogs in the clopidogrel and prednisone/clopidogrel groups were characterized as being controlled based on aggregometry, versus 1/6 dogs each in the placebo and prednisone groups. Dogs receiving prednisone/clopidogrel were 11 times (P=0.03) more likely to be over-controlled over time (day 14, 6/6; day 28, 6/6) than dogs receiving clopidogrel monotherapy (day 14, 5/6; day 28, 2/6).



Based on human monitoring standards, clopidogrel's therapeutic efficacy decreases during sustained administration in dogs. Prednisone co-administration increases antiplatelet effects of clopidogrel therapy, increasing the risk of overcontrol at dosages currently used for management of immune-mediated hemolytic anemia. Platelet reactivity should be monitored during sustained clopidogrel therapy to avoid over- or undertreatment.

#### Disclosures

No disclosures to report.

## ESVIM-O-9

# Retrospective analysis of presentation and diagnosis in 115 referred juvenile dogs presenting with pyrexia

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Establishing the cause of pyrexia in dogs can prove challenging. Previously studies have analysed diagnoses in dogs of all ages presenting with reproducible pyrexia. Immune-mediated disease has been identified to be most commonly responsible, in 22-33% of dogs, followed by infectious, neoplastic, and miscellaneous disorders. In human medicine pyrexia in children has been analysed separately, this has revealed an increased tendency for diagnosis of infectious and collagen disorders compared with adults.

The aim of this study is to describe the diagnoses in juvenile dogs presenting with pyrexia to a single UK-based referral centre (2013-2018).

Clinical records of dogs aged 1-18 months presenting with pyrexia (≥39.2°C), reproduced during hospitalisation, were retrospectively reviewed. Signalment, history including previous treatment, and diagnosis was recorded. Diagnoses were categorised as non-infectious inflammatory, infectious, congenital, neoplastic, and miscellaneous.

One hundred and forty cases were identified. Breeds commonly recorded were beagles (20), Border collies (17), and Labrador retrievers (12). Median age at presentation was 8 months (range 2-18 months). Prior to presentation 123 dogs had received combinations of antibiotics (71), non-steroidal anti-inflammatories (68) and steroids (5). Seventeen dogs had not received any medications at presentation.

Diagnosis was reached in 115 cases. In 20 cases pyrexia resolved during hospitalisation (<3days) without treatment, in four dogs prednisolone was commenced based on a high suspicion of immune-mediated disease, and the remaining dog died before investigations were completed. In the 115 dogs with a secure diagnosis, non-infectious inflammatory disease was identified in 91 cases (79%), infectious disease in 19 cases (17%), congenital disorders in 4 dogs (3%), and neoplasia in one dog (1%).

In the non-infectious inflammatory disease group steroid responsive meningitis-arteritis (SRMA) was diagnosed in 55 dogs (48% of total diagnosed cases). Immune-mediated polyarthritis (IMPA) was identified in 15 dogs, and metaphyseal osteopathy was diagnosed in eight dogs, 5 of these dogs had concurrent inflammatory dermatopathies. Infectious diseases diagnosed in more than 1 patient included abscess (5), aspiration pneumonia (4), pyothorax (2), and haemorrhagic gastroenteritis (2). Congenital disorders were trapped neutrophil syndrome with metaphyseal osteomyelitis (2) and congenital hypocobalaminaemia with opportunistic infection (2); all four dogs were Border collies. One dog was diagnosed with polyostotic lymphoma.

This study has identified that non-infectious inflammatory disease; in particular SRMA, IMPA and metaphyseal osteopathy was commonly diagnosed in this population of pyrexic juvenile dogs and should be considered in dogs that do not respond to antibiotics. Congenital disorders should be considered in Border collies.

#### Disclosures

Disclosures to report.

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# ESVIM-O-10

# Development of a frailty index to assess the risk of mortality in

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In humans, and in dogs alike, the risk of mortality increases with age. Such an increase is not uniform and not all the individuals of the same age have the same risk of mortality. In humans, the risk of mortality and other adverse outcomes in elderly people is increasingly being assessed by means of the so-called frailty index (FI). This index is calculated by considering a checklist of multidimensional health deficits (e.g. symptoms, signs, laboratory abnormalities) potentially accumulated by the aging individual. To calculate the FI, the actual number of deficits in an individual is counted and divided by the total number of deficits included in the checklist. In humans, the FI shows a moderate accuracy in the prediction of near term (less than 18 months) mortality. A specific FI has been developed also in mice. We developed a dogs-specific FI computed by considering 33 deficits, that was divided into two parts: the first 19 questions were focused on the general health status as reported by the owner, whereas the second 14 questions reported the results of the clinical evaluation of the subject along with the findings of the diagnostic and laboratory tests performed during the routine clinical evaluation. Dogs presented to our facility between January 2017 and August 2017 were included. Inclusion criteria included a complete clinical evaluation of the subject and recent (less that 2 months) blood analysis. Fatalities were excluded from the study. Mortality was assessed six months after the FI was administered. 124 dogs belonging to several different breeds matched the inclusion criteria (mean age: 8,26± 4,4 years). The developed FI had a high diagnostic accuracy in the prediction of near term mortality (six months) with an area under the curve of 0,93 (95% confidence interval (CI)= 0.87-0.97); using a cut-off value of 0,23 the sensitivity was 78,57% (95% CI= 49.2-95.3) and the specificity of 90,91% (95% CI= 83.9-95.6), the positive likelihood ratio was 8.64 (95% CI= 4.5-16.6), the negative likelihood ratio was 0.24 (95% CI = 0.09-0.6). There was a moderate (r= 0,454 - 95% CI= 0.301-0.583) but



significant (p= 0.001) correlation between the frailty index and age. A large-scale use of the FI could provide new interesting information regarding the aging process in the dog.

#### Disclosures

No disclosures to report.

#### ESVNU-O-1

# Inflammatory and pro-fibrotic pathway upregulation in an ischemic model of feline chronic kidney disease

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Chronic kidney disease (CKD) is a common, slowly progressive and irreversible disorder in domestic cats. Feline CKD is characterized histologically by tubulointerstitial inflammation and fibrosis; however, the molecular pathways associated with its progression have not been thoroughly characterized. Our group has recently described a model of feline CKD that utilizes unilateral in vivo ischemia to induce renal changes mimicking those of naturally occurring disease. The objective of this study was to characterize the renal transcriptome in an ischemic model of feline CKD using RNA sequencing. We hypothesized that 1) tissues from cats with experimental CKD would display increased genetic expression of molecular pathways associated with inflammation and fibrosis, and that 2) in cats having undergone unilateral renal ischemia, tissues from the contralateral (i.e., non-ischemic) kidney would show differential genetic expression when compared to tissues from control cats.

This study included bilateral renal tissue samples banked from cats that underwent temporary unilateral renal ischemia six months prior to tissue collection (n = 6) and from healthy cats (control group, n = 9). For the CKD model cats, tissues from both the ischemic kidney (IK group) and the contralateral, non-ischemic kidney (NIK group) were evaluated. Illumina short read sequencing technology was used to produce transcriptome sequences from the renal tissues. Reads were mapped to the reference feline genome (Felis\_catus\_8.0). Differential expression analysis was conducted with edgeR at a false discovery rate (FDR) £0.05. Gene ontology analysis was performed using the Panther classification system and the Fisher's exact test with FDR multiple test correction.

When tested against the control group, the IK group exhibited 1743 upregulated and 1196 downregulated genes, and the NIK group displayed 209 upregulated and 291 downregulated genes. Compared to the NIK group, there were 849 upregulated genes and 451 downregulated genes in the IK group. Genes associated with the gene ontology categories of collagen binding (e.g., transforming growth factor b1 and matrix metalloproteinase [MMP] 13), metalloendopeptidase activity (e.g., MMP-7, -9, -11, -13, -16, -23B and -28), chemokine activity, Tcell migration, and positive regulation of macrophages chemotaxis were overrepresented in the IK group vs. control. Genes associated with the extracellular matrix (e.g. tissue inhibitor of metalloproteinase 1 and connective tissue growth factor) were upregulated in both IK and NIK tissues.

The results of this study suggest that six months after renal ischemia, upregulation of inflammatory and pro-fibrotic pathways persists. Additionally, unilateral ischemic injury differentially alters gene expression in both kidneys.

#### **Disclosures**

Disclosures to report.

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#### ESVNU-O-2

# Symmetric Dymethylarginine evaluation and characterization of proteinuria in dogs with pituitary-dependant hyperadrenocorticism

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Various consequences of canine hyperadrenocorticism on renal function have been described. Urinary markers of glomerular and tubular dysfunction, urine protein-to-creatinine ratio [UPC] and glomerular filtration rate [GFR] are known to be increased in dogs with hyperadrenocorticism. Symmetric dimethylarginine [SDMA] may detect early kidney dysfunction in dogs with hyperadrenocorticism. The aim of the study was to compare renal function at the time of diagnosis among dogs with pituitary-dependent hyperadrenocorticism [PDH], elderly healthy dogs and control dogs (for which low dose dexamethasone suppression test was negative), using commercially available tests.

Following ethical committee approval, thirty-nine dogs were prospectively enrolled: 14 dogs with PDH, 9 control dogs, and 16 healthy dogs. Dogs with urinary tract infections or those receiving drugs potentially affecting kidney function were excluded. All dogs underwent physical examination, blood pressure measurements, routine urinalysis, UPC, urinary protein sodium dodecyl sulfate-agarose gel electrophoresis [SDS-AGE], as well as serum urea, creatinine [sCr], and SDMA measurements. Comparisons were made between groups using Mann-Whitney test for quantitative variables and Fisher test for distribution of categorical variables.

Prevalence of proteinuria and magnitude of UPC were significantly higher in PDH dogs than in healthy dogs (p0.01), but not significantly different from control dogs (p=0.16). Seven out of 13 dogs with PDH, 4/9 control dogs and no healthy dogs had a UPC0.5. sCr concentration was lower in PDH dogs than in healthy dogs (p0.01) but not from control dogs (p=0.15). No difference in SDMA values was detected between groups, nor between proteinuric and non proteinuric dogs. sUrea was not different between groups. Serum SDMA was normal in all but 1 healthy dog (16  $\mu$ g/dL) and sCr was normal in all but 1 dog with PDH (200 µmol/L). Among the seven dogs with PDH and proteinuria, moderate (160-180 mmHg) and severe hypertension (≥180 mmHg) were found in 3 and 1 dogs, respectively. Abnormal patterns were found on SDS-AGE in 9/13 (8 glomerular, 1 mixed



patterns) PDH dogs, 4/9 (3 glomerular, 1 mixed patterns) control dogs and in 0/16 healthy dogs.

Although PDH dogs were more likely to have proteinuria, renal dysfunction was not evident based on SDMA levels. Proteinuria was partially of glomerular origin in all proteinuric dogs as evaluated by SDS-AGE. These results need to be confirmed in a larger cohort and a longitudinal follow-up study is required to identify if SDMA increases after treatment of PDH and to quantitatively and qualitatively follow proteinuria especially in proteinuric dogs.

#### Disclosures

Disclosures to report.

M. Coyne and R. Murphy are employees of IDEXX Laboratories, Inc. Measurement of biochemical parameters was performed by IDEXX Laboratories free of charge. The residency program of the presenting author (MM) is sponsored by Royal Canin.

#### ESVNU-O-3

#### The clinical utility of glomerular filtration rate measurement in dogs

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GFR measurement is considered the gold standard for assessment of renal function given direct proportionality to functional renal mass. Although widely used in research settings, there is little published on clinical utility. The aim of this study was to describe the clinical utility of GFR measurement in dogs.

The Royal Veterinary College has offered a GFR service since 2013 using 3 sample iohexol clearance. In addition to serum sample submission, clinical data pertaining to the patients are requested as part of the laboratory submission facilitating GFR interpretation. GFR results and records were reviewed and submitting practices contacted in order to obtain outcome data. Descriptive analysis provides a preliminary assessment of clinical utility of GFR measurement.

Between 2013-2017, 132 canine GFR assessments were performed; the most common reasons for submission being screening for preazotaemic chronic kidney disease (CKD; n=103), cutaneous and renal glomerular vasculopathy (n=18) and carboplatin dose adjustment (n=3). Clinical and laboratory findings prompting GFR measurement included polyuria/polydipsia (n=74), urinary incontinence (n=20), proteinuria (n=14) and isosthenuria (n=12). Follow-up data were available for 78.8% and a final diagnosis in 84.6% of dogs. GFR confirmed pre-azotaemic CKD in 11% of dogs and psychogenic polydipsia in 13.6%. 84 dogs were alive at follow-up. In only 1/18 dogs that died/were euthanised was death attributed to CKD. Three dogs developed azotaemic CKD during the follow-up period, with median time to onset being 281 days.

This study suggests that GFR measurement is useful to distinguish dogs with pre-azotaemic kidney disease from dogs with psychogenic polydipsia.

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Disclosures to report.

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#### ESVNU-O-4

In vitro antimicrobial susceptibility patterns of urinary bacterial isolates in dogs with chronic kidney disease

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An increasing prevalence of antimicrobial resistance is reported in urinary tract infections in dogs, particularly in those receiving antimicrobials during the previous 30 days. A high rate (69%) of multidrug resistance (MDR) has been observed in urinary isolates from humans with chronic kidney disease (CKD). In dogs with CKD, the frequency of resistance among urinary isolates is unknown. The aim of this study was to describe the antimicrobial susceptibility patterns of urinary tract isolates found in dogs with CKD.

Aerobic bacterial isolates growth and susceptibility data were collected retrospectively from urine cultures of dogs with CKD and significant bacteriuria seen at two veterinary teaching hospitals between January 2010 and June 2016. A significant bacteriuria was defined as finding growth of at least 1000 CFU/mL of urine collected by cystocentesis. Treatment with antimicrobial within one month preceding sampling was an exclusion criteria. MDR was defined as resistance to one agent in at least three separate antimicrobial categories for which the wild type bacteria would normally be susceptible, whereas possible extreme-drug resistance (XDR) was defined as resistance to all except 2 or fewer antimicrobial categories tested.

Sixty-five dogs with a positive urine culture and CKD were recruited, representing a total of 70 isolates. Most common isolates were Escherichia coli (67% of isolates) and coagulase negative Staphylococci (9% of isolates). Among all isolates, 11% were susceptible to all tested antimicrobials, 54% were MDR, 19% were possible XDR and 3% were resistant to all tested antimicrobials. Escherichia coli was susceptible to all tested antimicrobials in 15% of cases, MDR in 53% of cases, possible XDR in 13% of cases and none was resistant to all drug tested. For Escherichia coli, percent in-vitro susceptibility was 96% for fluoroquinolones, 89% for sulfamethoxazole-trimethoprim and extendedspectrum cephalosporins, 80% for aminoglycosides, 64% for tetracyclines and penicillins + beta-lactamase inhibitors, 43% for penicillins, 38% for non-extended spectrum cephalosporins and 32% for polymyxins.

Results of the present study suggest that MDR is frequent in dogs with CKD and bacteriuria, even if a recent antimicrobial treatment has not been administered. This finding suggests that urine culture and susceptibility testing are indicated in dogs with CKD. A high rate of invitro resistance to penicillins and non-extended spectrum cephalosporins was found. Conversely, sulfamethoxazole-trimethoprim had a low frequency of resistance and could be considered as a first-line treatment.

#### Disclosures

No disclosures to report.



#### ESVNU-O-5

# Dogs as reservoirs of uropathogenic Klebsiella pneumoniae to

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Klebsiella pneumoniae is a major nosocomial pathogen and the second most common enterobactereaceae causing UTI in humans. Companion animals (CA) may become infected with K. pneumoniae high-risk clonal lineages, however little is known about their role as reservoirs. This study aimed to evaluate the K. pneumoniae faecal colonization in healthy CA and their household members, and to compare their clonal relatedness with clinical strains from humans and companion animals with UTI.

Faecal samples from 17 dogs and 8 cats belonging to 18 households (total of 24 humans) were collected into sterile containers, after informed consent. Samples were plated onto MacConkey agar plates (MCK) with and without 1.5 ug/mL cefotaxime or meropenem supplementation. Negative samples were enriched prior to plating. When positive, up-to-five K. pneumoniae colonies were isolated per positive plate. The clonal relatedness of 80% of faecal K. pneumoniae isolates was compared after Xbal restriction PFGE with a clinical K. pneumoniae from UTI (n=25 CA, n=57 human community-acquired UTI, n=19 human hospital-acquired UTI). Dice/UPGMA with 1.5% tolerance was used. K. pneumoniae colonization was detected in dogs (41.2%, n=7/17) and

humans (37.5%, n=9/24). All cefotaxime or meropenem supplemented MCK plates were negative for K. pneumoniae. Overall, most dogs and humans were colonized with a single strain. Two households (11.1%, n=2/18) had K. pneumoniae colonized humans and dogs simultaneously. PFGE analysis revealed that in one household, two dogs shared 100% similar K. pneumoniae with one human. Fourteen unique K. pneumoniae pulsetypes were obtained from faecal samples. Thirtysix percent faecal strains (n=5/14; three from human and two from dogs) clustered with UTI K. pneumomiae (Dice index >80%). All five faecal strains clustered with strains causing UTI in humans. One faecal strain from a dog (Dog A) was 80.3% similar to one faecal strains from an unrelated human (Human B). Furthermore, Dog A and Human B faecal strains clustered with two community-acquired and one hospital acquired UTI K. pneumoniae. Of note, the Dog A strain was 92.3% similar to the UTI hospital-acquired K. pneumoniae.

This study shows that dogs may become colonized by clonally related K. pneumoniae to uropathogenic strains from humans. Furthermore, dogs and humans may share K. pneumoniae within the same household. These results highlight the role of dogs as reservoirs of K. pneumoniae to humans, or viceversa.

#### Disclosures

Disclosures to report.

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# ESVNU-O-6

Urolithiasis prevalence in leishmania infantum-infected dogs treated with allopurinol in an endemic area of the South of France

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Allopurinol is a consensual leishmaniostatic drug that increases the risk of xanthine urolithiasis, a slight to non-radio-opaque (NRO) urolithiasis. Fews reports described urinary obstruction or pyonephrosis due to xanthine lithiasis. One retrospective study showed lithiasis or renal mineralization with xanthinuria in 10% of leishmanian dogs treated with allopurinol. To the authors' experience, this prevalence seems to be underestimated. The aim of this prospective controlled study is to evaluate the NRO-urolithiasis prevalence in Leishmania infantuminfected dogs (Leish-dogs) treated with allopurinol in an endemic area. Client-owned dogs were recruited. The inclusion criteria for Leishdogs (L-group) were a definitive diagnosis of leishmaniasis and an allopurinol treatment of at least one month. The controlled dogs (C-group) where clinically healthy, and had a negative serological test for Leishmania infantum. The exclusion criteria were a severe hepatic disease, breeds predisposed to NRO urolithiasis and low purine diets. Complete clinical and blood analysis data were collected. Abdominal ultrasonography was systematically performed, and if calculi above 2 millimeters were identified, abdominal radiographs completed the study. All images were recorded and secondly blindly reviewed by the same board certified radiologist in all dogs. Urine was collected for classical urinalysis, xanthine measurement, urine xanthine/creatinine (UXC) ratio and infrared spectrometry of the urine sediment.

Forty dogs were included, 30 in the L-group and 10 in the C-group. The median duration of allopurinol treatment was 3 years (range: 1 month to 9 years). Urolithiasis was present in 83.3% of dogs in the L-group (76% in the kidneys) vs. 20% in the C-group (all in the bladder). In the C-group, the stones were less than 2 mm with struvite crystals. In the L-group, lithiasis were highly suspected to be xanthine lithiasis because of their slight to NRO feature, a high xanthinuria, a high UXC ratio and identification of xanthine in the sediment by infrared spectrometry in some dogs. In the L-group, the amount of renal NRO urolithiasis seemed to increase with the duration of allopurinol treatment and all the dogs treated since at least 5 years had slight to NRO renal lithiasis.

Our study showed an unexpectedly very high prevalence of slight to NRO urolithiasis (highly suspected to be xanthine lithiasis) in Leishdogs treated with allopurinol. A close monitoring of this side effect is then highly recommended as some ureteral obstruction has been seen and as it could induce a treatment adaptation.

#### Disclosures

Disclosures to report.

A financial support was provided by Royal Canin.

# ESVNU-O-7

Evaluation of prescription, anticoagulation, and metabolic response to membrane-based therapeutic plasma exchange in dogs

A. Schweighauser, T. Francey Vetsuisse Faculty University of Bern, Bern, Switzerland Extracorporeal blood purification techniques have become standard procedures in small animal medicine. Membranecentrifugation-based therapeutic plasma exchange (TPE) techniques have been used increasingly to remove pathogenic large-molecular weight products from the circulating blood, including antibodies, pathogenic proteins, and protein-bound toxins. The aim of this study is to describe the technique of membrane-based TPE and the physiological changes associated with metabolic and treatments.

The medical records of all dogs treated with TPE at the Vetsuisse Faculty Bern (2011-2017) were reviewed retrospectively to collect data including signalment, treatment indication, prescription, and clinical and laboratory parameters associated with the procedure. For descriptive statistics, data are presented as median (interquartile range, IQR) and pre-post procedure comparisons were performed with a Wilcoxon-rank-sum test, using a cutoff P-value of 0.05 for statistical significance.

Thirty-four dogs have been treated with TPE, including 19 males (12 intact) and 15 females (5 intact), with an age of 5.2 y (IQR, 2.5-8.5) and a weight of 13.0 kg (IQR, 7.8-30.1). Main indications for TPE were immune diseases in 29 dogs (immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, leptospirosis-associated pulmonary hemorrhages and neuromuscular diseases). Other indications included polysystemic diseases in 5 dogs (sepsis, microangiopathy), and hyperviscosity in 3 dogs (leishmaniasis). Sixty-four treatments were performed with a duration of 125 min (103-163) and a treatment dose of 1.0 plasma volume exchange (0.94-1.04). Replacement fluid consisted of fresh frozen plasma (n=64, 49% of the exchange volume), 20% human albumin (n=59, 23% volume), 6% HES (n=21, 7% volume), and 0.9% NaCl (n=33, 21% volume. 45 treatments (70%) were performed as stand-alone procedures, the others combined with hemodialysis. Anticoagulation was provided with regional citrate (n=24, 38%), systemic heparinization (n=2, 3%), or a combination of both (n=38, 59%). Treatments resulted in mildly increased systolic blood pressure (+10 mmHg, P=0.008), mildly decreased total proteins (-12 g/L, P=0.01), moderately decreased plasma fibrinogen (-126 mg/dl, P=0.009) and mild-moderate signs of citrate accumulation with increased total calcium (+1.3 mmol/l, P<0.001) and decreased ionized calcium (-0.21 mmol/l, P<0.001). Minor complications associated with the procedures were observed in 14 treatments (22%) and included vomiting, diarrhea, urticaria, and technical difficulties due to small patient size. More severe complications were seen in 5 treatments (8%) and included transient laryngeal edema, chemosis, and early treatment discontinuation due to a technical problem.

In view of the severity of the underlying diseases, most complications were benign and membrane-based TPE therefore can be performed safely in dogs.

#### Disclosures

Disclosures to report.

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#### ESVNU-O-8

Comparative Performance of IDEXX SDMA® Test and the DLD SDMA ELISA for the Measurement of SDMA in Canine and Feline

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Kidney disease is common in companion animals, and traditionally diagnosed with serum creatinine concentration (sCr), blood urea nitrogen, and urine specific gravity. More recently, it has been demonstrated that the kidney biomarker Symmetric dimethylarginine (SDMA) correlates with glomerular filtration rate, increasing earlier than sCr with acute kidney injury and chronic kidney disease. This prospective study compared accuracy and precision of two commercial SDMA assays, the IDEXX SDMA® Test manufactured by IDEXX Laboratories, Inc. and the SDMA ELISA manufactured by DLD Diagnostika, GmbH. Accuracy and precision for both commercial assays was measured relative to the established reference method, liauid chromatography/mass spectrometry (LC-MS). Anonymized surplus canine and feline serum samples submitted to an IDEXX commercial reference laboratory were used in this study. Thirty canine and 30 feline serum samples were used to evaluate accuracy compared to LC-MS. Pooled canine samples with a low SDMA concentration and pooled feline samples with a high SDMA concentration were used to evaluate precision. Using a best fit linear model, the IDEXX SDMA® Test resulted in a slope of 1.06 and an intercept of 0.23, with  $R^2$  = 0.99, and the DLD SDMA ELISA resulted in a slope of 0.38 and an intercept of 11.23. with  $R^2 = 0.27$ . Estimated bias over a clinically relevant range for SDMA (10 - 45  $\mu g/dL$ ) was 1-3 $\mu g/dL$  for the IDEXX SDMA® Test, while the DLD SDMA ELISA showed considerable bias, 5 to -17μg/dL. Day-to-day precision analysis of the low SDMA concentration samples showed 7.7% total coefficient of variation (CV) for the IDEXX SDMA® Test and 31.1% for the DLD SDMA ELISA. For the high SDMA concentration samples, total CV was 2.3% for the IDEXX SDMA® Test and 28.2% for the DLD SDMA ELISA. In this study the IDEXX SDMA® Test was more accurate and more precise than the DLD SDMA ELISA when compared to the reference method of LC-MS, supporting the conclusion that the IDEXX SDMA® Test is more suitable for clinical use in the diagnosis and monitoring of kidney disease in dogs and cats.

# **Disclosures**

Disclosures to report.

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#### ESVNU-O-9

# Ultrasound-guided subcutaneous ureteral bypass and microsurgical ureterotomy in cats with obstructive ureterolith

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Subcutaneous Ureteral Bypass (SUB) is a novel treatment alternative that has become more and more popular over the last years to treat ureteral obstructions in cats. However, the currently described method requires fluoroscopic guidance. The aim of this retrospective study is to describe an ultrasound-guided method of SUB placement and a microsurgical procedure to perform an ureterotomy with stone removal.

Twenty-one cats with ureteral obstruction were treated with ultrasoundguided SUB placement. The correct placement was intraoperatively confirmed by ultrasound-guided flush of the device. In all but 2 cases, the SUB device was placed without complication. In the first case, the string of the nephrostomy tube lead to urine leakage in the subcutaneous tissue, corrected surgically 9 days later. In the second case, the nephrostomy tube tip ended up subcapsular and led to a communication when pulled back into the pelvis, causing a subcapsular accumulation of urine, resolved after 2 months. Thirteen microsurgical ureterotomies were performed on 12 cats at the time of SUB placement. One ureterotomy was complicated by extrusion of purulent urine. The ureterotomy allowed stone removal in all but 2 cases. All 11 ureteroliths analysis identified calcium oxalate monohydrate. Two of the 8 cultures on pyelocentesis were positive while the preoperative cultures on cystocentesis were negative.

The most frequent postoperative complications were transient worsening of the azotemia and severe polyuria. The most serious complications related to the SUB placement included a severe postoperative acute kidney injury that required 8 days of peritoneal dialysis for the case with communication between the pelvis and subcapsular space. The case of subcutaneous leakage was discharged the day after its surgical correction. Another case with bilateral obstruction was euthanized 4 days post surgery for persistent severe azotemia, hypotension and coagulopathy. One case of dysequilibrium syndrome was suspected secondary to a severe diuresis with rapid decrease of the azotemia. Fifteen of the 21 cats are still alive at the time of writing. All but 2 of their SUB device remain patent. The 2 cases with a non patent device did not present with recurrent hydronephrosis, leading to the suspicion of a patent ureter secondary to the ureterotomy performed at the time of SUB placement.

This study suggest that ultrasound-guided SUB is a safe procedure to perform on a pelvis under pressure. The microsurgical ureterotomy with stone removal may avoid recurrence of hydronephrosis in case of SUB device obstruction.

#### Disclosures

No disclosures to report.

# ESVNU-O-10

#### The utility of charcoal bacteriology swabs for storage of urine prior to culture

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Bacterial urinary tract infections (UTIs) affect 14% of all dogs during their lifetime. In veterinary patients, collection of urine, by cystocentesis, for quantitative bacterial culture (QBC) is the current gold standard for diagnosing UTIs. To ensure accurate results, immediate culture is recommended. However, most general practitioners do not perform urine culture in-house and samples must be sent to an external laboratory. Refrigeration or the addition of preservatives, such as boric acid, have been traditionally used but previous veterinary studies suggest that these methods can significantly increase the number of false-negative results.

An effective method of preserving urine at room temperature is therefore desirable. The use of charcoal bacteriology swab has been suggested by some laboratories to store and transport urine when immediate culture is not possible. The aim of this study was to investigate the use of standard bacteriology swabs for storage of canine urine at room temperature. We compared urine stored at room temperature for 48h, either in a sterile tube, or stored in a bacteriology swab, to a reference QBC on a fresh sample.

Canine urine samples obtained by cystocentesis at AHT between April 2017 and February 2018 were prospectively included in the study. Samples were inoculated within 4h of collection for reference QBC. A bacteriology swab was immersed in the urine and returned to its original tube. The urine samples were kept in the original sterile container and both samples, sterile container and bacteriology swab, were stored at room temperature. After 48h, the urine samples with a positive culture were inoculated for standard QBC, again. A total of 213 urine samples were included, 16.4% (35/213) of the initial cultures were positive and 14.2% (5/35) of those had a mixed bacterial growth. Urine stored in a sterile container and bacteriology swab cultured 48h after sample collection had identical sensitivity of 94.6% and a 95% CI [81.81%-99.34%] for UTI detection. Considering all the colonies isolated in the initial samples, the sensitivity for bacterial species detection of urine stored in a sterile container and a bacteriology swab was 91.1% and 93.1% respectively. No samples showed growth of a bacterial species not isolated from the initial sample. In contrast to previous studies, the results of the current study suggest that the use of a urine soaked bacteriology swab or sterile containers are both reliable methods for detecting UTI after storage of canine urine for up to 48h at room temperature.

#### **Disclosures**

No disclosures to report.

#### ESVONC-0-1

Tumor-specific microRNA expression in canine intestinal T-cell lymphomas and analysis of microRNA function in a canine T-cell lymphoma cell line

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The histopathological differentiation between canine intestinal lymphoma and lymphoplasmacellular enteritis can be difficult and often requires additional diagnostic procedures. Specific microRNA expression patterns have already been detected in different tumor types in humans and animals. Studies on the function of microRNAs in tumor



cells may help to understand tumor pathogenesis. The aim of this study was to compare microRNA expression patterns in samples of intestinal T-cell lymphoma, lymphoplasmacellular enteritis, and healthy gut tissue. Additionally, microRNAs that were highly differentially expressed in lymphomas were selected, and their effect on cell death and cell proliferation was studied in the canine T-cell lymphoma cell line CL-1.

Formalin-fixed paraffin embedded samples of canine T-cell lymphomas, lymphoplasmacellular enteritis, and normal gut tissue were used (n=8 each group). The samples were selected in retrospect from material submitted for diagnostic purposes. Total RNA was extracted and the microRNAs were reverse transcribed. In a pilot study, the expression of 192 different microRNAs was compared on custom-made arrays for two samples per group. Twelve microRNAs that were highly differentially expressed in the lymphoma group were selected and their expression was compared in all 24 samples using single micro-RNA qPCR assays. Five of these microRNAs were transfected as microRNA-mimics and microRNA-inhibitors into the T-cell lymphoma cell line CL-1. Cell viability and cell toxicity were measured after 48h using the Multitox-Fluor Multiplex Cytotoxicity Assay (Promega).

Intestinal T-cell lymphomas display a microRNA expression pattern that differentiates them from healthy gut tissue and lymphoplasmacellular enteritis. In the tumor samples, a significant up-regulation of the microRNAs miR-18b, miR-20b, miR-363 was found, which are part of the miR-106~363 cluster. A significant down-regulation was found for the tumor-suppressing microRNAs miR-192 and miR-203. The transfection of these microRNAs in CL1-cells had distinct effects on their proliferation.

MicroRNA expression patterns could prove useful in the diagnosis of canine T-cell lymphomas, and microRNAs from the miR-106~363 cluster have effects on the proliferation of canine T cell lymphoma cells. Overexpression of the miR-106~363 cluster has been shown to induce T-cell lymphomas in mice, and high expression has been found in human T-cell leukemia. These microRNAs may play a role in tumor formation of T cells in several species and may represent a suitable target for translational cancer research.

#### Disclosures

No disclosures to report.

#### ESVONC-O-2

Retrospective comparison of first line adjuvant anthracycline versus metronomic-based chemotherapy protocols in the treatment of stage i-ii, canine splenic haemangiosarcoma

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Treatment for canine splenic haemangiosarcoma (HSA) includes surgery and adjuvant chemotherapy. Adjunctive metronomic chemotherapy (MC) has been shown to result in similar survivals when compared to anthracyclines (AC), whilst there are discordant results on the use of MC at the time of completion of AC-based protocols. The aim of this study was to assess time to progression (TTP) and median survival time (MST) in dogs with stage I-II splenic HSA and to compare different treatment protocols.

Medical records of 10 institutions were searched for dogs diagnosed with surgically excised stage I-II splenic HSA treated with adjuvant AC or MC. Patients receiving MC following completion of AC-based protocols were assigned to an additional group (anthracycline/metronomic, AMC). The MST and TTP were assessed and compared; prognostic variables included breed, gender, age, body weight, stage, mitotic index, treatment type and number of chemotherapy cycles for the AC/AMC groups.

Ninety-four dogs were included: median age was 9.7 years (range 3.9-15) and median body weight 27.25 kg (range 4.8-54.5). All patients underwent splenectomy followed by adjuvant chemotherapy: 50 dogs received anthracycline-based protocols (40 doxorubicin [DOX], 5 epirubicin [EPI], 5 pegylated liposomal encapsulated doxorubicin [PG-DOX]), 20 dogs received MC (18 cyclophosphamide, 2 chlorambucil) and 24 dogs received EPI (13), DOX (10) or PG-DOX (1), followed by MC once the protocol ended (AMC group). The overall MST was 193 days (range 47-3352) and the overall median TTP was 131 days (range 27-909). The MST was 149 days for the AC group (range 47-3352), 225 days for the MC group (range 57-911) and 338 days for the AMC group (range 79-1623). Survival was significantly longer in the AMC group vs the AC group (p= 0.0029) as well as TTP (183 vs 125 days, p=0.029), although no difference could be found between the MC group vs the AC or AMC group. Dogs receiving > 3 treatments with anthracyclines in the AMC vs the AC group survived significantly longer (338 vs 172 days, p=0.0015). No other factors appeared to affect survival or TTP.

This study suggests that the efficacy of adjuvant MC may be comparable to that of AC alone or followed by MC and the use of MC at the time of completion of AC-based protocols may be able to significantly prolong survival and TTP when compared to AC alone. Further largescale studies are necessary to define the role of MC in the treatment of canine HSA.

#### Disclosures

No disclosures to report.

#### ESVONC-O-3

Incidence of post-operative complications and outcome of 48 dogs undergoing surgical management of insulinoma

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Insulinoma is the most common canine pancreatic tumour and is likely to metastasise to regional lymph nodes, liver and lungs. Surgery is currently the preferred treatment for insulinoma, with dogs treated surgically surviving significantly longer than those treated conservatively (median survival 381 days versus 74 days). Prognostic factors for insulinoma are poorly-described. In one study, postoperative



hypoglycaemia was a negative prognostic factor, however significance of other factors such as postoperative complications is currently unknown.

Aim of this retrospective multicentre cross-sectional study was to report post-operative complications and their significance in survival following surgical management of insulinoma and to identify potential prognostic factors that could predict subsequent development of diabetes mellitus.

Clinical records were reviewed to identify patients diagnosed with insulinoma based on histopathology and a total of 48 dogs were enrolled from three referral hospitals in Europe. Cox's regression was used to determine factors associated with post-operative survival and relapse, whilst logistic regression was used to determine factors associated with the development of diabetes mellitus.

Median age at diagnosis was 7.5v (ranging from 4.5v to 11v) and various breeds were represented, with West Highland (8/48) white terriers and Boxers (8/48) the most common. Clinical signs at the time of diagnosis were mainly associated with hypoglycaemia, with seizures and collapse occurring most frequently. The median duration of clinical signs before diagnosis was 60 days (ranging from 1d to 282d). Diabetes mellitus and pancreatitis were the most common post-operative complications, occurring in 9 and 5 dogs, respectively. The diabetes mellitus was persistent in all cases (9/48, 19%), whilst a further 7 dogs (15%) developed short-term post-operative hyperglycaemia (average duration 2.5d), which did not require exogenous insulin administration.

Using logistic regression no pre-operative factors were found to be significantly associated with the development of post-operative diabetes mellitus. Presence of seizures at diagnosis (P 0.03), stage of disease (P 0.04), development of post-operative hypoglycaemia (P 0.004) and pancreatitis (P 0.0004) were all independently and negatively associated with survival. The likelihood of relapse was positively associated with the stage of disease, post-operative hypoglycaemia and pancreatitis.

In the current study, post-operative diabetes mellitus developed more common than previously reported, but no factors were identified that might be useful predictors. Stage of the disease, post-operative hypoglycaemia and pancreatitis were all associated with a greater likelihood of relapse and decreased survival time, and could be used when advising clients about prognosis

#### Disclosures

No disclosures to report.

#### ESVONC-O-4

# Owners' perceptions of the treatment of Osteosarcoma in large breed dogs

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Osteosarcoma is the commonest primary canine bone tumour. An increased incidence is seen in Irish Wolfhounds (IWH), Deerhounds, Greyhounds and Rottweilers. The current gold-standard treatment for osteosarcoma is amputation with adjunctive chemotherapy. To our knowledge there are no veterinary studies specifically surveying owners' thoughts regarding treatment of canine osteosarcoma. The aim of this

study was therefore to review the attitudes and determine owners' opinions of the treatments available for canine osteosarcoma. As part of this study, we aimed to understand the motivations behind selecting a particular treatment and particularly the owners' attitude to amputation. This retrospective study surveyed dog owners using bespoke online questionnaires. IWH, Deerhound, Rottweiler and Greyhound owners with and without experience of osteosarcoma were surveyed alongside owners of any dog without experience of osteosarcoma. There were 154 owners of dogs affected by osteosarcoma and 96 owners without experience of osteosarcoma.

Of the 154 respondents with experience of osteosarcoma: 23 selected chemotherapy and amputation, 14 selected amputation alone, 12 selected chemotherapy alone. The remaining 105 dogs received combination palliative care (which included 10 dogs receiving radiotherapy, as well as those receiving conventional analgesia, bisphosphonates and glucocorticoids) or were euthanized soon after diagnosis. Of those owners choosing to treat their dogs, 83% felt their dog tolerated amputation 'well' and 74% would recommend amputation to others. Furthermore, 80% felt their dog tolerated chemotherapy 'well' and 71% would recommend chemotherapy to others. Of the respondents that selected radiotherapy as part of their palliative care protocol, 78% felt their dog tolerated the therapy 'well'.

Owners were significantly less likely to select amputation for osteosarcoma than non-cancerous reasons (P<0.0001). Additionally, owners of older dogs were significantly less likely to select amputation (P<0.0001). We can conclude that treatments available for osteosarcoma are well received by owners who consider them to be well tolerated by their dogs. This study indicates a good quality-of-life in large breed dogs that have undergone amputation and thus goes some way to dispel the dogma that amputation is poorly tolerated in these breeds. These results provide an evidence-base which veterinary surgeons can use to help owners make therapeutic decisions in large-breed dogs suffering with osteosarcoma. Larger studies involving greater numbers of dogs and a wider range of breeds will help expand this dataset and determine whether these opinions are shared by all owners of dogs affected by osteosarcoma, ultimately providing a more extensive evidence-base for therapeutic decision-making.

#### Disclosures

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# **ESVONC-0-5**

#### Assessing serum clusterin as a potential biomarker for canine lymphoma

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Clusterin (apoliprotein J) is a heterodimeric glycoprotein, widely expressed throughout the body, with an important role in tumourigenesis, apoptosis, and immunoregulation. In humans, clusterin expression has been associated with anaplastic large cell and Hodgkin's lymphoma. The objective of this study was to determine if clusterin expression differed significantly between dogs with high grade multicentric lymphoma (MLSA), and a healthy control population and had potential as a biomarker. We hypothesised that serum clusterin

expression would be higher in untreated dogs with lymphoma in comparison to controls, and that clusterin would subsequently decrease with clinical remission, following successful treatment.

Twelve dogs with untreated, high grade, MLSA were compared to twelve control dogs and to a third population of twelve dogs, with MSLA, treated with chemotherapy (Cyclophosphamide -C, doxorubicin- H, vincristine-O, prednisolone-P protocol) and in complete remission. Dogs in the untreated population were stage IV (n=4) and stage V (n=8); substage a (n=4), or substage b (n=8). Those in the treated population, were stage II (n=1), stage III (n=3), stage IV (n=7) and stage V (n=1); substage a (n=5), or substage b (n=7). Serum clusterin levels in all 36 dogs were determined using Western Blot analysis (AbCam antibody, 104652) and with a commercial canine clusterin ELISA kit (BioVendor).

Western blot analysis of serum detected clusterin at 37kDa and optical density values determined using Image J software. Clusterin expression levels generated from Western Blot analysis and ELISA showed a significant correlation (Pearsons r = 0.6271). Statistical analysis (Student's t-test) of the relative clusterin level in the control population found clusterin to be significantly higher than both untreated (p=0.001) and treated (p=0.0097) MLSA populations when assessed by Western Blot analysis. In addition clusterin levels were statistically significantly higher in the treated compared to untreated MLSA populations (Student's t-test, p=0.0167). When all three populations were directly compared via ELISA, both untreated and treated groups were significantly lower than the control (Kruskal-Wallis test, p=0.0019). In conclusion, serum clusterin expression was reduced in MLSA com-

pared to a control population, with partial recovery in response to treatment. Clusterin expression varies widely between individuals especially within the control group and a single measurement may be insufficient for diagnosis. However, in combination with other biomarkers, clusterin may be of use for diagnosis and to monitor treatment response and relapse.

# Disclosures

No disclosures to report.

# ESVONC-O-7

# TGFβ, FOXP3 and angiogenesis in malignant canine mammary tumors: association with clinicopathological parameters and prognosis

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In human breast cancer the transforming growth factor- $\beta$  (TGF $\beta$ ) and FoxP3 regulatory T cells (Treg) shared signaling pathways have a crucial impact in several tumor hallmark steps, including angiogenesis, facilitating nutrient exchange and metastasis that result in tumor progression. In canine mammary tumors (CMT) this topic is not well documented yet.

This study included 67 malignant CMT obtained by surplus material after regular diagnosis for clinical purposes. Here we studied, by immunohistochemistry, the tumoral TGFβ (TGFβ1 antibody, Santa Cruz Biotechnology; diluted to 1:100), FoxP3 (Clone eBio7979, eBioscience, diluted to 1:100), VEGF (Clone JH121, Thermo Scientific, diluted to 1:100) and CD31 (Clone JC70A, Dako, diluted to 1:20) expression and its association with several clinicopathological characteristics. Additionally female dogs were followed-up for a 2 years period, to elucidate the potential association of TGFß and FoxP3 with angiogenesis and clinical outcome in malignant CMT.

The high levels of TGFB were associated with skin ulceration (p = 0.018), tumor necrosis (p = 0.024), high mitotic index (p < 0.001), marked nuclear pleomorphism (p = 0.001), poor tumor differentiation (p < 0.001), high histological grade of malignancy HGM (p < 0.001), presence of neoplastic intravascular emboli (p < 0.001) and presence of lymph node metastases (p < 0.001). The levels of TGF $\beta$  were positively correlated with intratumoral FoxP3 (r = 0.719; p < 0.001), VEGF (r = 0.378; p = 0.002) and CD31 (r = 0.511; p < 0.001). Tumors with concurrent high expression of TGFB/FoxP3, TGFB/VEGF and TGFB/ CD31 markers were associated with parameters of tumor malignancy (high HGM, presence of neoplastic vascular emboli and presence of lymph node metastasis). Additionally tumors with abundant TGFB and with concurrent high expression of TGFβ/FoxP3, TGFβ/VEGF and TGFβ/CD31 were associated with shorter overall survival (OS) time (p < 0.001). Interestingly TGFβ/FoxP3 class retained the association with shorter OS in multivariate analysis, arising as independent predictor of poor prognosis (9.731 hazard ratio, p < 0.001).

In conclusion, results of this study suggest that  $TGF\beta$  and Treg cells share important pathways contributing to tumoral angiogenis and CMT progression and malignancy.

#### **Disclosures**

No disclosures to report.

#### ESVONC-O-9

A combination of gene electrotransfer of canine IL-12, electrochemotherapy and cytoreductive surgery in the treatment of canine oral malignant melanoma

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Oral malignant melanoma (OMM) is a locally aggressive nonodontogenic oral tumor in dogs that usually metastasizes to the regional lymph nodes and lungs. Radical surgical excision is considered the golden standard of treatment, but multimodal approach to the treatment is recommended. Chemotherapy, radiation therapy and immunotherapy have all been employed in the treatment of canine OMM with some success. In the present study, we evaluated the efficacy of a multimodal treatment protocol for canine OMM combining cytoreductive surgery, electrochemotherapy with bleomycin (ECT) and gene electrotransfer with plasmid encoding canine IL-12 (IL-12 GET).

Nine dogs with OMM (clinical stage I to III), where clients declined radical surgery or previous surgery resulted in an incomplete excision, were included. Five dogs (Group I) with primary OMM underwent intracapsular excision of the tumor and ECT (bleomycin 0.3 mg/kg bw IV), followed by IL- 12 GET (2mg/patient, injected in two sites close



to the tumor submucosally). For ECT plate electrodes were used (8 pulses, 1300 V/cm, pulse duration 100µs, frequency 5 kHz), whereas for GET, multi-array electrodes (MAE) were chosen (1 pulse, 60 V, pulse duration 150 ms). Four dogs (Group II) with recurrent/ incompletely excised OMM were also treated with ECT and GET.

The results show a 66% (6/9) objective response rate (ORR) with 44% (4/9) complete response (CR) 1 month after the treatment. Although progressive disease (PD) occurred in 8/9 patients at the end of observation period (2-21 months, median 5 months), we achieved a mean survival time (MST) of 7 and 12 months in Groups 1 and 2, respectively. Three patients with stage III OMM survived from 5 months to 1 year and 2 of them were euthanized due to OMM-unrelated reasons. The achieved survival times are longer than the reported survival times for stage III OMM treated with radical surgical excision alone (MST 3 months) or combined with radiotherapy (MST 5 months). In addition, we observed a decline in the percentage of regulatory T cells (T<sub>reg</sub>) in the peripheral blood in the course of treatment, which could be attributed to a systemic antitumor response due to IL-12. No major side effects of the treatment were noted and the therapy was tolerated well by all patients.

In conclusion, the combination of cytoreductive surgery, ECT bleo and IL-12 GET may be beneficial for dogs with OMM, especially when other treatment approaches are not acceptable due to their invasiveness (major surgical procedure) or cost.

#### Disclosures

No disclosures to report.

# ESVONC-O-10

#### Evaluation of angiogenesis using semiguantitative RT-PCR for endothelial markers in various canine tumors

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Angiogenesis is essential for tumor progression, and microvessel density is known as a prognostic factor for many solid tumors. Recently, anti-angiogenic therapy has been a potential therapeutic strategy for various types of tumor. Histological assessment has only been reported for evaluating the angiogenesis in current veterinary medicine. For this reason, evaluating the angiogenesis is difficult in patients without surgery. Therefore, a novel evaluation method is needed. The aim of this study was to assess whether histological measurements of blood vessels is associated with mRNA levels of endothelial markers such as vWF and endoglin in spontaneous canine tumors. Additionally, we investigated the relationships between endothelial markers and angiogenic factors such as vascular endothelial growth factor (VEGF) and VEGF receptor2 (VEGFR2).

This study was included 25 various spontaneous canine tumors. For evaluation of angiogenesis, paraffin-embedded sections were stained with antibodies to vWF. The entire tumor section was first observed at low-power field (x40) to select the most vascularized areas (hotspots) and then under a high-power field (HPF) (x400) the stained microvessels were counted. The sections were divided into two groups: high microvessel counts (≥ 12 vessels / HPF) and low microvessel counts (< 12 vessels / HPF). mRNA expression levels of vWF, endoglin, VEGF, VEGFR2 and GAPDH were measured by semiquantitative RT-PCR. Statistical analysis was performed by Mann-Whitney U test and Chi-squared test, with statistical significance set at P < 0.05.

The groups with high microvessel counts showed significantly higher mRNA levels of vWF (P = 0.02) and endoglin (P = 0.01) than that with low microvessel counts. VEGFR2 mRNA level was significantly correlated with vWF mRNA level (P = 0.004), and tended to associate with the microvessel counts (P = 0.08). VEGF mRNA level was not correlated with the microvessel counts (P = 0.50) and vWF mRNA level (P = 0.10)

This study showed that mRNA levels of endothelial markers were associated with histological measurements of microvessels in spontaneous canine tumors, although the difference between two markers, vWF and enoglin, against microvessel counts was not clear. Our results suggest that mRNA measurements from samples taken by fine needle aspiration would be alternative evaluation method of prognostic factors. In addition, VEGFR2 mRNA level was associated with the amount of microvessels. This result indicates that measurement of VEGFR2 mRNA would be useful as a therapeutic predictive factor for VEGFR2-targeted drug, such as toceranib.

No disclosures to report.

# ISCAID-O-1

# Prevalence of feline coronavirus shedding and associated risk factors

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Feline coronavirus (FCoV) infection is common in multi-cat households. The exact prevalence of FCoV in German catteries, however, is unknown, as are factors influencing FCoV prevalence. Aim of the study was to determine FCoV prevalence in German catteries and to evaluate risk factors for FCoV infection.

Faecal samples of 239 cats from catteries across Germany were examined for FCoV by polymerase chain reaction (PCR). Owners had to complete a questionnaire to identify risk factors concerning husbandry and breeding management. Additionally, faeces were examined for the presence of other intestinal pathogens (feline panleukopenia virus, Giardia species (spp.), Tritrichomonas foetus, Toxoplasma gondii, Clostridium perfringens alpha toxin and enterotoxin, Salmonella spp., Cryptosporidium spp., Campylobacter jejuni, Campylobacter coli) by PCR and helminth infestation by faecal flotation. Fisher's exact test and multivariate logistic regression analysis were used to detect correlations between FCoV infection and risk factors.

The prevalence of FCoV in catteries was 61.9% (148/239). Factors with significant influence after multivariate analysis on the prevalence of FCoV shedding were regularly performed faecal examinations for endoparasites (p=0.032; Odds Ratio (OR): 1.884; 95% confidence interval (95%CI): 1.057-3.360) and detection of Clostridium perfringens enterotoxin (p=0.036; OR: 3.036; 95%CI: 1.078-8.556). Other factors,



such as the number of cats housed together, did not have significant influence on the presence of FCoV infection.

Approximately two thirds of all cats in German catteries are infected with FCoV. Cats regularly examined for endoparasites are less likely to be infected. The presence of Clostridium perfringens enterotoxin seems to have an influence on FCoV shedding.

#### Disclosures

Disclosures to report.

Dr. Christian Leutenegger is the Head of Molecular Diagnostics at IDEXX Laboratories, Inc. Dr. Nikola Pantchev is employed at IDEXX Laboratories, Ludwigsburg. This laboratory performed the PCR testing in this study. IDEXX played no role in the study design, in the collection and interpretation of data, or in the decision to submit the manuscript for publication. There is no commercial conflict of interest as the information generated here is solely for scientific dissemination. The authors declare that they have no competing interests.

#### ISCAID-O-2

Quakers Hill, NSW, Australia

# Novel hepatitis B-like hepadnavirus identified in a feline immunodeficiency virus-infected domestic cat

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Feline immunodeficiency virus (FIV), a pathogen of domestic cats worldwide, is classified alongside human immunodeficiency virus (HIV) in the genus Lentivirus, family Retroviridae. Like HIV infection in people, FIV causes progressive immune dysfunction. Unlike HIV, the natural clinical course of FIV is unpredictable. Most HIV-AIDSdefining illnesses in humans result from copathogens. The role of copathogens in natural FIV infection is poorly understood.

The aim of this study was to identify potential viral copathogens from FIV-associated lymphoma using transcriptome analysis. TruSeq paired-end libraries were prepared from ribosome-depleted RNA from 13 high-grade B-cell lymphomas arising in FIV-infected cats. Total RNA sequencing yielded 40-50 million reads per library. Bioinformatics analyses identified eight hepatitis-B virus-associated reads sharing 73-94% amino acid identity with the core protein, surface protein, and polymerase of hepadnaviruses, suggesting a novel virus. The presence of genome fragments was validated by PCR and sequencing of tumour DNA. The entire 3187bp genome was subsequently obtained by PCR. Polymerase, surface, core and X ORFs typical of orthohepadnaviruses were identified. Analyses of polymerase protein revealed 63.2% to 68.7% amino acid identity with known orthohepadnaviruses. This genetic distance merits assignment of a new species within the genus Orthohepadnavirus, tentatively named domestic hepadnavirus.

This is the first hepadnavirus infection identified in a felid and the first report of a hepadnavirus in a companion species. Domestic cats frequently interact with humans as well as other pets and wildlife. The epidemiology and pathogenic potential of this novel hepadnavirus for cats and other mammals, including humans, should be investigated.

#### Disclosures

No disclosures to report.

#### ISCAID-O-3

Long-term follow-up and prognostic factors in canine leishmaniasis in a non-endemic country, a retrospective cohort study

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Leishmania infantum is an intracellular protozoan parasite which is endemic in countries in the Mediterranean basin often with high prevalence. Leishmaniasis is increasingly diagnosed in non-endemic areas due to relocation and travelling of dogs from endemic geographic areas. Prognosis of Leishmaniasis in these dogs may differ from those in endemic areas due to the absence of an appropriate vector. The aims of this study were (1) to determine the Kaplan-Meier estimated survival time for dogs with Leishmaniasis in the non-endemic Netherlands and (2) to determine if clinical staging and clinicopathological variables at time of diagnosis can predict survival of these dogs.

The database of the University Clinic of Companion Animals of the Faculty of Veterinary Medicine, Utrecht University was interrogated for Leishmaniasis patients. Included were dogs with a diagnosis of Leishmaniasis based on the clinical presentation (e.g. non-pruritic cutaneous disease, clinicopathologic changes such as cytopenias, proteinuric renal disease and hyperglobulinemia) combined with confirmation of Leishmaniasis by a positive anti-Leishmania titer, or the presence of Leishmania amastigotes in biopsies. Patient records were reviewed for signalment, clinicopathological data at moment of diagnosis, incl. complete blood count, biochemistry, protein spectrum and urine analysis, and lastly the Canine Leishmania Working Group clinical staging system class. Follow-up was performed at the time of the study by phone contact and included received treatment, and date and cause of death if deceased. Kaplan-Meier survival curves were compared between groups using a Log Rank test. Multivariate analysis was performed using the COX proportional hazards regression model. In total 47 dogs were included. The overall estimated Kaplan-Meier survival time was 6.4 years (95% CI 1.8-11 years). The estimated Kaplan-Meier survival times did not differ between class C and class D dogs. In the univariate analysis increases in monocytes, plasma urea and creatinine concentrations and urine protein to creatinine ratio were all significantly associated with shorter survival time. The multivariate model identified a decrease in hematocrit and an increase in plasma creatinine concentration and total protein as negative prognostic factors.

It can be concluded that dogs diagnosed and treated against Leishmaniasis in the Netherlands in general have a long survival time in comparison to a comparable cohort in a German study. The presence of protein-losing renal disease was a strong negative predictor of survival. Interestingly, clinical staging at time of diagnosis did not seem to predict survival in this population.

#### Disclosures

Disclosures to report.

Both M.K. de Jong and C.J. Piek are employed by the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.



#### ISCAID-O-4

# Reduction in incidence of canine leptospirosis in Switzerland correlates with the introduction of a new quadrivalent antileptospiral vaccine

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A marked increase in incidence of acute canine leptospirosis was documented in Switzerland between 2003 and 2012 with 70% of infected dogs showing serologic evidence of infection with serogroup Australis. In 2013, a new tetravalent killed whole cell anti-leptospiral vaccine (Nobivac® L4, MSD Animal Health) was introduced onto the Swiss market. This vaccine includes serogroups Australis and Grippotyphosa in addition to serogroups Canicola and Icterohaemorrhagiae present in the previously available bivalent vaccines (L2). It had shown excellent protection against experimental challenge of dogs with heterologous strains from the same serogroups. Aim of this retrospective longitudinal case control vaccine efficiency study was to examine whether the introduction of L4 is associated with a reduction in hospital incidence of canine leptospirosis.

Dogs with leptospirosis (n=211) or acute kidney injury not due to leptospirosis (AKI-nL, n=119) treated at the Vetsuisse Faculty Bern between 2011 and 2017 were included if they had a complete vaccination history. A diagnosis of leptospirosis was confirmed based on compatible clinical findings and the presence of at least one of the following: positive single MAT titre ≥1:800 (n=66), MAT seroconversion (n=102), positive IgM lateral flow assay (n=16), positive urine RT-PCR (n=4), or strong clinical suspicion not confirmed serologically due to early death (n=23). Control dogs were diagnosed with AKI-nL based on a convincing alternative diagnosis and at least a negative serology. Annual incidences and vaccine effectiveness were compared between groups, the latter being expressed as odds ratio (OR) for being diagnosed with leptospirosis.

The control AKI-nL group showed a marked decrease in dogs vaccinated with L2 (from 100% in 2011 to 19% in 2016), and a steep increase in dogs vaccinated with L4 (from 0% to 81%), with stable numbers of dogs current on their vaccines (63  $\pm$  13%) or unvaccinated (2  $\pm$  3%). Simultaneously, the number of cases of leptospirosis significantly decreased from 63 (2011-2012) to 14 cases/y (2016-2017), while the number of control cases with AKI increased from 17 to 39 cases/y. The OR for a dog with AKI to be diagnosed with leptospirosis while being vaccinated vs not being vaccinated was 1.12 (95% CI, 0.89-1.42, P 0.33) for any anti-leptospirosis vaccine; 2.66 (95% CI, 1.87-3.79, P < 0.001) for a L2-vaccine; and 0.28 (95% CI, 0.17-0.47, P < 0.001) for L4.

These results indicate that the introduction of L4 was associated with a marked reduction in incidence of severe acute canine leptospirosis in Switzerland.

# Disclosures

Disclosures to report.

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# ISCAID-O-5

#### Leptospira seroprevalence in owned dogs from Spain

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Leptospirosis is a re-emerging and complex zoonotic bacterial disease. caused by pathogenic serovars of Leptospira with multiple clinical signs in dogs. Nowadays, the prevalence of canine leptospirosis in Spain is undetermined.

Our aim was to determine the seroprevalence of anti-Leptospira serum antibody in dogs in Spain, to know most common serovars and if there is any relationship between area and serovars.

This is a cross-sectional study with 1310 records of canine Leptospira testing data from all Spain since 2015 to 2017. Idexx's microscopic agglutination test (MAT) was performed. Inclusion criteria was a positive MAT (≥1/100) result for at least one serovar (Bratislava, Copenhageni, Icterohaemorrhagiae, Australis, Pomona, Grippotyphosa, Autumnalis, Canicola and Saxkoebing) and to have data of zip code.

Of the 1310: 338 (25.8%) were positive. According to biogeographic areas, North had the highest percentage (38.9%) followed by Center (29.6%), South (28.4%), Mediterranean (22.8%) and Northwest (22.2%), Highest incidence was detected in autumn (n=82), while an average of 57 cases in other seasons. Gender was available for 68/338 positive with 49 (72.0%) males and 19 (18.0%) females. Age was available for 49/338 positive, and incidence was 8.2% (4/49) in <2y, 28.6% (14/49) from 2 to <6y, 46.9% (23/49) from 6 to <10y, and 16.3% (8/49) in ≥10y.

Of the 338 samples: 254 (75.1%), 111 (32.8%), 94 (27.8%), 84 (24.8%), 59 (17.4%), 57 (16.8%), 44 (13%), and 11 (3.2%) were positive to Icterohaemorrhagiae, Bratislava, Grippotyphosa, Australis, Pomona, Autumnalis, Canicola, and Saxkoebing, respectively.

One hundred twenty-two (36.1%) had MAT titer ≥1:400 with 72 (59.0%), 54 (44.3%), 35 (28.7%), 30 (24.6%), 29 (23.8%), 25 (20.5%), 13 (10.6%), and 8 (6.5%) positive to Icterohaemorrhagiae, Bratislava, Grippotyphosa, Pomona, Australis, Autumnalis, Canicola, and Saxkoebing, respectively.

When dogs with MAT titer ≥1:400 were divided by serovars and biogeographic areas, Icterohaemorrhagiae was the most prevalent in Mediterranean 33.9% (38/112) and South 35.7% (10/28), Australis 33.3% (2/6) in Northwest, Bratislava 27.2% (9/33) in North, and Grippotyphosa 22.5% (20/89) in Center, respectively.

These results demonstrate that Spain's dogs have a high exposure to diverse Leptospira serovars. Male dogs older than 5 years seem to be riskier to be positive. Diagnosis increases in autumn with higher seroprevalence in the North area. Most prevalent are Icterohaemorrhagiae, Bratislava, and Grippotyphosa. However, the most prevalent serovar varies between geographic areas if we only included MAT titer ≥1:400, in order to limit the effect of unknown vaccination status presents in this study.

#### Disclosures

Disclosures to report.

Indirect benefits because an author could be benefit from the results of the study.

# ISCAID-O-6

# Urine shedding of pathogenic Leptospira spp. in cats in Southern Chile

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After experimental infection, cats can intermittently shed pathogenic Leptospira spp. in urine for several weeks. So far, only shedding of DNA of pathogenic Leptospira spp. has been documented in naturally infected cats in few countries, but not urinary shedding of infectious bacteria. The climate in Southern Chile is temperate rainy with high annual precipitations which represents ideal preconditions for survival of Leptospira spp. in the environment. The aim of the study was to investigate the shedding of pathogenic Leptospira spp. in outdoor cats in Southern Chile. Urine and blood samples of 231 outdoor cats from rural and urban areas were collected. Urine samples were investigated for pathogenic Leptospira spp. by immune magnetic concentration followed by real time PCR targeting the LipL32 gene. Urine samples were cultured in Ellinghausen-McCullough-Johnson-Harris medium at 29 °C for 13 weeks. Positive urine cultures were confirmed by PCR. MLST was used to molecularly characterize strains obtained from positive cultures. PCR products were sequenced afterwards.

Of all 231 urine samples, 30 (13,0%; 95% confidence interval (CI): 8.7-17.3%) were PCR-positive with a median of 288,000 genomic equivalents/ml (IQR 2,145-614,500; range 5 to 9,440,000). In 7/231 (3.0%; 95% CI: 0.5-4.5%) urine samples Leptospira spp. could be cultured. Applying MLST and sequencing of PCR products, L. interrogans copenhageni icterohaemorrhagiae was identified in 6 cats and L. interrogans autumnalis autumnalis in 1 cat.

Outdoor cats in Southern Chile shed strains of pathogenic Leptospira spp. that can be a possible source of infection for humans, dogs, and livestock animals.

#### Disclosures

Disclosures to report.

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# ISCAID-O-8

#### Patterns of antimicrobial use for selected feline diseases in Switzerland in 2016

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Antimicrobial resistance is an emerging problem in human and veterinary medicine. Antibiotic use is the main driver for resistance development. The goal of this study was to evaluate antimicrobial prescription in cats in Switzerland with acute upper respiratory tract disease (aURTD) and feline lower urinary tract disease (FLUTD). &#13Cases presented to two University hospitals and eight private veterinary practices during 2016 were retrospectively evaluated. Clinical history, diagnostic work-up and antimicrobial therapy (class, dosage, duration) were assessed. A justification score (JS) was used to assess the agreement of antimicrobial prescription with current guidelines (1=appropriate, 2=incorrect dosage/duration, 3=inappropriate antimicrobial, 4=overall wrong treatment decision). &#13Of 142 cats with aURTD, 107 (75%) received antibiotic therapy of the following classes: potentiated aminopenicillins (51%), third generation cephalosporins (22%), aminopenicillins (21%), tetracyclines (17%), fluoroguinolones (5%), amphenicoles (2%), first generation cephalosporins and macrolides (1% each): 16% received combination or serial therapy. The cases were treated for 4 to 37 (median 10) days. When assessment of prudent use was possible (120/142), antimicrobial therapy was judged appropriate (JS-1) in 35 (29%) and inappropriate in 85 (71%) cases (JS-2=2; JS-3=38; JS-4=45). Antibiotic therapy was significantly associated with the presence of lethargy, anorexia or fever (p=0.003).&#13Of 252 cats with FLUTD, 150 cases (60%; 50 with bacterial cystitis, 100 with other/unknown diagnosis) received antibiotic therapy of the following classes: potentiated aminopenicillins (67%), third generation cephalosporins (18%), fluoroquinolones (13%), aminopenicillins (7%), first generation cephalosporins (4%), amphenicoles (1%) and tetracyclines (1%); 10% received combination or serial therapy. The cats were treated for 1 to 56 (median 11) days. When assessment of prudent use was possible (152/252), antimicrobial therapy was judged appropriate (JS-1) in 76 (50%) and inappropriate in 76 (50%) cases (JS-2=1; JS-3=28; JS-4=47); inadequate diagnostic work-up (97/100) was the main reason to preclude judgment. Bacteriuria was significantly associated with antibiotic therapy (p<0.001). &#13Overall, frequency of antimicrobial therapy was not significantly different between university hospitals and private practices, but critically important antibiotics (third generation cephalosporins and fluoroquinolones) were significantly more often used in private practices (aURTD, p=0.009; FLUTD, p<0.001).&#13Our results suggest that overprescription of antibiotics in cats with aURTD and FLUTD is common. Third generation cephalosporins are frequently used. The study highlights the need to promote antimicrobial stewardship in small animal medicine. To support this, an online tool (www.antibioticscout.ch) based on current veterinary guidelines was launched in 2017. The impact of this tool on prescribing habits will be assessed in the future.

# **Disclosures**

Disclosures to report.



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# SCH-O-1

Development, validation, and application of an LC-MS/MS method for the quantitative determination of trans-4-hydroxy-I-proline in dogs with chronic hepatitis

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Trans-4-hydroxyl-1-proline, a nonproteinogenic amino acid, is an important constituent of the structural protein collagen. Increased trans-4-hydroxyl-1-proline concentrations in urine or serum are associated with degradation of connective tissue. Recently trans-4-hydroxyl-1-proline has been used as a biomarker of hepatic fibrosis in humans. The objectives of this study were to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantitative determination of endogenous trans-4-hydroxy-l-proline in dog serum, to analytically validate this method, and to asses serum trans-4-hydroxyl-1-proline concentrations in dogs with chronic hepatitis.

Serum trans-4-hydroxyl-1-proline concentrations were quantified on a Quantiva Triple Quandrupole Mass Spectrometer (ThermoFisher Scientific). The following validation variables were assessed: linearity by dilutional parallelism, precision by intra-assay variability, reproducibility by inter-assay variability, and accuracy by recovery (mixing samples of known concentration). This validated method was used to quantify the concentration of trans-4-hydroxyl-1-proline in serum from 24 dogs with histopathologically confirmed chronic hepatitis and 24 healthy control dogs.

A method for quantification of trans-4-hydroxy-l-proline in dog serum was successfully develop. Observed to expected ratios for dilutional parallelism (3 samples) ranged from 72.7% to 111.5% with a mean  $\pm$  SD of 91.3%  $\pm$  19.6%. Intra-assay (10 samples) and inter-assay (10 samples) coefficients of variation (%CVs) ranged from 0.3% to 4.0%, and <0.1% to 4.8%, respectively. Observed to expected ratios for mixtures of two serum samples of known concentrations (10 pairs of samples), ranged from 52.3% to 82.4% with a mean  $\pm$  SD of 66.1%  $\pm$  14.4%. Trans-4-hydroxy-l-proline concentrations were significantly decreased in the serum of dogs with chronic hepatitis versus healthy controls, with medians (minmax) of 0.28 ng/mL (0.071-0.79) and 1.85 ng/mL (0.074-4.88; P =0.037), respectively.

The developed method was shown to be precise and reproducible for the quantification of trans-4-hydroxy-l-proline in canine serum. The recoveries were limited which affected the accuracy. Dogs with chronic hepatitis had significantly lower serum trans-4-hxdroxy-lproline concentrations than healthy controls possibly due to abnormal hepatic amino acid metabolism.

# Disclosures

No disclosures to report.

#### SCH-O-2

#### Copper-associated Hepatitis in dogs: a retrospective study of 17 clinical cases

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Copper-associated hepatitis is a well-recognized chronic hepatic disease in dogs. While in some breeds the disease is due to a genetic defect on copper metabolism, in others it is still unclear whether its accumulation is a primary or a secondary condition. Nowadays, reports of non-genetically predisposed dogs are increasing.

This retrospective study aims to describe epidemiologic factors and clinical findings of dogs with copper-associated hepatitis.

All the dogs presented to a French referral center from May 2010 to March 2017, with positive rhodanine staining on liver biopsies were included. Medical records were retrospectively analyzed for: age of presentation, sex, breed, main clinical complaints, abdominal ultrasound (US) findings and rhodanine staining pattern.

A total of 17 dogs were included. Median age at presentation was 8 years (range 4-11). Eleven dogs were female and six were males. No sex predisposition was found (Chi Square test p=0.23). All dogs were pure-breed, namely: German shepherd (3/17), Labrador Retriever (2/17), Cocker Spaniel (2/17), Beauceron (2/17), American Staffordshire Terrier (2/17), Cavalier King Charles Spaniel (2/17), Mongrel (1/17), Jack Russell Terrier (1/17), West Highland White Terrier (1/17) and Dalmatian (1/17). Main clinical complaints were: an incidental finding of increased liver-enzymes (5/17), anorexia/decreased appetite (5/17), weight loss (4/17), polyuria/polydipsia (3/17), vomiting (3/17) and jaundice (2/17). Abdominal US was performed in all the dogs. Main findings included: an heterogenous mottled liver (5/17), hepatomegaly (4/17), hypoechoic nodules (3/17) and microhepatia (3/17). Rhodanine staining pattern was centrilobular (zone 3) in 8/17 and peri-portal (zone 1) in 3/17. In 6/17 the pattern was considered multifocal.

Similarly to previous studies, German Shepherd and Terriers were overrepresented. Less common breeds were also reported namely Beauceron and American Staffordshire which, despite being a terrier, has not been previously associated with this disease. The biochemical incidental finding of increased liver-enzymes emphasizes the silent progression of the disease. Although US abnormalities were inconsistent, the heterogenous mottled liver was a common finding. Centrilobular pattern of rhodanine staining observed in the majority of cases strengthens the primary condition of the disease. In the three cases in which a peri-portal distribution was observed, copper-deposition was believed to be secondary to cholestasis.

To the author's knowledge, this is the first study describing copperassociated hepatitis in Beauceron and American Staffordshire Terrier dogs. This study increases the number of reported breeds affected by copper-associated hepatitis, emphasizing a possible multifactorial etiology.

#### Disclosures

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## SCH-O-3

Plasma renin activity and aldosterone concentration in primary hypoplasia of the portal vein with portal hypertension in dogs

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The renin angiotensin aldosterone system (RAAS) is a physiological regulator of blood pressure, electrolyte balance, and fluid homeostasis. Studies of humans with hepatic cirrhosis have shown that the RAAS is activated by increased sympathetic nervous activity, hyponatremia, reduced renal perfusion, and hypovolemia. A possible therapeutic approach to the treatment of patients with portal hypotension is suppression of the RAAS. In dogs, the relationship between RAAS and cardiac or renal disorders has been studied; however, to our knowledge, there are no studies of the relationship between the RAAS and hepatic disease with portal hypotension.

To evaluate the relationship between portal hypertension and RAAS in dogs, we selected dogs histopathologically diagnosed with primary hypoplasia of the portal vein (PHPV) with portal hypertension. Diagnosis of portal hypertension was based on the presence of acquired portosystemic collaterals (APSCs). Dogs with congenital portosystemic shunt (CPSS) are reported to have similar histopathological and hemodynamic alterations compared to PHPV, without the evidence of portal hypertension. Thus dogs diagnosed as CPSS were evaluated as controls. Diagnosis of CPSS and APSCs was determined by computed tomographic angiography and gross evaluation by laparoscopy. For RAAS assessment, we measured plasma renin activity and plasma aldosterone concentration using radioimmunoassay technique. All blood samples were obtained at diagnosis and stored at -20°C until analyzed.

Twelve dogs diagnosed as PHPV with portal hypertension and nine dogs with CPSS were included in this study. There was a significant difference in the median plasma aldosterone concentration between CPSS (median 1 pg/ml) dogs and dogs with PHPV (median 89.5 pg/ml) (P < 0.001). No significant differences were found in the median plasma renin activity between the two groups.

This is the first study to evaluate the relationship between RAAS and portal hypertension in dogs. The inactivation of RAAS in CPSS dogs indicates that the RAAS is not activated by the hemodynamic changes by portosystemic shunting. The RAAS system is activated in dogs with portal hypertension. Thus, suppression of the RAAS may be a therapeutic option for portal hypertension in dogs.

#### Disclosures

No disclosures to report.

# SCH-O-4

Serum vitamin D, C-Reactive Protein, and von Willebrand's factor in dogs with inflammatory hepatopathies

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Biomarker investigation in canine hepatopathies is still in its infancy. Decreases in serum 25-hydroxyvitamin D (25(OH)VD) and increases in C-Reactive Protein (CRP) and von Willebrand's factor (vWF) are associated with disease severity in human chronic inflammatory hepatopathies. This current prospective and observational study of dogs with chronic inflammatory hepatopathies investigated whether changes in serum concentration of 25(OH)VD, CRP, or vWF are present in these disorders, and these biomarkers are associated with hepatic copper levels, liver injury markers (i.e. alanine aminotransferase (ALT)), a cholestasis marker (i.e. total bilirubin), or markers of more advanced liver disease (i.e. fibrinogen, albumin)

Serum 25(OH)VD, CRP, and vWF were determined prior to the liver biopsy which was performed either percutaneously with ultrasound guidance, or surgically via laparoscopic surgery or laparotomy. Data were analyzed for normality and log transformation was performed if the data was non-parametric. Correlations were tested using Pearson correlation test and p < 0.05 was considered significant.

Twenty five dogs met the inclusion criteria. Seventeen different breeds were represented, with Labrador retriever (4/17; 23.5%) and terrier cross (3/17; 17.6%) being common breeds. Median age was 8.3 vrs (range 2-14vrs).

CRP (median 8.6 ug/l, range 1-28.3) was increased in 52% (13/25) of dogs. The 25(OH)VD level (median 213 nmol/L, range 42-374) was low in 12% (3/25) of dogs. No dogs had increased vWF activity (median 69%, range 23-116%), but the activity was low in 24% (6/25) of dogs. Fibrinogen (median 150 mg/dL, range,57-377) was low (< 100 mg/dl) in 3 dogs. No dogs had increased fibrinogen. Albumin and vWF as well as albumin and 25(OH)VD had significant (both p<0.0001) strong positive correlations (r=0.68 and r=0.67, respectively). No significant correlations were found.

In dogs with chronic inflammatory hepatopathies, CRP is elevated in over 50% of dogs but vWF activity and 25(OH)VD serum levels are most often in the normal range. 25(OH)VD levels decrease as serum albumin decreases, which may reflect loss of hepatic synthetic in late stage inflammatory disease. Additional comparisons of biomarkers with histopathological scoring and grading as well as treatment outcome should be evaluated in the future to assess their ability to predict the disease severity or outcome.

#### Disclosures

Disclosures to report.

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#### SCH-O-5

# Serum melatonin concentrations in dogs with congenital portosystemic shunts

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Hepatic encephalopathy (HE) is a syndrome of neurologic dysfunction present in several liver diseases and an important contributor to patient morbidity. Canine HE is most commonly reported in association with congenial portosystemic shunting (cPSS). Although not completely characterised, several factors are known to influence the pathogenesis of

HE, namely increased ammonia, inflammation and manganese, as well as alkalosis, hyponatraemia and hypokalaemia. Melatonin is a hormone secreted by the pineal gland in the brain and by the enterochromaffinlike cells in the gastric mucosa, with actions linked with regulation of the circadian rhythm, enterocyte integrity, and free oxygen radical scavenging, amongst others. The majority of melatonin is metabolised in the liver and recently, melatonin levels were noted to be significantly increased in human alcohol-induced liver cirrhosis, correlating with both disease severity and with the severity of HE, hence hypothesised as a potential contributor to the development of the latter. To the authors' knowledge, melatonin homeostasis in canine liver disease is currently unknown. The aim of this study was to investigate whether melatonin concentrations could be altered in dogs with cPSS, with the hypothesis that higher levels would be present when compared to healthy controls. Medical records were retrospectively reviewed for inclusion into two cohorts: dogs with a confirmed diagnosis of cPSS (n = 24) and healthy dogs examined through wellbeing appointments (n = 15). A canine competitive enzyme-linked immunosorbent assay was used to measure serum melatonin prospectively. 100uL of sample was used from an archive of surplus samples retained after clinical diagnostic purposes. Informed owner consent for surplus retention and use for research had been obtained at the time of clinical sampling. Melatonin concentrations in each group were assessed for normality with the Anderson-Darling test. Both groups were not normally distributed, therefore data was described as median (minimum - maximum ranges) and differences between groups compared with the Mann-Whitney U test. Statistical significance level was set at P < 0.05.

The concentrations of melatonin in the cPSS group (25 pg/mL [18.5 -244.9 pg/mL]) did not differ significantly (P = 0.7839) from the healthy controls (27.2 pg/mL [19.8 - 161.5 pg/mL]).

This study suggests that serum melatonin is not increased in dogs with cPSS and is unlikely to play a role in HE pathogenesis.

#### Disclosures

No disclosures to report.

# VBPS-O-1

Comparison of systolic arterial blood pressure between low stress and common handling conditions in cats using the Doppler method and High Definition Oscillometry

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In cats, systolic arterial blood pressure (SBP) measurements can be influenced by the white coat effect and are commonly misinterpreted as arterial hypertension. The aim of this study was to compare the SBP measurements of cats examined under two different handling conditions and to investigate possible correlations with lifestyle and cohabitation with other animals, using the Doppler method and High Definition Oscillometry (HDO).

Seventy-nine cats, with an age ranging from 6 months to 17 years, were included in the study. Thirty-one of them were clinically healthy and 41/79 had underlying diseases unrelated to arterial hypertension. Historical data regarding lifestyle (indoors, outdoors and indoors/outdoors) and cohabitation with other animals (dogs or cats) was initially collected. SBP measurements were performed using two different handling protocols, in a randomized fashion. In the low stress handling, SBP values were measured with both HDO and Doppler after ten minutes of acclimatization in a quiet and isolated consultation room with a Feliway® diffuser (Ceva Animal Health Ltd), without any previous clinical handling of the cats or contact with other animals during the visit. In the typical handling, SBP was measured in the same consultation room using both devices, after the cats previously went through the clinic's normal admission procedure (common waiting room, clinical examination as part of a routine exam or during the diagnostic process), and without a period of acclimatization. All measurements were conducted by the same observer (OMI), with minimal restraint of the cat, from the coccygeal artery.

There was not any significant difference between the two different handling conditions' SBP measurements, when measured by either Doppler or HDO (p=0.2 and p=0.4, respectively). A Bland and Altman comparison analysis of the two measuring methods indicated that Doppler provided systematically higher SBP values compared to HDO in both handling conditions (95% CI). Using a linear mixed effect model, significantly higher SBP values were observed only in indoors/outdoors cats, regardless of handling conditions, cohabitation with dogs or cats, underlying clinical condition or method of SBP measurement (p<0.03). Cats with an underlying disease had significantly higher SBP during low stress handling but only when SBP was assessed using Doppler (p=0.004).

The results of this study suggest that SBP measurements in cats are apparently similar in a feline-friendly environment and during typical handling during a visit to the clinic. Furthermore, an indoors/outdoors living lifestyle seems to result in consistently higher SBP of these cats, compared to other lifestyles.

# **Disclosures**

No disclosures to report.

We wish to confirm that there are no known conflicts of interest associated with this publication / presentation and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. On behalf of the authors Olympia-Maria Ioannidi, DVM T: 0044 07547845899 Email: maroly9@gmail.com.

#### VBPS-O-2

Comparison of Doppler ultrasonography and oscillometry with or without proprietary optimizations for non-invasive blood pressure measurement in conscious cats

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Non -invasive blood pressure (BP) measurement in conscious cats is a vital part of monitoring clinically healthy older individuals, patients on

medication and with diseases that affect blood pressure. The aim of this study was to compare Doppler ultrasonography and oscillometry with or without proprietary optimizations for non-invasive BP measurement in conscious cats.

Twenty seven feline in-patients from the Feline Clinic of a University veterinary hospital were included in this prospective study. All measurements were obtained according to the guidelines in American College of Veterinary Internal Medicine consensus statement to achieve standardized conditions. Both devices were operated according to the manufacturer's guidelines and used alternatively as the first device.

Five systolic blood pressure (SBP) measurements were obtained by the Doppler and oscillometric (with and without proprietary optimizations) devices and were averaged prior to statistical analysis. Bland Altman analysis was used to determine the agreement. The differences in SBP were plotted against the mean of each pair of measurement. The bias was defined by the mean differences between two methods. The precision was calculated from the standard deviation (SD) of the differences and limits of agreement was calculated from the bias  $\pm$  1.96 x SD. A paired sample T test was run to determine whether there was a statistically significant mean difference in the SBP measurement of the two methods. Overall statistical analysis was achieved using Excel with Minitab 17 Software. Comparison of the methods gave an average bias of +26.99 mmHg with 95% CI between 19.30 and 34.69 mmHg. The lower limit of agreement (LLA) was set at -11.13 mmHg and 65.12 for the upper limit of agreement (ULA); the difference between the upper and lower limit was 76.25 mmHg. Comparison produced an average bias of 20.83 mmHg with 95% CI between 14.85 and 26.81 mmHg. The limits of agreements were set at -8.80 for LLA and 50.45 ULA; the difference between LLA and ULA was 59.25 mmHg. As indicated by the bias, the oscillometry (with or without proprietary optimizations) overestimated the SBP. The mean difference between the two NIBP devices was significantly different.

The findings of this study suggest that Doppler ultrasonography and oscillometry with or without proprietary optimizations cannot be used interchangeably. Different methodology should be taken into an account before using non-method specific reference ranges and starting antihypertensive treatment or substaging patients with kidney disease.

#### Disclosures

No disclosures to report.

# VBPS-O-3

# Comparison of two Doppler ultrasonography devices for measurement of systolic blood pressure in conscious dogs

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Serial blood pressure measurements are of vital significance for the assessment of dogs with diseases or on medications that are known affect the blood pressure. The Doppler ultrasonography device that was used for the establishing reference ranges for blood pressure was recently discontinued and data regarding the agreement of different Doppler ultrasonography devices are limited. The aim of this study was to compare two Doppler devices, Parks Medical Doppler and the Mano Médical Vet BP Doppler, for the measurement of systolic blood pressure (SBP) in conscious dogs.

Forty canine in-patients of a University veterinary hospital were included in this prospective study. SBP was measured indirectly using the standardised process according to American College of Veterinary Internal Medicine guidelines. Both devices were operated according to the manufacturer's guidelines and used alternatively as the first device.

Statistical analysis was performed using Matlab software, along with Bland-Altman analysis and scatter graphs to determine agreement, standard deviation (SD) and for each device's ability to detect hypertensive animals. Finally, least-squares linear regression fit data was evaluated for significance in relation to age, cuff size and heart rate.

The mean difference between the two devices was 0.61 suggesting good correlation. A linear rank correlation test was also performed on the SBP readings. The R correlation coefficient was found to be 1, which suggests strong correlation between devices. A mean of -0.61 indicates that the overall difference between the mean systolic BP differences between the two devices is -0.61mmHg. This shows that statistically, the devices are agreeable. No significance in relation to age, cuff and heart rate was identified.

This study suggests that the SBP measurements from these two Doppler devices correlate well, allowing different device measurement comparison and the use of the same SBP reference range.

#### Disclosures

No disclosures to report.

# ESCG-P-1

#### Proteomics of canine feces from healthy Boxer dogs: a pilot study

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The aim of this work was to detect and identify proteins of canine feces from healthy Boxer dogs by a proteomic approach. Dog proteins detected in feces may be of help in investigating (e.g. pathogenesis, diagnosis, follow-up) canine gastrointestinal (GI) diseases. As a first step, an attempt to define the "healthy" proteome pattern of canine feces has been performed. Feces sampled from two groups (8 and 7 patients each) of healthy (no GI signs + no pre/probiotic administration + no change in diet within the last 3 months) Boxer dogs, were included in the study. Two-dimensional polyacrylamide gel electrophoresis (2DE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were performed. Eight spots form 2DE were considered interesting due to their expression pattern and therefore were selected as candidates for LC-MS/MS analysis. By consulting SwissProt & NCBInr databases, MASCOT and SONAR results allowed us to find the origin species of 4 out of 8 proteins: Canis lupis familiaris, Flavobacteriaceae bacterium, Prochlorococcus marinus, Candida glabrata. Especially the protein from Canis lupis familiaris resulted to be very interesting, cause owing to the hosts (dogs). It was identified as Immunoglobulin lambda-1 light chain isoform X36. If confirmed, this datum



could be very interesting for the future perspectives of this kind of studies, being a protein involved in the immune response of the host, but also in some kind of tumor, and other pathological conditions.

#### Disclosures

No disclosures to report.

#### ESCG-P-2

# Elevated blood urea nitrogen and thrombocytopenia during treatment as prognostic factors for canine acute pancreatitis

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Canine acute pancreatitis (AP) has been easily diagnosed via the canine pancreas-specific lipase (Spec-cPL) test in recent years. Although several blood tests related prognostic factors for AP have been identified, prognostic factors during treatment are still unclear. In this study, we aimed to identify the prognostic factors of AP during treatment

The subjects included were 128 patients with AP who underwent hospitalization treatment. AP was diagnosed on the basis of clinical signs and the abnormal Spec-cPL result (> 400 μg/L), concurrently with exclusion of other gastrointestinal diseases via ultrasonography. At the end of treatment, 95 patients were alive, while 33 died. In patients with elevated blood urea nitrogen (BUN) levels at the first visit, the risk for death was high (P = 0.001, odds ratio [OR]: 4.53; sensitivity, 87%; specificity, 40%). In patients with Spec-cPL > 700 μg/L at the first visit, the risk for death tended to be high (P = 0.06, OR: 3.03). However, there were no significant differences in clinical symptoms, other blood test markers, and abdominal ultrasonography findings at the first visit. Furthermore,he risk for death was very high in patients in whom elevated BUN levels were noted after 3-5 days of treatment (P < 0.0001, OR: 75; sensitivity, 88%; specificity, 91%). In addition, the risk for death was high in patients with thrombocytopenia (platelet count <  $148 \times 10^3 / \mu L$ ) during treatment (P = 0.002, OR: 10; sensitivity, 91%; specificity, 50%).

It is important to monitor BUN levels and platelet count periodically as prognostic factors of AP.

#### Disclosures

No disclosures to report.

# ESCG-P-3

# Expression of leucine rich alpha-2-glycoprotein (LRG) in the duodenal mucosa of dogs with chronic enteropathy

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Canine chronic enteropathy (CE) is an idiopathic gastrointestinal disorder characterized by chronic gastrointestinal signs. Leucine rich alpha-2-glycoprotein (LRG) has recently been identified as a novel biomarker of human inflammatory bowel disease, Crohn's disease, and ulcerative colitis (UC). Serum LRG concentrations correlate well with the clinical and endoscopic disease activity in UC. Unlike other acute-phase proteins, such as C-reactive protein, LRG is reportedly expressed not only by hepatocytes, but also by intestinal epithelial cells. The expression of LRG protein is elevated in the intestinal mucosae from patients with UC. Therefore, the aim of this study was to examine the expression of LRG mRNA and protein in the duodenal mucosae of dogs with CE.

Twenty-four dogs diagnosed with CE were enrolled, and left-over biopsy samples from the duodenum were evaluated for LRG expression, with both quantitative polymerase chain reaction (gPCR) and immunohistochemistry. As a control, cryopreserved biopsy samples from the duodenum of ten healthy control dogs with no clinical signs of gastrointestinal disease were used. The Wilcoxon rank sum test was used to compare the LRG mRNA expression in the two groups. The relationship between LRG mRNA expression and the canine chronic enteropathy clinical activity index (CCECAI) or a histopathological score graded according to the guidelines of the WSAVA International Gastrointestinal Standardization Group was evaluated with Spearman's rank correlation coefficient. Statistical significance was defined as P < 0.05.

The expression of LRG mRNA was significantly higher in CE dogs than in the healthy controls. The expression of LRG mRNA was significantly higher in the high CCECAI (≥ 12) group than in the low CCECAI (< 12) group. In contrast, there was no correlation between LRG mRNA expression and the WSAVA score. On immunohistochemistry, LRG protein expression was higher in the intestinal epithelial cells of the duodenal mucosae of CE dogs than in those of the healthy controls. In conclusion, this study demonstrates that LRG mRNA and protein expression is increased in the duodenal mucosae of dogs with CE. LRG mRNA expression is also associated with a high clinical score. Future studies will evaluate the clinical utility of measuring serum LRG concentrations in dogs with CE.

#### Disclosures

No disclosures to report.

# ESCG-P-4

#### Differential diagnosis of ileocolic masses associated with intestinal obstruction in cats: A retrospective study of 8 cases (2015-2017)

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Few studies report ileocolic masses in cats and their etiology. In this context, the main causes described include lymphoma, feline infectious peritonitis, feline gastrointestinal eosinophilic sclerosing fibroplasia, feline intestinal mast cell tumor (2 cases), hemangiosarcoma (1 case), actinomycetoma (1 case) and intussusception. Moreover, they are often associated with a poor prognosis.

Inclusion criteria of this retrospective study were: (1) presence of an ileocolic mass (ICM) diagnosed by abdominal ultrasonography, (2) a surgical excision or biopsy of the ICM by laparotomy after standard preoperative investigations and (3) a histological evaluation of the ICM performed. Eight cats (2 males, 6 females) were recruited in the study, mostly domestic shorthair (6 versus 1 Maine Coon, and 1 Chartreux). Six were neutered (1 male, 5 females). The median age was 2.5 years old [range 1 - 12] and the median weight was 3.1 kilograms [range 2.3 - 6.8]. The main clinical signs at presentation were chronic



enhancement may be predisposed to portal vein thromboses and that CTA more accurately identified these findings.

\*Adrian AM, et al. J Vet Intern Med 2015 Jan; 29(1); 97-103.

RIdexx laboratories

#### Disclosures

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#### ESCG-P-6

# Canine jejunum and enteroids derived from healthy dogs are useful models to evaluate the gastrointestinal side effects of NSAIDs

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Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is a prostanoid playing important homeostatic functions, yet is also responsible for regulating pain and inflammation, and has been implicated in the development and progression of various cancers. The deleterious actions of PGE<sub>2</sub> are inhibited in varying degrees by Non-steroidal anti-inflammatory drugs (NSAIDs); however, administration of these drugs also leads to significant gastrointestinal (GI) side effects. Grapiprant is a new anti-inflammatory drug from the piprant class that functions as a selective EP4 prostaglandin-receptor (EP4R) inhibitor and is proposed to be associated with less GI side effects. The aim of this study was to determine the value of canine jejunal tissue as well as a novel in vitro 3-dimensional model of canine intestinal epithelium (enteroids) for future in vitro and ex-vivo investigation of possible GI side effects of drugs in the priprant class. Briefly, ten-centimeter tissue pieces were acquired from the jejunum of 7 healthy dogs which had been euthanized for an unrelated project. Full thickness tissues were fixed in 10% formalin saline, routinely processed and embedded in paraffin. For enteroid culture, minced samples were washed and crypts were enriched, using EDTA chelation, embedded in matrigel, and grown in intestinal stem cell media until full epithelial differentiation (day 7 of culture). RNA in situ hybridization (RNAscopeÒ, ACDBio) was used to evaluate full thickness samples of canine healthy jejunum as well as enteroids derived from the same tissues. To quantify RNAscopeÒ signals in tissue sections, an advanced digital pathology image analysis system (HALO, Indica Labs) was utilized. Data was expressed as copy number for EP4R with that signal compared to the housekeeping target (B-actin, positive control gene). In 7/7 jejunal samples evaluated, universal positive expression of EP4R was identified in the epithelium as well as in lamina propria immune cells (copy number 0.1 to 2.6 on average per cell). Expression of EP4 in enteroids showed similar expression in the epithelium when compared to epithelial expression in full thickness samples (Wilcoxon signed rank test, p=0.6).

These results report the mRNA expression of canine EP4R in jejunum and enteroids of healthy dogs and confirms the utility of canine enteroids to investigate effects of NSAIDs on the GI epithelium.

#### Disclosures

No disclosures to report.

digestive disorders such as vomiting (63%), diarrhea (88%), dysorexia (25%), and weigh loss (38%). Ultranosographic features lacked specificity and etiology could not be determined in all cats: considered previous differentials were lymphoma or feline infectious peritonitis in 7 cases. Both ileum wall and ileocolic lymph nodes were enlarged. The ileocolic lymph nodes median size was 5 mm [range 4.7-14,5 mm]. Six of 8 cats underwent a complete surgical removal (enterectomy via ileocolic junction resection) and two cats were submitted to biopsies by laparotomy. The histopathological analysis of the ICM revealed feline gastrointestinal eosinophilic fibroplasia (1), inflammatory lesions and fibrosis secondary to a presumed prior foreign body (4), inflammatory lesion associated with campylobacteriosis and tritrichomonosis (1), fibrosis (1), postoperative granuloma (1). Two cats died in the post-operative period (septic peritonitis and disseminated intravascular coagulation). Six months after the surgery, all the 6 others cats were still alive and didn't show any digestive disorders.

To our knowledge, this is the first study reporting eight cases of feline ICMs. The main purpose of this case series is to highlight that ICMs in cats are neither always associated with a neoplastic etiology nor with a desperate prognosis. An unjustified euthanasia should not be considered before any etiologic characterisation.

#### Disclosures

No disclosures to report.

# ESCG-P-5

#### Comparison of computed tomographic angiography and ultrasonography in the diagnosis of acute canine pancreatitis

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Computed tomographic angiography (CTA) is considered highly accurate in the diagnosis of human pancreatitis due to its ability to completely evaluate the pancreas, its contrast enhancement patterns, regional vasculature, along with rapid acquisition times. These properties can be valuable in dogs due to the morbidity of acute pancreatitis and under-diagnosis with current methods. The aim of this study was to compare CTA to ultrasonography as a diagnostic tool in canine acute pancreatitis. Twenty-five dogs met inclusion criteria: 15 prospectively enrolled and 10 included from a pilot project\* in this cross-sectional study. Inclusion was based on fulfilling two of three criteria: strong clinical suspicion of pancreatitis, ultrasonographic evidence of pancreatitis, and/or SNAP<sup>R</sup> positive canine pancreatitis lipase (cPLI) test. A Spec<sup>R</sup> cPLI was obtained and an abdominal ultrasound was performed followed within 24 hours by a sedated abdominal three phase CTA. The results showed that CTA visualized the entire pancreas in 100% of dogs; ultrasonography visualized the entire pancreas in 73% of dogs. 10/25 dogs had heterogeneous contrast enhancement of the pancreas. CTA significantly identified more portal vein thromboses than ultrasound; CT= 10/25 and ultrasound= 1/25 (p value < 0.01). Dogs with heterogeneous contrast enhancement were significantly more likely to have portal vein thrombosis (p value <0.01). This study suggests that dogs with pancreatitis and heterogeneous pancreatic contrast acute



# ESCG-P-7

#### Characterization of the fecal microbiome of cats with chronic enteropathy

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Chronic enteropathy (CE) is very common in cats. The intestinal microbiome is recognized as an important contributor to intestinal inflammation in people and dogs with CE. The aim of this study was to perform an untargeted analysis of the fecal microbiome of cats with CE.

Fecal samples were obtained from 30 cats with histologically confirmed CE and 42 healthy control cats. Amplification and sequencing of the V4 variable region of the 16S rRNA gene were performed utilizing the Illumina Sequencing (Illumina MiSeq) platform. The software Quantitative Insights Into Microbial Ecology (QIIME v2.0) was used for data processing and analysis. Differences in the species richness between groups were calculated using a Mann-Whitney U test. Differences in microbial communities between groups were evaluated with the phylogeny based unweighted UniFrac distance metric and statistics were performed with the Analysis of Similarities (ANOSIM). Linear discriminant analysis effect size (LEfSe) was utilized to identify bacterial populations associated with CE.

Results showed a significantly lower species richness in cats with CE compared to healthy control cats (p = 0.004). Chronic enteropathy was associated with bacteria of the families Streptococcaceae and Enterobacteriaceae. Analysis of beta-diversity showed significantly different clustering between cats with CE compared to healthy control cats (p= 0.035).

Similar to results in people and dogs, our results show a decreased bacterial diversity in cats with CE. Further studies to evaluate differences between different forms of CE and to develop a dysbiosis index to characterize global changes of the microbiome are warranted.

#### Disclosures

Disclosures to report.

S Marsilio, R Pilla, JA Lidbury, JM Steiner, and JS Suchodolski are affiliated with the Texas A&M Gastrointestinal Laboratory that offers tests for evaluation of the fecal microbiome and other tests for gastrointestinal function on a fee-for-service- basis.

#### ESCG-P-8

Serine proteases activity in intestinal tissue of dogs with inflammatory bowel disease (IBD) - a new insight into the pathogenesis of IBD?

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Canine inflammatory bowel disease (IBD) is a frequent and sometimes challenging condition. Although implications of the immune system

and intestinal microbiota are documented, intestinal proteolytic activity has never been measured in canine IBD. The objective of this study was to compare intestinal activities of elastase and trypsin in IBD and healthy dogs.

Eight IBD and 7 healthy dogs were recruited. All dogs had complete chemistry panel and blood count, fecal flotation, trypsin-like immunoreactivity, folates and B12, and duodenal and ileal biopsies with endoscopic biopsy forceps. Disease activity was quantified using CCECAI score. Tissue elastase (Du-Elas and II-Elas) and trypsin (Du-Tryp and II-Tryp) activities were quantified on intestinal tissue samples using quenched fluorescent substrate-based assays and reported in mU/mg of proteins. Wilcoxon and Spearman's rank tests were used for statistical analysis. Results are presented as median [range].

IBD dogs had a median CCECAI of 9, [4-16] and compared to healthy dogs, significantly higher Du-Elas (0.68mU/mg [0.12-1.61] vs 0.12mU/mg [0.09-0.43], p=0.040), Du-Tryp (5.91mU/mg [0.56-18.38] vs 2.21mU/mg [0.60-4.15], p=0.013), II-Elas (0.60 mU/mg [0.13-7.19] vs 0.06mU/mg [0.03-0.13], p<0.001) and II-Tryp (7.69mU/mg [1.29-11.69] vs 0.50mU/mg [0.12-0.80], p<0.001). Positive correlations between CCECAI and DuTryp (r=0.77, p=0.002) and IITryp (r=0.71, p=0.007) and negative correlations between folates and IIElas (r=-0.61, p=0.03), and also albumin and DuTryp (r=-0.76, p=0.003) were observed.

Intestinal proteolytic activities in canine IBD are upregulated, and correlate with clinical severity. Proteases are likely involved in the pathogenesis of canine IBD and may represent a possible therapeutic target in cases that are non-responsive to standard therapy.

No disclosures to report.

#### ESCG-P-9

## Hypercobalaminemia in 144 samples from cats and dogs

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Hypercobalaminemia is underestimated, classically considered as not clinically significant. However, hypercobalaminemia has been found in people with serious underlying pathologies (solid neoplasm, myeloproliferative blood disorders, liver disease and kidney failure). Recently, it has been linked to hepatic and neoplastic disease in cats, and hepatic disease in dogs. The aim of the present study was to evaluate the prevalence and significance of hypercobalaminemia in small animals.

Results of serum cobalamin measurements were retrospectively reviewed from samples from animals evaluated for different diseases from two Veterinary Hospitals. Samples were measured in an outside laboratory (Idexx Barcelona) with using a chemiluminescent assay (reference range 275-590 ng/L-dogs and 270-1000 ng/L- cats).

The study included 347 samples from 221 dogs and 59 cats (41 patients with ≥ 2 measures). There were 108 females and 171 males. Median age was 7 years (0.25-17 years). Four feline breeds and 48 canine breeds were represented.

The prevalence of hypercobalaminemia was 41,49% (144 out of 347 samples): 36,26% in dogs (99 out of 273) and 60,81% in cats



(45 out of 74). Among them, five patients have received previous cobalamin injection. In eighty-six patients underlying disease were confirmed and included IBD (n=35), pancreatitis (n=13), liver disease (n=10), antibiotic responsive enteropathy (n=9), food responsive enteropathy (n=6), hyperthyroidism (n=4), protein losing enteropathy (n=2), gluten intolerance (n=1), cardiac disease (n=2), inmunemediated thrombocytopenia (n=2), leishmaniasis (n=2), gastroduodenal ulcers (n=2), exocrine pancreatic insufficiency (n=1), ulcerative colitis (n=1) and foreign body (n=1). In the 34 patients with only presumptive diagnosis, the most likely underlying disease was chronic enteropathy (n=29).

The underlying diseases found in animals with hypercobalaminemia from the present study included some previously described etiologies. Interestingly, the most common encountered diseases were IBD, ARD and pancreatitis. A potential explanation for this finding could be the presence of reactive hepatic disease. In fact, those animals were examined for suspected cobalamin deficiency. Although there is no consensus in human medicine on the interpretation of this findings, it should be emphasized that methylmalonic acid values (not measured in the present study) better reflects cobalamin status and functional cobalamin deficiency can still occur. Another limitation was its retrospective design with previous cobalamin administration in referred patients.

This study highlights the importance of further evaluate patients with hypercobalaminemia, a warning sign requiring exclusion of other underlying pathologies.

# Disclosures

No disclosures to report.

#### ESVCN-P-1

# Studies on a new body condition scoring by morphometric methodin dogs (2nd report)

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The body condition score (BCS) is the most commonly used system for the assessment of nutritional status of dogs. It estimates body fat stores subjectively and semi-quantitatively by visual inspection and palpation. Its accuracy and reliability, however, are inherently limited. For a better assessment of overall body condition, we have previously developed a BCS palpation model, but it did not help the visual inspection part of the process. A morphometric method to estimate the percent body fat has been developed. However, this method cannot be applied to short-legged breeds. The purpose of the present study was, therefore, to develop a clinically feasible, new morphometric method for the assessment of body condition of the dog.

Forty-two dogs with varying BCS were used. The percent body fat was determined by the deuterium oxide dilution method. The following three lengths were measured using a ruler: A from the episternum to the ischial tuberosity; B from the cranial angle of the scapula to the base of the tail; C from the cranial angle of the scapula to the sacral tuber of the ilium. Body fat percentage of 20% was adopted as ideal body fat percentage. A 5-point BCS scale was defined by the ideal body weight (IBW ) and current body weight (BW) as follows: BCS of 5. BW > IBW×1.21: BCS of 4. BW= IBW ×1.11 to 1.20: BCS of 3. BW = IBW  $\times$ 0.91 to 1.10; BCS of 2, BW = IBW  $\times$ 0.90 to 0.81; and BCS of 1, BW < IBW ×0.80. The correlation between body length and IBW was examined by Pearson's correlation coefficient test.

A high correlation was found between IBW and each of the three measuring positions. The correlation coefficients between IBW and body length measured at A, B and C were 0.945, 0.932 and 0.910, respectively. We used position A as the body length, since the highest correlation coefficient was found between the position A and IBW. The regression express wasy=0.009x<sup>2</sup>-0.359x+5.162. Using the regression equation for this measurement position, we can estimate the IBW of a dog from its body length. For example, the IBW of a dog with a body length of 40cm would be 5.2kg. If this dog's current BW is 6.0kg, it is 1.15 times the IBW. This gives us a BCS of 4. When this method was tried by several clinical veterinarians, they replied that this method was identical or well matched to result of the conventional BCS assessment.

#### Disclosures

No disclosures to report.

#### ESVC-P-1

#### Left shift of the ventricular mean electrical axis in healthy Doberman **Pinschers**

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In electrocardiography, the ventricular mean electrical axis (MEA) is defined as the average direction of the myocardial activation vectors during ventricular depolarization. In dogs, the reference interval of the MEA on the frontal plane is between +40° and +100°. MEA values can be influenced by the shape of the thorax, since narrow-chested dogs have a more vertical cardiac axis compare to broad chested dogs. Breed differences in ECG values has been described in previous studies.

The aim of this study was to evaluate the MEA in healthy Doberman Pinschers to describe a normal reference range in this breed, hypothesizing that some of healthy Dobermans have a left shift of the MEA based on standard values.

This was a retrospective, multicenter, observational study. Dogs were included if considered healthy based on history, physical examination, chest x-ray, echocardiography and standard 6-lead surface ECG. Exclusion criteria consisted of any cardiac or systemic diseases. MEA was calculated using the isoelectric lead method. A MEA less than +40° was defined as left shift, and a MEA greater than 100° as right shift. Morphology of the QRS complex on lead II was also analysed.

A total of 41 healthy Doberman Pinschers were included, with 26 females and 15 males. The mean age was 5.2  $\pm$  2.7 years. The median MEA was +45°, and ranged from -45° to +90°. MEA was deviated in 16/41 dogs (39%). All dogs with a MEA deviation presented a left axis deviation, with a median value of 0° and a range from -45° to + 30°. No influence of sex and body weight on MEA was detected. Dogs older than 7 years had a significant left axis deviation in

comparison to younger dogs (P=0.037). Regarding the QRS complex morphology, 15/41 dogs (37%) had a "QR" morphology, 13/41 (32%) had a "qRs" morphology, 9/41 (22%) had a "QRs" morphology, 2/41 (5%) had a "QRS" morphology, 1/41 (2%) had a "qR" morphology and 1/41 (2%) "qrs" morphology. Among dogs showing left axis deviation, 9/16 dogs (56%) had "QRs" or "QR" morphology.

Reference range of the MEA in Doberman Pinschers differs from the general canine population, with a significant number of healthy dogs presenting a left shift of the MEA based on standard values, especially in older dogs. Many dogs with MEA deviation showed a deep Q wave in lead II. This data should be considered when electrocardiographic evaluation is performed in this breed.

#### Disclosures

No disclosures to report.

# ESVC-P-2

Effects of a structured exercise program in sedentary dogs with asymptomatic myxomatous mitral valve disease: quality of life, and radiographic and echocardiographic parameters

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According to American Heart Association guidelines, exercise can reduce all-cause mortality and hospital readmission, improving symptoms and health-related quality of life. The current evidence-based information on the effects of exercise on the management of myxomatous mitral valve disease in dogs, is limited. This study aimed to evaluate the effects of a structured exercise program in sedentary dogs with asymptomatic myxomatous mitral valve disease.

Fifteen indoor dogs (six in the control and nine in the exercise groups) with asymptomatic myxomatous mitral valve disease (ACVIM stage B1) were included. A thorough physical examination, routine blood work-up, non-invasive arterial systolic blood pressure measurement, lateral and ventrodorsal thoracic radiography, electrocardiography, and echocardiography were performed to exclude other cardiac and systemic diseases. The owners in the exercise group were instructed by a certified canine rehabilitation therapist on guiding their dogs through the exercise program. The exercise protocol combined aerobic and resistant exercises at the level of low to moderate intensity interval training. The owners in the control group were instructed to maintain their dogs' usual routine. For all dogs, a five-point quality of life scale (appetite, demeanor, exercise tolerance, fainting, respiratory effort, cough, and nocturnal dyspnea/coughing), six-minute walk test, chest radiographic, electrocardiographic, and echocardiographic examinations were then assessed every two months for six months. Multiple multivariate models of repeated measures were used for multiple comparisons before and after exercise in the study subjects and between the groups.

The vertebral heart sum and echocardiographic variables (fractional shortening, and left atrial-to-aortic ratio) showed no significant difference between the control and the exercise groups, and before and after the exercise intervention (day 0 and 180: control: VHS  $9.9\pm0.7$ and  $9.9\pm0.8$ , FS  $42\pm7\%$  and  $44\pm11\%$ , LA/Ao  $1.2\pm0.1$  and  $1.3\pm0.1$ ; exercise: VHS 9.9 $\pm$ 0.6 and 9.8 $\pm$ 0.4, FS 41 $\pm$ 10% and 46 $\pm$ 10%, LA/Ao  $1.3\pm0.2$  and  $1.3\pm0.2$ ). After six months of exercise, the quality of life score was significantly decreased in the exercise group (before:  $11.6\pm2.7$ , after:  $9.1\pm1.3$ ; P=0.26), but not in the control group (day 0:  $10.5\pm2.8$ , day 180:  $10.2\pm2.0$ ); and the six-minute walk distance was significantly increased in the exercise group (before:  $300.2\pm101.0$  m, after:  $362.4\pm112.4$  m; P = 0.008), but not in the control group (day 0:  $346.7 \pm 117.7$  m, day 180:  $378.3 \pm 135.0$  m). To conclude, a six-month structured exercise program was well-tolerated in sedentary dogs with asymptomatic myxomatous mitral valve disease. This program may improve the quality of life without impairment of the selected radiographic and echocardiographic parameters.

#### Disclosures

No disclosures to report.

# ESVC-P-3

#### Indeterminate mean electrical axis of the QRS complex in the dog

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The mean electrical axis (MEA) of the QRS complex is the average of all the instantaneous mean electrical vectors occurring sequentially during ventricular depolarization, and it ranges from +40° to +100° in healthy dogs. In humans, indeterminate ORS axis (iORS axis) is defined as the impossibility to calculate the MEA of the QRS due to biphasic complexes with equal or nearly equal positive and negative components in all the six limb leads. The iQRS axis is a rare condition in humans and it occurs when the MEA is perpendicular to the frontal plane. In humans, iQRS axis has been found in healthy subjects and in patients with ventricular hypertrophy. The aim of this study was to describe the prevalence of the iQRS axis in dogs, looking for associations between iQRS and age, body weight, thoracic morphotype as well as presence of cardiac diseases.

Standard 6-leads ECGs were retrospectively analysed from the clinical database of the Istituto Veterinario of Novara. MEA of the QRS complex was calculated with the isoelectric lead method. A total of 1333 dogs were included. IQRS axis was identified in 8/1333 dogs, with an overall prevalence of 0.6%. Among the 8 dogs presenting iQRS axis, 6 did not have any cardio-structural abnormality, and the remaining 2 dogs had severe right ventricular enlargement secondary to pulmonic stenosis (n=1) and tricuspid valve dysplasia (n=1). Among healthy dogs presenting iQRS axis, 6/6 (100%) had a deep-chested thoracic morphotype. No significant differences were found between dogs with and without iQRS axis regarding age (4.3 years, range 1-12 years versus 9.1 years, range 2-13 years; P=0.171) and body weight (24 kg, range 13-29 kg versus 21 kg, range 23-34 kg; P=0.646).

In conclusion, iQRS axis is a rare condition in dogs and it seems to be more frequent in deep-chested dogs. In this thoracic morphotype, the more vertical cardiac axis, perpendicular to the frontal plane, might be the reason of iQRS axis. In addition, according to our results, right ventricular enlargement might be a cause of iQRS axis. Further studies are needed to better identify the association between iQRS axis and cardiac diseases in dogs.

#### Disclosures

No disclosures to report.

#### ESVC-P-4

Clinical narrative analysis of clinical findings associated with measurement of N-terminal pro-B-type natriuretic peptide in dogs

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N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a biomarker of cardiac stretch. The best characterised indication for NT-proBNP measurement in dogs and cats is differentiation of cardiac and noncardiac causes of respiratory distress. Other indications include prognostication, screening for cardiomyopathies and cardiac disease monitoring. The aim of this study was to investigate clinical signs associated with measurement of NT-proBNP, as recorded by veterinarians in the free text clinical narrative (CN) of the Electronic Health Record (EHR) in dogs and cats presenting to the veterinary practices from the Small Animal Veterinary Surveillance Network (SAVSNET) in Great Britain.

The CNs of 3510 individual appointments (including 346 dogs and 272 cats) were evaluated from 99 veterinary practices across England and Wales between April 2014 and May 2017. Reasons for NTproBNP measurement were categorised as follows; disease Investigation, disease Monitoring, Discount (laboratory promotion) and Unknown (reason for measurement not stated). Key phrases were extracted using a simple text filtering based on keywords from the CN of each patient to determine the clinical signs associated with measurement of NT-proBNP.

NT-proBNP measurements in dogs were categorised as Investigation (60%, n=219), Monitoring (10%, n=36), Discount (9%, n=32) and Unknown (21%, n=75). In Investigation, a heart murmur (136/219, 62%) was the most commonly reported clinical sign. Signs commonly accompanying a murmur in Investigation were coughing (n=46/136,34%), exercise intolerance (n=13/136, 10%) and panting (n=11/136, 8%). Coughing was reported in 11% (n=25) of dogs with no murmur or arrhythmia in Investigation. The following were infrequently reported; tachypnoea (n= 5/219, 2%), dyspnoea (n=2/219, 1%), lethargy, "discomfort and unspecified malaise" and "gagging and retching" (each n=1/219, 0.5%). Pre-anaesthetic testing was the sole stated reason for NT-proBNP measurement in 1 dog.

NT-proBNP measurements in cats were categorised as Investigation (74%, n=210), Monitoring (8%, n=24) and Unknown (18%, n=50). In Investigation a heart murmur was the most commonly reported clinical sign (150/210, 71%). The following were infrequently reported; dyspnoea (n=8/210, 4%), weight loss, tachypnoea and lethargy (each n=1/210, 0.5%).

CN analysis of EHRs allowed summarisation of motivators for NTproBNP measurement in first opinion practice. Investigation of a heart murmur appears to be the most common reason for NTproBNP measurement in dogs and cats. NT-proBNP measurements were not commonly used for disease monitoring in either dogs or cats. Despite being the best characterised indication, NT-proBNP was rarely measured in dogs or cats presenting with tachypnoea or dyspnoea.

#### Disclosures

Disclosures to report.

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#### ESVC-P-5

Adverse Effects of amlodipine on the treatment of heart failure in dogs with myxomatous mitral valve disease: preliminary results

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Myxomatous Mitral Valve Disease (MMVD) is the most common heart disease in dogs of small breeds. Amlodipine is a calcium channel blocker that can be beneficial on the treatment of heart failure (HF), due to arterial vasodilator effect, but the adverse effects in patients with MMVD are unclear. The aim of this study is evaluate the adverse effects of amlodipine on the treatment of dogs with MMVD stage C. This is a clinical trial randomized double-blinded placebo-control study, that all dogs receive conventional therapy to MMVD stage C (furosemide, spironolactone, enalapril and pimobendan), and randomized in two groups: amlodipine or placebo. The amlodipine mean dose is 0.3 mg/kg once a day. The dogs are followed during 10 months, with clinical assessment, echocardiogram, measurement of systemic arterial blood pressure (SABP) and serum biochemistry. The main side effect of amlodipine is the hypotension that may lead to increase of serum creatinine and BUN, therefore the adverse effects of amlodipine have been assessed by measurement of systolic SABP (by vascular Doppler method) and serum creatinine and BUN. Systolic SABP was measured at the first day of treatment with amlodipine or placebo (T0), seven days (T7D), one month (T1M) and three months (T3M) after the beginning of the treatment. Serum creatinine and BUN were measured at times TO and T7D. Up to the present time it have already been enrolled 14 dogs in this study. There was no difference in systolic SABP between amlodipine and placebo groups at the four times assessed (p>0.05). There was no difference in systolic SABP in the placebo group among the four times too (p>0.05). The amlodipine group presented a significant increase of systolic SABP between T0 and T7D (p=0.003) and significant decrease between T1M and T3M (p=0.003). No dog (of the both groups) presented hypotension (systolic SABP < 90 mmHg) in the four times of evaluation. Creatinine and BUN serum values didn't present difference in the amlodipine group between T0 and T7D (p>0.05) and the same result was observed in the placebo group (p>0.05). There was also no difference to creatinine and BUN values between the groups for T0 and T7D (p>0.05). This preliminary results showed that amlodipine at the used dose don't induce hypotension or decrease of systolic SABP when compared to placebo group, and don't increase serum creatinine and BUN values during the seven first days of treatment.



#### Disclosures

No disclosures to report.

#### ESVC-P-6

# Assessment of pulmonary hypertension in dogs with angiostrongylosis before and after treatment

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Pulmonary hypertension (PH) is a common comorbidity in dogs infected with Angiostrongylus vasorum. The aim of the present descriptive study was to assess echocardiographic changes indicative of PH and outcome in dogs treated for angiostrongylosis. Dogs with a positive Angio-detect (IDEXX) or Bearmann analysis and echocardiography performed at diagnosis (T0) were retrospectively included (n=23, median age 1.9 years, range 0.3-15.8). After treatment, follow-up echocardiography was performed in 16 dogs either within short-term (n=12) (< 5months after diagnosis; median 1month) (T1) and/or long-term (n=7) (> 5months; median 15.9months) (T2). Tricuspid regurgitation pressure gradient (TRPG), main pulmonary artery to aorta ratio (MPA/Ao), right pulmonary vein to pulmonary artery ratio (PV/PA), right pulmonary distensibility index (RPAD), and acceleration to ejection time ratio of the pulmonary flow (AT:ET) were retrospectively measured and compared over time using a mixed linear model. Dogs were treated with fenbendazole for 1 to 21 days (median 7 days) followed by a moxidectin spot-on (n=19). Additionally, 14 dogs received cardiac treatment for PH including sildenafil (n=14), pimobendan (n=5), and furosemide (n=2). A significant improvement of PVPA in M-mode (0.56 at T0 vs. 1.02 at T1 vs. 0.90 at T2, P=0.0002) and in 2D-mode (0.55vs.0.82vs.083, P=0.0031), MPA/Ao (0.95vs.0.80vs.0.83, P=0.03) and AT:ET (0.37vs.0.47vs.0.48, P=0.0005) was observed between T0 and T1 and T0 and T2. Differences were not significant between T1 and T2 for these parameters. There was no significant change for RPAD (21.4vs.29.1vs.23.8%, P=0.385) nor TRPG (76.6vs.49.2mmHg, P=0.0842) over time. TRPG was measured in 16/23 (69.6%) dogs at T0, 7/12 (58.3%) at T1 and 1/7 (14.2%) at T2. TRPG indicated absent, mild, moderate and severe PH in respectively 1 (4.3%), 3 (13%), 2 (8.7%), and 10 (43.5%) dogs at diagnosis. Of 23 dogs included, 17 (74%) were alive at the time of writing, while 6 (26%) died; with all deaths related to cardio-respiratory disease. Individual survival times from these dogs were 1, 3, 36, 93, 148 and 320 days (median 64.5 days) after diagnosis. PH was moderate and severe in 2 and 4 non-survivors respectively. Individual echocardiographic parameters in non-survivors did not improve at short-term recheck (n=3). Results of this study demonstrated a mortality rate of 26% among dogs with angiostrongylosis that underwent an echocardiography at diagnosis. Non-survival seems to be associated with a persistent moderate to severe PH. In general, an improvement of indirect parameters of PH was noted at short- and long-term rechecks, despite no significant difference for TRPG at short-term.

# Disclosures

No disclosures to report.

#### ESVC-P-7

# Serum uric acid concentrations in dogs with chronic heart failure and

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Atrial fibrillation (AF) is the most common heart rhythm disturbance associated with poor prognosis and outcome encountered in clinical practice in dogs. While structural and electrical remodeling of the atria is a well known factor facilitating initiation and maintenance of AF, little is known about the role of oxidative stress in this process. Uric acid (UA) is the terminal enzymatic product of purine metabolism. In humans, the serum UA level is strongly correlated with cardiovascular risk factors, including the risk of atrial fibrillation. It is also considered a marker of increased oxidative stress via the xanthine oxidase pathway.

The purpose of this study was to investigate the concentrations of uric acid in the serum of dogs with advanced chronic heart failure, dogs with atrial fibrillation and healthy controls.

Fifty-three client-owned dogs with complete clinical, electro- and echocardiographic examinations were enrolled in the study. A fasting blood sample was collected for diagnostic purposes and sent to a commercial veterinary laboratory where CBC and general biochemistry, including the serum uric acid concentration, were evaluated. Dogs with normal kidney and liver function were then assigned to the chronic heart failure group (CHF group, 17 dogs), CHF with AF group (CHF+AF group, 20 dogs) and the control group (16 dogs). The nonparametric Kruskal-Wallis analysis followed Dunn's post-hoc multiple comparisons test were applied to test for statistically significant differences between the groups.

The serum UA concentration in the CHF+AF group (median: 24.85 μmol/L, range 6.76-54.9) was significantly higher than in the CHF group (median: 11  $\mu$ mol/L, range 7.96-24.3) and the control group (median 9.7 µmol/L, range 5.68-18.4; P<0.001). The ROC analysis for UA had an area under the curve of 0.92. A cut-off point of >16.6 μmol/L was associated with a sensitivity and specificity of 85% and 87.9%, respectively.

The results of this study suggest that AF in dogs is associated with increased oxidative stress via xanthine oxidase pathway. Moreover, the serum UA level could be a potential biomarker for AF in dogs.

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# ESVC-P-8

#### Oxidative stress in cats with asymptomatic and symptomatic hypertrophic cardiomyopathy

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Oxidative stress, which is an imbalance between the production of endogenous reactive oxygen species and antioxidant defence, is an



important contributory factor in the development and progression of numerous cardiovascular diseases. Little is known about the role of oxidative stress in hypertrophic cardiomyopathy (HCM), the most common heart disease in cats. Therefore, this study aimed to evaluate the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in serum and total antioxidant capacity (TAC) in plasma of cats with symptomatic and asymptomatic hypertrophic cardiomyopathy and in healthy controls.

Thirty client-owned cats with complete clinical, electro- and echocardiographic examinations, the Doppler systolic blood pressure below 160 mmHg and normal creatinine and thyroxine concentrations were enrolled in the study. Samples were collected for diagnostic purposes with research material appropriated from the excess of what was needed for diagnostics. The patients were assigned to the clinical HCM group (HCM, n=8), asymptomatic HCM group (A-HCM, n=11) and the control group (n=11). The activity of oxidative stress enzymes and plasma TAC were determined using commercially available assay kits. After testing for normality with Shapiro-Wilk test data were analyzed between the groups with one-way ANOVA followed by Tukey post test or the non-parametric Kruskal-Wallis analysis followed by the post hoc Dunn's multiple comparisons test to assess statistically significant differences.

There were no statistically significant differences among the groups with respect to age, weight and sex ratio. Each group consisted of various cat breeds. Serum SOD activity was significantly lower in the HCM group (mean 0.99±0.35 U/mL) and A-HCM group (mean  $1.39\pm0.4$  U/mL) compared to the healthy controls (mean  $2.07\pm0.76$ U/mL, p<0.01). Serum CAT activity was significantly lower in the A-HCM group (mean 19.4±4.2 nmol/min/mL) compared to the HCM group (mean 23.6±5.9 nmol/min/mL) and healthy controls (mean  $30\pm7.5$  nmol/min/mL, p<0.01). There were no significant differences in the serum activity of GPx (p=0.21) or plasma TAC (p=0.89) between the groups. Serum SOD activity correlated with the CAT activity (r=0.44, p<0.05).

Increased oxidative stress in the symptomatic and asymptomatic HCM cats was evident based on the statistically significant decrease in the serum SOD and CAT activity. The CAT activity also tended to be lower in symptomatic cats compared to healthy controls, although did not reach significance.

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# ESVC-P-9

# Is there an association between environmental factors and cardiovascular disease in dogs?

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Ambient air pollution has been found to increase cardiovascular risk in many human epidemiological studies. However, little is known about such risk in pet dogs. The aim of this study was to investigate the

association between environmental factors and cardiovascular disease in dogs living an indoor lifestyle. A retrospective case-control study was conducted in 103 client-owned dogs. Physical examination, blood pressure measurement, and other clinical information (thoracic radiography, echocardiography, and/or previous diagnosis) obtained from their medical records were used to determine the presence or absence of cardiovascular disease. The environmental information of the dogs' households (the presence/absence of indoor air pollution, ambient temperature, and household humidity) was obtained from the database of a previous project involving an indoor air pollution survey. Forty-seven dogs with cardiovascular disease (chronic valvular heart disease, pulmonary hypertention, aortic insufficiency, arrhythmia, or borderline/sustained systemic hypertension) and 56 control dogs were compared. Except for household humidity (64.8% vs 68.3%, P = 0.048), exposure to several well-known indoor air pollutants (second-hand smoke, P = 0.72; cooking fumes, P = 0.29; incense burning, P = 0.61), the average PM2.5 concentration in the pet house (P = 0.53), and ambient temperature (P = 0.054) were not statistically different between dogs with and without cardiovascular disease. Among signalment factors, dogs with cardiovascular disease were significantly older (P < 0.001) and were more likely to be overweight (BCS ≥7/9) (P = 0.013) than control dogs. Multivariable logistic regression analysis showed aging (adjust OR = 1.27, P = 0.004) and overweight (adjust OR = 4.34, P = 0.017) were associated with increased risk of cardiovascular disease in dogs. In conclusion, the association between environmental hazards and cardiovascular disease was not found in pet dogs.

#### Disclosures

Disclosures to report.

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# ESVC-P-10

Longitudinal right ventricle strain and strain rate by 2-dimensional speckle tracking echocardiography in dogs with pulmonary hypertension

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Longitudinal right ventricular (RV) function, as estimated by the deformation variables strain and strain rate, has been evaluated by speckle tracking echocardiography (STE) in healthy dogs and dogs with myxomatous mitral valve disease (MMVD), but not dogs with pulmonary hypertension (PH). Therefore, we evaluated the longitudinal RV strain and strain rate in dogs with PH.

We acquired 2D echocardiographic cineloops from the left apical 4-chamber view optimized for the RV, and analyzed longitudinal RV strain and strain rate in 82 dogs (32 healthy dogs, 24 MMVD dogs without PH and 26 dogs with PH) using Xstrain<sup>TM</sup> software. Dogs with PH were classified based on the tricuspid regurgitation (TR) jet velocity (> 3 m/sec) and degree of MMVD.

In healthy dogs, strain rate showed a moderate negative relationship with heart rate (r2=0.35) and a weak positive relationship with body weight (r2=0.13); strain showed no relationships with heart rate or



bodyweight. Strain and strain rate showed weak positive relationships with LA:Ao in MMVD dogs without PH (r2=0.2 and r2=0.18). Strain and strain rate showed strong positive relationships with TR velocity in dogs with PH (r2=0.7). Only TR velocity was retained in a multivariable model to predict strain and strain rate in dogs with PH; heart rate, bodyweight and LA:Ao were excluded. Both strain and strain rate showed good sensitivity (100%), but modest specificity (70%) for identifying severe PH.

Our data suggest that longitudinal RV deformation analysis using STE can identify dogs with severe PH. Body weight, heart rate and MMVD can influence the strain and strain rate values and should be carefully considered in dogs with PH.

#### Disclosures

No disclosures to report.

# ESVC-P-11

Epidemiological, clinical, echocardiographic features and prognosis of dogs with a thrilling murmur related to degenerative mitral valve disease: 374 cases (2006-2017)

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Degenerative mitral valve disease (DMVD) is the most common acquired heart disease in small-breed dogs, resulting in poor apposition of the mitral valve (MV) leaflets during systole. This leads to MV regurgitation, which is clinically detected as a left apical systolic heart murmur, the intensity of which is classically classified according to a 6-grade scheme. Past studies have shown that an increase in heart murmur intensity over time is associated with worsening of DMVD lesions, with thrilling murmurs (TM, i.e., of grade V/VI and VI/VI) associated with more severe disease. The objectives of this retrospective study were to characterize the epidemiological, clinical, and echocardiographic features of dogs with TM related to DMVD, and to identify parameters associated with survival. The case records of small-breed dogs (under 15 kg) diagnosed with TM related to DMVD were reviewed. The study population consisted of 374 dogs (median age at detection of TM=10.7 years [IQR=8.6-12.5], male-to-female ratio=1.8). The most represented breeds were Cavalier King Charles Spaniel, Yorkshire Terrier, Poodle and Chihuahua. Thirty-six dogs had a concurrent hemodynamically compromised systemic or cardiac disease and were therefore excluded from the subsequent analysis. According to the ACVIM classification, 24%(82/338) were in stage B2, 61% (207/338) in stage C and 7% (22/338) in stage D. Chordae tendineae rupture was identified in 75% (254/338) of the included dogs, severe pulmonary hypertension (systolic pulmonary arterial pressure >75 mmHg) in 27% (90/337), and tachyarrhythmias in 46% (157/338). Regurgitation fraction (RF), assessed using the PISA method, was >30% for all dogs (median value=70%[IQR=59-76]), which is consistent with moderate to severe MV regurgitation.

Among the 265/338 dogs for which a follow-up was available, 173/265 (65%) died. 68% (118/173) of deaths were cardiac in origin (CD) with a median time to CD of 25.1 months after diagnosis [IQR=8.3-52.7]. Univariate analyses revealed that RF>70%, left atrium to aorta ratio≥1.5 at end-diastole, left ventricular dilation, ACVIM

stages, presence of arrhythmias, and collapse were significantly associated with time to CD. Dogs from ACVIM stage B had a median time to decompensation of 15.9 months [IQR=8.2-45.0], and a median time to CD of 42.9 months [IQR=19.1-56.1] versus 17.8 [IQR=5.4-38.4] for dogs from stage C and D.

In conclusion, this study confirms that dogs with TM related to DMVD, including dogs from ACVIM stage B, usually have a severe form of the disease, with a majority of dogs presenting with chordae tendineae rupture, pulmonary hypertension, and severe MV regurgitation.

#### Disclosures

Disclosures to report.

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#### ESVC-P-12

A retrospective multi-centre study investigating evidence of thrombosis in dogs diagnosed with atrial fibrillation

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Atrial fibrillation (AF) is a common canine cardiac arrhythmia. Left atrial appendage thrombus development and associated risk of thromboembolism is a common complication of AF in humans. There are no largescale studies investigating the incidence of thromboembolic disease in dogs with AF. The aim of this retrospective study was to identify any evidence for thromboembolic events in dogs diagnosed with AF and whether this affected outcome.

One-hundred and twenty one dogs were included in this study following assessment by a cardiologist. Enrolment criteria required dogs to have a diagnosis of AF confirmed by electrocardiography. Direct or indirect evidence for thrombus formation or a thromboembolic event was identified for individual cases based on analysis of clinical notes and diagnostic investigations. Statistical tests included Chi-squared, unpaired student T-tests, Mann Whitney, Kruskal-Wallis and ANOVA testing, with significance set at P<0.05.

Intracardiac masses consistent with thrombi were identified echocardiographically in 5 dogs (4.1% of cases). Clinical signs potentially attributable to thromboembolic disease were identified in 15 dogs (12.4% of cases), however these cases had no intracardiac thrombus detected by echocardiography. There was no association between the presence of a thrombus and gender (P=0.73), type of AF (lone vs. AF secondary to structural heart disease) (P=0.76) or antiarrhythmic treatment (P=0.23).

Anti-platelet therapy was instituted in four cases (3.3%). Three of these cases had intra-cardiac thrombi confirmed by echocardiography and received clopidogrel. Two of these were euthanased due to progressive, refractory congestive heart failure; the third died naturally from an unknown cause. The fourth case received aspirin following a suspected thromboembolic event with neurological signs consistent with a central vascular accident (CVA); this dog was still alive at time of study.

Of the cases where circumstances at time of death were known (n=53), four dogs (7.5%) died from suspected thromboembolism. No significant association was identified between the median age at time of death (P=0.48) and median survival time after AF diagnosis (P=0.06) in the dogs suffering thromboembolic complications compared to those without, suggesting limited impact on outcome.

In conclusion, this study found thrombi or thromboembolic events in dogs diagnosed with AF were infrequent, although the low sensitivity of screening methods used may have underestimated the frequency of thrombi. Given the low-incidence of confirmed thromboembolic complications in dogs with AF, further work is required to understand the different factors influencing hypercoagulability and thrombus formation in dogs and humans.

#### Disclosures

No disclosures to report.

# ESVE-P-1

# The use of ACTH-depot for ACTH stimulation test in dogs with hyperadrenocorticism under trilostane therapy: changes to the conventional protocol

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Several studies have been performed in order to find the best functional test for clinical monitoring of dogs with hyperadrenocorticism (HAC) under trilostane therapy. Despite being controversial, ACTH stimulation test is one of the therapeutic tools currently used for this purpose. When using short-acting exogenous ACTH (tetracosactide), adrenal reserve stimulation is estimated to be maximal 1h after its administration. However, short-acting ACTH is not widely available in Europe and in several countries, only the depot formulation is purchasable. To author's knowledge, only one study has evaluated ACTH depot concluding that, for a better stimulation of adrenal reserve in dogs with HAC, blood for peak cortisol should be drawn 3h post stimulation with ACTH, instead of 1h (used in the conventional protocol). To date, little is known about the use of ACTH depot in dogs under trilostane therapy.

This study aims to assess whether blood cortisolemia is higher at 3h rather than at 1h after stimulation with ACTH depot in dogs with HAC under trilostane therapy.

A prospective study was performed including dogs with HAC under twice-daily trilostane therapy. The ACTH stimulation test was performed 2-4h after the morning pill and using the depot formulation administered intramuscularly. Blood was sampled at TO (basal cortisol), 1h (T1) and 3h (T3) after stimulation. Statistical analysis was performed using non-parametric tests (Friedman test and Wilcoxon for paired samples).

Eight dogs were enrolled. The mean ( $\pm$ SE) level of cortisol at T0, T1 and T3 was respectively : 1.90  $\mu$ g/dl ( $\pm$  0.44), 6.45  $\mu$ g/dl ( $\pm$  2.42) and 8.95  $\mu$ g/dl ( $\pm$  3.17). Differences on cortisolemia among these three time points were statistically significant (p=0.006). The cortisol level at T3 was significantly higher when comparing with T0 (p=0.03) and T1 (p=0.018).

This study confirms that cortisolemia is higher at 3h rather than 1h after stimulation with ACTH depot in dogs with HAC under trilostane therapy. These results are in agreement to what was previously published concerning the use of this compound for the diagnosis of HAC in suspected cases. This is probably due to the longer action of this formulation and, consequently, a latter stimulation of adrenal reserve, when compared to the short-acting ACTH.

In summary, when ACTH depot is used to assess adrenal function in dogs with HAC under trilostane therapy, the ACTH stimulation test protocol should be adapted and post-stimulation cortisol level should be measured 3h instead of 1h after stimulation.

#### **Disclosures**

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#### ESVE-P-2

# Predictive model to estimate ionized calcium from routine serum biochemical profiles in cats

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lonized calcium measurement is not readily available in practice and total calcium (tCa) may not be a reliable indicator of calcium status in cats.

The objectives of this cross-sectional study were to predict ionized calcium from cats with biochemical panels and T4 values and compare the diagnostic performances of predicted ionized calcium (piCa) and tCa.

Cats with both ionized calcium and biochemistry panels  $\pm$  T4 measurements performed within 24h were screened over 6 years. Cases with both a complete biochemical profile and T4 available were included in the training set (570 cats) and used to create a multivariate adaptive regression splines model to calculate piCa. Diagnostic performances of tCa and of piCa and its prediction interval (PI) were compared in 652 cats that had not been included in the training set. The final model included creatinine, BUN, albumin, tCa, potassium, chloride, gamma-glutamyltransferase, cholesterol, age and body condition score as independent variables. For hypercalcemia, piCa was significantly more specific (specificity: piCa=0.99, tCa=0.93, p<0.001) but less sensitive (sensitivity: piCa=0.30, tCa=0.59, p<0.001) than tCa. However, the upper limit of the PI was more sensitive than tCa (sensitivity=0.82, p<0.001). For hypocalcemia, piCa was also significantly more specific (specificity: piCa=0.82, tCa=0.74, p<0.001) but less sensitive (sensitivity: piCa=0.54, tCa=0.70, p<0.001) than tCa, and the lower limit of the PI was more sensitive than tCa (sensitivity=0.98, p<0.001).

In conclusion, piCa may help confirm suspected hypercalcemia in cats, owing to its high specificity. Its PI may be used to screen hypercalcemia and hypocalcemia, owing to its high sensitivity.

#### Disclosures

No disclosures to report.



#### ESVE-P-3

# Episodic nocturnal hypoglycaemia in healthy New Zealand working dogs

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Hypoglycaemia has not been reported in healthy dogs. The goal of this study was to determine if healthy working dogs experience hypoglycaemic episodes at night. Twenty-two apparently healthy New Zealand working Huntaway dogs (median age 4 years, IQR 2-6, range 1-10) were fitted with continuous blood glucose monitors (4 readings per hour) for 4 days. During that time, the dogs were worked according to their usual regimen and were fed complete balanced diets once daily between 6pm-8pm. Nocturnal hypoglycaemia was considered when serum glucose was < 3.5 mmol/L at any point between 12am-6am. Nine of 22 dogs (median age 3 years, IQR 2-5, range 1-8) experienced a median of 4 (IQR 2-22, range 1-79) hypoglycaemic (<3.5mmol/L) episodes (median total readings 96, IOR 72-96. range 72-96) of which 6 dogs experienced a median of 2 (IQR 1-14, range 1-78) severe hypoglycaemic (<2.5mmol/L) episodes (median total readings 96, IQR 78-96, range 72-96). In 5, 2, and 2 of the dogs, glucose was <3.5mmol/L for 1, 2, and 4 nights, respectively; whereas, in 5 and 1 of the dogs, glucose was <2.5mmol/L for 1 and 4 nights, respectively. One dog with a glucose of <3.5mmol/L and a second dog with a glucose of <2.5mmol/L had 9 and 1 glucose readings > 6.66 mmol/L, respectively. To our knowledge this is the first study that documents episodic nocturnal hypoglycaemia in apparently healthy dogs. Our findings suggest that episodic nocturnal hypoglycaemia may be a normal physiological phenomenon in dogs.

## Disclosures

No disclosures to report.

# ESVE-P-4

# Efficacy of orally administered anti-thyroid drugs for the treatment of hyperthyroid cats

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The most frequently applied treatments in hyperthyroid cats are orally administered anti-thyroid drugs. There are currently no studies evaluating and comparing the treatment success of different thioamide preparations.

In this retrospective study, treatment outcome of 107 hyperthyroid cats was evaluated. Cats were either treated with the oral anti-thyroid drug Methimazole (Felimazole®, n=61), Carbimazole retard (Vidalta®, n=25), or Carbimazole licensed for the use in humans (CHM, n=21). Data concerning signalement (age, gender, breed), concurrent disease, number of dose adjustments, time period until an euthyroid state was reached, occurrence of adverse effects, survival time, and cause of death were evaluated in all cats and compared between treatment groups.

No significant difference was found for all analysed parameters between the three groups. Adverse effects were frequently observed

and included gastrointestinal signs, cytopenias, facial excoriations, and increase in liver enzymes. In the Felimazole® group 31.2%, in the Vidalta® group 40.0%, and in the CHM group 28.6% of treated cats developed adverse effects. Median survival times were 540 days in the Felimazole®, 365 days in the Vidalta®, and 524 days in the CHM group. The only parameter influencing survival time was presence of chronic renal disease (CRD) prior to therapy. Those cats had a significantly shorter survival time (203.5 days) compared to cats in which CRD occurred during therapy (619 days) and cats without CRD (396 days).

In conclusion, all three antithyroid drugs were equally effective for the treatment of feline hyperthyroidism. Presence of CRD prior treatment was the most important prognostic parameter.

#### Disclosures

No disclosures to report.

## ESVE-P-5

# latrogenic hypoadrenocorticism following treatment with trilostane for hyperadrenocorticism in dogs: a description of 10 cases

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Trilostane is currently the medical treatment of choice for hyperadrenocorticism in dogs. latrogenic hypoadrenocorticism is considered infrequent, with most cases being transient. Only 8 cases of permanent hypoadrenocorticism have been described; adrenocortical necrosis was suspected based on post-mortem examination or adrenal changes on ultrasonography. The goal of this study was to report findings from 10 cases of iatrogenic hypoadrenocorticism following treatment with trilostane for hyperadrenocorticism.

Medical records of dogs treated with trilostane since 2008 were reviewed. Dogs were included if they had clinical signs suggestive of hypoadrenocorticism and compatible ACTH stimulation test results and/or serum electrolytes abnormalities. Cases were considered as permanent if long term treatment of hypoadrenocorticism was required.

Ten dogs met the inclusion criteria (5 males and 5 females) with an age ranging from 6.5 to 13 years. Nine dogs had a suspected pituitary-dependent hyperadrenocorticism. Clinical signs at the time of hypoadrenocorticism diagnosis were: lethargy (10/10), anorexia (10/10), vomiting (7/10), diarrhea (3/10), tremors (3/10) and polyuriapolydipsia (1/10). Time between beginning of trilostane treatment and hypoadrenocorticism occurrence ranged from 4 days to 13 months; trilostane dose ranged from 1 to 12 mg/kg/day. Five dogs had a suspicion of concurrent infectious disease at the time of hypoadrenocortism diagnosis. Sodium/potassium ratio was under 24 in 5 dogs and under 28 in 8 dogs. All 8 dogs having an ACTH stimulation test performed had pre- and post-stimulation cortisol concentration <55nmol/L. Trilostane dosage was decreased in 2 cases; trilostane was withdrawn in 1 case without further relapse of clinical signs of hyperadrenocorticism; 1 dog died at the time of diagnosis; glucocorticoids +/- mineralocorticoids supplementation was prescribed in 6 cases. Three out of these 6 dogs were lost to follow-up, the other

3 had a diagnosis of permanent hypoadrenocorticism. Adrenal gland ultrasonogaphy in these 3 dogs showed a progressive reduction in glands size with a heterogeneous echogenicity.

latrogenic hypoadrenocorticism was also rare in our population, with 10 cases identified over 10 years. Dose and duration of treatment were variable. One dog died and at least 3 suffer from permanent hypoadrenocorticism. Progressive adrenal changes on ultrasonography were seen in 3 cases of permanent hypoadrenocorticism. The suspicion of concomitant infection in half of our cases at hypoadrenocorticism occurrence is an interesting finding. An impaired ability to synthesize cortisol in response to infection might trigger clinical signs appearance in dogs with previously subclinical hypoadrenocorticism.

#### Disclosures

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#### FSVF-P-6

Evaluation of free T4 (fT4) measurement after equilibrium dialysis (FT4ED) and a chemiluminescent enzyme immunoassay (FT4CEIA) in hyperthyroid cats before and after treatment with radioiodine (131I)

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The combination of measuring total T4 (TT4) and free T4 (fT4) can be useful in cats with clinical suspicion of hyperthyroidism but with normal to only mildly increased TT4. Moreover, measurement of fT4 can play an essential role in monitoring hyperthyroid cats after treatment. Although measurement of fT4ED is considered the gold standard, it is time consuming, expensive and not widely available. Measurement of fT4CEIA is more time and cost effective but studies assessing its accuracy are scarce.

The objective of this study was to correlate measurement of fT4ED (Free T4 by Equilibrium Dialysis, Antech Diagnostics, Irvine, CA, USA) and fT4CEIA (IMMULITE® 2000 Veterinary Free T4, Siemens Healthcare Diagnostics Products Ltd., Lanberis, Gwynedd, UK) in cats before and one month after treatment with 131I.

FT4CEAI was consistently lower than fT4ED (median difference -5.4, P<0.001). Spearman correlation between fT4CEAI and fT4ED was 81.0% and 87.5% at TO and T1, respectively. FT4ED and fT4CEIA were within the reference interval (9-33pmol/L and 9-33.5pmol/L, respectively) in 27/45 and 16/45, below reference interval in 16/45 and 27/45 and above reference interval both in 2/45 at T1, respectively. No cats with fT4CEIA within the reference interval had decreased fT4ED (T1). Based on fT4CEIA, 10/45 (22%) were classified as having suboptimal thyroid function while having fT4ED within reference interval (T1).

Taking the consistently lower fT4CEIA value but good correlation between two techniques in consideration, adaptation of the current reference interval for fT4 measurement by CEIA could justify the routine use of this assay for determination of fT4.

# Disclosures

Disclosures to report.

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#### FSVF-P-7

#### Prevalence of hypokalemia in feline diabetes

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Feline diabetes mellitus is related with several clinic-pathological alterations, especially in cats with diabetic ketoacidosis (DKA). Electrolyte unbalances are frequent, mainly hypokalemia; however, the role of hypokalemia in non-DKA diabetic cats at diagnosis is unknown. The aims of this study were to calculate the prevalence of hypokalemia in non-DKA cats diagnosed with diabetes mellitus (DM) and to evaluate the relation between potassium plasma blood level (PBL) and clinical parameters at first visit. Adequate dosage of oral potassium supplementation in hypokalemic cats to return to normal reference range (NRR) was also evaluated.

Twenty-five cats diagnosed with DM without DKA at the Veterinary Teaching Hospital Complutense between 2013 and 2016 were included. Age, sex, reproductive status, weight, body condition score (BCS), PBL, systolic blood pressure (SBP), concurrent diseases, presence of neuropathy and previous corticosteroid administration were recorded. In hypokalemic cats, oral potassium supplementation and time needed to return PBL to NRR were also recorded. Data were analysed using SPSS program. Statistical significance was set at  $p \le 0.05$ .

Age ranged between 2.6 and 16.1 years (mean±SD; 7.8±3.7 years); 5 were females, 20 males and 24 were neutered. Body weight varied from 2.4 to 8.3 kg (4.9 $\pm$ 1.4 kg). According BCS animals were classified in cats  $\leq$  3/9 (n=4), cats between 4/9 and 6/9 (n=19) and cats  $\geq$  7/9 (n=2). PBL ranged from 2.9 to 5.1 mEq/L ( $3.6 \pm 0.5 mEq/L$ ). Fifteen cats were considered hypokalemic (PBL ≤ 3.5mEq/L). SBP ranged from 110 to 200mmHg (145.8±26.24mmHg). No association between PBL and different parameters evaluated was observed. SBP was significantly higher (p=0.038) in hypokalemic (151.1  $\pm$  26.5 mmHg) than in normokalemic patients (125.8  $\pm$  14.2 mmHg) as described in human medicine.

In eight hypokalemic patients follow-up was available. Six were treated with oral supplementation (potassiun gluconate or potassium glucoheptonate) and 2 did not received any supplementation treatment due to owner reject. Time to normalize PBL ranged from 5 to 270 days (mean 62.8±86.4days), which was negatively correlated (Rho= -0.829, p=0.042) with the initial oral potassium supplementation dose. The medium dose required to normalize BPL was 0.77mEq/kg/d (potassium gluconate) and 0.37mEq/kg/d (potassium glucoheptonate).

Hypokalemia occurs in 60% of non DKA diabetic cats and it is related to systemic hypertension. A significant reduction in SBP with increased dietary potassium has been demonstrated in humans. Oral potassium supplementation at high doses in cats with DM might be a useful and safe tool for the management of hypokalemia and systemic hypertension.

# **Disclosures**

No disclosures to report.



#### ESVE-P-8

# Evolution of trilostane dosage in dogs with pituitary dependent hyperadrenocorticism during the first year of

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Trilostane is the drug most frequently used in dogs diagnosed with pituitary-dependent-hyperadrenocorticism (PDH), but dosage recommendations are variable amongst the literature.

The objectives of this study were to assess the evolution of trilostane dosage (TD) in dogs with PDH over a one-year follow-up and its relationship with clinical variables.

Thirty-five dogs with naturally occurring PDH initially treated with low doses of trilostane (range 0.27-1.14mg/kg/12h) were included. TD was adjusted based on clinical signs and the results of an ACTH stimulation test (3-4 hours post-trilostane). Rechecks were performed at day 7 and 30 and thereafter every 3 months. Several parameters (age, sex, clinical and laboratory findings, concurrent diseases and clinical signs at diagnosis) were evaluated in relation with the evolution of TD. Dogs were divided into two groups according to the initial TD: Group-A (0.27-0.6mg/kg/12h; n=18) and Group-B (0.61-1.14mg/ kg/12h; n=17). Sex, age, clinical and laboratory findings and concurrent diseases were not significantly different between the 2 groups (p>0.05).

During follow-up, 40% (14/35) of the dogs needed an increase in TD, 20% (7/35) needed a decrease, and 40% (14/35) maintained the initial dosage. Among the 21 dogs in which TD was modified, 48% (10/21) needed a single adjustment and 52% (11/21) several. Eighty-percent (4/5) of diabetic dogs with PDH needed and increase in TD compared with 33.3% (10/30) of non-diabetic dogs (p=0,049). No significant differences were observed between the 2 groups regarding TD adjustments.

At one year of follow-up, adequate control of HAC was obtained in 34/35 dogs. Dosage at that time (mean  $\pm$  SD; 1.0 $\pm$ 0.87mg/kg/12h) was correlated with the initial TD (rho=0.502; p=0.002) Percentage of variation between initial and final dose through follow-up was obtained for each dog, ranging from -78.00% to 90.67% (44.44±90.67%) (n=35) and was not significantly different between groups (Group A:  $45.73\pm80.91\%$ ; Group B:  $43.09\pm102.52\%$ ; p=0.98). Basal cortisol (rho=0.766; p=0.002) and body condition score at diagnosis (rho=0.510; p=0.021) were positively correlated with TD at one year. Dogs with panting at diagnosis needed proportionally a higher increase in TD despite not being statistically significant (p=0.054)

Using low dosages of trilostane for the treatment of dogs with PDH, TD needed to be adjusted in 60% of PDH cases. Dogs with DM, higher basal cortisol at diagnosis and/or elevated body condition score are more likely to need an increase in TD.

# Disclosures

Disclosures to report.

Royal Canin kindly supports research in hyperadrenocorticism at the Veterinary Teaching Hospital Complutense.

#### ESVE-P-9

#### Survival of dogs with naturally occurring hyperadrenocorticism

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Hyperadrenocorticism (HAC) is a common endocrine disease in dogs characterized by a chronic endogenous cortisol excess that results in a progressive deterioration in untreated patients.

The objectives of this study were to assess the median survival time (MST) of dogs with HAC from diagnosis and its potential relationship with age, sex, type of HAC (adrenal [ADH] or pituitary [PDH]), concurrent diseases, systolic blood pressure, body condition score (BCS) and clinical and laboratory findings at diagnosis.

Sixty-six newly diagnosed dogs with HAC between January 2013 and December 2016 at the Veterinary Teaching Hospital Complutense were included. Diagnosis of HAC was based on clinical and laboratory abnormalities and adrenal functioning tests according to the ACVIM Guidelines. Four dogs had been previously misdiagnosed as hypothyroid and treatment was discontinued. All dogs were initially treated with trilostane (0.5-1.0mg/kg/12h). Rechecks were performed every 3 months. For survival analysis all causes of death were included. Kaplan Meier curves and Cox-regression test were obtained for all the variables reviewed.

At time of study closure 29/65 dogs were alive (44.6%). One dog was lost for follow-up. The overall MST was 777±264days; 44/65 (68%), 23/65 (35%) and 13/65 (20%) of dogs were alive at one-year, twoyears and three-years respectively. MST of dogs diagnosed with PDH was higher than those with ADH (mean±SD; 1108±252days and  $81\pm12$ days respectively; p=0.012). BCS was correlated with survival (p=0.01); dogs with a BCS <3/9 had a lower MST (124 $\pm$ 78days) than dogs with BCS 4-6/9 (1108±373) and obese dogs (>6/9) (976±209days). Dogs with calcinosis cutis at diagnosis (MST 441±233days) lived shorter than those without (1108±310days; p=0.029).

Age (HR:1.298, CI95% 1.129-1.493; p<0.0001) and post-ACTH cortisol concentration (HR:1.036, CI95% 1.012-1.061; p=0.03) at diagnosis were negatively correlated with survival.

Using a multivariate model, age at diagnosis (HR:1.322, CI95% 1.121-1.561; p=0.001), ADH (HR 2.713, CI95% 1.075-6.843; p=0.035), iatrogenic hyperthyroidism (HR:5.422, CI95% 1.420-20.848; p=0.013) and calcinosis cutis (HR:4.17, CI95% 1.616-10.786; p=0.003) were negatively correlated with survival, whilst a BCS>6/9 (HR:0.169, CI95% 0.41-0.687; p=0.013) was positively correlated with survival.

In the present study, survival time of dogs with PDH is slightly higher than previously reported. Dogs with ADH had reduced survival, probably due to the presence of metastases at diagnosis in 2/9 dogs. Age and post-ACTH cortisol were negatively correlated with survival as previously reported. To our knowledge this is the first time that reduced BCS, presence of calcinosis cutis and iatrogenic hyperthyroidism are reported as poor prognostic factors in HAC.



#### Disclosures

Disclosures to report.

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#### ESVE-P-10

# Efficacy and safety of toceranib phospate in dogs diagnosed with beta cell neoplasia

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Canine B cell neoplasms (insulinomas) are the most frequent neuroendocrine tumor in the pancreas. They secrete excessive amounts of insulin which leads to hypoglycemia and the subsequent clinical signs. In humans, a multitargeted tyrosine kinase inhibitor (sunitinib) has proven efficacy in patients with pancreatic neuroendocrine tumors. Anecdotal reports suggest an antitumor activity of toceranib phosphate in dogs with insulinomas.

The aim of this study was to evaluate the efficacy and safety of the tyrosine kinase inhibitor (toceranib phosphate-Palladia®) in dogs with insulinomas in stages III or IV.

Eighteen dogs diagnosed with insulinoma at the Veterinary Teaching Hospital Complutense of Madrid were included. Diagnosis was based on clinical signs, serum glucose and insulin levels, abdominal ultrasonography (AU) and computed tomography (CT). Insulinomas were classified with the TNM staging system of the European Neuroendocrine Tumor Society (ENETS). Dogs were surgically or medically treated depending of the clinical stage and were followed up until death. Efficacy of medical treatment was based on RECIST criteria.

At diagnosis dogs were classified in stage I or II (n=13) and III or IV (n=5). Dogs with stages I/II underwent pancreatectomy (n=12), the remaining refused surgical procedure. In 6 out of these 12 dogs, recurrence of the tumor was observed during the follow up. A total of 11 dogs with stages III/IV (at diagnosis or during follow-up after surgery) were treated with palliative treatment (n=6) (control group-CG) or toceranib phosphate (n=5) (toceranib phosphate group-TG). Palliative treatment consisted in diet, exercise restriction, prednisone (n=6) and octreotide (n=1). Dogs in TG received additionally toceranib phosphate at a mean dosage of  $2.41\pm0.14$  mg/kg/48h (2.22-2.56).

In dogs with stages I/II surgically treated (n=12), recurrence occurred at a median disease-free survival time (DFS) of 20±49days (n=6); in the remaining 6 dogs recurrence was not observed until death. Among dogs with stages III/IV (n=11), the median overall survival time (OST) in TG was significantly higher (282 $\pm$ 73days) than the median OST of dogs in CG (59±18days) (p=0.020). In TG, a stable disease (n=5) and partial response (n=1) was observed at 180 days of treatment. Toceranib phosphate was maintained until progressive disease (n=1) or severe adverse effects (n=4) occurred. Grade 1 and 2 (n=3) gastrointestinal signs occurred initially (3-4 weeks), and Grade 4 (n=4) after months causing treatment withdrawal.

Toceranib phosphate might have a role in the treatment of dogs with insulinoma in stages III/IV, increasing survival of dogs and providing adequate quality of life.

#### Disclosures

No disclosures to report.

#### ESVE-P-11

#### Neural network based model for glycemic dynamics predictions in feline diabetes mellitus

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Diabetes mellitus is a disorder of persistent hyperglycemia which can be induced by a series of underlying causes. It is one of the most common endocrine diseases diagnosed in cats, with a variable prevalence worldwide, ranging from 1 in 200 cats to up to 1 in 80. Extensive research in all aspects of feline diabetes mellitus (diagnosis, etiopathophysiology, therapy, etc.) has led to large amount of data, thus motivating our research to develop an artificial neural network to be used for predicting the glucose concentration in feline diabetes mellitus.

For this study, the data was obtained from 25 diabetic cats undergoing routine blood glucose monitoring for insulin treatment. Continuous glucose monitoring was performed with a CGM sensor. For each cat the following were recorded: glucose level at a 5 minutes sample period, number of units of insulin input (three types of insulin denoted by A, B, C, were used) and food intake (in kcal). A visual inspection of the data revealed that no patient had an ideal stable behavior which would imply a 55 to 160 mg/dl constraint evolution of the glucose level over time. The quality of the data was statistically evaluated to identify possible outliers in the patients' population.

Neural based methods in biosciences proved to be a powerful tool in transforming intelligently available information into valuable knowledge, such as models. This study considered two types of ANNs: Multi-Layer Perceptron (MLP) and Radial Basis Function (RBF), known as universal approximators for bounded continuous functions. The training data for both networks was built by adding recordings with low intercorrelation and relevant for the entire dataset. The input neural vector was formed considering adjustable lags in data samples to capture the dynamic behavior of the modeled process. Each neural network was designed with single hidden layer and a number of neurons inducted by experimental tuning.

Multiple tests were conducted, the training data varying between 8 and 15 patients. The experimental results revealed a confidence over 95% for both types of ANN's, regardless of the type of insulin. Accuracy performance obtained on training and validation datasets proved that the use of the adopted neural network vector is relevant to the dynamics of the glycemic evolution in feline diabetes mellitus. Results suggest predictive modeling is feasible for establishing and adjusting insulin dose, improve treatment effectiveness, increase diabetes remission rate and consequently obtain a better quality of life of diabetic cats.

#### Disclosures

No disclosures to report.



#### ESVE-P-12

# Use of tandem mass spectrometry (LC-MS/MS) for the measurement of thyroid hormones in dogs with spontaneous hypothyroidism

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In human medicine liquid chromatography tandem mass spectrometry (LC-MS/MS) is actually considered the "gold standard" for measurement of many hormones concentration and it is widely used in clinical practice; its diagnostic performance has never been investigated in dogs with hypothyroidism (DWH).

The aim of this study was to determine whether serum concentrations of fT<sub>4</sub>, fT<sub>3</sub>, rT<sub>3</sub>, 3.3-T<sub>2</sub>, 3.5-T<sub>2</sub>, measured with LC-MS/MS, were able to differentiate DWH (n=13) from dogs with non-thyroidal illness (DNTI) (n=12), septic dogs (SD) (n=12) and healthy dogs (HD) (n=12). Hypothyroidism was diagnosed based on consistent clinical signs, laboratory findings, total T<sub>4</sub> (TT<sub>4</sub>) and cTSH concentrations below and above the reference interval (RI), respectively; in dogs with normal cTSH, a rhTSH stimulation test was performed to confirm the diagnosis. In DNTI, hypothyroidism was excluded upon a negative result of a rhTSH stimulation test. SD were diagnosed based on alteration of temperature, cardiac and respiratory frequency, differential leukocyte count and C-reactive protein concentration above RI. HD were considered healthy upon history and physical examination. Hormones evaluation were performed with LC-MS/MS on surplus serum stored at -80°C. TT₄ and cTSH were measured using a validated immunoassay (Immulite®).

Non-significant differences considering signalment, age and body weight were found between groups.

Median TT<sub>4</sub> and fT<sub>4</sub> serum concentrations were significantly higher (p<0.001) in HD compared to DNTI, DWH and SD. Median fT<sub>3</sub> serum concentration was significantly lower in DWH and DNTI compared to SD (p<0.001 and p=0.0091, respectively) and HD (p<0.001 and p=0.0024, respectively). Median rT<sub>3</sub> serum concentration was significantly lower in DWH compared to SD (p=0.0141) and HD (p=0.0128). Median 3.3-T<sub>2</sub> serum concentration was significantly higher in DWH compared to DNTI (p=0.0038) and HD (p=0.0447). There were non-significant differences regarding median 3.5-T<sub>2</sub> serum concentrations among the dogs of the four groups.

Using the ROC curve analysis to differentiate DWH from DNTI+SD an AUC of 0.86 (p=0.003), 0.76 (p=0.009) and 0.75 (p=0.012) was obtained for fT<sub>3</sub>, fT<sub>4</sub> and TT<sub>4</sub>, respectively. Values of fT<sub>3</sub> <0.61pmol/L better discriminated hypothyroidism with 69% sensitivity (95%CI: 39-91%), 83% specificity (95%CI: 63-95%) and accuracy of 0.86 (95% CI: 0.74-0.98).

Although serum fT<sub>3</sub> and fT<sub>4</sub> (LC-MS/MS) have shown better performances than the serum TT<sub>4</sub> (Immulite®) in identifying DWH, the overlap between DWH and DNTI+SD was unfortunately relevant also for the thyroid hormones measurements with LC-MS/MS. Despite the introduction of new analytical methods, the use of dynamic tests (e.g. rhTSH stimulation test) remains the better method to discriminate DWH from DNTI.

#### Disclosures

No disclosures to report.

# ESVE-P-13

#### Accuracy of a flash glucose monitoring system in dogs with diabetic ketoacidosis

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A factory-calibrated flash glucose monitoring system (FGMS) (FreeStyle Libre, Abbott, UK) was recently evaluated in stable diabetic dogs. The aims of this retrospective study were to assess the performance of the FGMS in dogs with diabetic ketoacidosis (DKA) and to determine the effect of body condition score (BCS), perfusion, severity of ketosis and acidosis on the accuracy of the device.

FGMS was placed in a clipped and clean area on the dorsal part of the neck of dogs with DKA within 14 hours from the presentation. The interstitial glucose measurements were compared with blood glucose (BG) measurements, obtained by a validated portable glucometer (Optium Xceed, Abbott, UK). Overall accuracy was determined by fulfillment of ISO 15197:2013 criteria, calculating mean absolute difference (MAD), mean absolute relative difference (MARD), median absolute relative difference (mARD), mean relative difference (MRD), percentage of results within  $\pm 15$  mg/dL of the BG value for glucose <100 mg/dL and within  $\pm15\%$  of the BG value for glucose ≥100 mg/dL. Clinical accuracy was also illustrated using Parkes error grid and Bland-Altman plot. Sensor performance during changes in metabolic variables (lactate, β-hydroxybutyrate, pH and bicarbonate) was evaluated using Spearman's rank correlation.

Four hundred eighty-five paired results from 14 diabetic dogs with DKA were available for analysis. Good agreement between interstitial glucose measurements and BG was obtained (r=0.86: slope 0.88. intercept=18.37 mg/dL, r<sup>2</sup>=0.72). Clinical accuracy of FGMS was demonstrated, with 63.9% of results in zone A and 99.8% of results in zones A and B. Overall MARD was 18.9%, mARD was 16.6%, MRD was -4.4%; the percentage of values within 15 mg/dL or  $\pm 15\%$  was 48%. In the low glucose range, BG<100 mg/dL (n=26), MAD was 24.9 mg/dL; in the higher glucose range, BG≥100 mg/dL (n=459), MARD was 18.4%. Variations of lactate, β-hydroxybutyrate, pH and bicarbonate did not affect sensor performance. A significant interpatient variability in the accuracy of the device was observed (Kruskal-Wallis test, P<0.0001); FGMS tends to overestimate the glucose level in dogs with BCS≤3 and to underestimate in dogs with BCS≥7.

Despite the ISO 2013 requirements were partially fulfilled, FGMS provides clinically accurate estimates of BG in dogs with DKA. Accuracy of the system was apparently unaffected by metabolic variables making it suitable, not only for stable diabetic dogs, but also for dogs with DKA.

#### Disclosures

No disclosures to report.



# ESVE-P-14

# Predictors of response to pituitary radiotherapy in dogs with and without pituitary dependent hyperadrenocorticism

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Pituitary radiotherapy (RT) is a treatment for macroadenoma induced neurological complications. A previous study reported concurrent pituitary dependent hyperadrenocorticism (PDH) did not affect outcome for dogs undergoing RT. However, as PDH is associated with multiple comorbidities, authors hypothesised dogs with PDH would have poorer survival than those without PDH. This retrospective study recruited dogs who underwent pituitary RT at a single centre between 01/01/2008 and 01/01/2018. Signalment, time to RT post-diagnosis, tumour characteristics, RT protocol and PDH status were recorded. Thirty-one dogs were enrolled: 16 males and 15 females. Median age was 9 years (IQR 4.7) and 14 dogs had PDH; twenty-six underwent definitive RT. Median tumour size was 2.60 cm<sup>3</sup> (S.D. 1.47) and median radiation dose was 48 Gy (IQR 4). Follow-up intra-cranial imaging was available for four dogs: all experienced tumour volume reduction (38,45,72 and 78%). Median survival times of patients with and without PDH were 750 (IQR 915) and 709 (IQR 761) days, respectively (Kaplan-Meier log-rank P=0.386). Variables with P<0.2 on univariate Cox regression analysis (gender and age at diagnosis) were included in multivariate analysis to assess patient characteristics associated with survival. Only gender remained significant (male HR 2.722, 95% CI 1.089-6.804, P=0.032). Male and female survival times were 360 and 957 days, respectively (Kaplan-Meier log-rank P=0.074).

RT resulted in pituitary tumour reduction for the four patients with follow-up intra-cranial imaging. Tumour characteristics, patient age, time to RT or PDH status did not affect outcome. Gender might influence survival of dogs undergoing pituitary RT.

#### Disclosures

No disclosures to report.

## ESVE-P-15

## Telmisartan versus benazepril on the management of systemic hypertension in dogs with hyperadrenocorticism

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Systemic hypertension is present in approximately 70% of dogs with hyperadrenocorticism (HAC) and must be managed independently of HAC management.

The objective of the study was to compare two antihypertensive treatments in dogs with HAC.

Eleven hypertensive dogs diagnosed with HAC were prospectively included at the VTHC of Madrid. Systolic blood pressure (SBP) was assessed by Doppler method and hypertension was defined as SBP ≥ 160 mmHg.

Dogs were randomly included into 2 groups: Telmisartan group (TG) included 5 dogs treated with initial dose of 1mg/kg daily (up to 2 mg/kg daily) and Benazepril group (BG) included 6 dogs treated with initial dose of 0.2-0.3mg/kg daily (up to 0.5 mg/kg twice daily). In both groups, amlodipine was added at 0,1mg/kg (up to 0,25mg/kg) daily if SBP remained uncontrolled.

SBP was evaluated at 1, 3, 6, 12 and 18 months. Dogs were evaluated one week after any adjustment of antihypertensive medication. Mean initial SBP was similar: 208±22mmHg (TG); 209±21mmHg (BG).

In TG at 1 month, 4/5 dogs needed telmisartan dose increase up to 1.5-2mg/kg daily, while 1/5 dog achieved good control with initial dose. At 3 months, 2/5 dogs needed amlodipine (multitherapy). At 6 months, SBP remained controlled with telmisartan monotherapy (TM) (n=3). In the 2 dogs with multitherapy (with amlodipine at maximum dose), telmisartan was replaced by benazepril due to inadequate SBP control. At 12 months, amlodipine was added to telmisartan (n=1), and 2/3 dogs remained controlled with TM. At 18 months, 3/3 dogs were normotensive, with telmisartan (n=2) and telmisartan and amlodipine (n=1).

In BG, at 1 month, 6/6 dogs needed an increase in frequency to twice daily. At 3 months, 5/6 dogs had adequate SBP control, whilst 1/6 needed multitherapy with amlodipine. At 6 months, 2/6 dogs maintained an adequate SBP control with benazepril monotherapy, whilst in 3/6 dogs dosage of benazepril was increased. In multitherapy dog (1/6), amlodipine dosage was also increased. At 12 months, dogs needed an increase in benazepril (n=2) and in amlodipine dosage (n=1). SBP was controlled in all dogs at 18 months.

In conclusion, telmisartan used in monotherapy at a mean dosage of 1.0-1.8mg/kg daily provided good SBP control in 2/5 dogs while benacepril at 0.39 mg/kg twice daily did in 5/6. Telmisartan might be useful as antihypertensive treatment in hypertensive dogs with HAC, but better control was obtained with benacepril at the doses proposed. Higher doses of telmisartan must/can be assessed.

#### **Disclosures**

No disclosures to report

Boehringer Ingelheim- Merial has financed the drugs for the owners of the dogs included in the study

# ESVIM-P-1

# Meta-analysis of the association between Mycoplasma species and canine lower respiratory tract disease

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Mycoplasma species are readily isolated from healthy dog oropharynges and are accepted as commensal organisms in the oral cavity. However, its pathogenic role is debated in the lower respiratory tract (LRT), as it could be part of the normal flora or represent oral bacterial contamination.

A meta-analysis was performed on studies comparing the prevalence of Mycoplasma species between dogs showing signs of LRT disease and healthy controls. Studies were identified from 7 database. Mycoplasma presence was assessed by PCR or culture performed on samples from LRT, or seroconversion using paired samples. The quality of the evidence was rated using a modified Newcastle-Ottawa scale. The

meta-analysis was stratified by the four most common species of mycoplasma in dogs (M. cynos, M. spumans, M. canis, and M. edwardii). Of the 13,798 reports retrieved initially, 7 met inclusion criteria. Mycoplasma cynos was significantly more prevalent among symptomatic dogs than among controls (odds ratio (OR): 2.99; 95% confidence intervals (CI): 1.70-5.26; p<0.001). On the other hand, presence of M. canis and M. edwardii was not significantly associated with LRT signs (OR: 1.19; CI: 0.52-2.74; p=0.67 and OR: 1.57; CI: 0.43-5.66; p=0.48, respectively). Although a trend was detected for the association between M. spumans presence and LRT signs (OR: 2.69; CI: 0.92-7.92), this association was not statistically significant (p=0.07). These results suggest a pathogenic role of Mycoplasma cynos in canine LRT disease, as opposed to other mycoplasma species. Primers should be specific for M. cynos when PCR are performed on samples from LRT of dogs.

#### Disclosures

No disclosures to report.

# ESVIM-P-2

A questionnaire-based survey of owner-reported environment and care of West Highland white Terrier with or without idiopathic pulmonary fibrosis

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Canine idiopathic pulmonary fibrosis (CIPF) is a progressive parenchymal lung disease of unknown origin and poorly understood pathophysiology that mainly occurs in old West Highland white terriers (WHWTs). Not all dogs from the WHWT breed develop CIPF, which suggests the involvement of triggering factors in the onset and/or progression of the disease. To assess potential triggers, an online questionnaire-based survey was submitted to WHWTs owners. The questionnaire was accessible during a 3-month period in 3 different languages (English, French, and Dutch) and was distributed to WHWTs owners and breeders through social medial and emails. Questions were divided into sections regarding owner, dog (clinical and diagnostic data for CIPF), environment (house and surroundings, compost, ventilation, air conditioning, humidification, asbestosis, smoking, and household maintenance), grooming, and veterinary care (vaccination, deworming, and comorbidities). Completed questionnaires were received from 458 WHWTs owners from various countries including principally USA (n=193), Australia (n=62), France (n=56), UK (n=39), and Belgium (n=30). Thirty-eight questionnaires were discarded due to lack of essential data resulting in a total amount of 420 exploitable responses, 138 of which concerning a CIPF affected WHWT. Median age reported at CIPF diagnosis was 11.5 years (range 2- 6.8). Inspiratory crackles were noticed in 82.3% of dogs. Seventy-three out of 138 (52.9%) CIPF WHWTs were dead at the time of questionnaire completion. The overall survival time after diagnosis was 1.4 years (0-8.5). Cause of death was CIPF-related in 76.7% of cases. In order to assess potential triggers associated with CIPF, each CIPF dog was matched to one unaffected WHWT by age. Univariate logistic regression analysis was used for group comparison (CIPF vs. CTRL). Statistical significance was set at a P-value ≤ 0.05. Parameters significantly associated with CIPF included genetic relationship with another dog affected with CIPF (P=0.025), living in an old house (P=0.012), absence of a ventilation system (P<0.0001), and frequent grooming in dedicated facilities (P=0.001). CIPF dogs were at increased risk of cardiac disease (P=0.001), most likely due to the development of secondary pulmonary hypertension, and at decreased risk of endocrine disease (P=0.01) compared with CTRL. The preliminary results of this survey suggest an association between CIPF, genetic background and environmental factors such as old nonventilated houses or grooming habits. Further analysis of the results of the questionnaire are ongoing.

#### Disclosures

No disclosures to report.

#### ESVIM-P-3

Investigation of serum Kebs von den lungen 6 (kl-6) concentration as a predisposing factor and in the diagnosis of canine idiopathic pulmonary fibrosis in the West Highland white Terrier

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Canine idiopathic pulmonary fibrosis (CIPF) mimics human idiopathic pulmonary fibrosis (IPF) and affects old dogs from the West Highland white terrier (WHWT) breed. Due to difficulties to both diagnose early and follow the course of pulmonary fibrosis, numerous biomarkers have been studied in both species. In humans, the mucin Krebs Von den Lungen 6 (KL-6) has consistently been found to be increased in the serum from patients with interstitial lung disease, including IPF and is possibly involved in the IPF pathophysiological process. In CIPF, it has not yet been assessed.

In this study, we assessed the diagnostic value of serum KL-6 concentration in CIPF as well as the association between serum KL-6 concentrations and breed predisposition for the disease.

Blood was obtained from 5 groups of dogs: WHWTs affected with CIPF (n=20, 14 females (F)/6 males (M), mean age=11.0y, range [5.2-14.5]), healthy aged-matched WHWTs (n=18, 11F/7M, 10.5y [5.3-15.3]), and healthy dogs from other breeds not predisposed for CIPF: Jack Russel terriers (JR) (n=13, 8F/5M, 6.0y [3.0-9.1]), Malinois Belgian shepherds (MBS) (n=13, 5F/8M, 6.5y [2.8-10.8]) and King Charles spaniels (KCS) (n=13, 9F/4M, 6.5y [3.6-9.3]). Serum was stored at -80°C until batched analysis. KL-6 concentration was measured with a commercially available ELISA test (Canine Krebs Von den Lungen 6 Elisa kit, amsbio). Results were compared using a covariance analysis including the 5 groups defined above, gender and an interaction between groups and gender together with the effect of the age. The analysis was followed by post-hoc comparisons between groups using t-tests. Results show that only the effect of groups was significant (p=0.010). Serum KL-6 concentration was higher in healthy WHWTs (4.8+/-4.0 ng/ml) compared with KCS (1.6+/-1.8 ng/ml) and MBS (2.2+/-1.0 ng/ml) (p=0.001 and 0.013 respectively) and higher in JR (4.1+/-2.9 ng/ml) compared with KCS (p=0.016). There was no difference between CIPF (3.2+/-1.9 ng/ml) and healthy WHWTs (p=0.072).



In conclusion, differences in serum KI-6 concentrations were found amongst breeds in healthy dogs, with higher concentrations observed in terrier breeds. Whether this may reflect a predisposing factor for CIPF development merits further investigations. Unlike what has been found in human IPF. KL-6 is not a good serum biomarker for diagnosis as there was no difference between CIPF and unaffected WHWTs.

#### Disclosures

No disclosures to report.

#### ESVIM-P-4

Trends in serum cobalamin, folate and total iron binding capacity concentrations in pregnant bitches and their association with hematological parameters and neonatal survival

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This study evaluated cobalamin, folate and the iron status in pregnant bitches and their association with pregnancy-related anemia (PRA) and neonatal survival (NS). Complete blood count, serum folate and cobalamin concentrations and total iron binding capacity (TIBC) were measured in 48 bitches at mid- and late-pregnancy. The effects of the pregnancy stage, breed, age, parity and breeder on these variables were determined by generalized estimating equation test. Linear correlations between variables were assessed by Pearson's correlation test. Serum cobalamin (mean±SD) decreased (434±143 vs. 335±104; P<0.001), TIBC increased (3.6 $\pm$ 0.6 vs. 4.2 $\pm$ 0.7; P<0.001), while serum folate decreased insignificantly (13.4 $\pm$ 3.5 vs. 12.5 $\pm$ 3.3; P=0.1) at late pregnancy. The breed significantly (P<0.001) affected serum cobalamin and folate concentrations and TIBC, while the breeder significantly (P <0.001) affected folate concentration and TIBC. The neutrophil, platelet and leukocyte counts significantly increased, while the red blood cell count (RBC) significantly decreased (P<0.001 for all) at late pregnancy. The decrease in RBC was significantly, albeit weakly, correlated with the serum folate decrease (r=0.33; P=0.023) and the serum TIBC increase (r=0.40; P=0.004). None of the measures was associated with NS. In conclusion, although serum cobalamin had significantly decreased during pregnancy, this decrease was unassociated with PRA or NS. In pregnant bitches, contrary to pregnant women, TIBC increases as pregnancy progresses. Prospective studies are warranted to investigate whether prophylactic folate administration mitigates PRA, considering the significant correlation between the decreases of serum folate concentration and RBC with progression of pregnancy as noted herein.

#### Disclosures

No disclosures to report.

# ESVIM-P-5

Inter-clinician reliability of the respiratory physical examination in dogs and cats with abnormal breathing patterns

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In humans, consistent and repeatable respiratory physical examination is difficult, as reflected by poor to moderate agreement among clinicians

The aims of this prospective observational study were to assess interclinician reliability of the respiratory physical examination in dogs and cats, and determine the influence of time from study beginning and difference in clinical experience on inter-observer agreement.

Dogs and cats with abnormal breathing patterns presenting to Fregis, University of Missouri, or University of Pennsylvania veterinary hospitals were recruited over one year. Animals were included if they were evaluated by three clinicians before therapeutic intervention and/or diagnostic testing impacting respiratory sign recognition. Interobserver agreement was assessed via Fleiss' kappa coefficient. Influence of time from study beginning and clinical experience difference on agreement was evaluated via three-level mixed-effects logistic regression stratified by species.

Dogs (n=118) and cats (46) were enrolled. In dogs, overall interobserver agreement was moderate regarding breathing pattern recognition (K=0.46, varying from 0.28 to 0.55 depending on the pattern). Best agreement was obtained for respiratory noises (goose honking (K=0.84), stertor (K=0.8), and stridor (K=0.64)). Agreement was fair to moderate for abnormal auscultated lung sounds (crackles (K=0.5), wheezes (K=0.46), and expiratory snap (K=0.28)). Similar trends were observed in cats, with an overall lower inter-observer agreement than in dogs. Inter-clinician agreement did not improve with time. A lower difference in experience was associated with better agreement in stridor and tracheal sensitivity recognition in dogs.

Care should be taken when interpreting physical examination findings regarding respiratory signs with low inter-observer agreement.

#### Disclosures

No disclosures to report.

# ESVIM-P-6

#### Endoscopic bronchial anatomy in the dog

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Bronchoscopy is an important diagnostic procedure for the evaluation of many respiratory diseases and the removal of foreign bodies. During bronchoscopy, it is fundamental to know precisely the bronchial topography, in order to recognize abnormal anatomy or pathological changes. Few authors treat bronchial anatomy in the dog, and many of them limit the description to the lobar bronchi, without considering their branches. Currently, the endoscopic anatomy is based on a paper of 1986. Therefore, the aim of this study is to obtain a description of topographic anatomy and morphometric value, introduce a new standardised nomenclature and draw a correct bronchial map of the dog.

Twelve dogs, different in age, sex and breed, who died spontaneously for reasons other than pulmonary diseases, were included in the study, with owners' consent, and distinguished by weight in 3 groups (<10, 10-25, >25Kg). All the subjects were examined endoscopically in a systematic manner with a HD flexible endoscope (Ø6mm). The endoscopy focused on intracorpore (IC) and extracorpore (EC) exam. The EC was performed after the isolation of the lungs in order to obtain a better examination of cranial lobes, difficult in IC for their orientation. We considered the visualisation and the ability to pass through the lumen of each branch with the endoscope. After that, on the same lungs, casts of polyurethane foam were made and diameter and length of the bronchial branches were measured through a digital calliper. Furthermore, to name the structures and to draw the bronchial map, we defined them by looking at their direction and position. All the casts conformed to the orientation, the branching pattern and the topographic relationship of the bronchial system. For each lung lobe it was possible to define a new descriptive nomenclature for the first three series of bronchial division. The morphometric examination allowed to obtain a mean value of diameter and length of bronchi for each group of weight and to confirm the monopodial branching system. During bronchoscopy it was possible to locate and/or move in the principal, lobar and segmental bronchi, with significant differences between the groups.

After comparison with previous studies, we draw a new bronchial map and gave a descriptive nomenclature for the first three series of canine bronchial division. Moreover, we analysed the accessible airways with a 6mm diameter flexible endoscope in the different groups. Finally, our results provided accurate reference values useful in diagnostic imaging procedures, especially during bronchoscopy.

#### Disclosures

No disclosures to report.

#### ESVIM-P-7

#### A novel CMAH gene variant leading to blood type b in ragdolls

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In domestic cats, the AB blood group system consists of the three types A, B and C (also called AB), which vary in frequency among breeds and geographic regions. Mismatches cause acute hemolytic transfusion reactions and neonatal isoerythrolysis. The cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) is converting the N-acetylneuraminic acid (type B) to N-glycolylneuraminic acid (type A), and type C erythrocytes express both antigens. Sixteen variants in the CMAH gene were described to be associated with type B in different breeds, but in some breeds such as Ragdoll, Siberian and Turkish Angora, those variants did not correlate well with the phenotype.

We studied the feline CMAH coding regions of 70 blood typed Ragdolls (34 type A, 26 type B) by Sanger Sequencing and/or TagMan SNP genotyping and compared the sequences to published CMAH sequences.

Four novel CMAH variants (c.213A>G, c.593A>C, c.898A>G and c.1322delT) were identified beside seven previously reported variants. (c.142G>A, c.268T>A, c.993A>G, c.1269G>A, c.1392T>C, c.1603G>A and  $\Delta$ -53). The three previously described variants associated with blood type B (c.142G>A, c.268T>A and c.1603G>A) cosegregated in all Ragdolls. However, only seven cats were homozygous for those mutant alleles. The variant c.1322delT, causing an early stop codon at p.L411\*, was found homozygously in another 7 type B cats. The remaining 12 type B Ragdolls were compound heterozygotes for c.268T>A and c.1322delT. No type A cats were homozygous for any one of these variants.

In conclusion, type B in Ragdolls is caused by two CMAH variants: c.268T>A and c.1322delT in the homozygous or compound heterozygous state. General and particularly Ragdoll genotype screening should include both variants.

#### **Disclosures**

Disclosures to report.

Laboklin and PennGen are offering blood typing and blood compatibility testing. A patent has been submitted on the molecular genetic markers and panel testing discovered in this investigation and described in this abstract.

#### ESVIM-P-8

# Evaluation of diet's effects and ability of the Hemoccult assay for the detection of faecal occult blood in healthy dogs

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The guaiac-based faecal occult blood (FOB) test is widely used for colorectal cancer screening in humans. In dogs, it has been reported to be able to detect FOB after oral administration of 20 mg of haemoglobin/Kg body weight (mg<sub>hgb</sub>/kg<sub>bw</sub>) of autologous blood, but it is not routinely used.

The aims of the work were: i) to evaluate the ability of Hemoccult® to detect FOB in healthy dogs and to assess the influence of two diets: ii) to assess the influence of the time between faecal sampling and test results; iii) to find the lowest canine haemoglobin concentration to achieve all positive tests.

Initially, five healthy dogs were enrolled and each dog was fed with a meat-free protein diet (HA Purina®) then switched to gastrointestinal diet (EN Purina®) with 8 days of wash-out. No extra foods were permitted, apart from fresh or whey cheeses. The faeces of each dog were tested with Hemoccult® assay the day before starting HA diet and four- and five-day after. Starting from day six and every 4 days, progressive doses of autologous blood (5, 15, 20, 25 and 40 mg<sub>hgb</sub>/ kg<sub>bw</sub>) were administered orally and faeces were daily tested. Faeces were mixed with a wooden spatula before their collections. Thereafter, the same schedule described above was applied to each dog fed with EN diet. Then, the faeces of one dog were collected 6, 18 and 42 hours after a single 40  $mg_{hgb}/kg_{bw}$  blood-added meal. Seven test cards from each time-point faecal sample were prepared. Tests were assessed every two days until 14-day after collection. Finally, canine whole blood (18.0  $g_{hgb}/dL$ ) was progressively diluted in saline solution and directly applied on a set of three test cards until a negative result was found.

For the first aim, a total of 185 Hemoccult® tests were examined. Twelve (6,5%) were positive and no association between positive tests and administered amount of blood was found. None of the blood-free stool specimens was positive. Regarding the second set of



samples, only one resulted positive. Finally, 6.5 μg<sub>hgb</sub>/mL was the lowest concentration to achieve all positive tests.

In conclusion, Hemoccult® was not been influenced by both HA and EN diets, but its reproducibility to detect FOB in stools was unsatisfactory. Although, Hemoccult<sup>®</sup> was able to detect up to 6,5 μg<sub>hgb</sub>/mL when directly added to the card, the individual blood digestion and bowel transit time may be play a role on its poor reproducibility.

#### Disclosures

No disclosures to report.

#### ESVIM-P-9

#### Eosinophilic lung disease in 86 dogs (2006-2016)

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Eosinophilic lung disease in dogs can be associated with parasitic disease or can occur due to presumed primary hypersensitivity. The aim of this study was to report clinical variations in a large population of affected dogs.

In this retrospective study (2006 to 2016), 86 dogs were diagnosed with eosinophilic lung disease based on airway cytology (bronchoalveolar lavage eosinophils>14%) or histopathology. Records were reviewed for clinical and radiographic findings.

Mean body weight was 21.6+12.5kg with 5 dogs <5kg, 19 dogs 5-10kg, 10 dogs 10-20kg, and 50 dogs >20kg. German Shepherds (4), Labradors (4), and Standard Poodles (4) were most commonly affected. Clinical complaints included cough (80/87, 92%) and nasal discharge (25/87 dogs, 29%). Thoracic radiographs were normal in 14/86 (16%) dogs with variable pulmonary patterns in the remaining dogs. The most common bronchoscopic findings were hyperemia (61/86; 71%), increased airway mucus (57/86; 66%), airway collapse (24/86; 28%), and bronchiectasis (10/86; 12%). Inspissated intraluminal material was observed in 9/86 (10%) of dogs. One of these dogs had airway eosinophils of only 2% but demonstrated histologic evidence of eosinophilic infiltration. Median airway eosinophilia in the remaining 85 dogs was 39% (range 15-95%). Bronchoalveolar lavage total nucleated cells/µl ranged from 200 to 33,800/µl (median 1800). Neoplasia and pulmonary parasites were diagnosed in 1 dog each, and 2 dogs had concurrent bacterial infection. Aspergillus spp. were detected on aerobic culture in 2 dogs but were considered probable

In this group of dogs, eosinophilic lung disease was rarely associated with an identifiable underlying cause.

# Disclosures

No disclosures to report.

# ESVIM-P-10

#### Systemic and local immunoglobulin concentrations in Irish wolfhounds with recurrent bacterial pneumonia

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An increased incidence of bacterial pneumonia (BP) has been identified in the Irish wolfhound (IWH) and recurrence of BP occurs commonly in this breed. The etiology is largely unestablished; Immunoglobulin A (IgA) deficiency has been suggested as underlying factor. Purpose of this study was to investigate serum and bronchoalveolar lavage fluid (BALF) IgA, IgG and IgM concentrations in IWHs with recurrent BP as well as in healthy dogs.

A prospective, cross-sectional observational study included 11 IWHs (median age 6.3 years, IQR 5.7-7.0 years) with recurrent BP (median number of previous BPs 5, range 2-6). Healthy dogs were included as controls: 25 IWHs, 28 dogs of sighthound breeds and 16 dogs of other breeds (median age 6.6 years, IQR 6.3-8.9; 6.8, 5.3-8.7 and 6.5, 5.7-10.4 respectively). Six healthy laboratory beagles were included as controls for BALF immunoglobulin analysis. IgG, IgA and IgM were measured with ELISA method from serum and BALF. Statistical analysis was performed with an analysis of covariance models (ANCOVA).

Serum immunoglobulin concentrations (IgA in affected IWHs 104.8 mg/dl [IQR 76.0-238.6 mg/dl] in healthy IWHs 137.7, [92.5-168.5] in healthy sighthounds 87.3 [54.5-125.4] and in healthy dogs of other breeds 125.1 [75.9-226.9]; IgG 1000.4 [858.3-1368.0], 1227.0 [965.9-1482.5], 1349.5 [1042.8-1570.3] and 1164.0 [930.8-1266.3]; IgM 317.4 [251.1-378.3], 296.5 [220.0-404.1], 145.8 [131.9-219.6] and 187.9 [164.8-295.3] respectively) did not differ significantly between healthy and affected IWHs. BALF immunoglobulins did not differ significantly between affected IWHs and healthy beagles. These results indicate that immunoglobulin deficiency is unlikely a predisposing factor to recurrent BP in IWHs.

#### Disclosures

No disclosures to report

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# ESVIM-P-11

Evaluation of bronchoscopy and bronchoalveolar lavage findings in cats with Aelurostrongylus abstrusus in comparision to cats with feline bronchial disease

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The cat lungworm Aelurostrongylus abstrusus causes lower respiratory tract disease in cats worldwide. Bronchoscopy is an important tool for diagnosis of respiratory disease in cats; however, its role in the diagnosis of aelurostrongylosis remains unclear.

To investigate the usefulness of bronchoscopy in cat aelurostrongylosis, bronchoscopic and bronchoalveolar lavage (BAL) findings of 23 cats from Italy with A. abstrusus (Aa) were compared to those of 11 cats from the US with Feline Bronchial Disease (FBD).

Bronchoscopic lesions and bacterial isolation were recorded as present/absent, while inflammation type was classified by differential cytology of BAL. Data were analyzed using Mann-Whitney or Fisher's exact tests.

Age of Aa cats (13 males, 10 females) ranged from 12 to 65 months (median 34), while age of FBD cats (7 males, 4 females) ranged from 12 to 180 months (median 48). No bronchoscopic differences were detected between Aa and FBD cats for bronchial mucus (83 % vs 91%), nodular regions (4% vs 27%) and airways collapse (9% vs 9%), however airway hyperemia, epithelial irregularities, and stenosis were observed less frequently in Aa than FBD cats (30% vs 82%, 17% vs 82%, 13% vs 73%, p<0.01). Bronchiectasis was observed in 30% of Aa and none of the FBD cats (P<0.05). In Aa and FBD cats, neutrophilic. eosinophilic, and mixed inflammation (35% vs 27%; 35% vs 45%; 4% vs 28%, respectively) was reported. Lymphocytic inflammation was found in 9% of Aa versus 0% with FBD cats and cytology was normal in 17% of Aa cats. No significant differences in cytologic inflammation were detected between groups. Bacteria were isolated from BAL in 30% in Aa and 64% in FBD cats, with cytological evidence of sepsis in only 1 Aa cat. Larvae of A. abstrusus were detected cytologically in 22% of Aa cats.

This study suggests that bronchoscopic abnormalities in cats with various forms of lower respiratory tract disease are similar, although some lesions were more common in FBD. Interestingly, bronchiectasis was relatively common in cats with aelurostrongylosis. Cytological findings overlapped between inflammatory and lungworm disease, and the inability of BAL cytology to detect A. abstrusus larvae was confirmed. While bronchoscopy did not enhance the diagnosis of aelurostrongylosis, it represents a useful tool to detect bronchial abnormalities that might affect management or prognosis.

#### Disclosures

Disclosures to report.

This study was conducted as part of a project financed by Bayer Animal Health, Leverkusen, Germany, of which RS is an employee.

# ESVIM-P-12

#### Long term survival in two dogs with sterile multifocal osteomyelitis

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In veterinary patients, multifocal bone marrow lesions are typically neoplastic, with lymphoma and multiple myeloma the commonest causes. In this report, we describe two dogs with multifocal bone marrow disease in which histological analysis demonstrated osteomyelitis without evidence of infection or neoplasia. Both cases presented for further investigation of back pain and lethargy. Physical examination identified multifocal vertebral pain but there were no neurological deficits.

Magnetic Resonance Imaging (MRI) scans in both dogs revealed lesions in multiple vertebrae and in the ilium; the diaphysis of the humerus was also affected in case 1. All lesions were hyperintense on T2-weighted and STIR images and hypointense on T1-weighted images, with hyperintensity following contrast administration. Bone marrow aspirates and biopsies were evaluated cytologically and histopathologically, revealing pyogranulomatous osteomyelitis with no features of neoplasia and no infectious agents. Thoracic radiographs and abdominal ultrasonography were unremarkable and serological tests were negative for infectious diseases.

Case 1 was treated with an immunosuppressive dose of prednisolone and improved rapidly, with complete resolution of clinical signs. This was maintained throughout a period of dose tapering and there was no relapse following cessation of treatment at six months post-diagnosis. Case 2 was initially treated with doxycycline and carprofen, resulting in a temporary improvement followed by relapse on cessation of treatment. There was complete recovery following instigation of an immunosuppressive dose of prednisolone in this case, with no recurrence on dose tapering.

These cases demonstrate that sterile osteomyelitis is a rare but important differential diagnosis in dogs with multifocal bone marrow lesions and appears to carry a favorable prognosis.

#### Disclosures

No disclosures to report.

#### ESVIM-P-13

#### Retrospective comparison of cats with feline asthma and chronic bronchitis in a clinic population (2003 - 2016)

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Feline asthma (FA) and feline chronic bronchitis (CB) are common conditions in cats, often referred to as "feline lower airway disease". However, little is known about differences regarding signalment, clinical signs, and laboratory findings in cats with these two conditions.

Therefore, the aim of the study was to investigate potential differences between these two diseases that could be insightful in establishing a diagnosis of either condition.

The study population included 73 cats with feline asthma and 24 cats with chronic bronchitis. All cases were diagnosed based upon typical clinical signs and characteristic bronchoalveolar-lavage-fluid (BALF) cytology. Results of physical examination findings, laboratory values and radiographs were compared between both groups.

Patients with FA were presented with a median age of 6 years, cats with CB with 7.5 years. Thirty-two females and 65 male cats were included. Male animals were overall overrepresented with 70% in the FA and 58% in the CB group. The most common clinical signs were cough (FA 95%/CB 96%, p=1.000), abnormal respiratory sounds (FA 79%/CB 82%, p=0.766), and dyspnoea (FA 71%/CB 79%, p=0.601). In a laboratory analysis, 40% of cats with FA and 27% of cats with CB had an eosinophilia (p=0.026). Radiographic changes were detected in 94% of cats with FA and 91% of cats with CB (p=0.128).

The study showed that a differentiation of FA and CB by means of clinical signs, laboratory changes, or radiographic abnormalities is inconclusive. A reliable diagnosis would only be rendered possible by cytological examination of BALF.

# Disclosures

#### ESVIM-P-14

# Evaluation of long-term therapy in cats with feline asthma and chronic bronchitis

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Feline asthma (FA) and chronic bronchitis (CB) are common conditions in cats. It is still unknown, whether both diseases respond equally well to treatment, but therapy is commonly initiated without a clear distinction between these conditions.

The aim of the study was to compare the response to initial and longterm therapy, treatment success, adverse effects, and owner satisfaction in both groups, highlighting possible differences.

Study inclusion criterion was a cytological diagnosis of FA (eosinophilic inflammation) or CB (neutrophilic inflammation) in bronchoalveolar-lavage fluid (BALF) of cats with compatible clinical signs. Owners of cats meeting the inclusion criteria were thereafter asked to fill out a standardized questionnaire.

Owners of 35 cats with FA and 11 cats with CB completed the guestionnaire. Most cats were initially started on treatment with oral corticosteroids (FA 63%/CB 64%). In addition, oral bronchodilators (FA 43%/CB 46%) and antibiotics (FA 20%/CB 27%) were given to some patients. Treatment was later shifted to inhaled corticosteroids (FA 43%/CB 36%). Patients with CB were more likely to receive oral corticosteroids (FA 17%/CB 36%), bronchodilators (FA 6%/CB 27%), and antibiotics (FA 6%/CB 18%) than patients with FA in long-term therapy. Adverse effects (polyuria/polydipsia, fungal infection, diabetes mellitus) occurred in four patients with FA and one patient with CB. Most owners were satisfied with the long-term control of clinical signs in both groups (FA 74%/CB 82%).

The necessity of medications in both diseases can differ long-term; thus, knowledge of the underlying disease can be helpful in the management of cats with chronic bronchial diseases.

#### Disclosures

No disclosures to report.

#### ESVIM-P-15

# Matrix-metalloproteinase-7 activity in serum of West Highland White Terriers with idiopathic pulmonary fibrosis

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Canine idiopathic pulmonary fibrosis (CIPF), affecting mainly West Highland White Terriers (WHWTs.) is a chronic progressive interstitial lung disease sharing many similarities with human idiopathic pulmonary fibrosis (IPF). CIPF is characterized by exaggerated accumulation of extracellular matrix (ECM) in the lung parenchyma. Matrixmetalloproteinases (MMPs) are proteolytic enzymes capable of degrading ECM. In human IPF, MMP-7 has been found to be a potential diagnostic and prognostic biomarker. The aim of this study was to compare MMP-7 activities in serum of CIPF WHWTs, healthy WHWTs and other breed healthy dogs.

We analyzed the activities of MMP-7 using casein SDS-page zymography in the serum of WHWTs with CIPF (n=28), healthy WHWTs (n=27) and healthy dogs of other breeds (n=12). CIPF diagnosis was based on high resolution computed tomography or post-mortem lung histopathology (25/28 of CIPF WHWTs and 23/27 healthy WHWTs) or on typical clinical findings. The health of other breed dogs was verified by clinical examinations. MMP-7 activities were significantly higher in CIPF WHWTs (median 0.32, interquartile range [IQR] 0.17-0.67, range 0.039-2.16) compared to healthy WHWTs (median 0.16, IQR 0.12-0.29, range 0.029-0.55), p=0.0095. MMP-7 activity was detected only in one of the other breed healthy dogs (median 0, IQR 0, range 0-0.17). These results indicate that MMP-7 is a potential biomarker also in WHWTs with CIPF. However, we noted increased activities also in healthy WHWTs which can be connected to the breed susceptibility to CIPF.

#### **Disclosures**

No disclosures to report.

# ESVIM-P-16

#### Bronchoalveolar lavage lymphocytosis in dogs (2006-2016)

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Bronchoalveolar lavage (BAL) cytology and culture are used to characterize respiratory diseases in dogs, including inflammatory diseases such as eosinophilic lung disease and chronic bronchitis. Little is known about the clinical relevance or underlying disease processes associated with increased percentages of lymphocytes in BAL fluid. The aim of this retrospective study was to report clinical findings in a large population of dogs.

From 2006 to 2016, BAL lymphocytosis (>20% lymphocytes) was documented in 105/569 dogs. Records were reviewed for clinical and bronchoscopic findings. Respiratory diagnoses included eosinophilic lung disease, pneumonia, chronic bronchitis, aspiration injury, and airway collapse.

Lymphocytic inflammation in the absence of airway eosinophilia or neutrophilia was present in 33/105 (31%) dogs, with lymphocyte percentages ranging from 20-43% (median 24%). No bacteria were observed cytologically in any of these 33 dogs however pneumonia was confirmed in 3 dogs based on identification of pathogens in culture. Aspiration injury was suspected in 6/33 dogs. Concurrent eosinophilic airway inflammation (BAL eosinophils >14%) was documented in 15/105 dogs (lymphocytes 20-36%) and in 57/105 (54%) dogs, neutrophilic airway inflammation (>8%) was found (lymphocytes 20-52%), with pneumonia diagnosed in 13 dogs and chronic bronchitis in 18 dogs. Tracheobronchomalacia was documented significantly more often in dogs with lymphocytic inflammation alone (18/33) versus those with concurrent eosinophilic (2/15) or neutrophilic (18/57) inflammation, P = 0.01.

Mixed airway inflammation is common in infectious and inflammatory airway disease, however lymphocytic inflammation alone can be found in some dogs. The relationship between airway inflammation and tracheobronchomalacia requires further investigation.

# **Disclosures**



#### ESVIM-P-17

# Assessment of adverse effects of mycophenolate mofetil for treatment of immune mediated diseases in dogs

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The immunomodulatory drug mycophenolate mofetil (MMF) has been used for treatment of a number of immune mediated diseases in dogs and works through targeted antiproliferative effects on activated lymphocytes. MMF is gaining popularity for its perceived swift onset of action, easy accessibility and multiple preparations that include capsules, liquids, and intravenous formulations. MMF has been reported to cause gastrointestinal side effects, and there has been appropriate caution in its use due to the poorly characterised nature of these adverse events. The objectives of this study were to characterise the adverse events associated with the use of MMF when used for the treatment of immune-mediated diseases of dogs.

The study was a retrospective descriptive study which looked at dogs administered MMF from 2013 to 2016 at Davies Veterinary Specialists, United Kingdom.

Twenty seven dogs were available for analysis which were treated for IMHA (17), IMTP (3), Evans syndrome (3), IBD (2), IMPA (1), and MG (1). Fifty six percent of dogs that received MMF experienced adverse events, all of which were gastrointestinal in nature. Of the 27 dogs that received MMF, 44% (12) experienced diarrhoea, 19% (5) experienced melena or haematochezia, 11% (3) experienced vomiting, 7% (2) became anorexic,  $4^{\%}$  (1) was nauseous and  $4^{\%}$  (1) had nonspecified gastrointestinal signs.

There was no significant effect of the starting dose, or the dose frequency of MMF on the incidence of adverse events. A delay between diagnosis and beginning MMF had no significant effect on the likelihood of experiencing adverse events. Omeprazole was administered to nine dogs, all of which experienced adverse events that included diarrhoea, vomiting, haematochezia, melena and anorexia. In seven of these individuals, omeprazole was administered before the adverse events were documented. No other concurrent medications, including glucocorticoids, were associated with development of adverse events.

The incidence of adverse events experienced by this cohort of dogs treated with MMF for immune mediated diseases was high and exclusively gastrointestinal in nature.

#### Disclosures

No disclosures to report.

# FSVIM-P-18

Prevalence of subclinical Haemotropic Mycoplasmosis in healthy indoor cats eligible to become blood donors in the Iberian Peninsula

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Feline hemoplasmas are a group of Haemotropic Mycoplasmas known to cause feline infectious anemia. This group is compounded by

Mycoplasma haemofelis (Mhf), considered the most pathogenic, Candidatus Mycoplasma haemominutum (Mhm) and Candidatus Mycoplasma turicensis (Mtc), whose pathogenicity appears to be influenced by concurrent diseases and infections. They are pathogens with high prevalence worldwide, capable of causing a subclinical infection in feline blood donors, supposing a potential risk of infection in recipients after a blood transfusion.

This study aimed to widen the available information on the prevalence of subclinical infection by Haemotropic Mycoplasmas in healthy indoor cats eligible to become blood donors in the Iberian Peninsula. Blood samples of 1053 client-owned indoor healthy cats that were selected to be potential blood donors at the Animal Blood Bank in Spain and Portugal were obtained in the areas of Catalonia-Spain (231) and Portugal (822). Complete blood count, serum biochemistry. feline inmunodeficiency virus (FIV) and leukemia virus (FeLV), and species specific qualitative polimerase chain reaction for Mycoplasma were performed in each potential donor. All data was obtained from the routinary procedures performed at the animal blood bank, no unnecessary procedures were done to blood donors. All blood samples were collected after signed informed owner consent.

Fifty-one samples (4.84%) were positive for one of the Mycoplasma species, with a specific prevalence for Mhf, Mhm and Mtc of 2.37% (25/1053), 2.46 %(26/1053) and 0.19% (2/1053) respectively. Coinfection of different Mycoplasma species (Mhf and Mtc) was detected in 2 cats

From the 51 Mycoplasma positive samples, 2 were FIV positive, (one coinfected with Mhf and the other with Mhm); and one was FeLV positive (coinfected with Mhm). All infected cats presented a normal PCV, with a median value of 43.9% (26.8-49.9%) and were free of clinical signs or evidence of hemolysis in the blood smear evaluation. The previously reported prevalence of any feline mycoplasma in

healthy animals was 12% in Barcelona (Spain) and 43.43% in Portugal, with specific prevalences of 3.7% Mhf, 9.9% Mhm and 0.5% Mtc in Barcelona (Spain), and 12.81, 41.56% and 1.25% respectively in Portugal. The lower prevalences in this study may be due to the fact that only healthy indoor strict cats were enrolled, while the former studies admitted both stray and ill cats.

We can conclude that testing potential feline blood donors for feline mycoplasma is indicated even if no clinical signs are present and PCV value is within the normal range.

# **Disclosures**

No disclosures to report.

# ESVIM-P-19

Hyperplasia of the suprarytenoid pharyngeal fold in brachycephalic and nonbrachycephalic toy dogs: clinical presentation, treatment and outcome in 10 cases

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Hyperplasia of the suprarytenoid pharyngeal fold (HSPF) is a rare and unreported respiratory disease affecting brachycephalic and nonbrachycephalic toy dogs.



The dorsal pharyngeal fold might cause obstruction of the dorsal portion of the laryngeal aditus, covering the corniculate processes of arytenoid cartilages and limiting or blocking their abduction during inspiration.

The HSPF might be seen concurrently with laryngeal collapse or elongated soft palate.

The aim of this work is to describe the clinical, endoscopic, treatment and outcome in ten dogs diagnosed with HSPF.

Fourdogs were Pugs, two dogs Pomeranian, one mixed breed and three Chihuahua; seven dogs were females and three males, mean age was 6.2 years (1-12v). Main clinical signs were inspiratory dyspnea and inspiratory stridor in nine dogs, five dogs exhibited stertorous breathing and two dogs nocturnal apnea. Mean duration of clinical signs was 13 months (0.5-60 mos).

All dogs under general anesthesia underwent endoscopic evaluation of the upper and lower airways; main findings were HSPF with reduction of laryngeal abduction and laryngeal collapse in all dogs; three dogs had soft palate hyperplasia and three dogs stenotic nares; two dogs had bronchomalacia.

In all dogs the pharyngeal hyperplastic fold was lifted with a cotton swab improving laryngeal abduction during inspiration in 8/10 dogs.

Two Pugs underwent surgical correction of the HSPF with significant improvement of the inspiratory stridor and resolution of the nocturnal apnea. One dog died of unrelated causes four months after surgery. The other Pug was euthanized due to economic restraints.

Endoscopic alterations in the remaining seven dogs were considered mild and medical option with inhaled corticosteroids resulted in clinical improvement.

Mean follow-up was 16 months (4-24 mos).

HSPF might be an underestimated disease of the upper airways and clinician should be aware of this condition when evaluating brachycephalic and non-brachycephalic toy dogs with obstructive signs of the upper airways. It should also be considered that muscle relaxation induced by anaesthesia and mucosal oedema might overestimate the degree of HSPF and subsequently the degree of laryngeal collapse. When abduction of the pharyngeal fold by mean of a cotton swab results in significant improvement of laryngeal abduction, surgical correction of the HSPF should be considered in dogs with severe inspiratory dyspnea. In less severe cases conservative therapy can improve clinical signs.

A larger case series of dogs with HSPF is warranted to better characterize this condition and the indications for surgical versus conservative management.

#### Disclosures

No disclosures to report.

# ESVNU-P-1

Comparison of point-of-care (PocketchemTM UA) and reference laboratory protein/creatinine ratio measurements on canine and feline urine samples for diagnosis of proteinuria.

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The Pocketchem<sup>TM</sup> UA (Scil Animal Care Company) is a point-of-care instrument designed for routine analysis of canine and feline urine. Analysis is based on dipstick reading by dual wavelength reflectance.

The aims of this study were to compare Urinary Protein/Creatinine (UPC) ratio measurements by the Pocketchem<sup>TM</sup> UA with measurements done at a veterinary reference laboratory (gold standard: automated spectrophotometer) and to examine agreement between both methods in classifying dogs and cats as being non-proteinuric (NP), borderline proteinuric (BP) or proteinuric (P) according to IRIS guidelines for staging chronic kidney disease.

76 urine samples were collected via cystocentesis or free catch from 63 dogs and 13 cats that had been referred for varying disease conditions and as part of their diagnostic work-up. Routine urinalysis was done in-clinic while UPC ratio measurements were performed by a veterinary reference laboratory (Laboratoires Collard, Belgium). Simultaneously, for each urine sample (left-overs) a second UPC ratio measurement was done in-clinic using the Pocketchem<sup>TM</sup> UA.

The UPC ratios measured by the veterinary reference laboratory and the Pocketchem<sup>TM</sup> UA were significantly different, both for dogs (median 0.29, IQR 0.03-1.53, range 0-16.48 vs median 0.1, IQR 0-0.5, range 0-20; p<0.001) and cats (median 0.16, IQR 0.14-0.2, range 0-1.98 vs median 0.1, IQR 0.05-0.1, range 0-0.3; p<0.01). When each dog's UPC ratio was classified as NP (<0.2), BP (0.2-0.5) or P (>0.5) agreement between both methods was found in 48/63 cases (76.2%). 6 BP dogs were classified as being NP by the PocketchemTM UA while 4 and 3 P dogs, respectively, were classified as NP and BP by the same instrument. Additionally, 2 NP dogs were classified as BP by the Pocketchem<sup>TM</sup> UA.

When each cat's UPC ratio was classified as NP (<0.2), BP (0.2-0.4) or P (>0.4), agreement between both methods was found in 6/13 cases (46%). All 3 BP cats were classified as NP by the Pocketchem<sup>TM</sup> UA while 2/3 P cats and 1 P cat, respectively, were classified as NP and BP by the same instrument. Additionally, 1 NP cat was classified as BP by the Pocketchem<sup>TM</sup> UA.

Overall, Pocketchem<sup>TM</sup> UPC ratio classifications differed from the gold standard reference laboratory classifications in 28.9% of cases. The Pocketchem<sup>TM</sup> UA was not a reliable test for the measurement of UPC ratios in this cohort of dogs and cats.

#### Disclosures

No disclosures to report.

# FSVNU-P-2

### Evaluation of serum transforming growth factor beta 1 in cats with chronic kidney disease

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Chronic kidney disease (CKD) is a common problem in feline practice. Early diagnosis can help to influence the outcome and improve the prognosis. Currently there are no reliable biomarkers for the early diagnosis of CKD. Since fibrosis is the main tissue alteration during CKD, we assumed that the transforming growth factor beta 1 (TGFß1), an initiation molecule of tissue fibrosis may be used as an early indicator of CKD. The aim of this prospective study was to evaluate the diagnostic value of TGF-β1 in feline CKD. Twenty-five healthy cats and 26 cats with CKD were included in the study. TGF- $\beta$ 1 was measured in serum using a "Quantikine Human TGF-β1 Immunoassay"

(R&D Systems). In healthy cats the median TGF-β1 serum concentration was 27.37 ng/ml, while cats suffering from CKD had a median of 17.62 ng/ml, being the difference between both groups significant (p = 0.0013). CKD affected cats with International Renal Interest Society (IRIS) stage 2 had a median TGF-β1 serum concentration of 14.82 ng/ml. Cats with IRIS stage 3 had a median of 15.79 ng/ml and cats with IRIS stage 4 had a median of 19.24 ng/ml. There was no significant correlation between the serum TGF-B1 concentration and the different IRIS-Stages. Furthermore there was no significant correlation between serum TGF-β1 and the platelet count, even though these cells have the highest intracellular concentrations of TGF-β1 in blood. Cats diagnosed with CKD showed lower serum TGF-\(\beta\)1 concentrations than healthy cats. In another study serum TGF-β1 was also higher in healthy cats compared to cats with CKD. A higher expression of TGF-β1 in the kidneys accompanied by a higher synthesis of extracellular matrix can be detected histologically in humans. A missing rise of TGF-β1 in serum of cats with CKD could be attributed to the fact that this cytokine is synthesised directly in the kidneys for local fibrosis processes. The possible use of serum TGF-β1 as a diagnostic tool for CKD could not be validated in this study.

#### Disclosures

No disclosures to report.

#### ESVNU-P-3

Overweight and obesity did not predispose to subclinical bacteriuria in a mixed population of 152 middle aged and elderly cats - a prospective cross-sectional study

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Obesity has been implicated as a risk factor for development of asymptomatic/subclinical bacteriuria (SB) in humans and dogs. The aim of this study was to investigate whether overweight and/or obesity predispose cats to SB.

A cross-sectional study was carried out at the University Hospital for Companion Animals, Copenhagen, from October 2015 to March 2018. Cats ≥ 6 years presenting for causes unrelated to the lower urinary tract were eligible for enrolment. Body Condition Scoring (BCS) was performed on a 9-point scale with overweight being defined as BCS ≥6 and obesity as BCS ≥ 8. Urine was collected by cystocentesis and subjected to aerobic bacterial culture. The correlation between presence of SB and the variables sex, healthy/ill, age and BCS category was analyzed by binominal logistic regression. The study population consisted of 152 cats (72 females and 80 males) aged 6-18 years (median 10), of which 58 were healthy, and 94 were diseased. Fifty-nine cats scored as overweight and, among those, 14 as obese.

SB was identified in nine cats (5.9%) and Escherichia coli was the most commonly cultured bacterium (n=3). Female sex was a significant risk factor for SB (female OR 5.2 [1.0-27], RR 4.2 [1.2-12]; vs. male; p=0.04). Overweight was not a significant risk factor (overweight/ obese OR 0.38 [0.07-2.0], RR 0.40 [0.06-1.65]; vs. lean; p=0.25), and neither was obesity compared to lean and overweight cats (p=0.99).

Despite the few cases of SB detected, our data indicate that overweight and obesity do not predispose cats to SB.

#### Disclosures

No disclosures to report.

#### ESVNU-P-4

In-house culture and susceptibility reduce antibiotic overprescription in dogs with suspected cystitis - a randomized controlled trial

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Antibiotics are over-prescribed in dogs with suspected cystitis and the decision to prescribe may be affected by reliability of in-house diagnostics and coherence to test results.

The aim of the study was to investigate the impact of a commercial in-house culture and susceptibility test (C/S) on the decision to treat (DTT) with antibiotics and the choice of antibiotic agent (COT) compared to routine microscopy of stained urine sediment (UA).

The study was designed as a multi-center randomized controlled trial in primary veterinary practices. Dogs with clinical signs of cystitis were enrolled and randomized to one of two in-house diagnostic groups: C/S or UA. Antibiotics were withheld until culture results were available the day after the consultation in the C/S group. Excess urine was submitted to a reference laboratory for quantitative bacterial culture (QBC). Antibiotic prescription was considered an appropriate DTT in case of significant bacteriuria on reference QBC. Appropriate COT was defined as prescribing antibiotics with in vitro susceptibility and choosing first-line agents over second-line agents.

Fifteen clinics enrolled 66 dogs, of which 32 and 34 cases were randomized to C/S and UA, respectively. Cystitis was present in 41% of dogs according to the QBC. Appropriate DTT occurred in significantly more dogs (81%) of the C/S compared to the UA group (52%) (OR 5.92, 95%CI [1.57;28.9], P=0.014), but C/S did not improve COT (OR 0.70, 95%CI [0.07:8.05], P=0.73).

In conclusion, in-house C/S and withholding of antibiotics until culture results are available can reduce antibiotic over-prescription for dogs without cystitis.

#### Disclosures

No disclosures to report.

#### ESVNU-P-5

# Bacterial culture results from urine and ejaculates in healthy intact male dogs

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In a previous study performed at the Norwegian University of Life Sciences (NMBU), a large proportion of positive culture results from ejaculates from healthy male dogs was registered, and this prompted further examinations.

The present study was performed at the NMBU between December 2017 and February 2018. Fifteen healthy intact male dogs aged 1-6

years were included. Three urine samples were collected within eight hours and in the same order for each dog: 1) voided, 2) voided after cleaning the external genitals with tap water (clean voided), 3) cystocentesis. For each urine sample, a complete urinalysis was performed; including urine dipstick, microscopy of the sediment, and bacterial culture. After cleaning the prepuce, an ejaculate was collected and submitted for cytology and bacterial culture. The fluid obtained when cleaning the prepuce was also collected for bacterial culture. A boardcertified radiologist performed an ultrasound examination of the urogenital tract in 14/15 dogs. In one dog, ultrasound was not performed since the owner declined sedation.

Bacterial growth was found in 6/15 voided urine samples, and in 2/15 clean voided ones. All cystocentesis samples were culture negative. There was bacterial growth in 11/15 ejaculates, and a positive correlation between age and bacterial growth was detected (rs = 0.78, p = 0.006). Five of these dogs had negative cultures from all their urine samples. For 10/11 dogs with positive ejaculate cultures, similar culture results were obtained from the lavage-fluid. Seven dogs examined with ultrasound had changes compatible with benign prostatic hyperplasia; six of these had positive ejaculate cultures. The bacteria grown from the different samples (urine, ejaculates, lavage-fluid) were: Pasteurella spp., Mycoplasma canis, Staphylococcus pseudintermedius, Prevotella sp. Gram-negative anaerobic rods, Streptococcus canis, Coagulase-negative Staphylococcus spp. and mixed floras consisting of the ones mentioned.

The study revealed 13/15 negative cultures from clean voided urine samples. This is of clinical relevance since a negative culture is useful regardless of the sampling method. Further, the results suggest that one should be prudent when interpreting ejaculate cultures, since a large proportion of ejaculates from healthy dogs had bacterial growth. In addition, there were concordant culture results from the respective lavage fluids and ejaculates. Whether the bacteria grown from the ejaculates represent actual bacterial colonization of the prostate, or contamination from the prepuce and distal urethra, is yet to be determined. Further studies including prostate sampling (e.g. aspirates) are needed.

#### Disclosures

No disclosures to report.

#### ESVNU-P-6

### Retrospective study (2004-2017) of 137 uroliths diagnosed at the Complutense Veterinary Teaching Hospital

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In the present retrospective study 137 uroliths were removed and analyzed at the Minnesota Urolith Center. The data recorded were species, breed, gender, age, material, year of withdrawal, type and diagnostic images. Where the urolith had a struvite layer, the result of urine culture and sensitivity was recorded. Urine samples were collected by cystocentesis considering significant counts > 10 <sup>3</sup> CFU/mL. With regard to the species, canine uroliths were more frequent than feline (n = 116, 84.6% vs n = 21, 15.3%). Among dogs, males were over represented (n = 86, 74.13% vs n = 30, 25.86%). Male cats were twice as likely to suffer from urolithiasis as females (n = 14, 66.66% vs n = 7, 33,33%).

Concerning the frequency, the composition of canine uroliths was: calcium oxalate (56.89%, males = 81.81%, Yorkshire Terrier and Miniature Schnauzer = 53.03%, aged 3 to 14 yr); struvite (17.75%, females = 55%, Cocker Spaniel, crossbreed = 25%, aged 2 to 12 yr); urate (11.2%, males = 69.23%, Dalmatian = 38.46%, aged 1 to 14 yr); cystine (7.75%, males = 100%, Basset Hound = 44.4%, aged 2 to 12 yr); calcium phosphate (3.44%); xanthine (2.58%) and silicate (0.86 %). In cats, 66.66% of uroliths were composed of calcium oxalate (males =

64.28%, aged 1 to 15 yr); 23.8 % of struvite (males = 60%, aged 6 mo to 8 yr) and 9.5 % of urate. Mixed-breed were almost exclusively affected (90.47%)

In 26 (22 dogs and 4 cats) out of 35 struvite uroliths culture and sensitivity were performed, getting 21 positive results and 5 negative. Of these 5 negative, 3 were dogs and 2 cats.

The most frequent pathogens isolated were Staphylococcus pseudointermedius (n = 5, 23.8%) and Staphylococcus intermedius (n = 5, 23.8%), followed by Streptococcus canis (n =1, 4.76%), Staphylococcus spp. (n = 3, 14.28%), Staphylococcus aureus (n =2, 9.52%), Streptococcus spp. (n = 1, 4.76%), Escherichia coli (n = 1, 4.76%), Staphylococcus carnosus (n = 1, 4.76%), Pasteurellacanis (n = 1, 4.76%) and Proteus (n = 1, 4.76%).

Nitrofurantoin was the most effective antibiotic (n = 18 sensitivities, 90%). Staphylococcus spp. isolates were reported as resistant to cefovecin (31.25%), cephalexin (25%) and Amoxicillin-Clavulanic (6.25%).

To summarize, the study showed that canine uroliths were more frequent than feline affecting mostly males of both species. Calcium oxalate uroliths were more represented. A huge amount of urine isolates were susceptible to Nitrofurantoin.

#### Disclosures

No disclosures to report.

# ESVNU-P-7

#### Clinical efficacy of the Marbofloxacin usage in dogs and cats diagnosed lower urinary tract disorders

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Marbofloxacin is one of fluoroquinolones developed exclusively for veterinary medicine. The primary aim of the study is to identify and to assess the evidence which relates to the clinical efficacy of marbofloxacin in the treatment of urinary tract infections in small animal practice. 118 dogs and 123 cats were referred to Internal Medicine Department Polyclinics with lower urinary tract symptoms whom hadn't received antimicrobial or anti-inflammatory therapy previous 15 days, were included in the study. The diagnosis was confirmed by clinical findings, urinalysis and imaging. Rectal temperature, appetite, urinary signs and pain on abdominal palpation were monitored at two visits (day 0 and day 14). The timetable depending on diagnosis: two urinalyses and two bacterial examinations and antibacterial

susceptibility testing per case were performed. Bacterial UTI were confirmed of 36 dogs and 28 cats. Urine samples were collected on the first referral demonstrated the presence of various bacteria with a marked predominance of P. mirabilis and coagulase positive Staphylococci in canine and feline urine samples, respectively. Antimicrobial susceptibility test results revealed 25 (65.7%) of dog isolates and 24 (85.7%) of cat isolates were susceptible to marbofloxacin. Treatment of UTIs is generally challenging for the small animal practitioner. Because of long-term antimicrobial usage necessity, especially bacterial culture and susceptibility test are very important for treatment success. Marbofloxacin usage can be rewarding in the treatment of UTIs in dogs and cats. Thus, revealed the current status of Turkey for the first time. Key Words: antimicrobials; cat; dog; marbofloxacin; urinary tract infection

#### Disclosures

No disclosures to report.

#### ESVNU-P-8

### Moderate dietary phosphate restriction and fibroblast growth factor 23 in healthy older cats

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A previous prospective randomised placebo-controlled trial examined the effect of dietary phosphate restriction in healthy, non-azotaemic cats >9 years (Geddes et al., 2016). Cats were assigned to test or control diet (protein 76 g/Mcal, 86 g/Mcal; phosphate 1.6 g/Mcal, 2.6g/ Mcal). Blood samples were taken at baseline, 6 weeks, 6, 12 and 18 months on diet. No effect of phosphate restriction was observed on plasma fibroblast growth factor 23 (FGF23), a phosphaturic hormone shown to be predictive of development of azotaemia in cats > 10 years. Our understanding of normal plasma FGF23 concentration has developed. In a group of 46 healthy adult cats fed a maintenance diet, median concentration was 105 (81.4, 133) pg/mL. As a result, we reviewed data from the trial to determine whether feeding a senior diet reduced plasma FGF23 concentration in cats with FGF23 above the top 20 percentile of healthy adult cats.

Sixty-nine cats had baseline plasma FGF23 concentrations recorded. Cats were divided into test or control diet groups and further categorised into groups baseline FGF23 ≤ 150 pg/mL or >150 pg/mL. Data are presented as median [25<sup>th</sup>, 75<sup>th</sup> percentile] with nonparametric data being log transformed. FGF23 concentration was compared between timepoints using a one-way repeated measures ANOVA.

Thirty-two cats received control diet, 15 attended all visits. At baseline 9 cats had FGF23 ≤ 150 pg/mL (105 [61,115] pg/mL) and 6 had FGF23 >150 pg/mL (220 [207, 264 pg/mL). In both groups, mean logFGF23 did not differ significantly between time points.

Thirty-seven cats received test diet, 18 attended all visits. At baseline 9 cats had FGF23 ≤ 150 pg/mL (96 [86,131] pg/mL) and 9 FGF23 > 150 pg/mL (186 [163,241] pg/mL). In the group with baseline FGF23 ≤ 150 pg/mL, mean logFGF23 concentration did not differ significantly between time points. In the group with baseline FGF23 >150 pg/mL, mean logFGF23 concentration differed significantly between

time points (Wilks Lambda = .48, F (4, 5) = 24.56, P =.002). Post hoc tests revealed plasma FGF23 concentration significantly decreased between baseline and 12 months (139 [125, 161] pg/mL P< .05).

Plasma FGF23 concentrations deceased relative to baseline in cats with FGF23 > 150pg/ml when fed a senior diet after 12 months. These preliminary data suggest a positive effect of moderate phosphate restriction in these cats. Further studies are required to characterise disturbances in phosphate homeostasis in non-azotaemic cats and the long-term benefit of feeding a moderately phosphate-restricted diet.

#### Disclosures

Disclosures to report.

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# ESVNU-P-9

# Pattern of renal tubular damage and dysfunction in dogs with intrinsic acute kidney injury due to leptospirosis and other causes

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Leptospiral infection is associated with acute tubulo-interstitial nephritis and severe urine electrolytes wasting (particularly magnesium, phosphate and potassium) in humans. Tubular damage and dysfunction during acute canine leptospirosis are scarcely documented. This study aimed to evaluate urine chemistry and biomarkers of tubular damage in dogs with intrinsic acute kidney injury (iAKI) associated with leptospirosis (Leptospiral-iAKI) and with iAKI caused by different aetiologies (Other-iAKI), in order to potentially define peculiar diseaserelated patterns.

Dogs with history, clinical and clinicopathological signs consistent with iAKI (serum creatinine >1.6 mg/dL and/or urinary output <1ml/kg/h over 6h, in absence of fluid-responsiveness), as reported by AKI-IRIS staging guidelines, were prospectively included and grouped according to their aetiological diagnosis in Leptospiral-iAKI or Other-iAKI. Leptospirosis was diagnosed by positive microagglutination test (MAT; titre ≥1:800) upon admission or 4-fold increase in convalescent MAT titre and/or positive qPCR on blood/urine. Routine laboratory analyses, urine chemistry including fractional excretion (FE) of electrolytes (sodium, potassium, magnesium, phosphate, calcium) and urine neutrophil gelatinase-associated lipocalin (uNGAL), were performed upon admission. Data were reported as median, range (min-max), and reference interval (R.I.). Groups were compared using non-parametric statistics (P<0.05 considered significant).

UBig-ET1 concentration and uBig-ET1/UC ratio did not significantly differ between "at risk" and "CKD" cats.

UBig-ET1 was not significantly different between IRIS stages, whereas UBig-ET1/UC ratio was significantly higher (p=0.001) in IRIS 3-4 group.

Both uBig-ET1 and uBig-ET1/UC ratio did not differ significantly between "at risk" cats that remained stable and "at risk" cats that developed CKD (stage IRIS 1 or 2) during the monitored period.

Grouping according to SBP (higher or lower 150 mmHg), no significant difference was found for UBig-ET1 and UBig-ET1/UC ratio between normotensive and hypertensive cats.

UBig-ET1 did not differ significantly between samples sub-staged by proteinuria, according to IRIS guidelines. Conversely, UBig-ET1/UC was significantly higher in proteinuric cats when compared to non proteinuric cats (p=0.026).

These results suggest that SBP and proteinuria are not important determinants of the UBig-ET1 level in cats. This is not surprising, since in people urinary ET-1 is considered to reflect mainly renal production, instead of circulating levels, because of its autocrine and paracrine actions. No specific information is available about the physiology of ET-1 in cats and further studies are needed to explain its low serum concentration.

# Disclosures

Disclosures to report.

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# ESVNU-P-11

Evaluation of renal function in dogs infected by Dirofilaria immitis in relation to microfilaremia, parasite burden and pulmonary pressure

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Heartworm disease (Dirofilaria immitis) is characterized by intimal proliferation of the pulmonary arteries, pulmonary hypertension and heart failure. Furthermore, D. immitis also causes renal damage, primarily defined by the development of proliferative glomerulonephritis. However, few studies exist evaluating the prevalence of renal injury in dogs with heartworm.

The aim was to assess specific serum and urinary renal parameters in dogs infected by D. immitis and evaluate the impact of the parasite burden, microfilaremia and pulmonary pressure.

Twenty-two heartworm-infected dogs were evaluated. Microfilaremia was established by the Knott test while the parasite burden and pulmonary pressure were determined by echocardiography. Urinary and serum creatinine, microalbumin as well as serum urea were determined by a spectrophotometric system. Urine reactive strips were used to determine urinary parameters (Glucose, Bilirrubin, Urobilinogen, Ketones, Blood, pH, nitrogen, leucocytes, color, turbidity and density). Urinary protein concentration was determined by the Pyrogallol Red-Molybdate method. Microalbumin/urine creatinine ratio (A:C ratio) and urine protein/creatinine ratio (UP/C ratio) were calculated.

Microfilaremia was present in 40.9% of the dogs and pulmonary hypertension was present in 52.4% of them. Parasite burden was high

The study included 64 dogs: 31/64 were Leptospiral-iAKI and 33/64 Other-iAKI (non-infectious inflammatory disease n=10; toxic n=8; sepsis n=6; neoplasia n=3; trauma n=3; diabetic ketoacidosis n=3). Renal function was more severely compromised in Leptospiral-iAKI compared with Other-iAKI dogs (serum creatinine mg/dL: 6.9, 1.1-8.7 vs 4.3, 1.4-21.4, P=0.04, R.I. 0.65-1.40). Urine proteins and electrolytes loss was detected in both groups; however, no significant differences between Leptospiral-iAKI and Other-iAKI were identified for urine protein to creatinine ratio (UP/C: 1.7, 0.13-184 vs 2.2, 0.09-72, P=0.58; R.I. 0-0.5) and FE (%) of electrolytes (potassium: 92.3, 0.6-480 vs 61.5, 5.3-301. P=0.11. R.I. 3.7-20.4: magnesium: 8.9. 2.8-141 vs 10.6. 0.9-81, P=0.9, R.I. 0.5-3.9; phosphate: 31.9, 0.8-125 vs 32.5, 0.8-91, P=0.6, R.I. 5.7-24.6; sodium: 2.6, 0.08-64.3 vs 2.1, 0.04-69.3, P= 0.7, R.I. 0.05-0.92; calcium: 3.8, 0.12-70 vs 4.2, 0.14-56 vs, P=0.8, R.I. 0.05-0.33). Similarly, no significant differences were detected for uNGAL concentration (uNGAL pg/mL: 146960, 1204-308740 vs 22200, 637-1241800, P=0.18, R.I. 0-1200) and uNGAL to creatinine ratio (uNGAL/C x10<sup>3</sup> 159, 1.4-545 vs 40, 0.5-2057, P=0.33, R.I. 0-0.4).

Patterns of tubular damage and dysfunction in dogs with leptospirosis resemble the ones reported in humans. However, they are not disease-specific, as neither FE of electrolytes nor uNGAL were able to feature the infection.

#### Disclosures

No disclosures to report.

# ESVNU-P-10

#### Big endothelin-1 in cats with CKD: preliminary evaluation

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In human medicine the concentration of serum endothelin-1 (ET-1) increases in hypertension and CKD. Also urinary ET-1 correlates with the severity of renal disease and the magnitude of proteinuria. In dogs, increased concentration of ET-1, evaluated indirectly by the precursor Big Endothelin-1 (big-ET1), seems to be associated with the severity of CKD and hypertension.

The aim of this study was to gain information about serum and urinary levels of big-ET1 in cats with CKD, with and without hypertension and proteinuria.

Big-ET1 was measured with a solid phase sandwich ELISA developed for human big-ET1 (IBL international GmbH, Hamburg, Germany).

Twenty serum samples and 69 urinary samples obtained from 42 cats at different IRIS stages (12 sampled once, 24 sampled twice and 3 sampled thrice during a 6-12 months follow up) were assessed. The kit used to measure Big-ET1 was validated in urine (UBig-ET1) but not in serum since almost all the serum samples failed to yield results above the detection limit of the method. Results from the different groups of cats were statistically compared using Mann-Whitney U and Kruskal-Wallis tests.

Big-ET1 was virtually absent from most of the serum samples. UBig-ET1 and UBig-ET1/UC ratio were significantly positively correlated with serum creatinine (p=0.046; p=0.007).

in 13.6% and low in 86.4% of the animals. Pathological concentrations of microalbuminuria (>100 mg/L) were present in 22.7% of the dogs, while A:C ratio was above reference range (>0.2) in 36.3% of them. When the UP/C ratio was evaluated, 27.3% of the dogs were borderline proteinuric (0.2-0.5) and 18.2% presented overt proteinuria (>0.5). Serum urea and creatinine were within normal ranges in all dogs. Dogs with microfilaremia, high parasite burden and pulmonary hypertension showed higher incidence of abnormalities in the urine reactive strips. Also, microalbumin, A:C ratio and UP/C ratio were higher in these dogs, although the differences were not statistically significant for any of the results.

The study shows evidences of kidney damage in dogs with heartworm, and confirms that the measurement of urinary parameters was more sensible for the early detection of renal alterations and the best feasible assessment of clinically significant renal alterations, so the use of this methods should be considered as the first choice to evaluate the renal status in dogs infected by D. immitis. Although urinary parameters were higher in dogs with clinical alterations, the results were not significant; a larger sample should be studied to determine the influence of microfilaremia, parasite burden or pulmonary hypertension.

#### Disclosures

No disclosures to report.

# ESVNU-P-12

# Evaluation of acute kidney injury in dogs with Babesia rossi

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Dogs with babesiosis can present with multiple complications, including acute kidney injury (AKI). Our aim was to characterize AKI in Babesia rossi-infected dogs at presentation and after treatment, using routine and novel biomarkers of kidney injury and dysfunction.

Thirty-five dogs with Babesia rossi-infections and 10 healthy control dogs were enrolled in this prospective observational study. Blood and urine were collected in both groups at presentation (T0), in 10 Babesia-infected dogs after 24 hours (T24h), and in 7 dogs after 1 month (T1m). Routine renal biomarkers included serum creatinine (sCr) and urinary protein:creatinine (UPC). Novel kidney injury biomarkers, as determined by validated immunoassays, included urinary immunoglobulin G (ulgG), C-reactive protein (uCRP), retinol-binding protein (uRBP), urinary and plasma neutrophil gelatinase-associated lipocalin (u/pNGAL). Urinary biomarkers were normalized to urinary creatinine. Serum symmetric dimethylarginine (SDMA, IDEXX SDMA<sup>TM</sup> Test) was measured as a novel functional renal biomarker. Mann-Whitney tests compared biomarkers between dogs with and without babesiosis. Wilcoxon signed-rank tests compared biomarkers between T0, T24h, and T1m in infected dogs. P-values <0.05 were considered significant.

All urinary biomarkers and pNGAL were significantly higher in Babesia-infected dogs compared to healthy controls, while sCr and SDMA were not different. At T24h, UPC, uRBP, and uNGAL decreased significantly. Decreases were significant for all urinary biomarkers at T1m, reaching values not different from healthy controls. Significant changes in sCr, SDMA, and pNGAL were not seen after treatment. Babesia rossi-infected dogs showed transient kidney injury, which was detected by all urinary biomarkers and pNGAL, but remained undetected by functional biomarkers.

#### Disclosures

No disclosures to report.

#### ESVNU-P-13

#### Salt and sugar in your larder make your kidneys work harder

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Osmotic diuresis is the currently accepted mechanism of polyuria in diabetes mellitus and ostensibly leads to polyuria-mediated functional volume depletion. Consequently, a compensatory increase in glomerular filtration rate (GFR) is expected. The latter has not been documented, and we hypothesized that glucosuria would increase GFR and aimed to measure the GFR in a model of isolated renal glycosuria. We randomized eight cats in a crossover design to receive 10 mg dapagliflozin (a type-2 sodium glucose transporter inhibitor) or sham-treatment over four. 5-day treatment periods that were separated by 7-day washout periods. We assessed GFR and total body water content (TBWC) via iohexol clearance and deuterium-oxide tracer dilution method, respectively. The study had a power of 90% to detect a difference of 20±15 mL in daily urine output. We analyzed the results with a mixed effect model that included treatment and period of treatment as fixed effects. To control for the effect of repeated measures on the same cat, a random intercept for cat and an autoregressive process of order-1 correlation structure were fitted. Statistical significance was set at 0.05. Dapagliflozin induced profound glucosuria without an increase in daily urine output. The mean ( $\pm$ SE) iohexol clearance was 3.5 $\pm$ 0.3 and 4.2 $\pm$ 0.2 for the nontreated and sham-treated cats, respectively (P 0.003). The mean (±SE) TBWC, normalized to body weight, was 65.1±2.4% and 64.1±1.2%, for the sham-treated and treated cats, respectively (P 0.43). We conclude that in this model, the observed increase in GFR is associated with a concomitant preservation of TBWC.

# **Disclosures**

No disclosures to report.

# ESVONC-P-1

#### Expression of prostaglandin ep4 receptor in canine osteosarcoma

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Inflammation mediated by the cyclooxygenase enzymes supports the development of cancer by stimulation of angiogenesis, inhibition of apoptosis, and promotion of cell proliferation and motility. These enzymes convert arachidonic acid to prostaglandins, of which the most active product is PGE2. This prostaglandin drives cell proliferation, apoptosis, and angiogenesis through interaction with its specific receptors (EP1 receptor - EP4 receptor [EP1R-EP4R]). In particular,

the expression of EP4R is associated with the development of malignancy and poor prognosis in multiple human cancers. Expression of EP2R has been confirmed in canine osteosarcoma. Characterization of the canine EP4R has been completed, however expression of EP4R in canine osteosarcoma has not been evaluated.

The aim of this study was to characterize the mRNA expression of EP4R in canine osteosarcoma.

RNA in situ hybridization (RNAscope®, ACDBio) was used to evaluate 11 canine osteosarcoma samples for mRNA expression of canine EP4R. RNAscope technology is a modification of in situ hybridization for detection of RNA in formalin-fixed, paraffin-embedded tissue sections. To quantify RNAscope signals in tissue sections, an advanced digital pathology image analysis system (HALO, Indica Labs) was utilized. This software allows for determination of target RNA copy number at the cellular level. Data was expressed as copy number for EP4R with that signal compared to the housekeeping target (B-actin, positive control gene). No ethical approval was obtained as this study did not involve a prospective evaluation and utilized tissue samples leftover following patient clinical treatment (amputation) or diagnostic (biopsy) procedures for osteosarcoma.

In 11/11 canine osteosarcoma samples evaluated, universal positive expression of EP4R was identified (copy number 0.1 to 2.6 on average per cell).

These results confirm the mRNA expression of canine EP4R by canine osteosarcoma cells. Subsequent studies will evaluate EP4R protein expression (via immunohistochemistry) and in vitro effect of an EP4R antagonist, grapiprant, on canine osteosarcoma cell lines.

This data is clinically relevant as therapeutic blockade of EP4R with an antagonist (Galliprant® [grapiprant tablets], approved for use in dogs in the U.S.) may be a novel treatment option for dogs with osteosarcoma.

#### Disclosures

Disclosures to report.

M. Musser Aratana Therapeutics, Inc. (co-distributor for EP4R antagonist, Galliprant, in the U.S.) - Receives research support (unrelated to Galliprant). Elanco (primary distributor for EP4R antagonist, Galliprant, in the U.S.) - Receives research support (related to Galliprant but for different type of cancer, transitional cell carcinoma of the urinary bladder). C. Johannes Aratana Therapeutics, Inc. (co-distributor for EP4R antagonist, Galliprant, in the U.S.) - Former employee, speaking engagements, advisory board, consultant, receives honorarium, travel and research support (all unrelated to Galliprant). Elanco (primary distributor for EP4R antagonist, Galliprant, in the U.S.) - Receives research support (related to Galliprant but for different type of cancer, transitional cell carcinoma of the urinary bladder).

# **ESVONC-P-2**

#### Methadone potentiates the effect of doxorubicin on a canine transitional carcinoma cell line

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In various human cancer cell lines, opioid receptor activation enhances the efficacy of antineoplastic drugs. Concurrently, doxorubicin increases opioid receptors, and methadone enhanced cellular doxorubicin uptake. The aim of this study was to investigate a potential synergism between the opioid receptor agonist methadone and doxorubicin on a canine cancer cell line.

Canine transitional cell carcinoma (cTCC) cells were incubated with various concentrations of methadone and doxorubicin each, in order to test clinically achievable dose levels. Inhibition of cell proliferation was measured over 72 hours with CCK-8 cell proliferation assay for each drug alone and different combinations. Opioid receptor density was assessed in flow cytometry in drug native and doxorubicin pretreated cells.

Naloxon-fluorescein-stained flow-cytometric analysis revealed the presence of opioid receptors in the cTCC cell line. The receptor density increased mildly upon 24 hours pretreatment with doxorubicin. As intended, methadon given at a dose of 3µg/ml did not inhibit proliferation. Doxorubicin at 0.5µg/ml inhibited proliferation at all timepoints. When cells were pretreated with doxorubicin, and methadone added after 24 hours, no potentiation took place. Conversely, when methadone was given 24 hours before doxorubicin, a tendency of potentiation of doxorubicin's inhibitory effect on proliferation was

Opioid-receptors are present in cTCC cells and may be increased by pretreatment with doxorubicin. There is a tendency that methadone potentiates the response to doxorubicin in cTCC.

It will be intriguing to investigate further cell lines from diseases clinically treated with doxorubicin with combinations of methadone.

# Disclosures

No disclosures to report.

# ESVONC-P-3

Clinical manifestations and response to the therapy of extragenital intranasal form canine transmissible venereal sarcoma(CTVT): retrospective study 11 dogs in Ukraine

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CTVT is a frequent tumor in dogs in Ukraine without age, sex and breed predisposition. CTVT is most often transmitted by sexual contact and affects the genitals. Extragenital CTVT can be located on the skin, in the nasal and oral cavity, metastasize lungs and regional lymph nodes. The largest previously published study of nasal CTVT included an analysis of 6 cases. The aim of the study was to analyze and describe the clinical manifestations and response to therapy in patients with the nasal form of CTVT. 11 dogs with a naturally occurring CTVT were included in a retrospective study. Patients entered treatment from different regions of Ukraine and received therapy at the Zoovetservis clinic in Kiew in 2013-2017. All patients entered with visualized neoplasms, the diagnosis was made on the basis of cytological (in 4 cases additionally histology) investigations. 8 males and 3 females were included in the study. All of them were not neutered at the time of CTVT detection. None of the patients had genital CTVT lesions, 3 patients had owner, 8 stray dogs had guardians, 2 dogs were breed (bichon, spaniel), 9 dogs were mixbreed. The clinical



symptoms of nasal TVT included: sneezing, epistaxis, unilateral or bilateral purulent hemorrhagic discharge from the nose (in all 11 patients), hard skull infiltration and the formation of oronosal fistulas were observed in 9 of 11 patients. In 5 patients, skin fistulas were observed on the back of the nose, all of them spontaneously disappeared after treatment with CTVT. None of the patients had a CTVT planum nasals lesion. All dogs received monochemotherapy with vincristine weekly at a dosage of 0.7 mg / m2. The course consisted of 4-9 cycles. In patients with oronasal fistulas sneezing and discharge were maintained until the resolution of fistulas with negative cytological control. None of the patients had hematologic toxicity requiring the transfer of chemotherapy. 11 dogs responded to therapy with complete clinical remission. In 10 dogs a year later there was no relapse. There is no information about one dog (3 months after the treatment there was no relapse).

Intranasal CTVT can deeply affect the nasal cavity and hard palate, with skin fistel nut without planum nasalis involvement and it is well amenable to therapy, regardless of the severity of the disease.

#### Disclosures

No disclosures to report.

N.Ignatenko has presented lectures sponsored by Bayer Ukraine, but Bayer has no involvement in this study.

#### **ESVONC-P-4**

# The role of electrochemotherapy in management of tumours of the head in dogs

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This is a retrospective study of the efficacy of electrochemotherapy (ECT) in 60 dogs with tumours of the head including the oral cavity. Patients were privately owned pets. Tumours treated were mast cell tumours (19, 18 of which Paitnik grade 2, one grade 1) squamous cell carcinomas (15), spindle cell tumours (11), malignant melanomas (5), ameloblastomas (2), histiocytomas (2), basal cell tumour (1), carcinoma (1), epulis (3), plasmacytoma (1). Cases were treated using Bleomycin (15000 iu/sq m) intravenously with electroporation using either a Cytopulse PA 2000 or Cytopulse Oncovet signal generator, 8 pulses of 1000 V/cm at either 1 Hz or 5MHz applied starting 8 minutes after drug administration. Treatments were applied under general anaesthesia. ECT was applied either as sole therapy (n=42), to tumour margins during surgery (intra-operative, n=8) or to surgical scars roughly 2 weeks after surgery (adjuvant, n=9) whilst one case was initially treated by sole therapy with repeat during surgery. Two patients received second treatments whilst 58 received only a single treatment. All treatments were applied using Gehl array electrodes, two rows of needles 6 mm apart. Intra-operative treatment was applied after cytoreduction with no attempt to excise margins: in some cases some macroscopic tumour was present in the treated volume. Mast cell tumour cases receiving sole therapy ECT showed 10/12 (83.3%) enduring CR and those receiving intra-operative or adjuvant ECT showed 6/7 (85.7%) disease-free at censoring. Response to ECT of squamous cell carcinoma showed dependency on site. Those in the premaxilla or external nares (n=3) showing no responses. In other (all oral) lesions (n=12), 7 CR (58.3%), PR 3 (25%) and 2 surgically removed before ECT. Of these, 1 CR recurred and was treated for a second time leading to disease-free state at censorship, 3 PR recurred. Results for spindle cell tumours were disappointing: one small fibrosarcoma showing CR after sole therapy and overall response (CR and disease control) 5/12 (41.6%). Ameloblastomas and epulides showed 100% CR after sole therapy (n=5). Melanomas gave relatively poor results (25% CR to sole therapy – n=4). ECT showed good results in mast cell tumours and fair results in oral squamous cell carcinomas not involving the rostral portion of the mouth. Cosmetic and functional results were excellent with some scarring apparent in cases where skin was treated. Healing of oral lesions, even where bone was exposed, was rapid and complete.

#### Disclosures

Disclosures to report.

Both authors work in practice where the techniques described are carried out for gain.

#### **ESVONC-P-5**

# Feasibility, safety and diagnostic yield of ultrasound-guided fine needle aspiration of cardiac masses in dogs

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Hemangiosarcoma and chemodectoma represent the most frequent types of cardiac masses. Other tumors or non-neoplastic cardiac masses mimicking neoplasia, have also been reported. Establishing a definitive diagnosis has important prognostic and therapeutic implications. Cardiac ultrasonography has good specificity and sensitivity for the detection of cardiac masses in dogs with pericardial effusion. However, reported agreement between ultrasound mass location and definitive histological diagnosis is only of 65% (50% for hemangiosarcoma and 80% for chemodectoma). Cytological examination of pericardial fluid is also of limited interest. Cardiac masses cytology is a potential diagnostic tool, but information about its safety is sparse and its diagnostic accuracy has not yet been evaluated.

The aims of this study were to evaluate the feasibility and safety of ultrasound-guided fine needle aspiration (FNA) of cardiac masses, and to determine the correlation between cytological and histopathological diagnosis.

Dogs diagnosed with a cardiac mass between February 2016 and October 2017 were included. Inclusion criteria were an anatomic location allowing sampling, and achievement of owner consent. Ultrasound-guided FNA were performed under sedation. During procedure, all dogs were monitored with simultaneous electrocardiogram and blood pressure measurement. Dogs were kept hospitalized during 8 hours following FNA. Slides cellularity and quality were assessed, and diagnostic yield was evaluated as the percentage of cases in which a cytological diagnosis could be achieved. Accordance with histopathology was determined when possible. Cytological and histopathological slides were reviewed blindly, respectively by one clinical pathologist, and two pathologists.

Thirteen dogs were included, with a mean weight of 22 kg (range 7 – 45 kg): ten had masses at the right atrio-ventricular wall and three at heart-base. Ultrasound-guided sampling was possible in all cases. Time



sampling ranged from 10 to 25 minutes. No complications were observed. Cellularity of FNA smears was good to excellent, permitting to reach a diagnosis in 100% of cases. Ten masses were diagnosed as sarcoma (suspected hemangiosarcoma) and three as endocrine tumors (suspected chemodectoma). Post-mortem histopathological diagnosis was available in 7 dogs (4 hemangiosarcoma and 3 chemodectoma) and confirmed the cytological diagnosis in all cases.

In our study, ultrasound-guided FNA of cardiac masses was feasible and safe; its diagnostic usefulness was good with achievement of high-quality samples and perfect accordance with histopathology. Cytological evaluation could be considered in their diagnostic approach.

#### Disclosures

No disclosures to report.

#### ISCAID-P-1

#### Diagnostics of canine Leishmania infection by ELISA and/or PCR-a comparative study

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Leishmaniosis is one of prevalent parasitic canine diseases widely spread in temperate zones. Canine leishmaniosis may manifest as subclinical illness, a self-limiting disease or severe and chronic disease with multiple organ involvement. Infected dogs are considered the main reservoirs for human visceral Leishmaniasis.

Our study was aimed to evaluate the relationship between the two current diagnostic methods of canine Leishmania infection, namely anti-Leishmania antibody (Ab) assay by ELISA (Enzyme-Linked Immunosorbent Assav) and Leishmania-DNA assav by PCR (Polymerase Chain Reaction). Blood samples and, when available, other materials (tissue, swabs) were obtained from the dogs with suspected Leishmania infections or history of importation/travels in Germany.

In 2016 and 2017, we analysed a total of 15449 and 17789 blood samples, respectively, for Leishmania Ab assays and a total of 712 and 786 samples, respectively, for Leishmania DNA assays. In 2016, overall 19.6% and 19.8% of the samples were Ab-positive and DNA-positive. In 2017, overall 21.9% and 18.6% of the samples were Ab-positive and DNA-positive, respectively. Specifically concerning the samples subjected to both PCR and Ab assays (129/712 in 2016; 145/786 in 2017), we observed the following Ab profiles. In 2016 and 2017, seropositivities (by Leishmania unit or LE >1.1) were 97.0% and 93.5%, respectively, in PCR+ cohorts, versus 31.6% and 32.5%, respectively, in PCR- cohorts. Notably, the PCR+/Ab+ cohorts showed Ab titres (mean) ~2 times higher than those of the PCR-/Ab+ cohorts (3.1 LE versus 1.7 LE). In summary, (1) PCR-positive samples were almost 100% Ab-positive, (2) PCR-negative samples were ~32% Ab-positive, and (3) in Ab-positive cases, PCR-positive samples had approximately twice higher Ab titres than PCR-negative samples.

Our data suggested that PCR-positivity may be considered as the diagnostics generally confirming active Leishmaniosis which represented, in our study, an overall 20% of the tested samples. Other ~32% of the PCR-negative/sero-positive samples might be the cases where persistent/residual antibodies were present due to exposition to the parasite or past Leishmania infection, regardless of the clinical status of dogs.

#### Disclosures

Disclosures to report.

The authours Breu D and Guthardt J are empleyed at Laboklin GmbH & Co KG, Germany. Müller E is owner/mangare of the Laboklin GmcH & Co KG.

#### ISCAID-P-2

# Clinical and laboratory findings of sixty naturally Babesia gibsoni infected dogs

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Babesia gibsoni is increasingly recognized as an important canine tickborne disease worldwide, however, only a few clinical studies of naturally acquired infection are available. Besides, most of the published reports were mixed with a distinct species, i.e. B. conradae, which was assorted from B. gibsoni since 2006 by phylogenetic analyses of the 18S rRNA and the ITS-2 genes. Ticks are considered the most important vectors, although the parasite can also be transmitted during dog fighting, in particular by Pit Bull terriers as reported in the USA and also in Romania.

In this retrospective study, we enrolled 60 dogs diagnosed with B. gibsoni infection confirmed by PCR at the National Taiwan University Veterinary Teaching Hospital between January 2014 and December 2015. The average age of 60 dogs was 6.36  $\pm$  3.78 (95% CI, 5.39-7.34) years old. The 60 dogs included 35 males (58.3%) and 25 females (41.7%). Seventeen different breeds were included and the most observed species was mongrel (25.8%), followed by Maltese (16.1%), Miniature poodles (12.9%), Miniature schnauzer (8.1%), and Beagle (8.1%). None of the dogs were associated with dog fighting. The most common clinical signs observed in dogs were inappetence, apathy, pale mucous membranes, discoloration of urine, splenomegaly and hyperthermia. The predominant hematological abnormality was anemia (49 out of 60 dogs) (81.7%). The severity of anemia ranged from 18.3 % (11 of 60 dogs), 11.7 % (7/60), 30 % (18/60), 21.7 % (13/60) and 18.3 % (11/60) corresponding to the category of nonanemia (HCT >37), mild (30 ≤ HCT <37), moderate (20 ≤ HCT <30), severe (13 ≤ HCT <20), and very severe (HCT <13), respectively. Seventy percent of the dogs (42/60) carried an HCT of less then 30, whereas 40% (24/60) of the dogs had a life-threatening HCT below 20. Regenerative anemia was observed in 66.7% (38/57), whereas non-regenerative anemia occurred in 32.2% (11/57) of the cases, respectively. Thrombocytopenia was a further common hematological abnormality (37/60) (61.7%). The main biochemical abnormalities were hyperglobulinemia (28/53) (52.8%), hyperbilirubinemia (10/28)(35.7%) and elevated hepatic enzyme activities (7/48) (14.6%). In conclusion, the results of this study provide the detail clinical profiles of B. gibsoni infection in sick dogs. Infections through a tick bite play the important role in Taiwan.

# **Disclosures**



#### ISCAID-P-3

#### Blood Cultures and the detection of multidrug-resistant bacteria in companion animals

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The detection of bacteraemia through blood culture is essential for the definitive diagnosis and correction of the empirical therapy of sepsis. Sepsis is often associated with infections in the oral cavity, urogenital tract, skin, soft tissues, abdomen, respiratory tract, gastrointestinal tract and bone. The aim of this study was the early detection of bacteraemia namely multidrug-resistant bacteria (MDR) in the dog and cat associated with sepsis.

Between 2015 and 2017, 41 samples from dogs (n=35) and cats (n=6) were submitted for blood culture from several veterinary hospitals and clinics to the Genevet Laboratory. The samples were collected according to the proper asepsis rules (trichotomy and disinfection of the venipuncture site and bottle extremity), and blood was inoculated in a paediatric aerobic blood culture bottle. Bacteria were isolated after 24h of growth in the haemoculture bottles and then grew on selective media for methicillinresistant Staphyloccoccus spp. (MRS) and extended-spectrum betalactamases producer (ESBL) Enterobacteriaceae. Antimicrobial susceptibility testing was performed by the disc diffusion method and/or minimum inhibitory concentration. CLSI clinical breakpoints were applied.

From 41 blood cultures, 56% (23/41) were positive. In 3 blood cultures 2 bacteria strains were isolated. Diverse bacterial agents were found, mainly Staphyloccoccus spp. (8/27, 30%), Serratia marcescens (5/27, 19%), Klebsiella spp. (5/27, 19%), among others. The most frequent antimicrobial resistance was towards amoxicillin/clavulanic acid (18/27, 67%), tetracycline (14/26, 54%), trimethoprim/sulfamethoxazole (12/26, 46%) and fluoroguinolones (11/26, 42%). There was a high number of multidrugresistant isolates, i.e., resistant to more than 3 different antimicrobial classes (15/27 isolates, 56%) in the processed blood cultures. It should also be noted the isolation of 2 MRS (one methicillin-resistant Staphylococcus aureus and one methicillin-resistant Staphylococcus pseudintermedius, 2/27 isolates 7,4%) and 5 ESBL producer Enterobacteriaceae resistant to third generation cephalosporins (5/27 isolates, 18,5%).

In this study a high number of positive blood cultures was detected. The detection of multidrug-resistant, MRS or ESBL producer bacteria is a cause for great concern, with inherent therapeutic limitations. This study illustrates that blood cultures are a key tool for the early diagnosis of sepsis as well as its oriented antimicrobial therapy.

# Disclosures

No disclosures to report.

# ISCAID-P-4

# Use of neutrophil gelatinase-associated lipocalin (NGAL) in the early diagnosis of renal damage in canine leishmaniasis

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Diagnosis of chronic kidney disease (CKD) is usually achieved using urea and creatinine. However, it is well recognized that the disease is usually present before these biomarkers are elevated and other biomarkers such as neutrophil gelatinase-associated lipocalin(NGAL) have been designed for earlier diagnosis. NGAL has been linked to tubular damage. NGAL has been observed to be altered in dogs with acute renal failure and idiopathic CKD. Canine leishmaniasis (CL) is a highly prevalent disease in many mediterranean countries. The kidney is one of the organs affected and evaluation of kidney function by the assessment of creatinine, urea and the degree of proteinuria together with the presence and severity of clinical signs are the key aspects for disease classification (LeishVet classfication) and therapeutic guidelines.

The aim of this study was to evaluate whether dogs with CL with absence of azotaemia may suffer early renal damage (especifically tubular) and whether plasma or urine NGAL may be a potential useful biomarker in the characterization of dogs with CL.

Ethical approval and owners consent to use blood and urine samples from all dogs was granted. Three groups of dogs were established: Group 1 (G1) included 5 healthy dogs from staff members; Group 2 (G2) included 10 dogs with CL and CKD (Stage 3 and 4 LeishVet) and Group 3 (G3) included 10 dogs with positive quantitative serology, mild or absent clinical signs, mild or absent proteinuria (UPC <1) and absence of azotemia (Stage 1 and 2 LeishVet). Hematology, biochemistry and plasma NGAL were measured in blood. A full urine analysis including urinary protein/creatinine ratio, and measurement of urine NGAL was also carried out. NGAL was measured using the canine NGAL Elisa-kit (Abcam, USA) and results are expressed as NGAL/creatinine ratio.

Plasma NGAL was not significantly different between groups G1 (12.7  $\pm$  8.0 ng/mL) and G2 (14.3  $\pm$ 26.2 ng/mL; p>0.05) but was different between group G3 and the others G3 (59,00±48,76 (p<0,01). Urine NGAL in G2 was elevated (6.742.561,4  $\pm$  4.671.515,4 ng/g; p<0.001) when compared with G1 (1497,4  $\pm$  831,5 ng/g; p<0.001). In G3, urinary NGAL was also increased (142.245,8 ± 314.458,3 ng/g) compared to the other two groups (p<0.001).

In conclusion, urine but not plasma NGAL may be use as a marker of early renal damage in CL. The study also provides further evidence that CL dogs in LeishVet stage I and II may suffer from early renal damage.

#### Disclosures

No disclosures to report.

# ISCAID-P-5

# Seroepidemiology of feline leukemia virus (felv) and feline immunodeficiency virus (fiv) in cats in Greece

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Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are retroviruses causing significant morbidity and mortality in cats. Epidemiological data are necessary to design optimal strategies for the management and prevention of the infection by these viruses. Unfortunately, limited data are available on the seroepidemiology of retroviral infections of cats in Greece. The aim of this study was to determine the seroepidemiology of FeLV and FIV infections in different populations of cats from Greece.

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A total of 349 cats were prospectively included and divided into three groups (client-owned, stray and cats living in shelters or catteries). Signalment and historical data were collected using a standardized questionnaire and a thorough physical examination was performed for each cat. Blood samples were obtained by jugular venipuncture and serum was stored at -80°C until analysis. Seroprevalence for FeLV and FIV was determined using the SNAP FIV/FeLV Combo<sup>TM</sup> test (IDEXX) and was compared among the 3 groups, between males and females, between intact and neutered males or females, between clinically healthy and sick cats and between cats presenting or not at least one clinical sign compatible with FeLV and/or FIV ( $\chi^2$  test or Fischer's exact test) as well as for possible association with the age of the cats (student's t-test).

Of the 349 cats, 16 (4.6%) and 33 (9.5%) were found to be seropositive for FeLV and FIV, respectively. The risk of seropositivity for FeLV was significantly higher in sick than in clinically healthy cats (P=0.04) and in cats with at least one clinical sign compatible with FeLV (P=0.048). The risk of seropositivity for FIV was significantly higher in male than in female cats (P=0.003), in neutered than in intact male cats (P=0.005), in older cats (P=0.002), in sick than in clinically healthy cats (P =0.039) and in cats with at least one clinical sign compatible with FIV (P=0,007).

Due to the high prevalence of seropositivity for FeLV and FIV in the Greek feline population, it is necessary to define prophylactic and management strategies taking into particular consideration those groups of cats that are at increased risk of seropositivity and thus of infection.

#### Disclosures

No disclosures to report.

#### ISCAID-P-6

# Clinical Picture of Cats Seropositive for Dirofilaria immitis in a Hyperendemic Area: Is this Feline Disease Still Being Misdiagnosed?

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Feline dirofilariasis (Dirofilaria immitis) has progressively regained importance through the years due to a heightened awareness of the disease in this species and scientific progress which provided better diagnostic tools. However, the diagnosis is challenging and presents a huge difficulty, mainly due to non-specific or absent symptoms, and presence of low parasite burden or immature worms.

The objective was to determine the incidence of symptoms, presence of feline virus as well as radiographic and echocardiographic abnormalities in cats seropositive to D. immitis.

Blood samples from 146 cats living in a hyperendemic area were tested for the presence of anti-D. immitis antibodies, feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV). Antibodies test was positive in 32.9% of the cats; of them, 25% showed compatible symptoms of dirofilariosis versus 16.3% of the seronegative cats (P<0.05). Regarding feline virus, 5.5% were positive to FIV and 13.7% to FeLV. No statistically significant correlation was found between D. immitis seropositivity and presence of feline virus.

Half of the D. immitis seropositive cats (n=24) were further submitted to thoracic radiographies and echocardiography. Of them, lung radiographic changes compatible with heartworm were present in 79.2% of the cats (bronchial pattern, unstructured interstitial pattern, vascular pattern with enlargement of pulmonary arteries, lung hyperinflation) but no abnormalities were present in the cardiac silhouette in any of the animals. Radiographic abnormalities were displayed in 100% of symptomatic cats and 68.7% of the asymptomatic cats (P<0.05). Presence of adult worms was observed by echography in 6.25% (n=3) of the cats; of them, 2 were asymptomatic and 1 presented normal thoracic radiographies. One cat was indoor, one cat was outdoor and one cat was indoor/outdoor.

The results show the importance of image diagnosis to help in the correct diagnosis of feline dirofilariosis, indistinctly of the presence or absence of symptoms. Furthermore, a greater awareness of feline dirofilariosis is needed in cats, stating prophylaxis in kittens from 6-8 weeks. D. immitis infection should be included in the differential diagnosis of cats showing compatible clinical signs, and a combination of serological and image diagnosis techniques should be performed when feline heartworm is suspected, for a correct diagnosis or to properly rule out the disease.

#### **Disclosures**

No disclosures to report.

#### SCH-P-1

# Differentiation of different types of feline liver disease by clinical features, cytology and laboratory test results

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Feline liver diseases consist of different disease entities such as cholangiohepatitis (CG), hepatic lipidosis (HL), and neoplasia with lymphoma (L) and other neoplasia (ON) are a common feature in cats with variable clinicopathologic features, requiring different therapeutic strategies. Endoscopic liver biopsy is the current gold standard to establish a diagnosis. Owner compliance is limited because of invasiveness so that refinement of diagnostic criteria obtained by minimally invasive methods are needed to ensure best possible patient care.

Aim of this retrospective study was the identification of useful clinical and laboratory features to differentiate between inflammatory, metabolic and neoplastic hepatic disease.

Records of 196 cats (2010-2016) were retrospectively evaluated. Inclusion criteria were full physical exam, complete laboratory profile, fine needle aspiration (FNA) of liver and gallbladder and FNA bacterial culture. According to results and response to treatment, patients were allocated to 7 groups lymphoma (L), mixed cholangitis (MC), neutrophil cholangitis (NC), lymphoplasmacellular cholangitis (LPC), hepatic lipidosis (HL), other neoplasias (ON) and miscellaneous. Inferential statistics was performed to look for significant differences between groups, p< 0.05 was considered statistically significant.

Age, body temperature, neutrophilia, left shift and monocytosis were identified as potentially useful discriminators the disease entities with a p < 0.05 compared between the groups.

#### **Disclosures**