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ABSTRACT

# Research Communications of the 31st ECVIM-CA Online Congress

1-4 September 2021

*The European College of Veterinary Internal Medicine – Companion Animals (ECVIM-CA) Congress and the Journal of Veterinary Internal Medicine (JVIM) are not responsible for the content or dosage recommendations in the abstracts. The abstracts are not peer reviewed before publication. The opinions expressed in the abstracts are those of the author(s) and may not represent the views or position of the ECVIM-CA. The authors are solely responsible for the content of the abstracts.*

## LIST OF ORAL RESEARCH COMMUNICATIONS

### ESCG – European Society of Comparative Gastroenterology

Friday 3 September

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|---|---|---|---|
| 14.25-14.40 | ESCG-O-1 | Bernard | Comparative analysis of the distribution and severity of the lesions within the digestive tract in feline low-grade intestinal T-cell lymphoma or lymphoplasmacytic enteritis |
| 14.40-14.55 | ESCG-O-2 | Da Riz | Short-term survival and associated factors after surgical intestinal biopsies in cats with chronic enteropathy |
| 14.55-15.10 | ESCG-O-3 | Wu | Identification of bacteria in pancreas, liver, and bile of apparently healthy cats using next generation 16S rRNA sequencing and standard bacteriological culture |
| 15.10-15.25 | ESCG-O-4 | Collier | Investigating fecal microbial transplant in dogs with inflammatory bowel disease: A pilot study |
| 15.25-15.40 | ESCG-O-5 | Toresson | Clinical effects of fecal microbiota transplantation in dogs with chronic enteropathy |
| 15.40-15.55 | ESCG-O-6 | Kuijlaars | Faecal bile acid profiles in dogs with chronic enteropathies versus healthy controls |
| 15.55-16.10 | ESCG-O-7 | Sung | Fecal fatty acid, cholesterol, and bile acid concentrations in cats with chronic enteropathy |
| 16.30-16.45 | ESCG-O-8 | Walker | Metabolomic serum profiling in dogs with chronic enteropathy |
| 16.45-17.00 | ESCG-O-9 | Csukovich | Taking the next step: Modelling infectious diseases in canine intestinal organoids |
| 17.00-17.15 | ESCG-O-10 | Thomson | Retrospective analysis of the association between hepatic pathology and DGGR lipase in canines with histologically normal pancreas |
| 17.15-17.30 | ESCG-O-11 | Méric | Colorectal polypoid masses in dogs: Multicentre retrospective study of 53 cases |
| 17.30-17.45 | ESCG-O-12 | Dupont | Suspected acute hemorrhagic diarrhea syndrome in out-patients: A preliminary study of disease severity, treatment, outcome and client satisfaction |
| 17.45-18.00 | ESCG-O-13 | Tamura | Low-dose oral cobalamin supplementation ameliorated in serum cobalamin concentrations in dogs with chronic enteropathy when compared with small cell gastrointestinal lymphoma |
| 18.00-18.15 | ESCG-O-14 | Muradas | Assessment of visceral pain in dogs with chronic enteropathy and its' effect on behaviour and owner-observed quality of life |

**ESVC – European Society of Veterinary Cardiology**

Thursday 2 September

| | | | |
|---|---|---|---|
| 09.15-09.30 | ESVC-O-1 | Bagardi | Circulating miRNAs as potential biomarkers of early myxomatous mitral valve disease in Cavalier King Charles Spaniels |
| 09.30-09.45 | ESVC-O-2 | Wesselowski | Use of physical examination, electrocardiography, radiography and biomarkers to predict stage B2 myxomatous mitral valve disease in preclinical Cavalier King Charles Spaniels |
| 09.45-10.00 | ESVC-O-3 | Grosso | Prognostic significance of different echocardiographic parameters of left atrial size in dogs with asymptomatic myxomatous mitral valve disease |
| 10.00-10.15 | ESVC-O-4 | Partington | The effect of obesity and subsequent weight loss on cardiac structure and function in dogs |
| 10.15-10.30 | ESVC-O-5 | Safian | Performance of different echocardiographic measurements of left atrial size by observers with different levels of experience |
| 11.20-11.35 | ESVC-O-6 | Hezzell | Teaching vets to be EPIC: Validation of a focussed echocardiographic training program for general practitioners - the FEET-FIRST Study |
| 11.35-11.50 | ESVC-O-7 | Giraud | Evaluation of point-of-care ultrasound performed by non-cardiologists for diagnosis of degenerative mitral valve disease in dogs presented to the cardiology service |
| 11.50-12.05 | ESVC-O-8 | Saponaro | A new auscultatory finding confirmed by phonocardiography in cats with obstructive hypertrophic cardiomyopathy |

Friday 3 September

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|---|---|---|---|
| 09.15-09.30 | ESVC-O-9 | Malcolm | Reproducibility and repeatability of radiographic measurements of cardiac size in dogs |
| 09.30-09.45 | ESVC-O-11 | Kuo | An international survey of preferences for echocardiographic assessment of left atrial size in dogs: The BENEFIT Project |
| 09.45-10.00 | ESVC-O-12 | Lekane | Left Ventricular Eccentricity index for assessment of precapillary pulmonary hypertension in dogs |
| 10.00-10.15 | ESVC-O-13 | Oliveira | Continuous heart rate monitoring by Holter and validation of heart rate spot-checks to measure circadian variation in heart rate in dogs with atrial fibrillation |
| 10.15-10.30 | ESVC-O-14 | Saponaro | Holter characterization of ventricular and supraventricular arrhythmia in English bulldogs with presumed arrhythmogenic cardiomyopathy before specific antiarrhythmic therapy (94 cases) |
| 11.20-11.35 | ESVC-O-15 | Ward | Renin-angiotensin-aldosterone system activity in cats with systemic hypertension or cardiomyopathy |
| 11.35-11.50 | ESVC-O-16 | Fidanzio | Effect of the sampling time on urinary electrolytes following furosemide administration in dogs with myxomatous mitral valve disease |
| 11.50-12.05 | ESVC-O-17 | Patata | Echocardiographic variables predictive of clinical signs in dogs with pulmonary stenosis |
| 12.05-12.20 | ESVC-O-18 | Nisini | Bioelectrical Impedance Analysis in dogs with right congestive heart failure before and after treatment: A pilot study |
| 12.20-12.35 | ESVC-O-19 | Reimann | Platelet aggregation response to pimobendan in Cavalier King Charles Spaniels with myxomatous mitral valve disease |
| 12.35-12.50 | ESVC-O-20 | Franchini | The Longitudinal Outcome Of Canine (K9) myxomatous mitral valve disease (LOOK-Mitral) registry: Baseline treatment characteristics |

**ESVCN – European Society of Veterinary Comparative Nutrition**

Wednesday 1 September

| | | | |
|---|---|---|---|
| 12.05-12.20 | ESVCN-O-1 | Steffen | High intake of sodium chloride for 28 days causes no effect on serum FGF-23 concentrations in cats |
| 12.20-12.35 | ESVCN-O-3 | German | Beyond the scale: A retrospective observational study of cats and dogs presenting to a referral centre with severe obesity |

**ESVE – European Society of Veterinary Endocrinology**

Saturday 4 September

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|---|---|---|---|
| 08.15-08.30 | ESVE-O-1 | Tardo | Prospective evaluation of the prevalence of eunatraemic, eukalaemic hypoadrenocorticism in dogs with chronic gastrointestinal signs and risk of misdiagnosis in dogs with previous glucocorticoid administration |
| 08.30-08.45 | ESVE-O-2 | Aguiar | Development and characterisation of feline thyroid organoids as an in vitro model for feline hyperthyroidism research |
| 08.45-09.00 | ESVE-O-3 | Scheemaeker | Organoids of canine medullary thyroid carcinoma and feline thyroid adenomatous hyperplasia |
| 09.00-09.15 | ESVE-O-4 | Bree | Multicentre retrospective review of clinical features and short-term follow-up of 110 cases of canine primary hypoparathyroidism |
| 09.15-09.30 | ESVE-O-5 | Corsini | A prediction tool for diagnosis of canine hypothyroidism in clinical practice |

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| 09.30-09.45 | ESVE-O-6 | Carvalho | Diagnosis of naturally-occurring hypercortisolism by primary care veterinarians: A western European survey |
| 09.45-10.00 | ESVE-O-7 | Bunn | Association of proteinuria with fasting hypertriglyceridaemia and hyperadrenocorticism in Australian Miniature Schnauzers |
| 10.00-10.15 | ESVE-O-8 | Schofield | Role of internal medicine specialists in disseminating the evolving evidence base on Cushing's syndrome |
| 10.15-10.30 | ESVE-O-9 | Da Riz | Fibroblast Growth Factor-23 and phosphate metabolism in dogs with spontaneous hyperadrenocorticism (HAC) |
| 11.05-11.20 | ESVE-O-10 | Golinelli | Evaluation of clinical, ultrasonographic, and clinicopathological findings in dogs with pituitary-dependent hypercortisolism and poor trilostane response |
| 11.20-11.35 | ESVE-O-11 | Rapastella | Outcome in dogs with and without hyperadrenocorticism undergoing radiotherapy for pituitary macroadenomas |
| 11.35-11.50 | ESVE-O-12 | Miceli | Cabergoline treatment for feline hypersomatotropism |
| 11.50-12.05 | ESVE-O-13 | Callegari | Proteomic analysis in serum of cats with diabetes mellitus |
| 12.05-12.20 | ESVE-O-14 | Nivy | Effect of periodontal treatment on glycemic control in canine diabetic patients: A prospective, clinical study |
| 12.20-12.35 | ESVE-O-15 | del Baldo | the usefulness of different freestyle libre-derived metrics in assessing glycemic control in diabetic dogs |
| 12.35-12.50 | ESVE-O-16 | Jaffey | Effects of calcitriol on leukocyte cytokine production in dogs with diabetes mellitus |

**ESVIM – European Society of Veterinary Internal Medicine**

Wednesday 1 September

| | | | |
|---|---|---|---|
| 08.00-08.15 | ESVIM-O-1 | Lebastard | Association between bronchoalveolar lavage fluid quantitative bacterial culture results and antibiotic requirement in dogs with lower respiratory tract signs |
| 08.15-08.30 | ESVIM-O-2 | Gareis | Evaluation of pulmonary function by whole-body plethysmography for therapy monitoring in cats with chronic bronchial disease |
| 08.30-08.45 | ESVIM-O-3 | Fastrès | Serum and bronchoalveolar lavage fluid concentration of osteopontin and fibronectin in West Highland white terriers either affected with canine idiopathic pulmonary fibrosis or healthy and other terriers non predisposed to the disease |
| 09.00-09.15 | ESVIM-O-4 | Carluen | Incidence and characterization of penetration and aspiration in dogs using videofluoroscopic swallow studies |
| 09.15-09.30 | ESVIM-O-5 | Rodrigues | Antimicrobial discontinuation in dogs with acute aspiration pneumonia based on normal C-Reactive Protein and clinical improvement |
| 09.30-09.45 | ESVIM-O-6 | Machiels | Study of the use of IDEXX PROCYTE for total and differential cellular counts of bronchoalveolar lavage fluid in healthy dogs |
| 09.45-10.00 | ESVIM-O-7 | Kouki | Bile acids in saliva of dogs with respiratory diseases and of healthy dogs pre- and post-feeding |
| 10.00-10.15 | ESVIM-O-8 | Biénès | Effect of bronchoalveolar lavage on lung ultrasound and radiography in healthy dogs |
| 10.15-10.30 | ESVIM-O-9 | Lyssens | Gentamicin concentrations in bronchoalveolar lavage and serum in healthy dogs after inhalation therapy |
| 10.30-10.45 | ESVIM-O-10 | Biénès | Gentamicin concentration in nasal lavage in healthy dogs after inhalation therapy |
| 11.20-11.35 | ESVIM-O-11 | Vangrinsven | Comparison of culture- dependent and -independent methods on nasal swabs in dogs with nasal discharge |
| 11.35-11.50 | ESVIM-O-12 | Vangrinsven | Alterations of the nasal microbiota in dogs with sinonasal aspergillosis before and after cure and comparison with chronic idiopathic rhinitis |
| 11.50-12.05 | ESVIM-O-13 | Idalan | Comparison of immunohematological diagnostic tests including six different Coombs' test methods in dogs suspected to have immune-mediated hemolytic anemia |
| 12.05-12.20 | ESVIM-O-14 | Bouzouraa | Diagnostic utility of C-reactive protein on plasma and abdominal fluid in dogs with ascites |
| 12.20-12.35 | ESVIM-O-15 | Work | Shar Pei auto-inflammatory disorder (SPAID) in the United Kingdom - a retrospective survey |
| 12.35-12.50 | ESVIM-O-16 | Broughton | Diagnoses and outcomes associated with ionised hypercalcaemia in a referral population of cats |

Thursday 2 September

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|---|---|---|---|
| 08.00-08.15 | ESVIM-O-17 | Tumbarello | A prospective randomized trial of methylprednisolone with or without cyclosporine or mycophenolate mofetil for the treatment of immune-mediated haemolytic anaemia in 43 dogs |
| 08.15-08.30 | ESVIM-O-18 | Moretto | Suspected hypertensive encephalopathy in cats with systemic hypertension |

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| 08.30-08.45 | ESVIM-O-19 | Lamminen | Pregabalin alleviates feline anxiety and fear during transport and veterinary visits – a clinical field study |
| 08.45-09.00 | ESVIM-O-20 | Bunn | Prevalence and causes of fasting hypertriglyceridaemia in Australian Miniature Schnauzers |

**ESVNU – European Society of Veterinary Nephrology and Urology**

Friday 3 September

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|---|---|---|---|
| 09.45-10.00 | ESVNU-O-1 | Kongtasai | Urinary liver-type fatty acid-binding protein in cats with International Renal Interest Society (IRIS) stage 1 chronic kidney disease within a healthy elderly cohort |
| 10.00-10.15 | ESVNU-O-2 | Tang | Risk factors and implications associated with renal mineralisation in feline chronic kidney disease (CKD) |
| 10.15-10.30 | ESVNU-O-3 | Lippi | Erythrogram patterns in chronic kidney disease of dogs |
| 11.20-11.35 | ESVNU-O-4 | Scarpa | Evaluation of the clinical efficacy of benazepril in the treatment of renal proteinuria in dogs |
| 11.35-11.50 | ESVNU-O-5 | Rimer | Acute kidney injury in dogs: Etiology, clinical and clinicopathologic findings, prognostic markers, and outcome |
| 11.50-12.05 | ESVNU-O-6 | Brans | Effect of storage conditions and measurement device on serum Symmetric dimethylarginine in cats and dogs |
| 12.05-12.20 | ESVNU-O-7 | Harrer | Bacterial urinary tract infection and subclinical bacteriuria in dogs receiving chemotherapy: A prospective observational longitudinal clinical study |
| 12.35-12.50 | ESVNU-O-9 | Duperrier-Simond | Occurrence of cardio-vascular events in cats with acute urinary tract obstruction |

**ESVONC – European Society of Veterinary Oncology**

Saturday 4 September

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|---|---|---|---|
| 09.15-09.30 | ESVONC-O-1 | Troedson | Change of feline injection site sarcoma incidence and localization within the last 30 years |
| 09.30-09.45 | ESVONC-O-2 | Chalfon | An update on environmental risk factors for the development of feline oral squamous cell carcinoma |
| 09.45-10.00 | ESVONC-O-3 | Faroni | Possible association between anesthesia and recurrence in dogs with medium/large B-cell lymphoma in complete remission after chemo – immunotherapy |
| 10.00-10.15 | ESVONC-O-4 | Gedon | BRAF mutation status and its prognostic significance in 79 canine urothelial carcinomas: A retrospective study (2006-2019) |
| 11.20-11.35 | ESVONC-O-6 | Mason | Outcomes of Dogs with Anal Sac Gland Carcinoma Treated With Surgery and Adjunctive Radiotherapy in Ten 3.6Gy Fractions |
| 11.35-11.50 | ESVONC-O-7 | Kritsotalaki | Evaluation of outcome and toxicity in dogs undergoing "quad shot" radiation therapy for anal gland adenocarcinoma: A single center retrospective study of 17 cases |
| 11.50-12.05 | ESVONC-O-8 | Lappalainen | High–grade feline gastrointestinal lymphoma in 43 cases: One treatment does not fit them all |
| 12.05-12.20 | ESVONC-O-9 | Marconato | A phase 2, single-arm, open-label clinical trial on adjuvant active immunotherapy in dogs with appendicular osteosarcoma undergoing amputation and chemotherapy |
| 12.20-12.35 | ESVONC-O-10 | Busser | Impact of a 10% dose reduction and length of treatment delays in the management of chemotherapy-induced neutropenia in dogs: A single-centre experience |
| 12.35-12.50 | ESVONC-O-11 | Chavalle | Pharmacokinetic study of doxorubicin in cancer-bearing dogs: Validation of a simple high-performance liquid chromatography (HPLC) method in 10 dogs |

**ISCAID - International Society for Companion Animal Infectious Diseases**

Thursday 2 September

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|---|---|---|---|
| 14.15-14.30 | ISCAID-O-1 | Schwedinger | Results of a study about vaccination decision of dog owners |
| 14.30-14.45 | ISCAID-O-2 | Jähne | Detection of mutated and non-mutated feline coronaviruses in cats without feline infectious peritonitis |
| 14.45-15.00 | ISCAID-O-3 | Krafft | Report of one year surveillance of SARS-CoV-2 detection by PCR in dogs and cats with various exposure risk |
| 15.00-15.15 | ISCAID-O-4 | Walter-Weingärtner | Comparison of eight commercially available point-of-care tests to detect canine parvovirus in faeces of dogs |
| 15.15-15.30 | ISCAID-O-5 | Spiri | Modified-live FCV vaccination reduces viral RNA loads, duration of RNAemia and the severity of clinical signs after heterologous FCV Challenge |
| 15.30-15.45 | ISCAID-O-6 | Vahlenkamp | High prevalence of antibodies against feline morbillivirus type 1 and 2 and association with FLUTD and increased blood creatinine concentrations in domestic cats |
| 15.45-16.00 | ISCAID-O-7 | Griebsch | Emerging canine leptospirosis in NSW, Australia |

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| 16.00-16.15 | ISCAID-O-8 | Rigo | Experimental infection by leptospira australis in cats |
| 16.30-16.45 | ISCAID-O-9 | Taylor | Exploration of the role of *Leptospira* spp. in cats with chronic kidney disease (CKD) |
| 16.45-17.00 | ISCAID-O-10 | Bouzouraa | Epidemiological, clinical and biological impact of hemoplasmas in cancer-bearing dogs: A case-control study on 324 cases |
| 17.00-17.15 | ISCAID-O-11 | Schmitt | Dissemination of $bla_{OXA-48}$ carbapenemase- and extended-spectrum beta-lactamase-producing Enterobacteriaceae in a Swiss companion animal clinic |
| 17.15-17.30 | ISCAID-O-12 | Steffensen | Methylprednisolone induces neutrophil extracellular trap formation and enhances bactericidal effect of canine neutrophils |
| 17.30-17.45 | ISCAID-O-13 | McCartin | Evaluation of serum 25-hydroxyvitamin D and C-reactive protein as biomarkers in dogs with coccidioidomycosis |
| 17.45-18.00 | ISCAID-O-14 | Willi | Expanded geographic occurrence of *Cytauxzoon* sp. infection in domestic cats in Switzerland and detection of the infection in felid samples collected two decades ago |
| 18.00-18.15 | ISCAID-O-15 | Gentil | Prevalence of Taeniidae eggs and *Echinococcus multilocularis* in faecal samples of dogs in Europe |
| **SCH – Society of Comparative Hepatology** | | | |
| Thursday 2 September | | | |
| 11.05-11.20 | SCH-O-1 | Dröes | Prevalence of portal vein thrombosis in 153 dogs with chronic hepatitis: 2009-2019 |
| 11.20-11.35 | SCH-O-2 | Yamkate | Immunohistochemical expression of caspase-3 and malondialdehyde in archived liver specimens from dogs with chronic hepatitis |
| 11.35-11.50 | SCH-O-3 | Brunero | Investigation of risk factors for gallbladder mucocoele development in Border Terriers; a UK-based, online owner survey |
| 11.50-12.05 | SCH-O-4 | Jaffey | Clinical findings and prognostic variables in dogs from Asia with gallbladder mucocele |
| 12.05-12.20 | SCH-O-5 | Phillips | Dogs with congenial portosystemic shunts have altered amino acid profiles compared to healthy dogs |
| 12.20-12.35 | SCH-O-6 | Devriendt | Plasma amino acid profiles in dogs with closed extrahepatic portosystemic shunts improve but remain abnormal three months after successful gradual attenuation |
| 12.35-12.50 | SCH-O-7 | Devriendt | Serum vitamin concentrations suggest incomplete restoration of liver function three months after successful gradual attenuation of extrahepatic shunts in dogs |
| **Research reports** | | | |
| Thursday 2 September | | | |
| 14.25-15.05 | | Hazuchova | Feline diabetes mellitus - can we blame the genes? |
| 15.10-15.50 | | Aguiar | Unravelling the pathogenesis of feline hyperthyroidism |
| 16.30-17.10 | | Sargent | Phosphate homeostasis in early feline CKD |

## LIST OF POSTER RESEARCH COMMUNICATIONS

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|---|---|---|
| **ESCG - European Society of Comparative Gastroenterology** | | |
| ESCG-P-1 | Heilmann | Electrolyte imbalances in dogs with chronic inflammatory enteropathies |
| ESCG-P-2 | Zornow | Fecal S100A12 (Calgranulin C) Concentrations in Cats with Chronic Enteropathies |
| ESCG-P-3 | Werner | Alterations of fecal unconjugated primary bile acids and correlation with abundance of *C. hiranonis* in dogs with chronic enteropathy |
| ESCG-P-4 | Hanifeh | Correlation of intestinal histopathologic findings with mucosa-attached bacteria, clinical disease activity, and clinical outcome in dogs with chronic enteropathies |
| ESCG-P-5 | Gori | Evaluation of serum biochemical and urinary parameters suggesting renal involvement in a population of dogs with primary chronic enteropathy |
| ESCG-P-6 | Greil | First attempt at using Narrow Band Imaging-like endoscopy to differentiate chronic enteropathy and alimentary lymphoma in dogs |
| ESCG-P-8 | Busch | Evaluation of the efficacy of an ultra-hydrolyzed diet in the management of chronic diarrhea in dogs |

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| ESCG-P-9 | Ferriani | The microbiome composition in dogs with suspected antibiotic-responsive diarrhea and the effect of tylosin on it: A multicentric-perspective case-control study |
| ESCG-P-10 | Vezzosi | Primary Chronic Enteropathy in Dogs: What Cardiovascular Effects? |
| ESCG-P-11 | Blake | Serum amino acid concentrations in dogs with chronic enteropathies prior to treatment, and correlation with clinical activity index and gastrointestinal histopathology |
| ESCG-P-12 | Xenoulis | Serum concentrations of fPLI, fTLI, cobalamin, and folate in growing kittens |
| ESCG-P-13 | Herstad | Influence of canine-specific lactic-acid bacteria on the fecal microbiota and inflammatory markers in dogs receiving nonsteroidal anti-inflammatory treatment – a prospective, randomized, double-blinded placebo-controlled trial |
| ESCG-P-14 | Barko | Fecal bile acids and microbial amino acid metabolites in serum are correlated with fecal zonulin in dogs with exocrine pancreatic insufficiency |
| ESCG-P-15 | Cocci | Modification of fecal microbiota and disease activity index with only nutritional intervention in dogs with inflammatory bowel disease: A retrospective study in 17 dogs |
| ESCG-P-16 | Douay | Endoscopic resection of benign gastroduodenal polyp in six cats: Procedure, complications and outcome |
| ESCG-P-17 | Cocci | Specific Operational Taxonomic Units can be used as potential diagnostic markers for inflammatory bowel disease in dogs? A preliminary metagenomic analysis study |
| **ESVC- European Society of Veterinary Cardiology** | | |
| ESVC-P-1 | Claretti | Relationship between weight and aortic annulus in male and female Boxer dogs |
| ESVC-P-2 | Vezzosi | Diagnostic accuracy of the precordial lead system for the detection of right ventricular enlargement in dogs |
| ESVC-P-3 | van Israel | Laryngeal hemiplegia following surgical ligation of a patent ductus arteriosus in 4 dogs |
| ESVC-P-5 | Robledo | Effects of levomepromazine on left ventricular systolic function and blood pressure in propofol and isoflurane-anesthetized dogs |
| ESVC-P-6 | Novo Matos | Thin and akinetic left ventricular myocardial segments in cats |
| ESVC-P-7 | Hanås | Breed and sex affect serum cardiac troponin I concentration in healthy cats |
| ESVC-P-8 | Poissonnier | Clinical, echo-Doppler features and prognosis of dogs with chordae tendineae rupture related to degenerative mitral valve disease (500 cases, 2009-2019) |
| ESVC-P-9 | Alvarado Masis | Survival analysis and predictive value of global and regional right ventricular function variables assessed by conventional echocardiography, speckle tracking imaging and two-dimensional color tissue Doppler imaging, in dogs with congenital pulmonic stenosis: A prospective study of 75 cases (2013-2020) |
| ESVC-P-10 | Caivano | Two-dimensional echocardiographic estimates of left atrial volumes obtained from two different views in dogs are similar but not interchangeable |
| **ESVCN - European Society of Veterinary & Comparative Nutrition** | | |
| ESVCN-P-1 | Pires Gonçalves | Assessment of a proactive approach to pet obesity prevention by Portuguese veterinary nurses and technicians |
| ESVCN-P-2 | Pires Gonçalves | Are Portuguese veterinarians taking the initiative on preventing small animal obesity? – a survey-based study |
| ESVCN-P-3 | German | A cohort study to examine associations between initial weight loss outcomes and overall success of a controlled weight loss programme in dogs with obesity |
| ESVCN-P-4 | Bjornvad | Outcome and factors affecting success of a controlled weight loss program for dogs |
| ESVCN-P-5 | Dupont | Experiences with feeding a hydrolyzed protein diet to dogs with acute diarrhea: A prospective pilot study |
| ESVCN-P-6 | Nybroe | Outcome related to acquired Fanconi syndrome associated with ingestion of jerky treats in 30 dogs |
| ESVCN-P-7 | Andersson | Exploration of body weight in 115 000 young adult dogs of 72 breeds |
| **ESVE - European Society of Veterinary Endocrinology** | | |
| ESVE-P-1 | Knowlden | Use of a mobile phone application to evaluate quality of life of diabetic pets and their owners |
| ESVE-P-2 | Urbanschitz | Glycemic variability in non-diabetic, healthy beagle dogs |
| ESVE-P-3 | Jaffey | Phagocytic function, inflammatory phenotype, and serum 25-hydroxyvitamin D in dogs with diabetes mellitus |
| ESVE-P-4 | Rothlin-Zachrisson | The ones that did not make it: Owners' perceptions and reasons for why cats with diabetes mellitus were euthanized within four weeks of diagnosis |
| ESVE-P-5 | Roberts | Assessment of euthanasia rates of dogs diagnosed with diabetes mellitus in primary care practice in Australia |
| ESVE-P-6 | Corsini | Feline hypersomatotropism: The owners' point of view |
| ESVE-P-7 | Corsini | Feline hypersomatotropism: What is the veterinarians' approach to the disease? |

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| ESVE-P-8 | Oliveira | Feline Adrenomegaly in Clinical Practice – a retrospective study |
| ESVE-P-9 | Pérez-López | Evaluation of the ACTH stimulation test using a low-dose of a depot formulation in healthy dogs and in dogs with naturally occurring Cushing's syndrome |
| ESVE-P-10 | Golinelli | Myotonia associated with naturally occurring canine hypercortisolism: 30 cases (1984-2020) |
| ESVE-P-11 | Golinelli | Evaluation of serum electrophoresis in dogs with pituitary-dependent hypercortisolism |
| ESVE-P-12 | Carvalho | Treatment and monitoring of naturally-occurring hypercortisolism by primary care veterinarians: A Western European survey |
| ESVE-P-13 | García San José | Changes in systolic blood pressure in dogs with adrenal dependent hyperadrenocorticism during trilostane treatment or after adrenalectomy |
| ESVE-P-14 | Ottka | 1H NMR metabolomics identifies multiple metabolites associated with persistently elevated cortisol |
| ESVE-P-15 | Robredo Corta | Evaluation of ACTH-stimulation test, urinary cortisol to creatinine ratio and urinary specific gravity as monitoring tools in dogs with hyperadrenocorticism |
| ESVE-P-16 | Rebelo | The impact of low-dose dexamethasone suppression test patterns in clinical practice – a retrospective study |
| ESVE-P-17 | Kurtz | Prediction of azotemia in medically-treated hyperthyroid cats: An attempt to identify risk factors and to develop a scoring system |
| ESVE-P-18 | Schils | Investigation to detect of traces of methimazole in the urine of owners of hyperthyroid cats |
| ESVE-P-19 | Bree | Retrospective comparison of different vitamin D analogues in the management of canine primary hypoparathyroidism |

**ESVIM - European Society of Veterinary Internal Medicine**

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| ESVIM-P-2 | Olivares | Use of tranexamic acid in dogs with primary ITP. A feasibility study |
| ESVIM-P-3 | Bouzouraa | Usefulness of chlorambucil for treatment of canine Steroid-Responsive Meningitis-Arteritis |
| ESVIM-P-4 | Urion | Serologic testing and radiographic measurements of the tracheobronchial lymphoid soft tissue in dogs with pulmonary coccidioidomycosis |
| ESVIM-P-5 | Krautmann | T-cell dependent immune responses in cats treated with frunevetmab |
| ESVIM-P-6 | Théron | Autologous blood-patch pleurodesis treatment for persistent pneumothorax in 5 dogs |
| ESVIM-P-7 | Lin | Quantifiable features of the tidal breathing pattern in dogs with severe bronchomalacia |
| ESVIM-P-8 | Robin | Meta-analysis of complication rates of tracheal stenting in dogs |
| ESVIM-P-9 | Blasi Brugué | Evaluation of canine fresh frozen plasma CRI: Risk of contamination and effects on albumin and coagulation factors |
| ESVIM-P-10 | Enache | SARS-CoV-2 and the veterinary profession: A longitudinal-study in Italy (February 23-July 1, 2020) |
| ESVIM-P-11 | Herter | Alloimmunization in dogs following transfusion: A serial crossmatch study |
| ESVIM-P-12 | Schils | Hypertension in apparently healthy elderly dogs: Prevalence and comparison of in clinic versus at home measurement with Doppler ultrasonic technique |
| ESVIM-P-13 | Toone | Assessment of circulating inflammatory mediators in dogs with tracheal collapse |
| ESVIM-P-14 | Nagel | Stopping leaks in the chamber – differences in valve performance on inhalation and exhalation leakage for pet inhaler therapy |
| ESVIM-P-15 | Katsaouni | Evaluation of serum c-reactive protein, neutrophil-lymphocytic ratio and abdominal fluid cell count in dogs with septic and non-septic abdominal exudates |

**ESVNU - European Society of Veterinary Nephrology and Urology**

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| ESVNU-P-1 | Breu | Study of 4970 uroliths in cats over a five year period |
| ESVNU-P-3 | Jesus | Xanthinuria Secondary to Allopurinol Treatment in Dogs with Leishmaniosis: Current Perspectives of the Iberian Veterinary Community |
| ESVNU-P-4 | Mortier | Effect of storage time and temperature on feline urinary protein: Creatinine ratio |
| ESVNU-P-6 | Dias | Canine and Feline Urolithiasis in Portugal: New trends and findings - a retrospective study of 13 years (2007 – 2020) |
| ESVNU-P-7 | Gori | May urinary neutrophilic degeneration and intracellular bacteria predict urine culture outcome in canine suspected urinary tract infections? |
| ESVNU-P-8 | Tai | Sodium bicarbonate 8.4% as a potential alternative to unfractionated heparin as a dialysis catheter locking solution: A pilot study |
| ESVNU-P-9 | Marynissen | Comparison of Doppler ultrasonic technique and High Definition Oscillometric systolic blood pressure measurements in conscious apparently healthy elderly dogs |

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| ESVNU-P-10 | González | Evaluation of the safety of alogenic adipose tissue derived mesenchymal stem cells (aAMSC) therapy in dogs diagnosed with glomerular disease: Short- and medium- term |
| ESVNU-P-11 | Nelaton | Urolithiasis in dogs: A retrospective study of 6,700 canine urinary stones collected from 2016 to 2020 in France and analyzed by infrared spectrometry |
| ESVNU-P-12 | Mantelli | Longitudinal follow-up of renal function, hydration and electrolytic status of dogs affected by immune-mediated diseases and treated with oral prednisolone |
| ESVNU-P-13 | Afonso | Urinary clusterin as a potential biomarker for the early diagnosis of chronic kidney disease in cats |
| ESVNU-P-14 | Krafft | Plasma concentration of the activated and non-activated forms of the extrahepatic vitamins K-dependent protein Matrix Gla Protein in healthy dogs and dogs with chronic kidney diseases |
| ESVNU-P-15 | Lund | Long-term follow-up of Norwegian dogs with acquired Fanconi syndrome |
| **ESVONC - European Society of Veterinary Oncology** | | |
| ESVONC-P-1 | Pierini | Urinary bladder rhabdomyosarcoma in seven dogs |
| ESVONC-P-2 | Vilhena | Adenosine deaminase and uric acid in feline spontaneous malignant mammary tumors |
| **ISCAID - International Society for Companion Animal Infectious Diseases** | | |
| ISCAID-P-1 | Tabar | Prevalence of vector borne pathogens in owned dogs from mediterranean area with cranial cruciate ligament rupture |
| ISCAID-P-2 | Roels | Assessment of both IDEXX Angio Detect on bronchoalveolar lavage fluid and Angiostrongylus vasorum quantitative polymerase chain reaction on EDTA blood in dogs that tested negative for IDEXX Angio Detect on EDTA blood |
| ISCAID-P-3 | Carrasco Agulló | Honney-coomb spleen pattern in dogs and cats with leishmaniosis |
| ISCAID-P-4 | Machado | Identification of prognostic factors in dogs hospitalized due to acute *Leptospira* spp. infection |
| ISCAID-P-5 | Machado | Canine parvovirosis versus canine distemper: Risk factors, hospitalization course, and outcome |
| ISCAID-P-6 | Kohn | Autochthonous Babesia canis infections in 25 dogs in Berlin/Brandenburg (Germany) |
| ISCAID-P-7 | Schäfer | Infections with Babesia spp. in dogs living in Germany (2007-2020) |
| ISCAID-P-8 | González | Relationship between novel biomarkers of glomerular function and circulating antibody levels in advanced leishmaniosis stages in dogs |
| ISCAID-P-9 | Rodríguez-Sanz | Cytokine modulation of the innate immune response in natural canine leishmaniosis |
| ISCAID-P-10 | Baxarias | Use of serological screening tools to detect canine antibodies against Leishmania infantum in Spain |
| ISCAID-P-11 | Pomba | Rising trends on ESBL–producing Enterobacterales clinical strains from companion animals |
| ISCAID-P-12 | Vilhena | Serosurvey of Coxiella burnetii in companion animals from Portugal |
| **SCH - Society of Comparative Hepatology** | | |
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| SCH-P-2 | Tulone | Serum protein electrophoresis in dogs with chronic hepatitis |
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| SCH-P-4 | Gabriel | First Description and Preliminary Characterization of a Microfluidic Canine Liver-on-a-Chip |

## ESCG-O-1 - European Society of Comparative Gastroenterology

### Comparative analysis of the distribution and severity of the lesions within the digestive tract in feline low-grade intestinal T-cell lymphoma or lymphoplasmacytic enteritis

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Low-Grade Intestinal T-Cell Lymphoma (LGITL) is the first feline intestinal neoplasia. Differentiating LGITL from lymphoplasmacytic enteritis (LPE) is challenging. These two diseases show a heterogeneous distribution of the lesions whose severity may vary along digestive segments. Gastroduodenoscopy allows biopsies, but jejunal lesions may be missed by this technique, leading to misdiagnosis. In contrast, the whole digestive tract is reachable by surgery, even if rare life-threatening complications can occur. The aims of this study were 1) to evaluate the distribution of the lesions within the digestive tract in cats diagnosed with LPE or LGITL, 2) to evaluate the presence of concurrent inflammatory lesions in LGITL cases, and finally 3) to assess the severity of the lesions according to the digestive segment in LGITL cases.

This prospective cohort study enrolled cats presented with clinical signs consistent with LGITL or LPE. Cats underwent endoscopic biopsies of stomach, duodenum (ileum in some cases), and surgical full-thickness biopsies of jejunum and ileum for almost every cat, after a review of clinical, laboratory and ultrasonographic results. Histologic standard evaluation and immunohistochemical examination (CD3, CD20 and Ki67) were performed on each intestinal segment. Severity of histologic lesions was classified as mild, moderate or severe for all sampled segments.

Eleven LGITL cases (6 females, 5 males) and 8 LPE cases (3 females, 5 males) were enrolled. For cats diagnosed with LGITL, the jejunum was affected in 100% of cases (9/9), ileum in 91% (10/11), duodenum in 91% (10/11) and stomach in 18% (2/11). Jejunum, ileum, duodenum and stomach were all involved in one case. For cats diagnosed with LPE, jejunum and duodenum were systematically affected while 7 cats had an infiltrated ileum (87%) and 5 cats an infiltrated stomach (62%). In LGITL cases, the stomach showed less severe lesions than duodenum, jejunum and ileum ($p<0.001$, respectively), but no statistical difference between jejunum and duodenum or ileum was found ($p=0.265$ and 0.260, respectively). In 7/11 (64%) cats diagnosed with LGITL, signs of concomitant lymphoplasmacytic and neutrophilic inflammation were found in different segments infiltrated with neoplastic lymphocytes.

These results support that LGITL shows a heterogeneous repartition within the digestive tract. The jejunum seems systematically involved in cats presenting LGITL. Misdiagnosis is possible if biopsies are only performed by gastroduodenoscopy, supporting the relevance of jejunal surgical biopsies. Furthermore, signs of concurrent inflammation were found in LGITL cases, validating the *continuum* between these two entities, recently evoked in the scientific literature.

#### Disclosures

No disclosures to report.

## ESCG-O-2 - European Society of Comparative Gastroenterology

### Short-term survival and associated factors after surgical intestinal biopsies in cats with chronic enteropathy

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In cats with chronic enteropathy, differentiating inflammatory bowel disease (IBD) from low-grade intestinal T-cell lymphoma (LGITL) is challenging and requires histopathological analysis. There is an ongoing debate regarding the best sampling method as the lesions may heterogeneously affect different areas of the small intestine. Main limitations of surgical biopsies include increased morbidity and mortality due to prolonged anesthesia and surgical complications. Hypoalbuminemia and histopathological diagnosis of neoplasia have previously been described to be associated with dehiscence and mortality. The purpose of this study was to identify pre and per-operative factors associated with occurrence of all-cause death within 15 days from surgical intestinal biopsies in cats suspected with IBD or LGITL.

All cats from one referral center, who underwent full-thickness intestinal biopsies *via* laparotomy between 2010 and 2021 were retrospectively included in the study if their vital status was available within 15 days from surgery. Preoperative clinicopathological variables, ultrasonographic findings, both anesthetic and surgical procedure data as well as final diagnosis were retrieved. Quantitative variables were described by median and interquartile range [IQR]. Variables were compared between deceased and living cats using Chi-2 square, Fischer's exact test (qualitative and semi-quantitative variables), and Mann-Whitney test (quantitative variables). P-value <0.05 was considered significant and odds-ratios (ORs) were given with their 95% confidence interval.

A hundred and twenty-seven cats were included, among which 19 (15%) died within 15-days after laparotomy at a median of 5 days, [3-12 days]. Wound dehiscence was likely in 3 cases. Low body condition score (BCS≤3/9) was significantly associated with death within 15 days (OR=4.0 [0.9-20.4]; P=0.05). The presence of gallbladder ultrasound abnormality (OR=4.1 [1.3-13.1]; P=0.02) as well as concurrent hepatic biopsies performed during surgery (OR=9.2 [2.0-41.6]; P<0.01) and positive bacterial bile culture (OR=20.7 [2.6-163.8]; P<0.01) were significantly associated with death. Neither hypoalbuminemia (OR=1.6 [0.4-6.7]; P=0.53) nor neoplastic histopathological diagnosis (39% among deceased cats; 42% among living ones; OR=0.9 [0.3-2.4]; P=0.80) were significantly associated with death in this study. There was no significant association between anesthetic variables, number of digestive biopsies, cobalamin status, comorbidities and death.

This study is the second largest including cats undergoing GI biopsies, the mortality rate at 15 days was 15%, higher than previously reported (0 to 11%). Results suggest that poor BCS and concurrent hepatobiliary disease, as witnessed by ultrasonographic abnormalities and realization of liver biopsies, are associated with survival at 15 days suggesting that these factors should be evaluated for full-thickness intestinal biopsies decision.

**Disclosures**

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## ESCG-O-3 - ESCG - European Society of Comparative Gastroenterology

## Identification of bacteria in pancreas, liver, and bile of apparently healthy cats using next generation 16S rRNA sequencing and standard bacteriological culture

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Due to their close anatomical and functional relationship with the gastrointestinal tract, the pancreas and hepatobiliary system can be influenced by the gut microbiota. While studies have established the presence of bacteria within normal pancreatic tissues in mice, no similar studies have been reported in cats. The aim of this study was to identify bacteria in pancreas, liver, and bile of apparently healthy cats using next generation 16S rRNA gene sequencing (NGS) and standard bacteriological culture.

Pancreatic and hepatic tissue and bile were sterilely collected within 1.5 hours of death from 28 apparently healthy feral cats that had been euthanized for population control. Samples were divided between sterile molecular biology grade Eppendorf tubes, and aerobic and anaerobic culture swabs. In addition, a negative sampling control was collected for NGS from all 20 cats before collecting any tissue samples. Samples for NGS were transported with dry ice and stored at -80ÌŠ C until testing. Samples for culture were transported with ice packs and stored at 4ÌŠ C until plated onto standard media. Additional pancreatic and hepatic samples were collected for H&E histopathological examination and reviewed by a board-certified veterinary pathologist.

A total of 17/103 (17%) samples had detectable bacterial DNA using NGS, while 5/83 (6%) had identifiable bacteria using culture. Bacterial DNA was detected on 4/28 (14%) pancreatic and 5/28 (18%) hepatic tissue samples, 5/27 (19%) bile samples, and 3/20 (15%) negative sampling controls by NGS; the reported microbial compositions for each type of specimen varied widely. Bacteria were cultured from 2/28 (7%) pancreatic and 3/28 (11%) hepatic tissue samples and in 0/27 bile samples by standard bacteriological methods. Among 4 pancreatic tissue samples with detectable bacterial DNA, 1 showed the same bacteria as the negative sampling control (*Enterobacter cloacae*), and 1 had possible human skin commensal contamination (*Staphylococcus haemolyticus*, *Staphylococcus simulans*). However, no negative sampling controls were available for the other two cats. The only sample reported to have bacterial DNA/bacteria identified by both NGS and culture was a hepatic tissue sample. Bacteria or lesions related to possible bacterial infection were not identified by histopathology in any pancreatic or hepatic tissues.

Bacterial DNA and/or bacteria could be identified in pancreas, liver, and bile from some apparently healthy cats using NGS or standard bacteriological culture, respectively. The physiological and clinical relevance of bacterial DNA/bacteria within these specimens requires further study.

**Disclosures**

Drs. Wu, Pilla, Lidbury, Suchodolski, and Steiner are employed by the Gastrointestinal Laboratory at Texas A&M University, which offers laboratory testing on a fee-for-service basis. Dr. Jarvis is employed by MicroGenDx, which offers advanced DNA-based laboratory testing on a fee-for-service basis, and as a genomic diagnostics consultant to MicroGen Vet, which represents exclusively MicroGenDx laboratory testing in the veterinary diagnostic markets. Dr. Newman is the owner of Newman Specialty VetPath and provides various services, including histopathology reading on a fee-for-service basis. Drs. Wu and Lawhon are employed by the Clinical Microbiology Laboratory at Texas A&M University, which offers microbiology services, including standard bacteriological cultures on a fee-for-service basis. None of these relationships should have any impact on the data presented here.

## ESCG-O-4 - ESCG - European Society of Comparative Gastroenterology

### Investigating fecal microbial transplant in dogs with inflammatory bowel disease: A pilot study

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Inflammatory bowel disease (IBD) is a frequent cause of chronic vomiting, anorexia and diarrhea in dogs. The objective of this study was to assess if the addition of fecal microbial transplant to standard therapy (corticosteroids and a hypoallergenic diet) resulted in improved outcome versus standard treatment alone.

Thirteen client-owned dogs with IBD were enrolled in this double blinded, randomized clinical trial. All patients received corticosteroid therapy and a hypoallergenic diet; patients were randomized to receive either placebo or fecal microbial transplant. Measured outcomes included the canine chronic enteropathy clinical activity index (CCECAI) along with albumin, C-reactive protein, and cobalamin levels at 1 week, 1 month, and 3 months after enrolment. Fecal microbiota was analyzed after extracting DNA from fecal samples and profiling using 16S amplicon sequencing.

The CCECAI significantly decreased over time regardless of treatment group ($p = 0.001$). There was no difference between treatment groups in the CCECAI ($p = 0.735$), albumin ($p=0.43$), C-reactive protein ($p = 0.287$), or cobalamin ($p = 0.601$) after 90 days of treatment. No adverse effects were reported after FMT.

The alpha and beta diversity measurements (including community membership (Jaccard index), and structure (Yue and Clayton index)) were not significantly different between and within the treatment groups at all times ($P > 0.05$).

In conclusion, the addition of FMT did not change patient outcome as measured by the CCECAI, albumin, C-reactive protein, and cobalamin levels, as well as fecal microbiota diversity and composition, in dogs with IBD in this study.

#### Disclosures

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## ESCG-O-5 - ESCG - European Society of Comparative Gastroenterology

### Clinical effects of fecal microbiota transplantation in dogs with chronic enteropathy

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Fecal microbiota transplantation (FMT) is used to transfer intestinal microbiota from a healthy donor to a recipient with gastrointestinal disease to modulate the microbiome and decrease disease activity. In people, FMT is effective in treating recurrent *Clostridioides difficile*-infection. Repeated FMTs have also been shown to induce remission in people with ulcerative colitis in a placebo-controlled study. In dogs with chronic enteropathies (CE), a few case reports suggest decreased disease activity after FMT. The objective of this study was to report the clinical effects of FMT in a larger group of dogs with CE.

Retrospective data from dogs with CE treated with FMTs as adjunctive therapy were collected. Inclusion criteria were dogs with CE not responding satisfactorily to standard treatment and for which follow-up of at least 3 months post-FMT was available. Exclusion criteria were starting a new immunosuppressive treatment, or increasing the dose of current maintenance therapy, in parallel with FMT. FMT was given as a rectal enema (5-7 g donor feces/kg body weight of recipient). Two different donors were used, both having a dysbiosis index (DI) below -2.

Thirty-three dogs aged 0.6-13 years (median 6.8) were included. Dogs had been treated for CE for 1-110 months (median 21 months) at inclusion. Main presenting complaints were diarrhea (26/33) and lethargy (15/33). Thirty-two dogs were treated with corticosteroids at inclusion, and 20/33 dogs received various second line immunosuppressive drugs. Thirty-one dogs received 2-5 FMTs, with the majority (23/33) receiving 3 FMTs. Two non-responders only received one FMT each. Clinical improvement was noted in 24/33 dogs after FMT, most commonly characterized by increased activity level (18/33), improved fecal quality (17/33) and improved appetite and/or weight gain (7/33). Canine IBD Activity Index at inclusion was 2-17 (median 5), which decreased significantly to 1-9 (median 2) in the month after the last FMT ($p<0.0001$). Fecal samples for DI (reference interval ≤0) were available from 18 dogs at inclusion. Non-responders had significantly higher DI (range 0.8 to 8.9, median 4.2) compared to responders (range -2.4 to 6.0, median 0.7) ($p=0.031$).

A high DI has been previously shown to correlate with decreased microbial diversity. In humans, low microbial diversity is a negative prognostic factor for responding to FMT, which also appeared to be the case in this group of dogs. Our results suggest that FMT can be successfully used as adjunctive therapy in dogs with CE, but prospective longitudinal studies are warranted.

#### Disclosures

Dr Toresson's research is partly supported by the Swedish Veterinary Care Foundation. Drs Toresson and Ludvigsson are employed by

Evidensia. Dr Spillmann has no disclosures. Drs. Suchodolski, Steiner, and Lidbury are affiliated with the GI Lab at Texas A&M University, which offers the canine dysbiosis index (DI) and other diagnostic tests for gastrointestinal diseases on a fee-for-service basis.

## ESCG-O-6 - European Society of Comparative Gastroenterology

### Faecal bile acid profiles in dogs with chronic enteropathies versus healthy controls

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Bile acid diarrhoea (BAD) is frequently seen in people presenting with chronic diarrhoea of various etiologies. BAD can occur secondary to ileal inflammation and destruction of the apical sodium-dependent bile acid transporter (ASBT) or down regulation of ASBT expression (as seen in Crohn's disease) or in the absence of gastrointestinal morphological abnormalities or alternations in ASBT expression (as seen in irritable bowel syndrome-diarrhoea (IBS-D)). BAD in people is commonly associated with an increase in total faecal bile acids (FBA). Dysbiosis is present in people with BAD and likely plays a role in the pathophysiology. In veterinary medicine dysbiosis has been identified in dogs with chronic enteropathies (CE) and BAD has been proposed as a possible contributing factor to CE associated diarrhoea.

The aim of this study was to evaluate FBA profiles in dogs with CE in comparison with healthy controls (HC) to further explore the possibility of BAD in a cohort of CE cases.

Fifteen dogs diagnosed with CE and retrospectively classified as having food-response enteropathy (FRE=5), antibiotic-responsive enteropathy (ARE=1) or steroid-responsive enteropathy (SRE=9, 4 of which had protein-losing enteropathy (PLE)) and 10 HC were included in this prospective study. FBA were measured using HPLC-MS. Standards were included for Chenodeoxycholic acid (CDCA). Cholic acid (CA), Deoxycholic acid (DCA), Lithocholic acid (LCA), Taurocholic acid (TCA) and Taurolithocholic acid (TLCA). In addition to these 7 peaks, 24 peaks corresponding with known bile acids (BA) were identified (total FBA=31).

No significant difference in total FBA between the CE and HC group was found (p=0.277). Total primary BA (CA+CDCA) were significantly higher in the CE group compared to HC (p=0.009) but there was no significant difference in total secondary BA (DCA +LCA) between these two groups (p=0.869). Ratio of total primary BA: total secondary BA was significantly higher in dogs with CE compared to HC (p=0.033). Proportion of total primary BA to total BA was significantly higher in dogs with CE compared to HC (p=0.028).

BAD without an increase in total FBA has been reported in people with IBD and IBS-D and could be secondary to an altered FBA profile. In line with previous studies total primary bile acids are increased in dogs with CE compared to HC, the exact mechanism for this remains to be elucidated. The presence of BAD in CE cases could pose a useful therapeutic target.

#### Disclosures

No disclosures to report.

## ESCG-O-7 - ESCG - European Society of Comparative Gastroenterology

### Fecal fatty acid, cholesterol, and bile acid concentrations in cats with chronic enteropathy

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Chronic enteropathy (CE) in cats is a multifactorial gastrointestinal disorder, which includes inflammatory bowel disease (IBD) and small cell lymphoma (SCL). Changes in the gut metabolome have been documented in both humans and dogs with chronic enteropathies. However, the gut metabolome in cats with CE has not yet been widely studied. This study aimed to investigate differences in selected fecal metabolites in cats with CE compared to healthy controls using a targeted approach.

Surplus fecal samples from thirty cats with CE (13 cats with IBD and 17 cats with SCL) and 36 healthy control cats were enrolled. Fecal concentrations of selected fatty acids (i.e., myristic acid, palmitic acid, linoleic acid, alpha-linolenic acid, oleic acid, cis-vaccenic acid, stearic acid, arachidonic acid, gondic acid, docosanoic acid, and erucic acid), cholesterol, unconjugated primary bile acids (i.e., cholic acid, chenodeoxycholic acid), and unconjugated secondary bile acids (i.e., lithocholic acid, deoxycholic acid, and ursodeoxycholic acid) were measured by gas chromatography coupled with mass spectrometry. Mann-Whitney tests were used to compare the concentrations of each compound between cats with CE and healthy cats. Statistical significance was set at $p < .05$.

Fecal concentrations of all fatty acids, except alpha-linolenic acid, were significantly increased in cats with CE ($p$-values < .016), suggesting lipid malabsorption. Cats with CE had higher fecal cholesterol concentrations than healthy cats ($p = .0084$). 28% (10/36) of cats with CE had a percentage of fecal primary bile acids above the upper limit of the reference interval, indicating bile acid dysmetabolism. No significant differences between the targeted metabolites of cats with IBD and those with SCL were found.

Cats with CE had increased fecal concentrations of several fatty acids and cholesterol, suggesting lipid malabsorption. Cats with CE also had evidence of bile acid dysmetabolism. This is possibly due to decreased microbial conversion of primary to secondary bile acids. These findings are similar to those described for dogs with CE.

**Disclosures**

No disclosures to report.

## ESCG-O-8 - European Society of Comparative Gastroenterology

### Metabolomic serum profiling in dogs with chronic enteropathy

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Chronic enteropathy (CE), a common cause of morbidity in canine patients, remains a challenge to diagnose and prognosticate. Within human medicine, metabolic profiling has been extensively studied in idiopathic inflammatory enteropathies such ulcerative colitis and Crohn's disease. This has led to identification of several metabolites which consistently differ between these patients and healthy individuals, revealing promising new biomarkers, and insights into disease pathogenesis. Despite this expanding evidence base in human medicine, the impact of CE on the metabolic profile of dogs remains poorly understood.

The aim of this study was to compare the metabolic profile of dogs with histopathologically confirmed CE to that of a healthy population.

Serum samples from 55 dogs with CE and 207 healthy controls were included for this prospective cohort study. Dogs were classified as having CE if they had a >3-week history of gastrointestinal signs, confirmation of inflammatory infiltrates on histology of intestinal biopsies, and exclusion of other causative processes. Healthy controls were dogs presenting for annual vaccination with no history of gastrointestinal disease and no physical examination abnormalities.

Metabolic profiling was conducted using a canine-specific proton nuclear magnetic resonance ($^1$H NMR) spectroscopy platform. Forty metabolites and sixty-five lipoprotein analytes were measured. A two-sample T test was used to compare the two populations, with significance set at P <0.05. Bonferroni correction was done for each metabolite group.

Eighteen metabolites and 18 lipoprotein analytes were significantly different between the healthy and CE groups. Three metabolites were increased in dogs with CE compared to healthy controls: the amino acid phenylalanine ($P = 8.50^{-04}$); and the glycolysis related metabolites citrate ($P = 0.003$) and lactate ($P = 0.003$). Fifteen metabolites and 18 lipoprotein analytes were decreased in dogs with CE: including the fatty acids oleic acid ($P \leq 1.00^{-5}$), stearic acid ($P = 1.00^{-5}$), saturated fatty acids ($P \leq 1.00^{-5}$), polyunsaturated fatty acids ($P = 4.60^{-4}$), omega-6 ($P = 5.20^{-4}$), eicosapentaenoic acid ($P = 0.001$), and docosapentaenoic acid ($P = 0.002$); the amino acid glycine ($P \leq 1.00^{-5}$); and very-low-density lipoprotein cholesterol ($P = 0.001$).

This study highlights, for the first time, a significant difference in specific serum metabolites of dogs with CE. Many of the identified metabolites mirror perturbations identified in human gastrointestinal disease.

**Disclosures**

Claudia Ottka is an employee and Hannes Lohi is a shareholder and the chairman of the board of PetBIOMICS Oy, who developed and provides the metabolomics test. Silke Salavati: Current/ past employment: PhD funded by BBSRC & Protexin Ltd. Speaking engagement (last 5 y): Baltic Forum, Berliner Symposien, BSAVA, BSVP, DVG-DGK, Edra Publishing, EERVC, ESAVS, EVECC, Hill's, IDEXX, ISFM, London & Amsterdam microbiome congresses, SAMSoc, Silesian Vet Association, Synlab, VetPlus Consultancies (last 5 y): Waltham/ Mars Petcare Grants/ research (last 5 y): Pet Plan, CGS Gifts, hospitality, travel support (last 5 y): Baltic Forum, Berliner Symposien, DVGDGK, ESAVS, EVECC, Hill's, Silesian Vet Association, VetPlus This study was partially supported by PetBIOMICS Oy.

## ESCG-O-9 - ESCG - European Society of Comparative Gastroenterology

### Taking the next step: Modelling infectious diseases in canine intestinal organoids

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Gastrointestinal infectious diseases constitute a challenging problem in human and veterinary medicine due to their high incidence rates. Therefore, human and animal research have focused on elucidating the biological mechanisms underlying these disorders for many years. In this context, research still relies on rodent models, which do not mimic diseases in an authentic and species-specific manner. This led to the emergence of adult stem cell-derived organoids. However, veterinary research is only beginning to exploit organoid technology.

We generated canine small and large intestinal organoids and focused on simultaneous long-term expansion and concomitant cell differentiation using a modified growth medium. Organoids were shown to express cell type specific markers for stem cells, enterocytes, enteroendocrine and goblet cells, thus reflecting all major cell types of the primary canine tissue. To simplify disease modelling, the apical cell surface, which can be found on the inside of these organoids, needs to be accessed. Therefore, we created apical-out organoids via

complete removal of extracellular matrix proteins and 3D-cultivation in suspension which then allows for the addition of test compounds directly to the culture medium. Verifying this inside-out-model, we use a classical two-dimensional sulphorhodamine B assay, where organoids are dissociated into single cells and then seeded in 2D-cultures maintaining all major cell types before treatment.

As demonstrated in a toxin neutralisation-model, the addition of Toxin B from *Clostridioides difficile* (TcdB) reduced epithelial barrier integrity of apical-out organoids and induced cell death as observed in natural infections and *in vivo* models. This cell damaging effect can be rescued by the addition of Bezlotoxumab, a human monoclonal antibody designed to bind and neutralise TcdB.

We conclude that this technique further increases the applicability of intestinal organoids as a versatile *in vitro*model for canine gastrointestinal infections.

**Disclosures**

The Austrian Research Association FFG, project No 13610974, funded this work.

## ESCG-O-10 - ESCG - European Society of Comparative Gastroenterology

### Retrospective analysis of the association between hepatic pathology and DGGR lipase in canines with histologically normal pancreas

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1,2′′ o′′ dilauryl′′ rac′′ glycero′′ 3′′ glutaric acid" (6′′′ methylresorufin) ester (DGGR) is a substrate used to measure pancreatic lipase activity, with DGGR lipase assays commonly utilised in the diagnosis of canine pancreatitis. It is more specific than traditional 1,2-diglyceride assays and is reported to be as accurate as Spec cPL. Whilst renal disease, endocrinopathies, immune-mediated disorders and upper airway obstructions are associated with increased serum DGGR lipase activities, to the authors' knowledge, no studies have directly demonstrated if hepatic lipases released during hepatocyte injury influence serum DGGR lipase activity.

Our hypothesis was that serum DGGR lipase activity would be increased in dogs with histologically confirmed liver pathology without concurrent histopathological evidence of pancreatic pathology.

A post-mortem database from a single institution was searched for dogs with histologically confirmed liver pathology with concurrent unremarkable pancreatic histology between 2015 and 2020. Dogs with serum biochemistry results (including DGGR lipase) available within four months of death were included. Dogs that had comorbidities or exogenous factors known to affect DGGR lipase such as heart disease, azotaemia or steroid administration were excluded. A control group with no histological evidence of liver or pancreatic disease was identified for comparison and subjected to the same exclusion criteria as the hepatopathy group. Mann-Whitney U tests were performed to compare biochemical parameters between groups with results considered statistically significant when $P<0.05$. Data are presented as median [range].

Nine dogs were included in the hepatopathy group and 5 in the control group. Serum biochemistry was performed within 7 [0-162] days of death. Serum ALT and ALP activities (reference interval 14-67 and 26-107 IU/L respectively) were significantly greater in the hepatopathy group (149 [61-689] IU/L and 682 [289-3641] IU/L respectively) than the control group (38 [32-57] IU/L and 76 [27-119] IU/L; P=0.038 and P=0.006 respectively). There were no significant differences in serum DGGR lipase activity (reference interval <44 IU/L) between the hepatopathy (52 [27-85] IU/L) and control groups (37 [25-105] IU/L; P=0.947). Serum amylase activity (reference interval (256-1609 IU/L) was significantly higher in the hepatopathy group (830 [711-1210] IU/L) than the control group (541 [336-695] IU/L; $P=0.028$).

No association between serum DGGR lipase activity and hepatic pathology was identified in this study. However, serum amylase activities were higher (albeit within the reference interval) in dogs with hepatic pathology and without pancreatic pathology compared to dogs without hepatic or pancreatic pathology. The findings of this study suggest that DGGR lipase may not be increased secondary to hepatopathy.

**Disclosures**

No disclosures to report.

## ESCG-O-11 - European Society of Comparative Gastroenterology

### Colorectal polypoid masses in dogs: Multicentre retrospective study of 53 cases

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Sessile or pedunculated colorectal masses (hereinafter referred to as polypoid masses) are uncommon in dogs and may be benign inflammatory polyps, adenomatous polyps, carcinoma *in situ* polyps or any other tumor type (eg, adenocarcinoma, round cell tumor). Whether suspected before or discovered during endoscopic examination, the mass is often

resected and submitted to histological analysis. The aim of this study was to describe the epidemiology, clinical signs, excision method, histopathology, and prognosis of cases with colorectal polypoid masses.

Fifty-three cases were retrospectively included based on polypoid endoscopic appearance and availability of histopathological data. Poorly demarcated or "napkin-ring" masses were not included. Quantitative normally distributed variables are presented as mean and standard deviation (SD). Categorical variables are presented as percentage. Time from excision to recurrence or death was estimated by the Kaplan-Meier method.

Males represented 68% (36/53) of cases. Mean age was 7.9 years (SD, 14.5). West-Highland White Terrier (11%, 6/53), French Bulldog (9.4%, 5/53) and Boxer (9.4%, 5/53) were the most represented breeds. The main presenting complaints were haematochezia (92%, 49/53), visible masse at the anus (36%, 19/53), tenesmus (34%, 18/53), diarrhea (25%, 13/53), dyschezia (15%, 8/53), lethargy (9.4%, 5/53), abdominal pain (7.5%, 4/53) and mucoid stools (7.5%, 4/53). Physical examination data were available in 47 cases: a mass was detected in 89% (42/47) and blood was present on glove in 28% (13/47) of cases. Colonoscopy was performed in 77% (41/53) of cases. A unique mass was detected in 88% (36/41), and two masses in 12% (5/41) of dogs. Masses were excised by transanal pull-through in 58% (31/53), mucosal eversion in 21% (11/53), colectomy in 11% (6/53) and endoscopic polypectomy in 9.4% (5/53) of cases. Histopathology revealed adenoma in 47% (25/53), adenocarcinoma in 32% (17/53), carcinoma *in situ* in 9.4% (5/53), inflammatory polyp in 7.5% (4/53), leiomyoma in 1.9% (1/53) and leiomyosarcoma in 1.9% (1/53) of dogs. Recurrence occurred within first year in 20% of dogs. Median survival time was 54 months. No significant difference was observed regarding recurrence and survival times between tumor types.

In this study, males were more represented than females, as previously described. West Highland White Terriers were frequently involved, followed by French Bulldogs and Boxers. Haematochezia was prominent and constitutes a warning sign for clinicians. Recurrence frequency (20% at 1 year) was inferior in our study than in previously published case series (40%), and no significant association with excision technique or histopathology was observed.

**Disclosures**

Juan Hernandez: financial support from Royal Canin and Hill's Pet Nutrition.

## ESCG-O-12 - European Society of Comparative Gastroenterology

### Suspected acute hemorrhagic diarrhea syndrome in out-patients: A preliminary study of disease severity, treatment, outcome and client satisfaction

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Acute hemorrhagic diarrhea syndrome (AHDS) is a common emergency in veterinary practice which may present with varying severity from mild depression to severe life-threatening illness. Individual patient management is highly based on clinician's discretion and experience which may lead to variation in decisions on hospitalization and treatment protocols.

The aim was to investigate disease severity, treatment, outcome and client satisfaction in dogs with AHDS treated as outpatients in a prospective, observational study. Data were collected from medical records (signalment, disease history and severity at presentation, treatment prescribed) and through client interviews at least seven days after initial presentation.

One-hundred and fifty-seven dogs presented with diarrhea. Of these, 141 were excluded due to either non-hemorrhagic or only slightly blood-admixed diarrhea (N=60), signs of gastrointestinal disease for ≥7 days (N=27), NSAID or corticosteroid use (N=5), other disease known to cause hemorrhagic diarrhea (N=28) or hospitalization in conjunction with initial presentation (N=21).

The final population consisted of sixteen dogs with a median AHDS index of 12 (IQR 10-13), where 9/16 vomited >3x and 5/16 defecated >5x on the day of presentation. Five of the sixteen dogs were 5-6% dehydrated and 5/16 had no appetite. Five dogs had tachycardia (IQR 128-144 beats/minutes). None had hypo- or hyperthermia. All clients were instructed to contact the hospital in case of lack of improvement in the dog's condition. The option to hospitalize was discussed with all clients.

All dogs were prescribed a commercial gastrointestinal diet or home-cooked boiled rice and chicken/white fish diet. A single subcutaneous injection with maropitant (1mg/kg) was administered to 13/16 dogs in conjunction with the consultation. Nine dogs received either pre- and probiotics (Canikur Pro® N=6) or probiotics (SivoMixx®, N=2; ZooLac Propaste® N=1). None of the 16 dogs received antimicrobials.

Clinical signs resolved in 15 of the 16 dogs without further veterinary consultations or additional medication. One dog was hospitalized the day after initial presentation due to increased defecation frequency (every half hour) and slightly decreased activity level. The dog was treated with maropitant and intravenous fluid therapy, and was discharged the following day, where the defecation frequency had reduced markedly. All clients expressed satisfaction with the prescribed treatment on follow-up. The results of this study suggest that supportive nutritional and antiemetic treatment alone without antimicrobial therapy is sufficient in dogs with AHDS which can be managed as outpatients. Furthermore, high client satisfaction might be achievable despite refraining from antimicrobial prescription.

**Disclosures**

No disclosures to report.

## ESCG-O-13 - European Society of Comparative Gastroenterology

### Low-dose oral cobalamin supplementation ameliorated in serum cobalamin concentrations in dogs with chronic enteropathy when compared with small cell gastrointestinal lymphoma

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Hypocobalaminemia is a common complication of gastrointestinal diseases including canine chronic enteropathy (CE) and gastrointestinal small cell lymphoma (SCL). Previously, treatment for hypocobalaminemia was administered subcutaneously. In recent years, some studies have demonstrated that oral cobalamin supplementation for canine CE has the same effect as that of injections. However, efficacy of oral cobalamin supplementation in canine SCL with hypocobalaminemia has not yet been reported. Therefore, this study aimed to investigate the efficacy of oral cobalamin supplementation in canine SCL and compare cobalamin requirements between canine CE and SCL.

This prospective study included CE (n = 5) and SCL (n=3) with hypocobalaminemia. Diagnosis was based on chronic gastrointestinal diseases, physical examination, blood tests, fecal examination, urinalysis, abdominal radiography and ultrasound, and histopathological examination of endoscopic biopsy sampling. Serum cobalamin concentrations were measured by a commercial laboratory and hypocobalaminemia was defined by a serum cobalamin concentration falling below the reference interval (<252 ng/L). Oral vitamin B compound tablets containing 250 μg of cyanocobalamin each were used for treatment, along with diet therapy (elimination or low-fat diets), antibiotics (metronidazole or tylosin), prednisolone, or chlorambucil. Previously reported doses of oral cobalamin were more than sufficient after 28 days of treatment (median concentrations were higher than the reference interval); therefore, the starting dose in dogs with a body weight of 1-10 kg was 250 μg/day, and that in dogs >10 kg was 500 μg/day in this study. Serum cobalamin concentrations were measured at baseline and 28 ± 7 days after oral cobalamin supplementation. When serum cobalamin concentrations were above the detection limit (>1,000 ng/L), cobalamin doses were tapered by 50% and serum concentrations were measured at 28 ± 7 days after dose tapering, until serum cobalamin concentrations fell within the reference interval (252-908 ng/L).

Hypocobalaminemia was ameliorated by oral cobalamin supplementation in dogs with CE and dogs with SCL. Median dose of oral cobalamin requirements was significantly lower in dogs with CE (18 μg/kg/day; range, 6-30 μg/kg/day) than in dogs with SCL (44 μg/kg/day; range, 29-48 μg/kg/day).

These results revealed that oral cobalamin supplementation was effective for hypocobalaminemia in dogs with SCL, as well as dogs with CE. Moreover, this pilot study suggests that oral cobalamin requirements may be lower in dogs with CE than dogs with SCL.

### Disclosures

No disclosures to report.

## ESCG-O-14 - European Society of Comparative Gastroenterology

### Assessment of visceral pain in dogs with chronic enteropathy and its' effect on behaviour and owner-observed quality of life

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Validated clinical scores to assess the severity of canine chronic enteropathy (CE) do not include an assessment of pain or quality of life (QoL). Even though visceral pain (ViP) has been reported as a feature of canine and human CE, the prevalence of chronic ViP and its' impact on QoL and behaviour is unknown. We hypothesize that ViP impacts behaviour and reduces QoL in dogs with CE. Adult dogs diagnosed with CE (n=44) were assessed for ViP during physical examination (PE) by a veterinary surgeon using a modified chronic pain scale. Owners also completed a Canine Brief Pain Inventory (cBPI) questionnaire to evaluate ViP severity, ViP interference and QoL, with additional questions to assess interference with play and ability to settle, as well as observation of praying posture. A group of healthy dogs (n=21) underwent PE and ViP scoring to serve as controls. Video footage from all dogs was used for ethogram assessment by an observer blinded to the final diagnosis. Mann-Whitney U Test or Kruskal-Wallis (followed by Dunn test) were used to evaluate differences in behaviour frequency/duration, cBPI scores and pain scores between the CE and control groups, as well as between behavioural observations and pain score categories. P-values were adjusted using Benjamini-Hochberg method where appropriate. Frequency of facial expressions (frown, mouth tension and whale eye) were significantly higher in CE dogs than in controls (p=0.047). Wagging during palpation had a higher incidence in control dogs than dogs with CE (p=0.039), while tensing on palpation (ToP) was more frequently observed in the CE group (p=0.006). Pain scores were significantly higher in the CE group (p<0.001). ToP was more frequent in dogs with higher pain scores (p=0.011), while percentage of time wagging was higher in dogs with lower pain scores, (p=0.036). Regarding cBPI, pain severity (p<0.001), pain interference (p<0.001) and praying posture frequency (p<0.001) were significantly higher in the CE group, while QoL was significantly lower (p<0.001). This study provides evidence that dogs with CE may be experiencing chronic ViP, compromising their ability to engage with daily activities such as rest, exercise and play. Dogs with CE have a reduced QoL as perceived by the owner. The incorporation of assessment of ViP into a clinical scoring tool for CE might be prudent to assess severity of clinical signs more accurately and monitor response to treatment.

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## ESVC-O-1 - European Society of Veterinary Cardiology

### Circulating miRNAs as potential biomarkers of early myxomatous mitral valve disease in Cavalier King Charles Spaniels

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Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs and in Cavalier King Charles Spaniels (CKCS) is an important cause of cardiac morbidity and death, even at a young age. One of the main challenges in the study of MMVD in CKCS is the identification of biomarkers that that allow to discriminate the classes of the mitral disease and obtain an early diagnosis. MicroRNAs (miRNAs) are small non-coding RNAs, identified as post-transcriptional regulators of gene expression, both in physiologic and pathologic conditions that meet diagnostic and prognostic expectancies as biomarkers of MMVD.

In this study, we quantified the expression of 5 circulating miRNAs as being involved in MMVD in the plasma (left-over samples) of CKCS classified as ACVIM classes A (healthy) and B1 (affected by MMVD, asymptomatic and without cardiac remodelling). Forty-four CKCS were included in the study: 11 A, 11 B1 younger than 3 years, 11 B1 between 3 and 7 years, and 11 B1 older than 7 years. Total RNA was isolated from plasma, retro-transcribed and then the expression levels of miR-128-3p, miR-1, miR-30-b, miR-103, miR-191 were evaluated by RT-qPCR using TaqMan probes. The comparative analysis demonstrated that the concentrations of circulating miR128-3p and miR-30b were significantly greater in B1<3y ($p=0.013$ and $p=0.001$) and B1>7y ($p=0.049$ and $p=0.006$) as compared with A. The age of the subjects did not affect miRNAs' expression ($p>0.05$). ROC curves showed that miR-30b and miR-128-3p can discriminate between A and B1 under 3 years CKCS ($AUC_{[miR-30b]}$ 0.88, cut-off 23.56, Se 82%, Sp 82% and $AUC_{[miR-128-3p]}$ 0.80, cut-off 32.24, Se 54%, Sp 100%), and between A and B1 over 7 years CKCS ($AUC_{[miR-30b]}$ 0.86, cut-off 23.56, Se 80%, Sp 82% and $AUC_{[miR-128-3p]}$ 0.80, cut-off 13.33, Se 70%, Sp 82%). Combining two miRNAs, namely miR-30b and miR-128-3p, in a panel increased the efficiency of distinguishing between A and B1 over 7 years CKCS ($AUC_{[average]}$ 0.88, cut-off 0.52, Se 80%, Sp 91%).

The expression of miR-30b and miR-128-3p allows to discriminate ACVIM A and young B1 (< 3 years) CKCS, in most cases without heart murmurs. These miRNAs may be candidates as novel biomarkers in the disease characterization and may provide the basis for further investigations in the follow-up of the examined subjects, aimed to the characterization of the evolution of the disease in the CKCS.

## ESVC-O-2 - European Society of Veterinary Cardiology

### Use of physical examination, electrocardiography, radiography and biomarkers to predict stage B2 myxomatous mitral valve disease in preclinical Cavalier King Charles Spaniels

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Cavalier King Charles Spaniels (CKCS) are predisposed to developing myxomatous mitral valve disease (MMVD). The objective of this study was to develop predictive models to identify the best combination of history, physical examination and diagnostic tests to predict ACVIM Stage B2 MMVD in the absence of an echocardiogram in CKCS.

Two-hundred and thirty preclinical CKCS were prospectively enrolled. Dogs were staged by echocardiography using 2019 ACVIM MMVD consensus guidelines. Dogs underwent: physical examination, right lateral thoracic radiograph with vertebral heart size and left atrial size measurements (VHS, VLAS), systemic blood pressure measurement, 30-second electrocardiogram (ECG), and blood sampling to assess N-terminal pro B-type natriuretic peptide (NTproBNP), ultrasensitive cardiac troponin I, and a renal panel including symmetric dimethylarginine (SDMA). There were 185(80.4%) CKCS in Stage A or B1 and 45(19.6%) in stage B2.

Exploratory binary forward stepwise logistic regression analysis was used to develop prediction models. Variables were clustered initially by diagnostic test. Significant variables from each cluster were entered into a final model. Six variables were retained in the final model including (in order of significance): NTproBNP, VHS, P+QRS duration, murmur grade, ECG average heart rate. Validation was demonstrated by construction of classification tables to report specificity, sensitivity and percent of correct classifications using a cut-point threshold of 0.5. The area under the curve was 0.967 with a sensitivity, specificity and overall accuracy of 94.0%, 72.7%, and 89.8%, respectively. Discriminatory ability to identify Stage B2 was improved using variables derived from a combination of diagnostics compared to any variable alone.

**Disclosures**

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Laboratory. Dr. Sonya Gordon has done speaking engagements/consulting for IDEXX and Dr. Jordan Vitt received research support from IDEXX for an unrelated research project in the past 5 years.

## ESVC-O-3 - European Society of Veterinary Cardiology

### Prognostic significance of different echocardiographic parameters of left atrial size in dogs with asymptomatic myxomatous mitral valve disease

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Several echocardiographic parameters of left atrial size have been described in canine myxomatous mitral valve disease (MMVD). Among two-dimensional measurements, the left atrium-to-aorta root ratio (LA/Ao) has been the most used parameter in both clinical research and clinical practice. The left atrial diameter measured from right parasternal long-axis view (LAD) has been recently proposed as another useful parameter of left atrial size, showing higher reproducibility and less measurement variability in comparison to LA/Ao and left atrial volume. The aim of this study was to evaluate the prognostic role of LAD in asymptomatic MMVD dogs in comparison to the LA/Ao, also defining echocardiographic cut-offs predicting of cardiac mortality.

This was a retrospective, multicenter, observational study including asymptomatic dogs with MMVD imaged between 2011 and 2019. The LA/Ao, the LAD normalized for body weight (LADn) and the LAD indexed to the aortic annulus (LAD/Ao) were evaluated. Long term outcome was assessed by telephone interviews with the owners. ROC curve analysis and Youden index were used to define the best cut-offs predictive for cardiac mortality, and the relative risk (RR) with 95% confidence interval (CI) were calculated. Survival was analysed using Kaplan Meier curves and log-rank test.

A total of 444 dogs with asymptomatic MMVD were included. At the end of the study 76 dogs died for cardiac-related cause. LA/Ao>1.8 [AUC=0.73, sensitivity (Se) 62%, specificity (Sp) 81%, P<0.0001; RR=4.22, 95%CI 2.75-6.47], LADn>16 (AUC=0.72, Se 81%, Sp 58%, P<0.0001; RR=4.25, 95%CI 2.43-7.46) and LAD/Ao>2.6 (AUC=0.71, Se 77%, Sp 59%, P<0.0001; HR=3.53, 95%CI 2.11-5.90) were identified as the best cut-offs predictive of cardiac death. No significant differences between LA/Ao, LADn, and LAD/Ao were found in predicting cardiac mortality. Median survival time was not different between dogs with LA/Ao>1.8 (1228 days, 95%CI 814-1641 days), LADn>16 (1544 days, 95%CI 1282-1806 days) and LAD/Ao>2.6 (1587 days; 95%CI 1351-1822 days; P=0.37). Moreover, median survival time was not different between dogs presenting both LA/Ao>1.8 + LADn>16 (1180 days, 95%CI 885-1476 days) and LA/Ao>1.8 + LAD/Ao>2.6 (1295 days, 95%CI 822-1768 days) in comparison to those only presenting LA/Ao>1.8 (1228 days; 95%CI 814-1641 days; P=0.87).

Our results suggest similar prognostic significance of two-dimensional linear measurements of left atrial size in dogs with asymptomatic MMVD. LADn and LAD/Ao appears interchangeable as prognostic factors, and the combination with the LA/Ao does not significantly improve the risk stratification for cardiac death.

**Disclosures**

No disclosures to report.

## ESVC-O-4 - European Society of Veterinary Cardiology

### The effect of obesity and subsequent weight loss on cardiac structure and function in dogs

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In people, the cardiovascular effects of obesity include systemic hypertension, cardiac remodelling and both systolic and diastolic dysfunction, whilst weight loss can reverse myocardial remodelling and reduce the risk of subsequent cardiovascular disease. To date, variable results are reported in studies of the effect of obesity and subsequent weight loss on cardiovascular morphology and function in dogs. Therefore, this prospective cohort study aimed to assess cardiac function, heart rate variability, cardiac biomarkers and body composition before and after weight loss in pet dogs with obesity.

Twenty-four client-owned dogs with obesity participated, all referred for weight management. To assess the cardiac effects of obesity, body composition analysis (by dual energy x-ray absorptiometry, DEXA) and cardiovascular assessment (echocardiography, Doppler blood pressure, electrocardiography, cardiac biomarkers) were performed prior to weight management. To assess the effect of subsequent weight loss, cardiovascular assessment and DEXA were repeated in 12 of the dogs that reached target weight. A Wilcoxon-signed rank test was used to compare each variable pre- and post- weight loss. Median (inter-quartile range) duration of weight loss was 224 days (124-245 days), whilst percentage weight loss was 23% (18-31%) of starting weight. Median change in body fat mass was -50% (-44% to -55%; *P*=0.001), whilst median change in lean mass was -7% (+1% to -18%, *P*=0.077).

Borderline systemic hypertension (14/24 >150 mmHg, median 165 mmHg, 141-183), diastolic dysfunction and increased left ventricular wall thickness were common features in dogs with obesity prior to weight loss. However, systolic wall dimensions were the only variables that changed after weight loss, with a decrease in both the

systolic interventricular septum (before 13.4 mm, 11.9-15.9 mm; after 11.7 mm, 9.7-14.1, $P = 0.029$) and systolic left ventricular free wall (before 13.3 mm, 12.1-15.5; after 10.6 mm, 10.2-14.3; $P = 0.017$). There was no evidence of decreased heart rate variability in dogs with obesity ($P=0.367$) and no change in cardiac biomarker concentrations with weight loss (N-terminal proBNP, $P = 0.262$; cardiac troponin I $P = 0.657$).

Canine obesity results in diastolic dysfunction and left ventricular hypertrophy, the latter of which improves with significant weight and fat mass loss. Further studies are required to clarify the clinical consequences of these findings.

**Disclosures**

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## ESVC-O-5 - European Society of Veterinary Cardiology

### Performance of different echocardiographic measurements of left atrial size by observers with different levels of experience

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Assessment of left atrial (LA) sizes in dogs is important for clinical staging, risk assessment, treatment decisions, and prognosis. With widespread availability of ultrasound units, observers with different levels of experience are required to interpret LA sizes. The objective of this study was to assess diagnostic performance of operators with different levels of experience measuring the LA with different techniques. Echocardiographic images from 36 dogs with different degrees of left atrial enlargement (LAE) were retrospectively retrieved and measured by a veterinary student, a first-year cardiology resident, a third-year cardiology resident and two board-certified veterinary cardiologists. The LA to aortic root ratio (LA/Ao) and LA antero-postero diameter indexed to body weight ($LAi_{APD}$) were measured. Measurements obtained by the two cardiologists were averaged and used as criterion standard. Intraclass correlation coefficients (ICCs), Bland-Altman plots, and accuracy in identification of LAE were calculated for the three least experienced observers. Intra- and inter-observer ICCs were greater than 0.9 for every variable. The observer with least experience had significant positive bias and tendency to overestimation for larger measurements using LA/Ao, but not using $LAi_{APD}$. For both variables, the limits of agreement were narrower for observers with increased experience. The accuracy of identification of LAE also increased with the increasing level of experience and was higher for $LAi_{APD}$ compared to LA/Ao. Combining both methods for identification of LAE, further increased the accuracy. In conclusion, operator's experience is important when performing LA measurements, and using $LAi_{APD}$ or combining more than one can improve the identification of LAE.

**Disclosures**

No disclosures to report.

## ESVC-O-6 - European Society of Veterinary Cardiology

### Teaching vets to be EPIC: Validation of a focussed echocardiographic training program for general practitioners - The FEET-FIRST Study

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The EPIC study sets out criteria for dogs with preclinical myxomatous mitral valve disease (MMVD) likely to benefit from treatment with pimobendan. These include echocardiographic assessment of left atrial (LA) and ventricular (LV) size; access to echocardiography by a cardiologist is not universally available. The aim of this study was to evaluate a focussed training programme for the accurate identification of dogs fulfilling the EPIC criteria by primary care veterinarians (PCVs).

Six PCVs with no previous echocardiographic experience underwent the training programme. Each PCV subsequently evaluated ≤10 dogs that they believed to have preclinical MMVD. The evaluation was repeated by one of three board-certified cardiologists, blinded to the PCV's findings. The median time between PCV and cardiologist evaluation was 0 days (range 0-8). Fifty-two dogs were evaluated by PCVs; one dog was withdrawn from the study due to radiographic evidence of CHF.

In one dog the PCV's diagnosis of MMVD was incorrect (this dog had preclinical dilated cardiomyopathy (DCM)); preclinical MMVD was confirmed by the cardiologist in the remaining 50 dogs. Agreement between PCVs and cardiologists was fair to moderate for auscultatory findings (apex beat intensity (kappa=0.474; P<0.001), precordial thrill ($\kappa=0.510$; P<0.001), heart sound intensity ($\kappa=0.338$; P=0.012),

murmur intensity (κ=0.411; P=0.002) and murmur grade (κ=0.550; P<0.001). No differences in heart rate (P=0.454), vertebral heart scale (P=0.147; coefficient of variation (CV)=2.1%), interventricular septal thickness in diastole (P=0.979; CV=11.4%), LV free wall thickness in diastole (P=0.342; CV=13.4%), LV internal dimension in systole (P=0.280; CV=7.7%) or LA to aortic ratio (P=0.935; CV=7.1%) were detected between PCVs and cardiologists. LV internal diameter in diastole (LVIDd; 3.31cm (2.07-5.14) vs 3.45cm (1.42-5.80); P=0.024; CV=7.7%), LVIDd normalised for body weight (1.71cm/kg$^{0.294}$ (1.29-2.21) vs 1.73 cm/kg$^{0.294}$ (1.32-2.73); P=0.017; CV=5.7%), LA diameter (2.56cm (1.22-3.61) vs 2.76cm (1.68-4.55); P<0.001; CV=12.2%) and aortic root diameter (1.72cm (0.82-3.34) vs 1.73cm (1.26-3.13); P=0.016; CV=10.5%) were significantly lower when assessed by PCVs vs cardiologists; all differences were clinically insignificant. Agreement between PCVs and cardiologists regarding whether dogs fulfilled the EPIC criteria was almost perfect (kappa=0.828; P<0.001); both agreed in 47 cases, disagreed in 4 and the diagnosis was incorrect in 1 (DCM). Plasma N-terminal pro-B-type natriuretic peptide concentrations, measured in surplus samples, were significantly higher in dogs which fulfilled the EPIC criteria vs those that did not (1748pmol/L (449-3088) vs 571pmol/L (250-2172); P<0.001).

This study suggests that the focussed programme effectively trained PCVs to accurately assess EPIC criteria.

**Disclosures**

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## ESVC-O-7 - European Society of Veterinary Cardiology

### Evaluation of point-of-care ultrasound performed by non-cardiologists for diagnosis of degenerative mitral valve disease in dogs presented to the cardiology service

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Cardiac point-of-care ultrasound (POCUS) is a rapid screening method for gross cardiac pathology. Cardiac POCUS performed by non-cardiologists is used for diagnosis and management of human emergency patients or when a cardiologist is not on site. Degenerative mitral valve disease (DMVD) is the most common acquired canine heart disease. Being able to reliably diagnose, stage and identify complicating factors of DMVD would be of great benefit to general practitioners. This prospective study evaluated the ability of clinicians trained in POCUS to correctly diagnose and stage DMVD, and to identify post-capillary pulmonary hypertension (PH) in dogs presented to the cardiology service.

Dogs presented between March 2019 and December 2020 for evaluation or follow-up of heart disease were eligible for inclusion in this descriptive study. Two clinicians received an 8-hour course in thoracic and cardiovascular POCUS. Clinicians were informed about the dogs' presenting complaints but were blinded to the underlying heart disease, treatment and results of echocardiography and thoracic radiographs. They performed physical examination, thoracic and cardiac POCUS. More than 3 B-lines in a view was considered abnormal. Left atrial (LA), left ventricular (LV), and right heart (RH) size were subjectively scored as small, normal or enlarged. Caudal vena cava was subjectively scored as flat, normal or fat. Clinicians classified dogs as presumptive DMVD (Group 1), no cardiac disease (Group 2) or other cardiac disease (Group 3). For dogs with presumptive DMVD, they indicated ACVIM stage and screened for presence or absence of associated PH based on RH size. Results were compared with the final cardiological diagnosis based on history, clinical examination, full-Doppler echocardiography and thoracic radiographs.

Eighty-five dogs were enrolled, 56 with DMVD, 8 without cardiac disease and 21 with other cardiac disease. Clinicians classified 54 dogs into group 1, 10 into group 2 and 21 into group 3. Clinicians correctly identified DMVD in 96.4% of the cases (54/56). Dogs without cardiac disease were correctly identified in 75% of the cases (6/8). No dog without cardiac disease or with other cardiac disease was misclassified into group 1. Staging of DMVD was correct in 79.6% (43/54) dogs, overscored in 6 and underscored in 5. PH was not identified by clinicians in any dog from group 1, although mild to severe PH was present in 11.

Clinicians can accurately diagnose DMVD based on history, physical examination and POCUS, and assess stage with moderate accuracy, yet fail to detect pulmonary hypertension in DMVD patients.

**Disclosures**

No disclosures to report.

## ESVC-O-8 - European Society of Veterinary Cardiology

### A new auscultatory finding confirmed by phonocardiography in cats with obstructive hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is the most prevalent feline heart disease and represents a main cause of cardiovascular mortality in this species. Establishing the diagnosis of HCM is therefore of

major importance, and cardiac auscultation may help to identify cats with HCM. Auscultatory findings commonly associated with HCM include heart murmurs, gallop sounds, and arrhythmias. Systolic anterior motion of the mitral valve (SAM) is frequently reported in cats with HCM as a feature of the obstructive form. A SAM-associated sound (SAM-AS) related to systolic collision of the mitral valve leaflet with the interventricular septum has been reported in human patients but, to the best of our knowledge, not in cats.

The aim of this prospective study was to identify and characterize SAM-AS in HCM cats. For this purpose, 50 cats were prospectively enrolled, i.e., 20 HCM cats with SAM and 30 cats without SAM, as confirmed by echocardiography. Auscultation was first performed with a conventional stethoscope and then an electronic stethoscope with simultaneous phonocardiography recording on both sides of the chest wall in a quiet room, by one observer (A). Observer A (VS) had a higher level of experience and initially identified this new auscultatory finding, corresponding to an additional low-pitched systolic sound best heard over the left cranial sternal border, occurring immediately after S1 and appearing on phonocardiography as a smaller peak than S1, about 60 milliseconds after S1, before S2. Digital recordings of cardiac auscultation were then stored and played back for off-line auscultation in a random order by three observers blinded to signalment, medical history, and echocardiographic results: A, B (moderate level of experience and a short training to recognise the finding) and C (moderate experience, without training). For evaluating agreement between auscultation and echocardiography (the gold standard method for identification of SAM), Cohen's weighted kappa values were calculated for each observer. Kappa values were 0.92, 0.62, 0.08 for observers A, B and C (with detection of 20/20 [100%], 18/20 [90%], and 13/20 [65%] SAM-AS) respectively. Kappa value between observers A and B was 0.7. SAM-AS that were judged louder by A were recognised by two to three observers. In these cases the median trans-aortic gradient was 96 mmHg (versus 40 mmHg for softer SAM-AS).

In conclusion, we describe here a new auscultatory abnormality in cats with HCM. It could help a trained observer, independently from his level of experience, in identifying obstructive HCM in cats upon auscultation.

**Disclosures**

No disclosures to report.

**ESVC-O-9 - European Society of Veterinary Cardiology**

## Reproducibility and repeatability of radiographic measurements of cardiac size in dogs

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Objective measurements of radiographic cardiac size are reported in veterinary medicine including: vertebral heart size(VHS), vertebral left atrial size (VLAS), and manubrium heart score(MHS). The objectives of this study were to evaluate the reproducibility and repeatability of VHS, VLAS and MHS and to evaluate the effect of experience level. A set of 35 right lateral thoracic radiographs were selected to include a range of diagnostic quality and cardiac size. This set was duplicated five times resulting in a total of 165 radiographs that were measured in randomized order. Measurements were made using commercial digital radiology software (Metron-DVM). Observers received identical training. Reproducibility and repeatability were calculated for VHS, VLAS and MHS overall and by experience group. Fourteen observers participated in the study (veterinary students n=5, veterinary residents/interns n=6, Diplomates of the American College of Veterinary Internal Medicine, Cardiology (DACVIM-C) n=3). Measurements were recorded in 2310/2310 (100%), 2279/2310 (98.7%) and 1857/2310 (80.4%) images for VHS, VLAS and MHS, respectively. The overall reproducibility% (inter-observer variability) and repeatability% (intra-observer variability) were: VHS(5.3%, 0.4%), VLAS (14.6%, 12.2%), and MHS(6.2%, 0.4%), respectively. The associated 95% CI in vertebral bodies(v) was ±0.56v for VHS, ±0.52v for VLAS and ±0.7v for MHS. The DAVCIM-C group demonstrated the lowest reproducibility and repeatability for all measurements: VHS (1.3%, 0.4%), VLAS (4.6%, 3.4%), MHS (1.3%, 0.3%). Overall reproducibility and repeatability were very good for VHS and MHS, and acceptable for VLAS, however VLAS and MHS are not always measurable, and VHS had the smallest 95% CI.

**Disclosures**

All observers utilized a digital radiography software provided by Metron-DVM Veterinary Imaging Software.

**ESVC-O-11 - European Society of Veterinary Cardiology**

## An international survey of preferences for echocardiographic assessment of left atrial size in dogs: The BENEFIT Project

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Veterinary echocardiographers' preferences for LA size assessment in dogs have, to our knowledge, never been systematically investigated.

The aim of this international survey study was to investigate veterinary echocardiographers' preferences concerning LA size assessment in dogs; including positioning of the dog, acquisition views, indexing methods and timing.

A link to a 126 questions survey was distributed globally via e-mail sendouts to clinical practitioners.

A total of 671 responders, of which 55.9% worked in specialty practice and 37.2% in general practice, from 6 continents (Europe 49.6%, North America 19.6%, Asia 13.8%, South America 13.5%, Oceania 2.4%, Africa 1%) and 54 countries, completed the survey. Linear 2D (n=623/671, 93.3%) was the most commonly used method for LA size assessment, followed by subjective assessment (n=379/671, 56.4%), linear M-mode (n=149/671, 22.2%), volume (n=75/671, 11.2%), and area (n=45/671, 6.7%) methods, respectively. The most popular preferences for LA size assessment by linear 2D echocardiography included having the dog positioned in right lateral recumbency (n= 556/623, 89.2%), using the right parasternal short axis view (n=529/623, 84.9%), indexing the LA by the aorta (n=545/623, 87.5%), and using echocardiographic guiding (n=400/623, 64.2%) rather than ECG (n=223/623, 35.8%) for timing of measurements. Only 30.7% (n=191/623) respondents that used linear 2D echocardiography for LA size assessment had all the same preferences regarding positioning of the dog, acquisition view, indexing method and time point used for measurement.

In conclusion, the LA size in dogs was most commonly assessed by linear 2D echocardiography, but the veterinary echocardiographers' preferences for the assessment varied substantially.

**Disclosures**

No disclosures to report.

**ESVC-O-12 - European Society of Veterinary Cardiology**

## Left Ventricular Eccentricity index for assessment of precapillary pulmonary hypertension in dogs

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Ventricular septal flattening, frequently present in pulmonary hypertension (PH) can be quantified using the eccentricity index (EI), an index of left ventricular (LV) shape and septal curvature, reflecting the interaction between the two ventricles. In human medicine, EI correlates with invasive pulmonary pressure measurements and can accurately define patients with clinically important PH.

The aim of this study was to evaluate whether in dogs, EI is a quantitative marker of PH and correlates with other variables indicative of PH.

In this retrospective study, dogs with measurable tricuspid regurgitation (TRPG) or pulmonic insufficiency (PRPG), without evidence of right ventricular obstruction and in the absence of increased LA size, were included.

Dogs were divided into 4 classes (absent, mild, moderate and severe PH), according to TRPG (<30, 31-50, 51-75, >75 mmHg) and/or PRPG (<19,5, 20-25, 25-35, >35 mmHg) respectively. Quantitative parameters of right heart remodelling including right ventricular (RV) internal diameter (RVIDd/Ao), RV free wall (RVFWd/LVFWd), presence of interventricular septal flattening, right atrial size (RAD/LAD), main and right pulmonary artery size (MPA/Ao; PV/PA), pulmonary flow profile (AT/ET), RV function (TAPSE) were documented. Left ventricular end-diastolic (EId) and end-systolic (EIs) EI were measured at the mid-ventricular level from a parasternal short-axis view as the ratio of the latero-lateral and cranio-caudal LV cavity dimensions. Data were expressed as median and range [minimum-maximum].

Ninety-seven dogs were included: 29 absent, 13 mild, 25 moderate and 30 severe PH. EId and EIs were significantly higher in severe (1.51 [1.05-2.96];2.57 [1.25-9.72]) compared to absent (1.12 [0.94-1.30];1.11 [0.92-1.26]), mild (1.12 [1.01-1.28];1.17 [0.99-1.38]) and moderate (1.19 [1.02-1.72];1.21 [0.95-2.34]) PH (P<0.0005). EIs was also significantly higher in moderate compared to absent PH (p<0.01). All variables were significantly associated with EI. EIs was moderately associated with TRPG (r=0,62), Eid (r=0,56) RAD/LAD (r=0,54) and PV/PA (r=0,45) (p<0.0001). EId was moderately associated with TRPG (p<0.0001). Remaining variables were weakly associated with EI (r < 0,40; p<0.01). Area under the receiver-operating characteristic curve for EId and EIs were 0.78 and 0.87 respectively. Optimal cut-off values were 1,24 and 1,34, discriminating moderate to severe PH from absent and mild PH with a sensitivity of 60% and 67% and a specificity of 90 and 95%, respectively.

EI increased with severity of precapillary PH. EIs appears to be more sensitive and specific than Eid, possibly due the right ventricular pressure overload mechanism and may be useful for assessment of moderate to severe precapillary PH in dogs.

**Disclosures**

No disclosures to report.

## ESVC-O-13 - European Society of Veterinary Cardiology

### Continuous heart rate monitoring by Holter and validation of heart rate spot-checks to measure circadian variation in heart rate in dogs with atrial fibrillation

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Disrupted circadian variation (CV) increases cardiovascular risk in people. Atrial fibrillation (AF) is a negative prognostic indicator in dogs. This study determined whether CV in heart rate (HR) occurs in pet dogs with AF, and is identifiable by HR spot-checks.

Using 24-hour Holter studies from 21 AF dogs, mean and median HRs were calculated from every hour, every 4, 6 and 8 hours, and the first 30 and 60 seconds of every 4$^{th}$, 6$^{th}$ and 8$^{th}$ hour. Values were compared (correlation, Bland Altman analysis) with the mesor and amplitude from cosinor analysis.

Only mean hourly HR varied sinusoidally in 14/21 dogs (mesor 133.8±29.6bpm, amplitude 15.8±6.2bpm). Mean HR had higher levels of agreement with the mesor (r=1, p<0.0001, bias-0.12, SD0.7).

Four-hourly median HR had greater agreement with the mesor (r=0.77, p=0.001, bias-1.59, SD18.8), but 30 and 60-second mean HR was superior (30s, r=0.92, p<0.0001, bias5.4, SD11.9; 60s, r=0.95, p<0.0001, bias3.3, SD9.3).

Six-hourly median HR had greater agreement with the mesor (r=0.67, p=0.009, bias-2.67, SD22.1), including for 30-second intervals (r=0.96, p<0.0001, bias4.9, SD8.4). At 60-second intervals, mean HR was superior (r=0.98, p<0.0001, bias-1.5, SD6.3).

Eight-hourly mean HR had greater agreement with the mesor (r=0.57, p=0.035, bias-29.5, SD65), including for 30 and 60-second intervals (30s, r=0.86, p<0.0001, bias2.41, SD16.7; 60s, r=0.87, p<0.0001, bias1.94, SD15.7).

CV in HR occurs in pet dogs despite AF but is not identified by spot-checks, which accurately estimate 24-hourly HR. Continuous HR monitoring could help determine if lost CV in HR is a therapeutic target in canine AF.

#### Disclosures

This abstract is a part of a research project with the title ' Use of heart rate spot-check protocol to assess 24-hour average heart rate in canine patients with atrial fibrillation'. The ECVIM-CA and Purina Institute Resident Research Awards have awarded us with € 8000 to fund this project.

## ESVC-O-14 - European Society of Veterinary Cardiology

### Holter characterization of ventricular and supraventricular arrhythmia in English bulldogs with presumed arrhythmogenic cardiomyopathy before specific antiarrhythmic therapy (94 cases)

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Arrhythmogenic cardiomyopathy (AC) has been largely described in boxer dogs. It is due to a fibro-fatty replacement prevalently of the right ventricular myocardium even if other cardiac chambers can be involved. In English bulldogs (EB) AC has been reported for the first time in 2009 by Santilli and coll. A more recent study described the clinical and ECG features in a population of dogs with presumed AC with only three dogs undergoing Holter examination before starting the anti-arrhythmic therapy. The aim of the present study was therefore to describe Holter findings in a larger population of EB with presumed AC without specific anti-arrhythmic therapy. The database of a Holter referral centre was retrospectively searched for EB screened for AC. Dogs were included if they didn't receive any specific therapy for ventricular arrhythmia (e.g. sotalol, amiodarone, mexiletine). Reason for examination, main heart structural changes, clinical signs and ventricular (VA) and supraventricular arrhythmias (SVA), scored by number of events and maximal heart rate (HR), were collected. Morphology, coupling interval, and burden for VA (VAB = atypical/normal beats %), were also provided. Of the 135 EB undergoing Holter for AC indications, 94 didn't receive specific anti-arrhythmics (1 received digoxine and 1 diltiazem). VA were found in 90 dogs (96%), 50 of which showed also SVA (55%). Ventricular premature complexes were found in 99%, with a median coupling interval of 233 (160÷500) milliseconds, couplets in 64% and triplets in 20% of dogs, allorhythmic forms were also well represented especially by ventricular bigeminy (77%). Right bundle branch block morphology was observed in only 3%. Monomorphic ventricular tachycardia (MVT) was observed in 30% of dogs and in 14% it was sustained with maximal HR of 430 bpm. Median VAB was 1.4 (0.04÷99)%. A median of 214 (8÷18475) supraventricular premature complexes and a median of 164.5 (5÷10297) episodes of focal atrial tachycardia (FAT, median HR = 250 [200÷280] bpm) were respectively observed in 46 and 30% of dogs. Three other dogs showed atrial fibrillation, two atrial flutter and one focal junctional tachycardia. High grade of organisation of VA (couplets and MVT runs) was frequently observed. SVA, mostly FAT, was present in more than half of dogs (in the previous study this percentage was lower: 23%), especially when VAB <12%, while it was absent when VAB>30%. This information, along with the details provided about the VA, could be helpful in defining the Holter diagnostic criteria of EB AC.

**Disclosures**

No disclosures to report.

## ESVC-O-15 - European Society of Veterinary Cardiology

### Renin-angiotensin-aldosterone system activity in cats with systemic hypertension or cardiomyopathy

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Activity of the renin-angiotensin-aldosterone system (RAAS) has not been comprehensively characterized in feline systemic hypertension (SH) or cardiomyopathy (CM), and the effects of furosemide or amlodipine treatment on RAAS biomarkers have not been fully evaluated in cats. The purpose of this study was to document RAAS activity in cats with SH or CM compared to healthy cats and determine how RAAS profiles change with furosemide or amlodipine treatment.

Sixty-six client-owned cats were enrolled: 34 healthy, 15 with SH (8 untreated, 7 amlodipine-treated), and 17 with advanced CM (8 untreated, 7 furosemide-treated). Equilibrium levels of RAAS peptides were quantified in banked surplus serum samples by liquid chromatography-mass spectrometry. Variables were compared between groups using Kruskal-Wallis analysis with *post hoc* Holms-corrected Dunn's testing.

Compared with healthy cats, cats with CM had higher concentrations of angiotensin (Ang) I, aldosterone, and plasma renin activity (all $P < 0.01$), and these differences remained significant ($P < 0.03$) considering subgroups of untreated or furosemide-treated cats. Compared with healthy cats, untreated cats with SH showed no differences in RAAS biomarkers, while amlodipine-treated cats had higher levels of AngI, AngII, AngIII (2-8), AngIV (3-8), Ang (1-7), aldosterone, and plasma renin activity (all $P < 0.03$). Multivariate analysis revealed that furosemide and amlodipine treatment were independent predictors of elevated RAAS biomarkers.

Results suggest that cats with advanced CM demonstrate RAAS dysregulation prior to treatment, while cats with untreated SH do not. Furosemide activates the classical RAAS pathway, while amlodipine leads to nonspecific upregulation of both classical and alternative RAAS pathways in cats.

**Disclosures**

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## ESVC-O-16 - European Society of Veterinary Cardiology

### Effect of the sampling time on urinary electrolytes following furosemide administration in dogs with myxomatous mitral valve disease

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Furosemide is the first-choice treatment for congestive heart failure in dogs with myxomatous mitral valve disease (MMVD). Urinary electrolytes have received growing attention to estimate diuretic response in both human and veterinary medicine.

The aim of the study was to evaluate the impact of time elapsed between oral furosemide administration and sample collection on urine electrolyte concentrations in dogs with MMVD American College of Veterinary Internal Medicine (ACVIM) stage C.

Dogs with stable MMVD ACVIM C receiving oral furosemide twice daily (median daily dose 4 mg/kg), were prospectively enrolled in the study. Other standard cardiovascular treatments were allowed. Healthy untreated dogs (n=106) were included for comparison. MMVD dogs were grouped based on the time elapsed between furosemide administration and samples collection: MMVD Morning Group (MMVD-MG) from 1 to 6 hours; MMVD Evening Group (MMVD-EG) > 6 hours. Analogously, healthy dogs were divided in two groups according to the time of blood/urine sampling: Healthy-Morning Group (H-MG) between 9 a.m. and 1 p.m.; Healthy-Evening Group (H-EG) between 2 and 7 p.m. Laboratory variables including serum and urine electrolytes and fractional excretion (FE) of electrolytes were measured. Data were reported as median and minimum-maximum values and compared with nonparametric statistics (P<0.05 considered significant).

Seventy-three dogs with MMVD ACVIM C were included. No significant difference was observed between MMVD-MG and MMVD-EG for serum creatinine, urea and serum electrolytes. If compared to the MMVD-EG, MMVD-MG group had significantly higher urine sodium (71.1, 5.9-192.9 vs. 36.3, 4.6-103.9; p=0.021), urine sodium to creatinine ratio (uNa:uCr; 1.63, 0.09-118.22 vs. 0.50, 0.06-2.82; p=0.003), FE of sodium (FENa; 1.3, 0.08-10.7 vs. 0.45, 0.06-2.5; p=0.008), urine chloride (68.2, 6-148.1 vs. 32.5, 5-114.8; p=0.038), urine chloride to creatinine ratio (uCl:uCr; 1.51, 0.09-14.7 vs. 0.44, 0.06-2.99; p=0.008), FE of chloride (FECl; 1.54, 0.08-14.8 vs. 0.53, 0.07-4; p=0.015) and urine sodium to urine potassium ratio (uNa:uK; 1.67, 0.11-10.21 vs. 0.93, 0.14-3.77; p=0.016). No significant difference

was found between H-MG and H-EG. The MMVD-MG group had significantly higher FENa, FECl and uNa:uK compared to H-MG (p<0.0001, p=0.0004 and p=0.0001, respectively). No difference was detected between MMVD-EG and H-EG.

In conclusion, time of sampling should be standardized when evaluating urine chemistry in dogs with MMVD receiving furosemide. Blood and urine sampling at <6 hours from oral furosemide administration should be recommended in the clinical practice.

**Disclosures**

No disclosures to report.

## ESVC-O-17 - European Society of Veterinary Cardiology

### Echocardiographic variables predictive of clinical signs in dogs with pulmonary stenosis

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Pulmonic valve stenosis (PS) is one of the most common congenital heart diseases in dogs. Dogs affected by PS may develop clinical signs which can be even fatal. Echocardiographic study of the right heart (RH) can help in characterizing the severity of PS. The aim of this study was to evaluate RH echocardiographic variables in dogs affected by PS and to identify those associated with clinical signs.

This was a prospective observational study. Dogs were divided in three severity groups based on the pulmonary transvalvular pressure gradient (PG): mild (PG<50 mmHg), moderate (PG 50-80 mmHg) and severe (PG>80 mmHg). Clinical signs considered in the study were exercise intolerance, syncope and right-sided congestive heart failure. The following RH echocardiographic variables were evaluated: right atrial area indexed to body surface area (BSA) (iRAA), right ventricular end-diastolic area indexed to BSA (iRVAd), right ventricular free wall thickness indexed to body weight (BW) (iRVFWd), fractional area change of the right ventricle indexed to BW (FAC), tricuspid annular plane systolic excursion indexed to BW (iTAPSE), transpulmonic peak velocity ($PA_{Vmax}$), aortic and pulmonic velocity time integral ($VTI_{Ao}$, $VTI_{PA}$), $VTI_{Ao}/VTI_{PA}$ratio and severity of tricuspid valve regurgitation (TR). Tricuspid regurgitation was classified as follows: 0 absent, 1 mild, 2 moderate and 3 severe.

Fifty dogs with PS were included. iRAA, iRVFWd, $VTI_{PA}$ were significantly higher in dogs with severe PS compared to dogs with mild and moderate PS (P<0.001) and the iRVAd was higher in dogs with severe PS compared to dogs with mild PS (P=0.007). The $VTI_{Ao}/VTI_{PA}$ratio was lower in dogs with severe PS compared to dogs with mild and moderate PS (P<0.001). In the present population, iRAA >9.6 $cm^2/m^2$ [AUC=0.77, P=0.015, sensitivity (Se) 72%, specificity (Sp) 77%], iRVFWd >0.39 $cm/kg^{0.25}$(AUC=0.72, P=0.013, Se72%, Sp 70%), $PA_{Vmax}$>5.2 m/sec(AUC=0.80, P<0.001,Se 83%, Sp 75%), $VTI_{PA}$ >0.94cm (AUC=0.84, P<0.001, Se 80%, Sp 58%), $VTI_{Ao}$≤0.13 cm (AUC=0.73, P=0.004,Se 80%, Sp 58%), $VTI_{Ao}/VTI_{PA}$ratio ≤0.11 (AUC=0.89, P<0.001, Se 80%, Sp 94%) and the severity of TR (>0, AUC=0.72, P=0.003, Se83%, Sp 54%) were independent predictors of the presence of clinical signs. Conversely, iTAPSE and FAC were not associated with clinical signs.

In conclusion, this study supports the use of a multiparametric echocardiographic assessment of the RH in dogs with PS to better assess its severity and, possibly, prognosis.

**Disclosures**

No disclosures to report.

## ESVC-O-18 - European Society of Veterinary Cardiology

### Bioelectrical Impedance Analysis in dogs with right congestive heart failure before and after treatment: A pilot study

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Congestive heart failure (CHF) is a complex clinical syndrome characterized by fluid retention due to severe heart disease. Volume overload and fluid congestion are a fundamental issue in the assessment and management of patients with CHF. Signs of CHF are commonly evaluated and monitored by physical examination, medical imaging and biomarkers detection. Bioelectrical Impedance Analysis (BIA) is a simple, non-invasive, real-time diagnostic technique validated and routinely used in human medicine to assess body composition and hydration status. BIA is based on measuring the electrical resistance that tissues oppose to the flow of a low-intensity alternating current applied to the body. Body resistance is primarily and inversely affected by body water content. Therefore, we hypothesized that BIA could be helpful to detect and quantify fluid retention due to CHF. The aim of this study was to evaluate if BIA was able to detect changes in body water content in dogs with right CHF before and after treatment.

Six client-owned dogs with ascites of cardiac origin were referred to the Veterinary Teaching Hospital of Perugia University and prospectively recruited in a longitudinal study design. Three consecutive whole-body BIA measurements were performed before and after resolution of the ascites in each dog, using a bi-frequency (50-100KHz) bioelectrical device (Biosmart®), and bioelectrical variables of impedance, reactance, resistance and phase angle were recorded. All measurements were obtained in non-sedated dogs, gently restrained in standing position using a standard tetrapolar electrode configuration

with two emitting electrodes applied by crocodile clips on the skin dorsal to the right elbow and dorsal to the patella of the right hindlimb. Two receiving electrodes were positioned 3.5 cm dorsally to their respective emitting electrodes. For each dog morphometric measurements including body weight, length, rib cage and abdominal circumference were also recorded. Bioelectrical and morphometric variables of each dog obtained before and after resolution of the ascites were averaged and compared using a Student's t-test for paired data.

All BIA variables except reactance and phase angle measured at 100kHz showed a statistically significant increase (p<0,05), in accordance with an increase of whole-body impedance, while both body weight and abdominal circumference decreased (p<0,05). We concluded that BIA is effective in detecting decreases in total body water in dogs with right CHF after treatment and could be a useful tool in monitoring patients with fluid retention. Our preliminary results need to be confirmed by larger studies.

**Disclosures**

No disclosures to report.

## ESVC-O-19 - European Society of Veterinary Cardiology

### Platelet aggregation response to pimobendan in Cavalier King Charles Spaniels with myxomatous mitral valve disease

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Pimobendan is recommended for treatment of certain stages of canine myxomatous mitral valve disease (MMVD). The beneficial effect of pimobendan is mainly thought to be a result of the inotropic and vasodilatory properties. Interestingly, at high concentrations, an inhibitory effect on canine blood platelets has been described *ex vivo* using whole blood impedance aggregometry and thromboelastography.

This study aimed to determine the *ex vivo* effect of pimobendan on adenosine diphosphate (ADP)-induced platelet aggregation response at therapeutic (0.03μM) and high (10μM) concentrations using optical aggregometry; considered gold standard for platelet function assessment. Secondary aims were to investigate if the effect of pimobendan on platelets was influenced by age, sex, severity of MMVD, haematocrit and platelet count.

A total of 41 privately-owned Cavalier King Charles Spaniels (CKCS) were prospectively enrolled and grouped based on ACVIM consensus guidelines: Stage A (n=9), stage B1 (n=22) and stage B2 (n=10). None of the dogs received cardiac treatment. Aggregation response was assessed in platelet rich plasma using optical aggregometry. Area under the curve (AUC), maximal aggregation (Amax) and aggregation velocity (Vel) were included as measures of platelet aggregation response.

Pimobendan significantly inhibited platelet aggregation response (AUC, Amax and Vel) at a concentration of 10 μM ($P$<0.0001) but not at 0.03 μM. Age ($P$<0.03) but not sex, severity of MMVD, haematocrit or platelet count was associated with the effect of pimobendan.

In conclusion, this study confirmed an inhibitory effect of pimobendan at a high concentration on platelets in CKCS, but not at the therapeutic concentration. The effect of pimobendan was influenced by age only. Further studies are warranted to determine the platelet effects of the active pimobendan metabolite (UD-CG 212), a more potent phosphodiesterase inhibitor, in dogs.

**Disclosures**

No disclosures to report.

## ESVC-O-20 - European Society of Veterinary Cardiology

### The Longitudinal Outcome Of Canine (K9) myxomatous mitral valve disease (LOOK-Mitral) registry: Baseline treatment characteristics

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This study aimed to describe the medical treatment prescribed or modified by veterinary cardiologists at the enrollment visit of dogs included in The Longitudinal Outcome of Canine (K9) myxomatous mitral valve disease (MMVD) registry (LOOK-mitral registry), and to evaluate the influence of the EPIC trial and of selected echocardiographic variables on prescribing habits of cardiologists. The medical records of 6,102 enrolled in the LOOK-mitral registry were reviewed, and 6,016 dogs were included. Medical treatment was prescribed by the cardiologist to 2,599 dogs (15% Stage-B1, 90% Stage-B2, and to all dogs in Stage-C). Angiotensin-converting enzyme inhibitors (ACE-i) were the treatment most commonly prescribed to dogs in Stage-B1

(n=352, 9%). Pimobendan associated with an ACE-i represented the most common treatment in Stage-B2 dogs (n=367, 41%). Furosemide, an ACE-i, and pimobendan were the most common cardiac medical treatments prescribed to Stage-C dogs (n=57%). Within each stage, dogs with larger left atrial and left ventricular dimensions were more likely to receive ACE-i, pimobendan, or spironolactone. There was a four-fold increase in pimobendan and four-fold decrease in spironolactone prescriptions for dogs in Stage-B2 after the publication of the EPIC trial. Moreover, a 15% reduction in ACE_i prescription was also noted in these dogs. In 974 dogs, a medical treatment was prescribed by the referring veterinarian. This treatment was not changed, was modified, or was suspended by the cardiologist in, respectively, 12%, 74% and 14% of dogs. In conclusion, the EPIC trial and the echocardiographic assessment of left atrial and ventricular dimensions influence cardiologist prescription habits.

**Disclosures**

This project was funded by Ceva Animal Health.

**ESVCN-O-1 - European society of Veterinary & Comparative Nutrition**

## High intake of sodium chloride for 28 days causes no effect on serum FGF-23 concentrations in cats

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It has been demonstrated in humans that the phosphatonin FGF-23 also plays an important regulatory role in the adjustment of blood pressure and renal excretion of sodium (Na): Increased FGF-23 serum levels are positively correlated with Na retention. As demonstrated in mice, high intake of sodium chloride (NaCl) leads to a significant decrease of serum FGF-23 concentrations and consequently of Na retention. The aim of this study was to investigate the effect of high oral NaCl intake on serum FGF-23 and Na concentrations as well as on renal Na excretion in cats.

Eleven healthy adult European short hair cats were fed a balanced control diet (CON) for 28 days (18d adaption, 10d balance trial), followed by feeding an above-maintenance level of Na (diet HNaCl; 2.5g Na/1000kcal) by adding NaCl. On the last day of the trial, blood samples were obtained in the fasted animals (pre) and 3h postprandially (ppr) and analysed for serum FGF23 (sandwich ELISA by KAINOS Laboratories Inc., Tokyo, Japan) and Na (flame photometry). Urine was collected quantitatively during the balance trial and a pooled sample for each cat was analysed for Na. For statistical evaluation, Student's t-test was applied.

Feeding diet HNaCl caused a significant increase in renal Na excretion (CON: 29.0±4.4; HNaCl: 136.5±25.9 mg/kg BW/d) and a significant postprandial decrease of serum Na concentrations (157±2 vs. 146±4 mmol/l). Pre Na concentrations as well as pre and ppr serum FGF23 concentrations were not affected by NaCl intake.

In contrast to previous findings in other species, neither led high intake of NaCl to decreased serum FGF-23 concentrations nor were serum FGF-23 and Na retention correlated in cats. In alignment with these results, it was previously shown that unlike in humans, feeding high amounts of NaCl does not influence blood pressure in cats[5]. Comparatively low serum Na levels in trial HNaCl may be due to the significantly increased renal Na excretion. In cats, a high renal Na excretion due to high salt intake does not seem to be controlled by FGF-23 serum concentrations. Therefore, it is hypothesised that in cats regulatory effects of FGF-23 differ from that in other species like humans and mice.

**Disclosures**

No disclosures to report.

**ESVCN-O-3 - European society of Veterinary & Comparative Nutrition**

## Beyond the scale: A retrospective observational study of cats and dogs presenting to a referral centre with severe obesity

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Adiposity is usually semi-quantitively determined by body condition scoring (BCS). Using the 9-point system, each point between 5 and 9 equates to ~10% excess weight, with a score of 9 corresponding to ~40% excess weight. It is unclear how many dogs and cats have severe obesity (>40% overweight) and, therefore, are not accurately depicted by BCS. Since identifying such cases requires other methods of body composition analysis, the aim of the current study was to use dual-energy X-ray absorptiometry (DEXA) to determine the prevalence of severe obesity in dogs and cats at a specialist weight management referral clinic.

The records of dogs and cats attending a specialist weight management referral clinic for the investigation and management of obesity, between 2004 and 2021 were reviewed. After initial enrolment, a tailored controlled weight loss plan was designed for each dog or cat.

Body composition was analysed by fan-beam DEXA (Lunar Prodigy Advance; GE Lunar), with results being used to determine percentage overweight for every animal and whether they had 'severe obesity' (>40% overweight). Briefly, lean mass, fat mass and bone mineral content results were entered into a computer spreadsheet (Excel, Microsoft), and a mathematical formula was used to predict expected body composition after weight loss at different weights. The mathematical formula used was based upon typical body composition results from previous studies at the same clinic. The Kruskal-Wallis test was used to assess changes in median percentage overweight across four time periods: 2004-2008, 2009-2012, 2013-2016 and 2017-2021. Additionally, the Cochran-Armitage trend test was used to assess changes in the proportion of cases with severe obesity over the same time periods.

In total, 314 dogs and 124 cats met the eligibility criteria. Median (range) percentage overweight was 45% (11-191%) and 37% (0-133%) in dogs and cats, respectively, whilst 192 (61%) and 55 (44%) had severe obesity. In dogs, there was no difference in median percentage overweight ($P$=0.350) or proportion with severe obesity ($P$=0.182) over time; in contrast, for cats, the proportion with severe obesity increased over time ($P$=0.008), whilst median percentage overweight was different amongst time periods ($P$=0.001); on post-hoc analysis (Steel-Dwass method), percentage overweight was greater during 2018-2021 compared with both 2004-2008 ($P$<0.001) and 2009-2012 ($P$=0.016).

Based on results from a specialist weight management clinic, many dogs and cats have severe obesity (>40% overweight) and, for cats, the proportion of such cases has increased over time.

**Disclosures**

The study was funded by a grant from Royal Canin, a division of Mars Petcare, and this company manufactured the diets fed in this study. Vincent Biourge and John Flanagan are employees of Royal Canin. Alexander J. German and Georgiana R.T. Woods are employees of the University of Liverpool but their positions are funded by Royal Canin. Both have received financial remuneration and gifts for providing educational material, speaking at conferences, and consultancy work.

## ESVE-O-1 - European Society of Veterinary Endocrinology

### A prediction tool for diagnosis of canine hypothyroidism in clinical practice

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The diagnosis of canine hypothyroidism can sometimes be challenging, requiring a cohomprensive evaluation of clinical signs, clinicopathological abnormalities, and thyroid function tests. Consequently, the decision whether to treat or not, or to suggest further diagnostic testing, could be complicated. Predictive models could support clinicians in managing suspected hypothyroid dogs.

The aim of this cross-sectional study was to develop a prediction tool to assist the decision making in clinical practice.

The electronic database of the Veterinary Teaching Hospital was searched for dogs tested for hypothyroidism between January 2006 and June 2020. Hypothyroidism was diagnosed in dogs with compatible clinical signs and thyroid function tests (i.e.: low serum total thyroxine [T4] plus high serum thyrotropin [cTSH] concentrations or suggestive recombinant human TSH stimulation test). Dogs were excluded if medical records were incomplete or if a clear-cut distinction between hypothyroidism or euthyroidism was not possible. Eighty-two hypothyroid dogs and 233 dogs where hypothyroidism was suspected but then excluded, were included.

After data cleaning and comparison between the two groups, presence/absence of dermatological signs, serum concentrations of cholesterol, T4, and cTSH, haematocrit, were identified as relevant variables to insert in the dataset. Cholesterol, haematocrit, T4 and cTSH were expressed both as quantitative and qualitative variables, and combined with dermatological signs in four different models: reduced qualitative model (RQ), extended qualitative model (EQ), reduced quali-quantitative model (RQQ) and extended quali-quantitative model (EQQ). The extended models included serum T4 and cTSH concentrations, while reduced models did not. For each model different machine learning algorithms (CART classification tree, Random Forest, Gradient Boosting Machines [GBM], Support Vector Machine, Naive Bayes, Generalized Linear Model, K–Nearest Neighbor) were applied to assess the predictive performance. Each model was evaluated and internally validated by mean of bootstrapped training-test procedure: 500 times the dataset was randomly divided into a training-set and a test-set on which the predictive capacity was calculated and reported as Area Under the ROC Curve (AUROC). The implemented procedure yielded excellent performances, with different algorithms producing different results in the four models: the best performances were provided by the Naive Bayes in the RQ model (AUROC=0.847; 95% Confidence Interval [95% CI]=0.843-0.851]) and by the GBM in both quali-quantitative models (AUROC=0.987; 95% CI=0.986-0.988). A beta version of the software including the four models is currently undergoing an external validation phase.

Based on our results, implementation of this prediction tool in clinical practice could prove useful, particularly in primary care practice.

**Disclosures**

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## ESVE-O-2 - European Society of Veterinary Endocrinology

### Development and characterisation of feline thyroid organoids as an in vitro model for feline hyperthyroidism research

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Traditional 2D primary cell culture models have been extensively used to investigate the pathogenesis of various thyroid diseases, including feline hyperthyroidism, but are hampered by the low turnover rate of thyroid cells and the absence of follicular structures, integral to hormone synthesis. Thyroid organoids may overcome these limitations given their multicellular complexity resembling the original tissue's structure and function.

This project aimed to optimise a novel protocol for the development and characterisation of feline thyroid organoids, as an *in vitro* model for feline hyperthyroidism research.

Eight thyroids were obtained from thyroidectomy or post-mortem tissue collection of client owned cats (3 euthyroid and 5 hyperthyroid) attending a geriatric cat clinic. The Ethics and Welfare Committee at our institution approved data and tissue collection and storage from cats included in this study and informed consent was obtained from their owners.

Thyroid tissues were sliced and preserved in DMSO - containing cell freezing medium at -80°C, within 4 hours of collection, until processed. After thawing, thyroid tissue was minced and digested into a cell solution with collagenase type Ia in DMEM/F12 with Rock inhibitor Y-27632. After a series of centrifugations and filtering through a 70 μm cell strainer, the cell pellet was suspended in Matrigel® reduced basement membrane matrix and cultured onto organoids in droplets, at 37°C and 5% CO2. Matrigel droplets were bathed in complete growth medium composed of advanced DMEM/F-12 supplemented with B27 supplement, N-acetyl-L-cysteine, epidermal growth factor, fibroblast growth factor 10, R-spondin-1, noggin, Wnt-3a, TSH, glutamax, HEPES buffer and 1% antibiotic/antimycotic. Organoid growth was evaluated under the effect of various concentrations of TSH (0 – 20 mU/ml) and characterisation was performed by immunofluorescent cytochemical expression analysis of tissue markers, including thyroglobulin and vimentin.

Organoids were successfully developed from 7 of the 8 collected thyroids tissues and grew as spherical structures composed of multiple individual round to cuboidal cells. Organoids were subcultured every 12 to 14 days. Immunofluorescence cytochemistry proved thyroid origin with all organoids expressing thyroglobulin. Vimentin expression was also observed suggesting the concomitant presence of cells of a mesenchymal phenotype. Further work is required to continue feline thyroid organoids' characterisation.

This study optimised the first protocol for culture of feline thyroid organoids which can be replicated by other researchers and hopefully contribute to the discovery of the driving mechanisms behind the pathogenesis of feline hyperthyroidism, as well as to investigate new targets for therapy.

**Disclosures**

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## ESVE-O-3 - European Society of Veterinary Endocrinology

### Organoids of canine medullary thyroid carcinoma and feline thyroid adenomatous hyperplasia

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The aetiology and pathophysiology of canine medullary thyroid carcinoma (cMTC) and functional feline thyroid adenomatous hyperplasia (fTAH) remain poorly understood and 3D organoid culture could constitute an excellent *in vitro* model. The main goal of this study was to establish and characterize patient-derived organoid cultures of cMTC and fTAH. Secondary aims were to explore new therapeutic options for cMTC and evaluate the effect of TSH on growth and thyroid hormone (TH) production in fTAH organoids.

DMSO frozen (-150°C) cMTC (n=1) and fTAH (n=1) tissue samples were enzymatically digested to single cells. Cells were suspended in Cultrex® Basement Membrane Extract and TSH-containing growth medium and were incubated at 37°C (5%$C0_2$). Histology and immunohistochemistry for thyroglobulin, calcitonin, vimentin and Ki-67 were performed on cMTC organoids (37 days, passage 4 (P4)) and fTAH organoids (40 days, P4). Immunohistochemistry for thyroid transcription factor-1 (TTF-1) and synaptophysin was also performed on cMTC organoids. The effect of carboplatin, meloxicam and toceranib phosphate on cMTC organoid growth was evaluated using CellTiter-Blue® (CTB) cell viability assay. CTB assay was also used to evaluate the effect of TSH (0, 8, 16 and 32 mIU/mL) on fTAH organoid growth. TH production (T4, fT4, T3) by fTAH organoids was evaluated after incubation with sodium iodide (100 nM) and TSH (0, 8 and 32 mIU/mL).

Organoids from naturally occurring cMTC and fTAH were cultured for 99 (P9) and 65 (P8) days, respectively. Histologically, organoids resembled the primary tissue regarding cell morphology, follicular

organization, anisocytosis and anisokaryosis. TTF-1, calcitonin and synaptophysin immunolabeling in cMTC organoids confirmed thyroid, C-cell and neuroendocrine origin, respectively. 5-10% of fTAH organoid cells showed immunolabeling for thyroglobulin (100% in primary tissue), while no immunolabeling for calcitonin was present (negative in primary tissue). Immunolabeling for vimentin and Ki-67 was low in primary cMTC cells (10% and 1.42%, respectively) and fTAH cells (0% and 0.02%, respectively) in contrary to cMTC organoid cells (100% and 50%, respectively) and fTAH organoid cells (60% and 42.3%, respectively).

Carboplatin, meloxicam and toceranib phosphate had no effect on cMTC organoid growth within *in vivo* concentration ranges. TSH had no effect on growth of fTAH organoids and no TH production was detected.

In conclusion, organoids could be cultured from patient-derived cMTC and fTAH retaining histological features of the primary tissue. cMTC organoids also conserved immunohistochemical features of the primary tumor and could therefore be used to model cMTC.

**Disclosures**

No disclosures to report.

## ESVE-O-4 - European Society of Veterinary Endocrinology

### Multicentre retrospective review of clinical features and short-term follow-up of 110 cases of canine primary hypoparathyroidism

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Canine primary hypoparathyroidism (PH) is an uncommon endocrinopathy with 47 previously published cases.

This multi-centre retrospective study describes the history, clinical findings, clinicopathological data and therapy of dogs with PH from 23 European referral centres. Case records were collected using a data-capture platform (CastorEDC).

One-hundred-and-ten dogs of 28 breeds were represented, including 19 crossbreeds, 13 miniature schnauzers, nine Labradors and nine English Cocker spaniels. There were 56 females and 54 males, with a median (and range) age of six-years-old (0.6-12.3). Clinical signs were reported since the day of presentation in nine (8%), < 1 week in 60 (55%), and > 4 weeks in 17 (15%) dogs. Muscle tremors (61%), gait abnormalities (49%), seizures (49%), weakness (40%), agitation (32%), inappetence (31%), vomiting (29%), panting (27%) and facial rubbing (15%) were commonly reported. Less common signs included cataracts (7%) and aggression (5%).

Median ionised calcium concentration was 0.69 (0.25–1.2) mmol/L and median total calcium concentration 1.4 (1.4 – 3.1) mmol/L at diagnosis. Parathyroid hormone (PTH) was measured in 107 dogs; 50 dogs had undetectable PTH, 46 dogs had a PTH value < 50%, and ten dogs had a PTH value > 50%, of their respective reference interval (RI); one dog had a PTH value > the RI. There was no significant correlation between ionised calcium concentration and the adjusted PTH concentration at diagnosis, Spearman's correlation ($r_{s)} = -0.007$). All dogs received a vitamin D analogue. Of 99 dogs with available data, 86 received oral calcium supplementation. Ninety-three (85%) dogs received parenteral calcium gluconate. Ten dogs received injectable anti-epileptic therapy.

Median hospitalisation time was 5 (1–21) days after instituting vitamin D therapy. Median ionised calcium concentration at discharge for hospitalised dogs was 1.1 (0.57–2.59) mmol/L, and for dogs discharged the day of presentation was 0.84 (0.63–1.6) mmol/L. There was no correlation between initial ionised calcium concentration, and time-to-discharge, $r_s = -0.007$. One-hundred-and-eight (98%) dogs survived to discharge, 41 (38%) dogs had reported side effects of vitamin D therapy. For 104 dogs with short-term follow-up data, first recheck occurred a median of 6.5 (1–71) days post discharge. The dose of vitamin D was altered in 34 of these dogs (33%) at this reassessment.

Canine PH was most commonly seen in miniature schnauzers, Labradors and English cocker spaniels in this study. Despite presenting with marked hypocalcaemia and associated clinical signs, all improved with treatment, with the majority successfully discharged.

**Disclosures**

Fergus Allerton is the current chairperson of the Small Animal Medicine Society (SAMsoc), which provided funding for the Castor software platform.

## ESVE-O-5 - European Society of Veterinary Endocrinology

### Prospective evaluation of the prevalence of eunatraemic, eukalaemic hypoadrenocorticism in dogs with chronic gastrointestinal signs and risk of misdiagnosis in dogs with previous glucocorticoid administration

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Clinical signs of eunatraemic, eukalaemic hypoadrenocorticism (EEH) are non-specific, mimicking chronic gastrointestinal disease (CGD). Moreover, previous administration of glucocorticoids (PAG), commonly used in CGD dogs, can give false-positive results on the ACTH stimulation test (ACTHst). It is therefore important to exclude PAG and measure endogenous ACTH concentration (eACTH) to investigate the presence of EEH. This study aimed to determine the prevalence of EEH in dogs with CGD signs and identify clinical and clinicopathological features to recognize PAG in dogs with CGD.

A complete work-up, including basal serum cortisol (BSC) measurement, was performed in all dogs with CGD signs admitted to a single referral center between June 2019-February 2021. When BSC was <2 μg/dL and in all PAG dogs an ACTHst plus eACTH were performed. Dogs with ACTHst plus eACTH were divided into two groups: those who have not received glucocorticoids in the previous 3 months (non-PAG) and dogs with systemic or topical PAG, suspended from less than three months before admission. The overall EEH prevalence was determined. Fisher's exact and Mann-Whitney tests were used to compare clinical and clinicopathological variables between non-PAG and PAG dogs.

Eighty-nine dogs were prospectively enrolled, including 81 non-PAG and 8 PAG dogs. In non-PAG dogs, 32/81 (39.5%) had BSC <2 μg/dL and only one dog was diagnosed with EEH (post-ACTH cortisol 1.51 μg/dL; eACTH >1250 pg/mL). In PAG dogs, prednisolone was the most commonly used in 6/8 (median dose 0.5 mg/kg, range 0.14-0.9). The median time (range) of glucocorticoid treatment and discontinuation was 45 (2-210) and 25 (6-63) days, respectively. BSC was <2 μg/dL in all PAG dogs in which it was measured (6/8) and no EEH was diagnosed in this group. ACTHst provided a false-positive result in 2/8 dogs; in these dogs, eACTH was low-normal (5-17.5 pg/mL) and repeated ACTHst (after 14 and 33 days) was normal.

The overall prevalence of EEH was 1.1%. Polyuria/polydipsia was more commonly reported in PAG than non-PAG dogs (37.5% vs 3.7%,$p$=0.008). Moreover, PAG dogs showed lower BSC ($p$=0.01) and post-ACTH cortisol ($p$=0.02) and higher urea ($p$=0.03) and haptoglobin ($p$=0.04) concentrations. There was no significant difference concerning eACTH in both groups.

In conclusion, the prevalence of EEH in dogs with CGD signs was lower than previously reported. Due to the high risk of EEH misdiagnosis in dogs with CGD signs, the clinical and clinicopathological variables identified in this study may help clinicians to suspect PAG and establish an adequate diagnostic work-up.

**Disclosures**
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## ESVE-O-6 - European Society of Veterinary Endocrinology

### Diagnosis of naturally-occurring hypercortisolism by primary care veterinarians: A Western European Survey

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Several diagnostic tests have been described to screen for naturally-occurring hypercortisolism (HC) and differentiate the cause in affected dogs. This study aimed to determine testing protocols used by Western European primary care veterinarians (WEPCV) for diagnosis of canine HC.

An online survey translated into four different languages (Portuguese, Spanish, French and Italian) was developed using an electronic platform. Respondents were recruited through social network veterinary groups, mailing lists and talks. Questions focused on testing protocols for screening and differentiation.

Overall, 2021 responses from 8 European countries were included (Italy [n=1297], Portugal [n=261], France [n= 222], Spain [n=192], Belgium [n=41], Switzerland [n=4], Luxembourg [n=3] and Netherlands [n=1]). Of the respondents, 80.0%, 64.0%, 63.5%, 62.4%, 49.5%, 18.0% and 7.7% indicated always performing haematology, urinalysis, abdominal ultrasonography, electrolytes, biochemistry, blood pressure measurement and urine culture prior to adrenal function testing, respectively. When HC was suspected, 98.8% of respondents indicated performing adrenal function testing, while 1.2% relied

on a treatment trial. Among the former, 58.9% indicated they would screen a dog for HC without consistent clinical signs but with consistent clinicopathological abnormalities. Of 1996 respondents who performed adrenal function testing, 66% indicated always using the same initial screening tests, while 34% indicated using different screening tests depending on their pre-test suspicion. Among the former, tests used included ACTH stimulation test (33%), low-dose dexamethasone suppression test (LDDST) (32.2%), urine corticoid: creatinine ratio (UCCR) (5.5%), UCCR dexamethasone suppression test (2.4%), basal cortisol (1.1%) or a combination of tests (25.8%). Where there was no financial constraint, 1349 (67.6%) respondents always attempted differentiation while 417 (20.9%) and 229 (11.5%) never and sometimes did it, respectively. Differentiating tests included abdominal ultrasonography (82.8%), LDDST (48.3%), head CT/MRI (11.2%), endogenous ACTH (10.8%), high-dose dexamethasone suppression test (8.4%), UCCR dexamethasone suppression test (7.1%) and abdominal CT/MRI (6.6%). Overall, 68.5% of respondents indicated having offered referral to an internal medicine or dermatology specialist to ≤ 20% cases suspected or diagnosed with HC over the previous 5 years.

Testing protocols vary among WEPCV. Over 50% of respondents potentially screen for HC in dogs without consistent clinical signs, raising concerns for overdiagnosis. A proportion of WEPCV never attempt to differentiate the cause of HC, which likely affects management strategies and long-term prognosis. Cases are rarely referred to a specialist, reflecting that this disease is mainly managed in first-opinion practices. The results suggest that there is room for further education of WEPCV.

**Disclosures**

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## ESVE-O-7 - European Society of Veterinary Endocrinology

### Association of proteinuria with fasting hypertriglyceridaemia and hyperadrenocorticism in Australian Miniature Schnauzers

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Proteinuria has been described in Miniature Schnauzers with primary hypertriglyceridaemia. Hyperadrenocorticism can also cause fasting hypertriglyceridaemia and proteinuria with no overt progression to renal disease. The aim of this study was to investigate the association of proteinuria with primary hypertriglyceridaemia and hyperadrenocorticism in Australian Miniature Schnauzers.

Two hundred and fifteen healthy Miniature Schnauzers were recruited into a cross-sectional study. Each dog was assessed with an owner questionnaire and a physical examination. Triglyceride concentrations were measured after a 15-hour fast. If fasting hypertriglyceridaemia was identified, haematology, serum biochemistry, urinalysis including urine protein:creatinine ratio (UPCR), total thyroxine (including thyroid stimulating hormone if total thyroxine was low) and a low-dose dexamethasone suppression test were performed. Dogs with no underlying cause for the fasting hypertriglyceridaemia had an adrenocorticotrophic hormone stimulation test performed. Thirty of the dogs with normal triglyceride concentrations underwent the same testing to act as controls. Proteinuria was defined as UPCR ≥ 0.5.

Forty of 215 dogs (18.6%; 95% CI: 14.0%-24.3%) had fasting hypertriglyceridaemia. Thirty-nine hypertriglyceridaemic dogs underwent further testing and 31 (79.4%; 95% CI: 63.5%-90.7%) had hyperadrenocorticism, one (2.5%; 95% CI: 0%-13.4%) had hypothyroidism and seven (17.9%; 95% CI: 7.5%-33.5%) had primary hypertriglyceridaemia. Ten of the 30 control dogs (33.3%; 95% CI: 17.2%-52.8%) were diagnosed with hyperadrenocorticism. No other diseases were identified in the control group. The UPCR was assessed in 39 hypertriglyceridaemic dogs and 29 controls. Proteinuria was associated with hypertriglyceridaemia ($P<0.001$); an increased UPCR was detected in 18 of 39 hypertriglyceridaemic dogs and 0 of 29 dogs with normal triglycerides. Two dogs with proteinuria had primary hypertriglyceridaemia, 15 had hyperadrenocorticism and one had hypothyroidism. In hypertriglyceridaemic dogs, those with hyperadrenocorticism were 3.5 (95% CI: 1.1-10.9) times as likely to be proteinuric than dogs with hyperadrenocorticism and normal triglycerides. In contrast, there was no evidence of an association between primary hypertriglyceridaemia and proteinuria ($P=0.6$).

The estimated relative risk for proteinuria in hypertriglyceridaemic Australian Miniature Schnauzers diagnosed with hyperadrenocorticism was over 3 times that for dogs with hyperadrenocorticism without hypertriglyceridaemia. Proteinuria only occurred with hyperadrenocorticism when hypertriglyceridaemia was present. As proteinuria was not associated with primary hypertriglyceridaemia, rigorous investigation of hyperadrenocorticism is recommended in hypertriglyceridaemic Miniature Schnauzers with proteinuria.

**Disclosures**

Sue Foster is an Adjunct Associate Professor in Small Animal medicine at Murdoch University and provides consultancy services to Vetnostics, the laboratory which performed the majority of the laboratory tests. Sue Foster has no other disclosures. Doug Hayward is employed by Vetnostics, the laboratory that performed the majority of the laboratory tests.

## ESVE-O-8 - European Society of Veterinary Endocrinology

### Role of internal medicine specialists in disseminating the evolving evidence base on Cushing's syndrome

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The majority of diagnosed Cushing's syndrome (CS) cases remain under primary veterinary care, with just 3% of all cases involving referral care. Disparities are reported between the diagnostic guidelines for CS recommended by specialists and the typical decision-making carried out by primary-care practice veterinarians (PCPV). This study aimed to take a qualitative approach to better understand the experiences of PCPVs and the barriers faced surrounding the diagnosing of CS in dogs. These data will enable more tailored dissemination of evidence-based veterinary medicine (EBVM) on CS for greater clinical effect.

A purposive sample of UK PCPVs were recruited to participate in semi-structured interviews exploring CS diagnosis. The interviews were conducted with individual veterinarians via videoconferencing. Interviews were transcribed verbatim and reflexive thematic analysis was used to construct three key themes. This abstract reports on one of these themes; the implementation of EBVM in primary-care practice to formulate clinical decisions for the diagnosis of CS.

This study analysed 891 minutes of interview data from 20 PCPVs with a range of clinical experience. Veterinarians described the typical resources and applications of EBVM they used when diagnosing CS. The most important source of EBVM were from informal discussions with veterinarians in their professional network; from within their practice, internal medicine specialists at their local referral practice and representatives from pharmaceutical companies. The veterinarians interviewed rarely read or searched the peer-reviewed literature and few had undertaken recent training on CS. Veterinarians often acknowledged their knowledge of the EBVM surrounding CS could be improved but time and lack of available resources was a commonly stated limitation. Veterinarians expressed frustration at conflicting and frequently changing advice by specialists which often led to their decisions being based on the opinion(s) of one preferred colleague within their practice or external specialist. Veterinarians also expressed the opinion that current advice and/or guidelines are not realistic for the primary-care setting.

These findings suggest that specialist-led advice on CS diagnosis is a key form of EBVM used by PCPVs. Consideration should be made regarding the consistency of the information disseminated by internal medicine specialists and guidelines formed with primary-care practice in mind.

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## ESVE-O-9 - European Society of Veterinary Endocrinology

### Fibroblast Growth Factor-23 and phosphate metabolism in dogs with spontaneous hyperadrenocorticism (HAC)

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Increased plasma parathormone concentration ($[PTH]_{pl}$) and serum phosphate concentration ($[P]_s$) along with reduced phosphaturia have been previously demonstrated in dogs with HAC. However, mechanisms remain poorly understood. Fibroblast Growth Factor-23 (FGF23) is a phosphaturic bone-derived hormone, playing a central role in phosphate metabolism. One could hypothesise a reduction in FGF23 concentration or activity in HAC dogs. Proteinuria is another common complication of HAC in dogs. A relationship between proteinuria and increased tubular phosphate resorption, along with increased serum FGF23 concentration has been suggested in human studies. The first aim of our study was to further characterise phosphate metabolism profile in dogs with HAC compared with healthy dogs and dogs with non-adrenal illness (NAI), using plasma FGF23 ($[FGF23]_{pl}$), PTH, serum Vitamin-D concentrations. A second aim was to investigate potential correlations between proteinuria, FGF23 and phosphate concentrations in HAC dogs.

In this prospective study conducted between 2019 and 2021, dogs were included in the HAC group or in the NAI group depending on clinicopathological data, endocrine test results and final diagnosis. Dogs were age-matched with healthy dogs to create a Healthy-Control group. The study was approved by ethical committee. Samples of plasma, serum and urine were stored at -80°C for further dosage of FGF23, Parathormone, Vitamin-D (both 25(OH)Vitamin-D and calcitriol), urine protein-to-creatinine ratio (UPCR), and fractional excretion of calcium ($FE_{Ca}$) and phosphorus ($FE_P$). Results were statistically compared between groups using Mann-Whitney test with $p<0.05$ considered significant. Correlations between variables were assessed in the HAC group using Spearman test.

Twelve dogs were included in HAC group, 12 in NAI-Control group and 11 in Healthy-Control group. $[FGF23]_{pl}$ and Vitamin-D were not significantly different between groups. $[PTH]_{pl}$ was significantly higher in HAC dogs (118pg/mL) than in healthy controls (40pg/mL; $p<0.01$).

$[P]_s$ was significantly higher in HAC dogs (1.76mmoL/L) compared with NAI (1.08mmoL/L) and healthy controls (1.2mmoL/L; p<0.01). There was a weak positive correlation between $[FGF23]_{pl}$ and $FE_P$ (p=0.03; Rs=0.63), but not with $[P]_s$. Weak negative correlations were documented between UPCR and 25(OH)Vitamin-D (p=0.032; Rs=-0.62) as well as calcitriol (p=0.049; Rs=-0.59), but not between UPCR and $[FGF23]_{pl}$ or $FE_P$.

This study further supports hyperparathyroidism and increased phosphatemia in dogs with HAC. FGF23 may not be primarily involved in these biological pathways, however reduced receptor or cofactor activity was not assessed. Moreover, small sample size likely underpowered the study. A correlation between phosphate abnormalities and proteinuria was not demonstrated, although proteinuria seemed weakly inversely correlated to 25(OH)Vitamin-D.

**Disclosures**

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## ESVE-O-10 - European Society of Veterinary Endocrinology

### Evaluation of clinical, ultrasonographic, and clinicopathological findings in dogs with pituitary-dependent hypercortisolism and poor trilostane response

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Trilostane is reported to be effective in about 90% of dogs with spontaneous hypercortisolism. Factors able to predict trilostane efficacy have never been evaluated. This retrospective study aimed to compare different clinical, ultrasonographic (US), and clinicopathological findings between dogs with a good clinical response and dogs showing poor response to trilostane treatment.

Only dogs with pituitary-dependent hypercortisolism (PDH), diagnosed and monitored at a referral veterinary hospital and treated with trilostane twice daily, were included. Good responder dogs (GRD) were included if, after 6 months of treatment, they were receiving a trilostane dose lower than 3 mg/Kg BID, and all the clinical signs resolved. Poor responder dogs (PRD) were included if, after 6 months of treatment, they were receiving a trilostane dose higher than 3 mg/Kg BID and the clinical signs [polyuria and polydipsia (PU/PD) and dermatological signs] were still present. Clinical, ultrasonographic, and clinicopathological findings were compared between the 2 groups at diagnosis (T0) and after 6 months of treatment (T6).

Fourteen GRD and 17 PRD were included. Among the clinical signs, alopecia was the only clinical finding more significantly observed in PRD at the time of diagnosis (p<0.001). In GRD the following clinicopathological variables resulted significantly lower at T0 compared to the PRD: serum ALT (p=0.02), serum GGT (p=0.02), endogenous plasma ACTH (p=0.003), pre-ACTH serum cortisol (p<0.001), post-ACTH serum cortisol (p=0.02), 8 hours post dexamethasone serum cortisol (p=0.03). Serum creatinine was significantly higher in GRD (p=0.007). The number of GRD without bilateral symmetrical adrenomegaly on US was significantly higher compared to PRD (p<0.001). In GRD the following serum clinicopathological variables resulted significantly lower at T6 compared to PRD: ALT (p<0.001), GGT (p=0.001), ALP (p=0.009), cholesterol (p=0.007), phosphate (p=0.002), pre-ACTH cortisol (p<0.001), post-ACTH cortisol (p=0.006), pre-trilostane cortisol (p=0.008). Serum creatinine was significantly higher (p<0.001) in GRD (p=0.01).

In conclusion, this preliminary study showed that different routinely performed clinicopathological variables associated with some clinical and US findings might be useful to identify PRD. A larger population of dogs is needed to determine cutoff values and the predicting importance of these variables to early identify PRD.

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## ESVE-O-11 - European Society of Veterinary Endocrinology

### Outcome in dogs with and without hyperadrenocorticism undergoing radiotherapy for pituitary macroadenomas

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Pituitary radiotherapy is considered an effective treatment for pituitary space-occupying tumours in dogs presenting with neurologic signs. Previous studies reported that concurrent pituitary-dependant hyperadrenocorticism (PDH) did not affect the outcome in dogs undergoing pituitary radiotherapy. However, those authors hypothesed that dogs of the non-PDH group would have poorer outcomes since diagnosis of a macroadenoma might be expected to be delayed due to the absence of the clinical signs typically associated with hyperadrenocorticism.

The primary aim of this retrospective cohort study was to determine whether dogs with pituitary dependent hyperadrenocorticism (PDH

group) would have longer survival following pituitary radiotherapy for a macroadenoma, compared to dogs with non-hormonally active pituitary masses (non-PDH group). A secondary aim was to evaluate whether various clinical, diagnostic imaging or radiotherapy factors were associated with survival. Survival analysis was performed using Kaplan Meier with log rank tests and Cox proportional hazards analyses.

Ninety-five dogs undergoing radiotherapy for a pituitary macroadenoma between January 2008 and 2018 at five referral centres in Europe were included. Forty-eight had a diagnosis of hyperadrenocorticism (PDH group) and 47 had a pituitary mass with no evidence of concurrent endocrinopathy (non-PDH group). Median age was 8 years in both PDH and non-PDH group and median body weight was 16.75 kg in PDH and 19.2 kg in non-PDH dogs.

Survival was not different between groups (MST 710 days for non-PDH group vs 591 days for PDH group, p=0.488). Kaplan Meier analysis identified that definitive protocol was associated with longer survival compared to a palliative protocol (MST 605 vs 262 days, p=0.048). Factors associated with survival derived from the Cox proportional hazard analysis included total Gy delivered (p<0.05) and type of linear accelerator used, with Elekta Precise HR = 3.5, p=0.010, Siemens Onco HR = 6.7, p<0.001 compared to the reference machine, Varian 600C (chosen as reference as associated with longest survival time). Patient age, tumour volume and P/B ratio were not associated with survival.

The results of this study demonstrate that dogs with pituitary dependent hyperadrenocorticism did not have longer survival as we hypothesised. Factors statistically associated with survival were total Gy delivered, radiotherapy protocol and type of radiotherapy machine, which might reflect institution differences for the management of these patients. This is the largest multicentre study evaluating prognostic factors for dogs undergoing pituitary radiotherapy based on their hormonal status.

**Disclosures**

No disclosures to report.

## ESVE-O-12 - European Society of Veterinary Endocrinology

### Cabergoline treatment for feline hypersomatotropism

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Spontaneous hypersomatotropism is a chronic metabolic disease caused by hypersecretion of growth hormone. Hypersomatotropism is characterised by presence of diabetes mellitus (DM), which sometimes is insulin-resistant. Cabergoline is a long-acting dopamine agonist, with a high affinity for dopamine receptor 2 (D2R). The expression of D2R has been demonstrated in pituitary gland of cats with hypersomatotropism. The aim of this study was to evaluate the safety and efficacy of cabergoline to control DM and hypersomatotropism.

In this prospective study, 25 cats with hypersomatotropism were included. Cats were distributed into two groups: diabetic cats (n=19) and non-diabetic cats (n=6). The diagnosis of hypersomatotropism was made according to serum insulin-like growth factor 1 (IGF-1) and MRI. Cats received an oral dose of cabergoline (10 μg/Kg every 48 hours) for 6 months. Serum IGF-1 and fructosamine concentrations were measured at the time of diagnosis of hypersomatotropism, 3 and 6 months of cabergoline treatment. Periodic clinical controls (blood glucose curves and intense monitoring) were carried out to determine insulin requirements. Statistical analysis was performed by Wilcoxon test, expressed as median and ranges (p<0.05).

Serum IGF-1 concentrations did not show significant differences with cabergoline treatment in any group. Fructosamine concentrations had a significant decrease at 3 and 6 months after treatment with cabergoline in the diabetic group (p<0.001). Insulin requirements were significantly reduced at 3 and 6 months after cabergoline treatment (p<0.01). Seven diabetic cats (7/19=36.8%) achieved remission of DM within 3 to 6 months of cabergoline treatment. In 3 diabetic cats, clinical signs of hypoglycemia occurred. No additional adverse effects were observed during the study.

This study suggests that treatment with cabergoline, although it does not control IGF-1 excess, substantially improves diabetes control and allows remission of DM in 36.8% of diabetic cats with hypersomatotropism. Therefore, cabergoline should be considered as a possible treatment option for cats with hypersomatotropism and concurrent DM.

**Disclosures**

No disclosures to report.

## ESVE-O-13 - European Society of Veterinary Endocrinology

### Proteomic analysis in serum of cats with diabetes mellitus

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Mass spectrometry allows proteomic investigations to identify proteins and pathways associated with the development of certain diseases. Recent studies showed that humans with type 2 diabetes mellitus have abnormal concentrations of proteins related to lipid metabolism

(e.g., lower apolipoprotein A-1) and inflammation (e.g., increased haptoglobin protein-beta). In diabetic cats, proteomic investigations have not yet been performed. Hence, the study aim was to assess if the circulating protein profile differs between diabetic and healthy cats, and between diabetic cats achieving remission and cats not achieving remission.

Healthy cats and cats with newly diagnosed diabetes mellitus not affected by obvious concurrent diseases were enrolled. Diabetic cats received insulin glargine and a low-carbohydrate diet and were followed-up for 3-4 months. Remission was defined as euglycemia without insulin for >4 weeks. Blood was collected at first admission, and after 1-2 and 3-4 months (left-over samples). The proteomic profiles of plasma samples were obtained with liquid chromatography coupled to high-resolution mass spectrometry and database search. Differences between groups were investigated with non-parametric tests.

Six healthy cats and 18 diabetic cats were included; 8 diabetic cats achieved remission between 1 and 4 months after diagnosis. Overall, 245 proteins were considered for analysis. Compared to controls, diabetic cats had higher levels of afamin (fold change [FC]=1.33, p=0.002), inhibitor of carbonic anhydrase-like protein (FC=1.39, p=0.044), vitronectin (FC=1.47, p=0.044), and lower levels of tetranectin (FC=1.85, p=0.002) and zinc alpha2 glycoprotein (FC=1.70, p=0.004) at diagnosis. Cats with remission had higher concentrations of inter-alpha-trypsin inhibitor heavy chain H3 (FC=1.90, p=0.040) compared to cats that did not achieve remission, while inhibitor of carbonic anhydrase-like protein was lower (FC=1.44, p=0.005) at diagnosis. All above proteins are related to lipid metabolism and inflammation, with the exception of the inhibitor of carbonic anhydrase-like protein whose function is currently unknown.

In conclusion, proteomic analysis identified several proteins that are differentially expressed in diabetic cats, highlighting the potential involvement of lipid metabolism and inflammation in the pathogenesis of the disease. The role of the inhibitor of carbonic anhydrase-like protein, in particular in diabetic cats achieving remission, needs further investigation.

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## ESVE-O-14 - European Society of Veterinary Endocrinology

### Effect of periodontal treatment on glycemic control in canine diabetic patients: A prospective, clinical study

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Diabetes mellitus (DM) is a common endocrinopathy in dogs. Periodontal disease (PD) is a multi-factorial, bacterial disease of dental supporting tissues with potential systemic inflammatory ramifications. In humans, PD adversely affects glycemic control, but a similar effect has not been thoroughly investigated in dogs in the clinical setting.

Ten client-owned dogs with poorly-regulated DM and PD were prospectively enrolled. A complete blood count, chemistry, urinalysis and measurement of C-reactive protein (CRP), interleukin-6, TNF-α, HbA1c and fructosamine concentrations were performed on the day of periodontal treatment (PT), and monthly thereafter for 3 months. A periodontal severity score (PDSS) was determined during PT. Effects of time post-PT and PDSS on measures of inflammation and glycemic control were determined by generalized estimating equation analysis.

Hemoglobin A1c concentration (mean ±SE) decreased 3 months post-PT (6.2%±0.41 vs. 5.3%±0.56; P=0.013). Due to a significant (P<0.001) interaction between PDSS and time post-PT in the analysis of fructosamine, dogs with low (1-3)/high (7-9) PDSS were analyzed separately. Fructosamine (mean ± SE) significantly decreased 1-, 2-, and 3-months post-PT (572±57, 542±63 and 547±71, respectively, vs. 624±62; P≤0.03) in the high PDSS group, but not the low PDSS group. Fructosamine concentration upon enrollment was significantly correlated to PDSS (r=0.73, P=0.017). Neither CRP nor TNF-α concentration were increased during the study, while interleukin-6 concentration significantly decreased 3 months post-PT (P=0.002).

In conclusion, the findings herein support a potential detrimental interaction between PD and DM. The apparent beneficial effect of PT on glycemic control was most conspicuous in dogs with a more severe PD.

**Disclosures**

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## ESVE-O-15 - European Society of Veterinary Endocrinology

### The usefulness of different freestyle libre-derived metrics in assessing glycemic control in diabetic dogs

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Flash glucose monitoring system (FGMS, FreeStyle Libre®) is nowadays routinely used in diabetic dogs (DD) and a few studies have demonstrated its accuracy and clinical utility. However, successful utilization of FGMS data in routine clinical practice remains relatively low because there is a lack of agreement regarding the interpretation of FGMS data. This study aims to assess the utility of different metrics readily available with the use of FGMS to monitor DD.

Fourteen DD on insulin treatment (12 porcine lente insulin, 2 Neutral Protamine Hagedorn insulin) were retrospectively enrolled in the study.

A single evaluation for each patient was included. All dogs were monitored with FGMS, and data were collected after at least 7 days of continuous glucose detection. The glycemic control was classified according to the ESVE ALIVE clinical score (CS) that takes into account the stability of body weight, presence of polyuria/polydipsia, activity/attitude, and appetite. The clinical score range from 0 (optimal) to 12 (poor). The following metrics were evaluated: percent time in range (percentage of time glucose within 70-250 mg/dL; TIR%), percent time above range (percentage of time glucose above 250 mg/dL; TAR%), percent time below range (percentage of time glucose below 70 mg/dL; TBR%), median glucose (MG), percent coefficient of variation (CV%). Correlations between CS and TIR%, TAR%, TBR%, CV%, and MG were evaluated. Moreover, the correlation between CV% and MG was assessed. Mann-Whitney test was used to compare CV% in dogs with and without concurrent diseases, as well as in dogs with and without clinical hypoglycemia.

TIR%, TAR% and TBR% were significantly correlated with the CS (*rs*=-0.79,*P*=0.001; *rs*=0.79,*P*=0.001; and *rs*=*-0.56*,*P*=*0.04*; respectively). Moreover, a significant correlation between MG and CS was found (*rs*=0.79, *P*=*0.001*). CV% was inversely correlated with MG and CS (*rs*=-0.90, *P*<0.0001; *rs*=*-0.78*, *P*=*0.002*; respectively). CV% was 37.6% and 27.5% in dogs with concurrent diseases and dogs without concurrent diseases, respectively (*P*=*0.75*). Further, CV% was higher in dogs with clinical hypoglycemia compared to dogs that did not experience clinical hypoglycemia (48.7% vs 32.8%), although the difference was not significant (*P*=*0.10*).

In conclusion, this is the first study evaluating FGMS-derived metrics in DD. The strong correlation between TIR%, TAR%, TBR%, MG, and CS suggests the potential clinical usefulness of these metrics for monitoring DD. The CV% does not seem to reflect the short-term glycemic control. Although not significant, CV% seems to be higher in dogs with concurrent diseases and clinical hypoglycemia.

**Disclosures**

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## ESVE-O-16 - European Society of Veterinary Endocrinology

### Effects of calcitriol on leukocyte cytokine production in dogs with diabetes mellitus

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Oral vitamin D supplementation abrogates inflammation and improves glycemic control in humans with type-1 diabetes mellitus (T1DM). Dogs with T1DM have a proinflammatory phenotype and glycemic control can be difficult. Vitamin D supplementation could have a future role in T1DM dogs. We assessed the *in vitro* effect of calcitriol on stimulated leukocyte production of cytokines in dogs with T1DM and subanalyses based on glycemic control. Otherwise healthy dogs with T1DM as well as healthy controls were included. Dogs with T1DM were categorized as controlled (T1DM-C) or uncontrolled (T1DM-U) based on clinical signs and serum fructosamine concentrations. Whole blood was incubated with either calcitriol ($10^{-7}$ M) or ethanol (control) for 24 h, with subsequent exposure with either phosphate buffered saline (PBS), lipopolysaccharide (LPS), or lipoteichoic acid (LTA) for 24 h. Cytokines were measured using a canine-specific multiplex assay. Differences in cytokines (TNF-α, IL-6, IL-10, IL-8, and TNF-α:IL-10 ratio), the effect of incubation intervention (i.e., calcitriol, ethanol) and type of stimulant (i.e., PBS, LPS, LTA) between T1DM and control dogs were assessed using multivariable linear regression. The effect of T1DM on the action of calcitriol was assessed using a Kruskal-Wallis rank test, and Dunn's test for T1DM subtypes.

Twenty dogs with T1DM (T1DM-C, n=10; T1DM-U, n=10) and 20 age, sex, and breed matched controls were studied. Dogs with T1DM had higher leukocyte production of IL-10 (P = 0.004), IL-6 (P < 0.001), and IL-8 (P < 0.001) than control dogs, irrespective of stimulant or intervention. T1DM-C and T1DM-U dogs had higher leukocyte production of IL-10 (T1DM-U, P = 0.006; T1DM-C, P = 0.03), IL-6 (both subgroups, P < 0.001) and IL-8 (both subgroups, P < 0.001) compared to control dogs, irrespective of intervention or stimulant. Overall, incubation with calcitriol decreased leukocyte production of TNF-α (P = 0.03) and TNF-α:IL-10 ratio (P = 0.001), and increased IL-6 (P = 0.03), irrespective of group (i.e. T1DM, controls) or stimulant. Control dogs had a greater decrease in leukocyte production of TNF-α (P = 0.006), IL-6 (P < 0.001), and IL-8 (P < 0.001) after incubation with calcitriol than T1DM dogs, irrespective of stimulant. These data indicate that T1DM dogs have an exaggerated inflammatory response when stimulated and incubation with calcitriol modulates inflammatory phenotype.

**Disclosures**

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## ESVIM-O-1 - European Society of Veterinary Internal Medicine

### Association between bronchoalveolar lavage fluid quantitative bacterial culture results and antibiotic requirement in dogs with lower respiratory tract signs

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Lower respiratory tract infection (LRTI) is an important differential in dogs with respiratory signs. The diagnosis relies on positive bacterial culture of bronchoalveolar lavage fluid (BALF). A bacterial count above 1700 CFU/mL has been suggested as a highly sensitive and specific threshold to diagnose LRTI. Conversely, evidence of asymptomatic bacterial colonization, which does not require antibiotics, is growing in human and veterinary medicine. Overusing antibiotics is a critical concern and relying on this threshold for LRTI diagnosis therefore could be misleading.

This cross-sectional study hypothesized that BALF quantitative bacterial culture results would be poorly predictive of LRTI requiring antibiotics (LRTI-RA) in dogs.

Client owned-dogs with positive BALF quantitative bacterial culture were retrospectively collected between 2016 and 2021. Dogs with evidence of oropharyngeal contamination were excluded. Antibiotic use before BALF collection was recorded. Included dogs were divided into two groups: a group of dogs with LRTI-RA and a group of dogs that did not require antibiotics despite positive BALF culture (no-antibiotic-requirement [NAR] group). Dogs were placed in the LRTI-RA group if they had: (1) a positive BALF bacterial culture with neutrophilic inflammation on BALF cytology; AND (2) a radiographic pattern consistent with pneumonia OR intracellular bacteria on BALF cytology OR an unambiguous response to antibiotic therapy. Dogs were placed in the NAR group if they had: (1) a positive BALF bacterial culture with or without neutrophilic inflammation on BALF cytology; AND (2) no radiographic pattern consistent with pneumonia; AND (3) no intracellular bacteria on BALF cytology; AND (4) no response to appropriate antibiotics based on susceptibility profile OR an unambiguous response to an alternative treatment.

Twenty-eight dogs were included in the LRTI-RA group and 12 dogs in the NAR group. Prior antibiotic use was reported in 8 dogs from the LRTI-RA group and 2 dogs from the NAR group. No significant difference in bacterial colony number was detected between the two groups (median: 4600 CFU/mL [10–3*10^8] in LRTI-RA group and 10,000 CFU/mL [250–1.3*10^9] in NAR group, p=.18), regardless of prior antibiotic therapy (p=.32). The 1700-CFU/mL threshold for BALF culture interpretation seemed poorly predictive of LRTI-RA. Indeed, 13/28 dogs with LRTI-RA would have been missed using this threshold, and 8/12 dogs from the NAR group would have been misdiagnosed with LRTI-RA, inciting unnecessary antibiotic treatment.

This study suggests that LRTI-RA diagnosis should be based on clinical, radiographic, and cytological criteria and not on bacterial colony number retrieved from BALF culture.

**Disclosures**

No disclosures to report.

**ESVIM-O-2 - European Society of Veterinary Internal Medicine**

## Evaluation of pulmonary function by whole-body plethysmography for therapy monitoring in cats with chronic bronchial disease

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In human medicine, pulmonary function testing is a standard procedure for therapeutic monitoring of chronic bronchial disease. In cats with feline lower airway disease (FLAD), therapeutic success is commonly evaluated by clinical examination only.

The aim of the prospective study was to evaluate whether barometric whole-body plethysmography (BWBP) can be used for non-invasive assessment of therapeutic response in cats with FLAD, and to investigate the correlation with clinical improvement.

Cats diagnosed with FLAD (n=22), based on typical clinical signs, radiographic findings and bronchoalveolar lavage cytology, were included.

At three time points (day 0, 14, 60), a clinical evaluation of the patient according to a standardized examination protocol (clinical 12-point score) and BWBP were performed. Individual therapy was given to all patients from day 0.

While the clinical score improved significantly over the three time points ($p < 0.001$), the BWBP-parameters RR, TV/BW, Ti, Te, Penh, PAU, EIP, EEP, TP and PEF/PIF improved from day 0 to day 14; however, this improvement was not significant.

There was no significant correlation of any measurement parameter with clinical improvement.

Certain BWBP-parameters improve after initiation of therapy and may help in assessing a functional improvement. However, cats with FLAD should always be evaluated clinically to assess response to therapy.

**Disclosures**

No disclosures to report.

**ESVIM-O-3 - European Society of Veterinary Internal Medicine**

## Serum and bronchoalveolar lavage fluid concentration of osteopontin and fibronectin in West Highland white terriers either affected with canine idiopathic pulmonary fibrosis or healthy and other terriers non predisposed to the disease

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Overexpression of osteopontin (SPP1) and fibronectin (FN1), two molecules associated with pulmonary fibrosis in humans and mice, was recently

identified in pro-fibrotic bronchoalveolar lavage fluid (BALF) macrophage populations in West Highland white terriers (WHWTs) affected with canine idiopathic pulmonary fibrosis (CIPF) compared to healthy WHWTs. The aims of the present study were to confirm the overexpression of SPP1 and FN1 genes at the protein level and to assess the potential utility of those proteins as serum and BALF biomarkers of CIPF severity.

Serum and BALF concentrations of SPP1 and FN1 were measured using commercially available canine ELISA kits in CIPF WHWTs at diagnosis (n=24), healthy aged-matched WHWTs (n=13) and healthy terriers from other breeds (n=15). Values obtained were compared between groups using Kruskal-Wallis test. Correlations between SPP1 and FN1 concentrations and markers of disease severity (arterial partial pressure in oxygen ($PaO_2$) and 6-minute walked distance (6MWD)) were performed using Spearman test.

SPP1 serum concentrations were higher in CIPF WHWTs (median [interquartile range]: 2.15 ng/mL [0.87-5.13]) compared with healthy WHWTs (0.63 ng/mL [0.41-1.63]; P=0.013) and healthy terriers (0.31 ng/mL [0.19-0.51]; P<0.0001), and higher in healthy WHWTs compared with healthy terriers (P=0.002). Higher SPP1 BALF concentrations were found in CIPF (0.34 ng/mL [0.15-0.52]) and healthy WHWTs (0.25 ng/mL [0.14-0.40]) compared with healthy terriers (0.02 ng/mL [0.01-0.08]; P<0.0001 and P=0.002, respectively), while no difference was shown between CIPF and healthy WHWTs (P=0.962). SPP1 serum concentrations negatively correlated with $PaO_2$ (r=-0.502; P=0.007), but not with 6MWD. FN1 serum concentrations were lower in WHWTs either affected with CIPF or healthy (0.81 ng/mL [0.36-1.47] and 0.61 ng/mL [0.24-0.65]) compared with healthy terriers (2.72 ng/mL [2.15-5.21]; P<0.0001 and P=0.001, respectively). No difference was found between groups in BALF FN1 concentrations (P=0.077). No correlation was identified between serum and BALF FN1 concentrations and parameters of CIPF severity.

Results of the present study did not confirm the FN1 overexpression found in BALF pro-fibrotic macrophages neither in serum nor in BALF. Serum and BALF FN1 concentrations were not associated with CIPF severity parameters. SPP1 gene overexpression was confirmed at the protein level in serum but not in BALF in CIPF compared with healthy WHWTs. Moreover, SPP1 serum concentration could serve as a biomarker of disease severity as it correlates with $PaO_2$. Finally, the higher serum and BALF SPP1 concentrations found in the WHWT breed compared to other terriers could be linked to their predisposition to CIPF.

**Disclosures**

No disclosures to report.

**ESVIM-O-4 - European Society of Veterinary Internal Medicine**

## Incidence and characterization of penetration and aspiration in dogs using videofluoroscopic swallow studies

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Videofluoroscopic swallow studies (VFSS) utilizing a penetration-aspiration (P-A) scale have been used in people to assess airway protection. Penetration, aspirated material cranial to the vocal folds, and aspiration, material caudal to the vocal folds, are associated with increased risk of lung injury in people. P-A scales have been validated for use in animal models, but the incidence of P-A, clinical signs (CS), and dysphagic disorders associated with P-A in dogs are unknown. The objectives of this study were to identify the incidence of P-A, compare CS between dogs with and without P-A, and identify predisposing dysphagic disorders for P-A using VFSS. Fifty-three sequential VFSS and associated medical records from dogs presenting to the Auburn University College of Veterinary Medicine were retrospectively reviewed. Between group comparisons were made by Mann-Whitney Rank Sum Test, One-way ANOVA on Ranks, and Spearman Rank Order Correlation ($p$<0.050). The incidence of P-A was 47%. No differences in P-A were detected between dogs presenting exclusively with respiratory CS (20/53; $p$=0.924), exclusively GI CS (20/53; $p$=0.656), respiratory and GI CS (11/53; $p$=0.287), or other CS (2/53; $p$=0.169). Significant differences in P-A were detected between dysphagia groups (p <0.001). Oral-preparatory and pharyngeal, but not esophageal dysphagia occurred more frequently in dogs with P-A. Pharyngeal dysphagia was moderately positively correlated with P-A score ($p$<0.0001; r:0.619) In conclusion, P-A was commonly identified in dogs even in the absence of respiratory CS (i.e., occult P-A). Dogs with oral-preparatory or pharyngeal dysphagia should be considered at high risk for P-A.

**Disclosures**

No disclosures to report.

**ESVIM-O-5 - European Society of Veterinary Internal Medicine**

## Antimicrobial discontinuation in dogs with acute aspiration pneumonia based on normal C-reactive protein and clinical improvement

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Treatment guidelines for canine aspiration pneumonia (AP) recommend 4 to 6 weeks of antimicrobials. However, evidence regarding optimal treatment duration and the role for chest radiographs (CXR) and lung

ultrasound (LUS) in the follow up of canine AP is lacking. C Reactive protein (CRP) is a reliable acute phase protein to monitor treatment response in humans with community acquired pneumonia, and seems promising in canine bacterial pneumonia.

This study investigated safety of antibiotic discontinuation based on clinical improvement and CRP normalisation. The second objective of this study was to evaluate the usefulness of CXR and LUS for follow up.

Prospective observational study. Dogs diagnosed with AP based on compatible history, clinical signs, CRP concentration, CXR and LUS findings were included. All dogs were treated with amoxicillin / clavulanic acid, which was discontinued based on clinical improvement and CRP normalisation after 1, 3 or 5 weeks. At each time point a quality of life (QOL) questionnaire filled in by the owners, physical examination, CRP concentration, CXR and LUS were assessed. Clinical improvement was defined on QOL and physical examination. A follow up was performed 2 weeks and, at least 1 month after antimicrobial discontinuation assessing short and long term relapse, identified by recurring clinical signs and/or an increased CRP concentration. In case of relapse, treatment was reimplemented.

Thirteen dogs were included. Mean CRP concentration was 90,6 mg/L (ref < 9, range 24-267) at admission. Antimicrobial treatment was discontinued after 1 and 3 weeks in 11 (84%) and 2 (16%) dogs, respectively. Short-term relapse based on mild CRP increase without compatible clinical signs was observed in 1/13 dogs. Long-term relapse was observed in 2/10 dogs, 2 and 6 months after antibiotic discontinuation due to acute vomiting. CXR and LUS lesions improved over time. Complete short term resolution of CXR lesions only observed in 3/13 dogs. Despite improvement of the severity of LUS findings over time, abnormalities persisted during short term serial follow up in all dogs.

This study suggests that canine AP can be safely and effectively treated with a short antimicrobial regimen based on clinical improvement and CRP normalisation. The role of CXR and LUS in this setting for the monitoring of AP is questionable.

**Disclosures**

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**ESVIM-O-6 - European Society of Veterinary Internal Medicine**

## Study of the use of IDEXX PROCYTE for total and differential cellular counts of bronchoalveolar lavage fluid in healthy dogs

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Bronchoalveolar lavage fluid (BALF) analysis is routinely used in canine and feline respiratory medicine. BALF manual counting allows total and differential cellular count measurement (TCC and DCC, respectively), but is time-consuming and requires training and expertise.

Accordingly, the aims of the present study were (1) to assess usefulness of the IDEXX PROCYTE analyzer in comparison with manual counting for in clinic-BALF TCC and DCC measurement and (2) to assess the effects of a pre-treatment with either a mucolytic agent or a filtration on BALF TCC and DCC, in healthy dogs.

Ten healthy beagles were prospectively included. BALF was collected using three aliquots of 1mL/kg of saline solution. Manual TCC and DCC were calculated using a hemocytometer and a cytospin preparation of naïve BALF, BALF treated with dithiothreitol 0.15% (DTT) solution and BALF filtered through a 70µm Cell Strainer. For DCC, a total of 200 cells were counted at high power field, by 2 independent operators. Automatic TCC and DCC were also calculated using the IDEXX PROCYTE analyzer on DTT-treated and filtered BALF. Results were compared using intraclass correlation coefficients (ICC) (interobserver agreement), Bland-Altman plot analysis and Wilcoxon signed rank test (Procyte versus manual counting), and Friedman test (DTT and filtering effects).

A good to excellent agreement was found between observers for manual TCC and DCC in each BALF type (ICC: 0.9-0.77; $p<0.02$). There was no significant difference in manual TCC between naïve, DTT-treated or filtered BALF ($p = 0.741$) and also no significant difference in manual DCC between the different treatments ($p = 0.628$). There was no significant difference in TCC between manual and PROCYTE counting neither after DTT ($p = 0.373$), nor after filtering ($p = 0.477$) and a good agreement was found between the 2 methods (ICC: 0,87-0,82; $p \leq 0,001$). However, PROCYTE did not provide pertinent DCC, since macrophages were not identified.

Results of this study demonstrated that BALF TCC can be adequately measured using both manual and PROCYTE counting, and is not impacted by pre-treatment with DTT or filtering. On the contrary, IDEXX PROCYTE does not appear suitable for DCC measurement. Effects of DTT and filtration procedures on TCC and DCC measurements should be further analyzed in inflammatory samples from dogs with lower airway diseases.

**Disclosures**

No disclosures to report.

**ESVIM-O-7 - European Society of Veterinary Internal Medicine**

## Bile acids in saliva of dogs with respiratory diseases and of healthy dogs pre- and post-feeding

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In microaspiration, small amounts of gastric contents are inhaled into lungs. The presence of bile acids in saliva can be used to detect the risk of microaspiration.

Our aim was to evaluate the presence of bile acids in saliva and to study whether fasting affects the amount.

Six West Highland White Terriers (WHWTs) with canine idiopathic pulmonary fibrosis (CIPF), 9 dogs with inflammatory airway disease (IAD), 7 dogs with recurrent or acute airway infection (INF), 10 healthy WHWTs, 20 brachycephalic dogs (BD) and 25 healthy dogs (HD) were included in this prospective cross-sectional observational study. Saliva samples were collected after 12 hours fast from all dogs and one hour after feeding from 19 HDs. Total bile acid (TBA) concentrations were analyzed by liquid chromatography-tandem mass spectrometry.

TBA concentrations were above the limit of quantification in 100% of CIPF (6/6), 56% of IAD (5/9), 57% of INF (4/7), 70% of healthy WHWTs (7/10), 85% of BD (17/20), 48% of HD pre-feeding (12/25), 84% of HD post-feeding (16/19). TBA concentrations were significantly higher in CIPF group (median 0.1692 μM, range 0.0731-0.3460 μM) compared to INF (0.0116 μM, not quantifiable [n.q.]-0.1190 μM, P=0.034), healthy WHWTs (0.0115 μM, n.q.-0.0964 μM, P=0.029) and fasted HDs (0.0025 μM, n.q.-0.1550 μM, P<0.001). In HDs, pre-feeding TBA concentrations were significantly lower compared to post-feeding samples (0.0235 μM, n.q.-0.3601 μM, p=0.019).

These results show that reflux occurs both in healthy and diseased dogs. Higher TBA concentrations in CIPF WHWTs suggest a connection of CIPF and reflux. Feeding increases reflux.

**Disclosures**

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**ESVIM-O-8 - European Society of Veterinary Internal Medicine**

## Effect of bronchoalveolar lavage on lung ultrasound and radiography in healthy dogs

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Bronchoalveolar lavage (BAL) is a minimally-invasive procedure used in dogs with suspected lower airway diseases. Thoracic radiographs (TRx) are important in the work-up of such dogs. More recently, Lung Ultrasound (LUS) has gained interest in veterinary medicine, showing benefits for detection and follow up of pulmonary conditions. Mild and transient TRx lesions have been reported after BAL while LUS-findings have not been reported.

The aim of this study was to investigate the impact of BAL on LUS and TRx findings.

Nine healthy beagles were included. BAL was performed under anesthesia using a pediatric bronchoscope; three 20mL aliquots of saline were instilled, two into the right caudal lobe and one into the left caudal lobe. LUS and TRx were obtained the day before the BAL procedure (D-1), as well as within 2 hours (H2), and 1, 2, 3, 4, and 7 days afterwards (D1, D2, D3, D4, D7 respectively). For LUS, 5 second cineloops were recorded at 9 points on each hemithorax and lesions were scored by a trained and blinded clinician according to severity: up to 3 B-lines was scored 0; >3 B-lines 1, and coalescent B-lines were scored 2, respectively. The presence or absence of a shred sign was also recorded. TRx lesions were scored by a blinded diagnostic imager from 0 up to 3 (mild to moderate interstitial pattern up to alveolar pattern) in 4 pre-defined zones. Global LUS and TRx scores were calculated as the sum of scores of the different locations.

Only mild LUS lesions were observed, except for one dog showing a shred sign at D1. In 5/9 dogs, >3 B-Lines or/and coalescent B-lines appeared either at H2 (4/5) or at D1 (1/5) in variable locations while in the 4 other dogs, LUS global score remained 0. As of D3 all scores were 0. LUS global score did not change significantly. All dogs displayed TRx lesions at H2. Lesions had resolved by D2 in 4, D4 in 7 and at D7 in all dogs. Alveolar patterns with airway bronchograms disappeared in all by D3. TRx global score increased significantly from D-1 to H2 (P=0.001), and decreased significantly from H2 and D1 to D4 (P=0.005 and 0.027, respectively).

In conclusion, BAL may induce LUS and TRx findings up to 4 days and 7 days after the procedure respectively. POCUS and TRx findings should be interpreted accordingly, in the follow up of dogs with lower respiratory disease.

**Disclosures**

No disclosures to report.

**ESVIM-O-9 - European Society of Veterinary Internal Medicine**

## Gentamicin concentrations in bronchoalveolar lavage and serum in healthy dogs after inhalation therapy

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Inhalation therapy is frequently used in dogs while little is known about how multiple possible factors influence the delivery of drugs into the lower airways. Gentamicin nebulization has been shown to be

efficacious for treating dogs with *Bordetella bronchiseptica* infection that are poorly responsive to conventional antimicrobial treatment using an empirical protocol. In companion animals, a minimum inhibitory concentration (MIC) of gentamicin of approximately 1-4mg/L has been described for *Bordetella bronchiseptica* isolates.

The aim of this study was to determine the concentration of gentamicin in the lower airways and serum of dogs after nebulization with undiluted gentamicin, according to the duration of nebulization during spontaneous breathing.

Ten healthy beagles were prospectively included. A standardized bronchoalveolar lavage (BAL) procedure was performed in each dog after 1 week of administration of each of 2 different gentamicin nebulization protocols separated by a one-week wash out period. The 2 protocols consisted in nebulization of gentamicin (50mg/mL) twice daily either for 10 minutes per session (±95mg) (10-min-protocol) or for 3 minutes per session (±28.5mg) (3-min-protocol). BAL fluid (BALF) was obtained under general anesthesia using a bronchoscope within 15 minutes after administration of the last nebulization. Three 20ml aliquots of isotonic saline were instilled through the endoscope channel, 2 into the right caudal lobe successively and 1 into the left caudal lobe. Blood was obtained within 5 minutes after BALF. BALF and serum gentamicin concentration were determined by particle enhanced turbidimetric inhibition immunoassay. Concentrations between protocols were compared using a paired t-test.

The BAL procedure yielded 23 to 32mL of BALF (median volume of 27.85mL). BALF and serum gentamicin concentrations were higher after 10-min-protocol compared with 3-min-protocol (2.41±0.87mg/L; mean ± SD; versus 1.25±0.31mg/L; $p<0.0001$ and 1.05±0.55mg/L versus 0.34±0.24mg/L; $p=0.001$ in BALF and serum respectively) while the BALF to serum ratio did not differ between the protocols (5.1±4.9 versus 2.8±1.6; $p=0.214$).

Since the proportion of epithelium lining fluid in BALF is about 2 to 3%, we can assume that both protocols provide the minimum level gentamicin concentrations required to kill *Bordetella bronchiseptica* in the lower airways of dogs, while the serum concentrations remain below the toxic ranges (>2mg/L). Although the most adequate and efficacious nebulization protocols still need to be determined, treating dogs, diagnosed with a *Bordetella bronchiseptica* infection and reluctant to oral antimicrobial therapy, with a 3-min-nebulization of undiluted gentamicin should be as efficacious as the 10-min-protocol so far described.

**Disclosures**

No disclosures to report.

**ESVIM-O-10 - European Society of Veterinary Internal Medicine**

## Gentamicin concentration in nasal lavage in healthy dogs after inhalation therapy

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In human medicine, topical administration of antimicrobials is a common treatment of upper respiratory tract diseases and gentamicin inhalation has shown promising results for chronic rhinosinusitis management. Because of gentamicin's narrow therapeutic index, inhalation therapy is interesting to allow high local bioavailability in sinonasal cavities with less systemic impact. In canine upper respiratory tract disease, antimicrobials inhalation efficacy for treatment of rhinosinusitis has not been assessed. Nasal lavage (NAL) might be a useful diagnostic and therapeutic method, but a well standardized protocol has not been described.

This study's aims were (1) to describe a NAL standardized procedure and total cell count in healthy beagle dogs; (2) to compare gentamicin concentrations in NAL-fluid (NALF) obtained after administration of nebulization protocols of different duration.

Ten healthy beagles were used. A standardized NAL procedure was performed in each dog prior to gentamicin administration, as well as after one week of administration of two different nebulization protocols. Gentamicin (50mg/ml) was nebulized twice daily either for 10 minutes per session (± 95mg) (10-min-protocol) or for 3 minutes per session (± 28.5mg) (3-min-protocol), separated by a one-week wash out period. NAL was performed under anesthesia, within 30 min after last nebulization. Gentamicin determination was performed on NALF by particle enhanced turbidimetric inhibition immunoassay.

Dogs were placed in ventral recumbency, the nasopharynx was manually obstructed and a 12 Fr fenestrated catheter was introduced in the first third of left nasal cavity for administration of 20 mL of isotonic saline and retrieval. NAL procedures yielded 5 to 16mL of NALF (mean ± standard deviation of 11.1 ± 2.55mL) with poor cellularity (17.50 ± 19.70 cells/μL), mainly consisting in keratinized (51.1%) and epithelial cells (46.4%), with no or few neutrophils. NALF gentamicin concentration was higher in the 10-min-protocol compared with the 3-min-protocol (13.66 ±8.28mg/L and 6.28 ± 3.23mg/L; $p=0.013$). In spite of an obvious dilution effect related to the NAL procedure, NALF gentamicin concentrations were correlated with the duration of inhalation and were high, compared to the minimum inhibitory concentration reported for aminoglycoside-sensitive bacteria (2-4mg/L).

This study shows that, (1) the NAL method seems reproducible and yields samples that can be used for measurement of local drug concentration as a surrogate of intranasal drug concentration and (2) gentamicin nebulization appears as reproducible therapeutic procedure with interest for the management of bacterial rhinosinusitis in dogs, although the most adequate and efficacious nebulization protocol still needs to be determined.

**Disclosures**

No disclosures to report.

## ESVIM-O-11 - European Society of Veterinary Internal Medicine

### Comparison of culture- dependent and -independent methods on nasal swabs in dogs with nasal discharge

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The role of bacterial communities in the pathophysiology of canine nasal disease is still unclear. How and when to treat dogs with rhinitis when a secondary bacterial infection is suspected and on which test to rely before making a decision to treat with antimicrobials has not been clearly established.

The aim of the study was to compare, in dogs with nasal discharge clinically suspected to be of bacterial origin, regardless of the primary etiology of the rhinitis, the results of culture with 16S rDNA sequencing results.

Thirty client-owned dogs referred for rhinoscopic examination were prospectively included. Dogs were diagnosed with sinonasal aspergillosis (n=11), chronic idiopathic rhinitis (n=8) and other nasal or extranasal diseases (n=11). Secondary nasal bacterial infection was suspected based on the presence of mucopurulent-like nasal discharge. Four dogs had ongoing systemic antibiotic treatment at the time of sampling. Two swabs (eSwab and FLOQSwab) were successively collected in the distal third of the same affected nasal cavity using a sterile technique. The eSwab samples were streaked on 4 agar media (Columbia blood Agar, MacConkey, Chapman and Edward's) that were incubated overnight in aerobic conditions. FLOQSwabs were stored in a sterile cryotube and banked at -80°C until bacterial total DNA extraction. Extracted DNA underwent PCR targeting the V1-V3 region of the 16S rRNA gene.

An average of 1.6±0.9 bacterial isolates were cultured while between 13 and 225 distinct operational taxonomic units were detected by sequencing. There was no significant effect of antibiotic treatment on the number of taxa detected by culture or sequencing. The vast majority (93%) of dogs had a microbial profile compatible with dysbiosis. Three dogs had negative culture results. The dominant sequence types corresponded to culture results in 10 (33%) dogs, in association with marked predominance of one taxon (>80% RA) in 6/10 cases. In 12 (40%) dogs the cultured isolates were rare (<10% RA) or undetected components of the corresponding sequence libraries. A negative culture in the face of bacterial predominance (>50% RA) of a potentially pathogenic bacteria detected by sequencing occurred in 23% (n=7) of cases and mostly with anaerobic/difficult/slow-growing bacteria, however use of other agar media may have decreased this percentage. In conclusion, culture results of nasal swabs must be interpreted with caution and cannot be used as a single tool to guide antimicrobial treatment. Culture-independent techniques provide a more comprehensive profile of taxa present in dogs with nasal disease and helps identifying difficult-to-culture pathogens.

#### Disclosures

No disclosures to report.

## ESVIM-O-12 - European Society of Veterinary Internal Medicine

### Alterations of the nasal microbiota in dogs with sinonasal aspergillosis before and after cure and comparison with chronic idiopathic rhinitis

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Pathogenesis of canine sinonasal aspergillosis (SNA) is, to date, not fully understood. Treatment is still challenging and even after cure, intranasal remodelling may be associated with clinical disease mimicking chronic idiopathic rhinitis (CR) and recurrence can occur. Previous studies demonstrated significant alterations of the nasal microbiota in dogs with SNA including decreased abundance of Moraxellaceae, that was also described in dogs with CR. The objectives of the present study were (1)to confirm microbiota alterations in dogs with SNA; (2) to characterise the microbiota present after successful treatment of the disease; (3)to compare the microbiota in dogs with SNA and CR.

Forty dogs diagnosed with SNA, 14 dogs with CR and 29 healthy control dogs were included. Nine of the SNA dogs were resampled after successful treatment with enilconazole infusion. A sterile swab was introduced in the distal third of the nasal cavity under general anaesthesia. After total DNA extraction, a PCR targeting the V1-V3 hypervariable region of the 16S rDNA was performed and amplicons were sequenced on a MiSeq Illumina sequencer. Taxonomical assignation and microbiota community analysis were done with MOTHUR V1.41.0 with an OTU clustering distance of 0.03. Kruskal-Wallis with FDR correction in STAMP (2.1.3) was used to identify differences in relative abundance between groups. Ecological parameters (Chao1 index, Simpson and inverse Simpson index) and b-diversity (using AMOVA and HOMOVA) were also calculated.

Major alterations were observed in dogs with chronic nasal diseases including a decrease in *Moraxella* (SNA 5.4±18%, CR 4.6±8.7%, control 51.8±39.7%) and an increased richness and a-diversity at species level. A difference in b-diversity was significant only between SNA and control dogs. Unique alterations further differentiated SNA (increase in Neisseriaceae, Porphyromonadaceae and Staphyloccaceae among others) and CR (increase in Pasteurellaceae and Lactobacillaceae). In SNA dogs at cure, only 1 dog recovered a high

relative abundance of Moraxellaceae while in others the nasal microbiota was either unchanged (n=2) or dominated (>50%) by a single other bacterial family (n=5).

In conclusion, results of the present study confirm major alterations, such as the decrease of *Moraxella*, of the nasal microbiota in dogs affected with SNA and CR, identifying *Moraxella* as a guardian for nasal health stability. Beside this common alteration, a specific dysbiotic profile differentiated SNA from CR. Whether nasal health is associated with recolonization with microbes of the Moraxellaceae family, while other dogs ineluctably progress toward CR or SNA recurrence is unknown and warrants further investigation.

**Disclosures**

No disclosures to report.

## ESVIM-O-13 - European Society of Veterinary Internal Medicine

### Comparison of immunohematological diagnostic tests including six different Coombs' test methods in dogs suspected to have immune-mediated hemolytic anemia

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The direct Coombs' or antiglobulin test (DAT) is used to diagnose immune-mediated hemolytic anemias (IMHA) in humans and animals, albeit its value has been questioned for IMHA in dogs. A 2019 ACVIM consensus statement on the diagnosis of IMHA in dogs lacks evidence on immunodiagnostic test performances. This prospective study compares agglutination, spherocytosis, and six different DAT methods.

During a 13-month period, left-over EDTA-blood from 126 samples submitted for a DAT to a veterinary diagnostic laboratory and 28 samples from clinically healthy dogs were tested with a microtiter plate test, an in-clinic immunochromatographic strip test, two gel tests (in-clinic and in laboratory), a microcapillary test, and flow cytometry – some with different antiglobulins and at different temperatures, and with and without washing erythrocytes prior to testing.

Overall 67 dogs were DAT+ with at least four methods, and agreements between DAT methods were (very) good (κ-value>0.6; p<.002). Spherocytosis was seen in 58 cases with 93% being DAT+ (p<.001). Autoagglutination was seen in 48 samples before washing, and 20% were DAT-. Persistent mild autoagglutination was only observed in four samples; they were DAT+. Among the 12 DAT+ dogs followed up during treatment, ten were still DAT+ when tested 1-24 weeks after initial diagnostic assessment.

Based upon this extensive comparative prospective survey, the various in-clinic and laboratory DAT techniques produced similar immunodiagnostic results when performed by a trained person. They can be recommended for detection of antibody-coated erythrocytes and for the immunohematological diagnosis of IMHA in dogs as well as monitoring of therapeutic responses.

**Disclosures**

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## ESVIM-O-14 - European Society of Veterinary Internal Medicine

### Diagnostic utility of C-reactive protein on plasma and abdominal fluid in dogs with ascites

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C-reactive protein (CRP) is an important acute phase protein in dogs. Its plasmatic concentration increases with any systemic inflammation and rapidly decreases once the inflammation resolves. In dogs, ascites can be broadly divided into transudates and exudates. The different types of effusion involve varying degree of inflammation that could possibly be assessed through CRP measurement directly on ascitic fluids.

The objectives were to evaluate the diagnostic utility of CRP on plasma ($CRP_p$) and abdominal fluid ($CRP_f$) then $\Delta_{CRP}$ ($CRP_p$ – $CRP_f$) in dogs with abdominal effusion.

Client-owned dogs were prospectively enrolled in 2 centers, between January 1$^{st}$ and June 30$^{th}$ 2020, only if surplus-discarded blood, abdominal fluid (sampled for diagnostic purpose) and complete medical data were available. Categorical (sex, survival) and continuous variables (age, weight, $CRP_p$, $CRP_f$ and $\Delta_{CRP}$) were estimated with percentages and medians (min–max), respectively. Fluids were classified using classical markers (glucose, lactate, total proteins, hematocrit, fluid total nucleated cell count and density). Comparisons of $CRP_p$, $CRP_f$, and $\Delta_{CRP}$ between transudates and exudates, then between septic and non-septic effusions were performed using Mann-Whitney Wilcoxon test. Receiver Operating Characteristic (ROC) curves were generated to assess the performance of each parameter.

Fifty cases were included (26 females and 24 males), with a median age of 9 (0.5–15) years and median weight of 21 (2–65) kg. Effusions were classified as transudates (36%), exudates (64%), non-septic (80%) and septic (20%). Diagnosis included cardiogenic ascites (28%), hemoabdomen (26%), septic peritonitis (20%), non-septic exudates

(12%), carcinomatosis (6%), protein-rich transudates (4%), uroabdomen (2%) and pure transudates (2%). Median $CRP_p$ (56.2 mg/l vs 14.2 mg/l, p=0.003), $CRP_f$ (27.2 mg/l vs 9.3 mg/l, p = 0.028) and $\Delta_{CRP}$ (20.5 mg/l vs 4.0 mg/l, p=0.022) were significantly higher in dogs with exudates than with transudates. Median $CRP_p$ (94.5 mg/l vs 23.8 mg/l, p<0.005) and $CRP_f$ (56.4 mg/l vs 11.7 mg/l, p<0.005) were significantly higher in dogs with septic effusions than in dogs with non-septic effusions, while $\Delta_{CRP}$ (32.4 mg/l vs 7.5 mg/l) did not differ between the 2 populations (p=0.169). ROC curves indicated that a cutoff value of 32.4 mg/l for $CRP_f$ was 100% sensitive and 90% specific for the diagnosis of septic effusion, with an area under the curve of 0.97.

$CRP_p$, $CRP_f$ and $\Delta_{CRP}$ could aid for the diagnosis of abdominal effusions in dogs. $CRP_f$ appeared to be the most discriminating parameter.

**Disclosures**

No disclosures to report.

## ESVIM-O-15 - European Society of Veterinary Internal Medicine

### Shar Pei auto-inflammatory disorder (SPAID) in the United Kingdom - A retrospective survey

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Shar Pei autoinflammatory disorder (SPAID), also known as Shar Pei Fever, is an inherited auto-inflammatory disorder. Few descriptions of episodes of SPAID have been reported and treatment recommendations are based on anecdotal experience.

A retrospective survey was performed to estimate the prevalence of SPAID among UK Shar Pei (SP), to characterise episodes of SPAID, and identify commonly used treatments. Clinical data was collected from owners and veterinary surgeons via an electronic data capture system (CastorEDC). A diagnosis of SPAID was based on veterinary assessment and ≥1 presentation with unexplained pyrexia (>39.2°C). Frequencies of previously proposed risk factors (wrinkled skin, muzzle conformation, and certain comorbidities) were compared between the SPAID and non-SPAID groups using the Fischer's exact test. Significance was set at p <0.05 in all analyses.

132 SP were enrolled; 26 were excluded for incomplete records. At least one episode of SPAID was reported in 52/106 (49%) SP. Nine SP had fever episodes consistent with SPAID reported by their owners but not diagnosed by their veterinarian. Median rectal temperature at presentation for SPAID was 40.0°C (39.3-41.2°C); 73% of episodes lasted < 36 hours. Owners reported associated hyporexia (n=33) and vomiting (n=8) more frequently than veterinary records (22 and 0 respectively). The median number of veterinary appointments for SPAID was 2 per dog (range 1-15), while owners reported a median 4 SPAID episodes per year.

Treatments prescribed for SPAID included non-steroidal anti-inflammatory medications (NSAIDs) (38/52), antimicrobial therapy (17/52), and paracetamol (9/52); three SP required hospitalisation for intravenous fluid therapy. Colchicine was prescribed in ten SP; eight of these owners reported reduced frequency or severity of fever episodes. 4/10 reported self-limiting diarrhoea with colchicine therapy.

The SPAID and non-SPAID groups contained similar numbers of SP with 'meat-mouth' conformation (thickened, fleshy muzzle), moderately- and extremely-wrinkled skin (owner assessment); none of these phenotypic variants were significantly associated with SPAID. No significant differences in frequencies of dietary sensitivities, allergic skin disease or recurrent otitis externa were found between groups.

The prevalence of SPAID in this population was higher than in previous reports. Episodes of SPAID were reported approximately twice as frequently by owners compared to veterinary records, suggesting the burden of this condition may be underestimated by veterinary surgeons. Specific risk factors for SPAID were not identified. A potential role of colchicine as a preventative therapy is suggested, however the efficacy of this treatment cannot be assessed due to infrequent use in the UK.

**Disclosures**

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## ESVIM-O-16 - European Society of Veterinary Internal Medicine

### Diagnoses and outcomes associated with ionised hypercalcaemia in a referral population of cats

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Ionised calcium (iCa) is the biologically active fraction and more sensitive for detecting calcium disturbances than serum total calcium. Published evidence on clinical associations with ionised hypercalcaemia in cats are limited.

Cats seen 1/1/09 - 1/1/19 at a UK tertiary referral hospital with [iCa] above the machine reference interval were identified.

From 11,431 cats, 442 had an elevated [iCa] recorded. Presenting complaints included: inappetence/anorexia (37.3%), lethargy (33.7%), blood-work abnormalities (33.7%), weight loss (24%), vomiting (22.2%), polyuria and/or polydipsia (13.1%), neurological signs (10.9%), ionised and/or total hypercalcaemia (9.1%). 207/442 cats (46.8%) had transient, inconsequential or iatrogenic hypercalcaemia, or were young patients. The remaining 235 cases had [iCa] >1.4mmol/l (considered clinically significant). final diagnoses for these

235 cases included: acute kidney injury (17.4%), neoplasia (15.7%), idiopathic hypercalcaemia (13.6%), CKD or renal diet-associated (6.8%), primary hyperparathyroidism (2.1%), vitamin D toxicity (2.1%) and granulomatous disease (1.2%). 94 cases (40%) had no single diagnosis (insufficient work-up (n=90) or multiple concurrent diagnoses (n=3)).
156/442 cats underwent multiple [iCa] measurement(s); 33.9% remained hypercalcaemic, 37.8% became normocalcaemic and 28.2% had fluctuating [iCa]. Cats with fluctuating [iCa] had longer follow-up (median 417 days (range 1-2299), versus 78 days (0-1077) for persistent, versus 85 days (1-2237) for resolved; P=0.005). No difference was identified in the proportion of cats with concurrent total hypercalcaemia (48.9% resolved, 44.0% persistent, 46.1% fluctuating hypercalcaemia; P=0.833).
Ionised hypercalcaemia was deemed clinically significant in just over half of cases and most commonly due to kidney diseases, neoplasia or idiopathic hypercalcaemia. Longer-term calcium status was highly variable.

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## ESVIM-O-17 - European Society of Veterinary Internal Medicine

### A prospective randomized trial of methylprednisolone with or without cyclosporine or mycophenolate mofetil for the treatment of immune-mediated haemolytic anaemia in 43 dogs

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Immune-mediated haemolytic anaemia (IMHA) is associated with high mortality rate in dogs. Glucocorticoids alone or in combination with second-line immunosuppressive drugs are reported as treatment options. However, the benefit of adding these drugs to a standard steroid protocol has not been established yet.
The aim of this open label, unblinded, randomized, prospective study was to evaluate haematological response (HR) and clinicopathological outcomes in dogs affected by non-associative IMHA (naIMHA) treated with three different immunosuppressive protocols.
Diagnosis of naIMHA was based on the presence of haemolytic anaemia (HCT<37%) and at least one of the following criteria: positive Coombs' test, saline erythrocyte agglutination and spherocytosis. Dogs were excluded if underlying diseases, triggering factors or previous administration of immunosuppressive drugs were present.
Immunosuppressive doses of methylprednisolone (M) were administered to all patients; then dogs were randomized to receive one of the following: only M (M group), M plus Cyclosporine (MC group), M plus Mycophenolate Mofetil (MM group).
Clinical and clinicopathological data were evaluated at admission and at different times (T7, T14, T30, T60, T120, T180 and T365 days). HR was assessed at T14, T30 and T60. Complete recovery (CR) was defined as: HCT >37%, negative signs of both immune-mediated destruction and haemolysis; partial recovery (PR) was documented by HCT<37%, negative signs of both immune-mediated destruction and haemolysis.
Forty-three dogs were diagnosed with naIMHA. Two dogs were excluded after discharge, due to lost follow up. HR, number of transfusion treatments, length of hospitalization, frequency of relapse and complications were not significantly different among treatment groups.
At T14, only 7% of dogs achieved a PR; at T30, 7% of dogs showed CR and 18% of dogs a PR. Finally, at T60, 25% of dogs displayed a CR and 19% of dogs a PR. Ten dogs (24%) developed infectious complications and 17% of dogs had thrombotic complications; three dogs (7%) had a relapse. Overall survival rate was 93%, 78% and 75% at discharge, at T60 and at T365, respectively. Survival rate at T60 and T365 was significantly higher for dogs belonging to the MC group compared with M and MM groups (P= .03 and P= .01, respectively).
The results of this study did not show a beneficial effect of the combined immunosuppressive regimens over methylprednisolone therapy alone in the HR of naIMHA in dogs. Nevertheless, in this population, the combination of cyclosporine and methylprednisolone is associated with a higher survival rate at 60 and 365 days.

**Disclosures**

No disclosures to report.

## ESVIM-O-18 - European Society of Veterinary Internal Medicine

### Suspected hypertensive encephalopathy in cats with systemic hypertension

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Hypertensive encephalopathy in cats has long been recognized, but in our perception this is still widely underestimated in clinical practice. Part of an explanation maybe that the individual clinical signs are quite variable and may be subtle. Therefore, the objective of this study was to characterize clinical manifestations of hypertensive encephalopathy in cats.

All cats with suspected systemic hypertension (SHT) due to age, underlying predisposing disease or suggestive clinical presentation, neurological or non-neurological, were prospectively enrolled over a 2-year period. Confirmation of SHT was based on at least two sets of measurements of systolic blood pressure >160mmHg by Doppler sphygmomanometry.

Thirty cats with SHT and neurological signs were identified. Eight patients presented with seizures, 6 with generalized and 2 with focal seizures. Nine cats showed altered behavior, including increased and inappropriate vocalization (n=4), hiding (n=2), disorientation (n=2), inappropriate urination (n=1), sleeping in unusual locations (n=1), increased appetite (n=1), paresthesia on the head (n=1). Fifteen cats showed ataxia; of these eight cats had vestibular signs. An additional 7 cats had ataxia of non-vestibular origin; of these 3 were blind, which was considered a causing or contributing factor. Four cats showed variable paresis, i.e. hemiparesis (n=1), hemiplegia (n=1), paraparesis (n=1), tetraparesis (n=1). Three cats showed pleurothotonus, 2 ventroflexion (1 hypokalemic due to hyperaldosteronism, 1 normokalemic), 1 cat was stuporous. In 16 of the 30 cats, the primary presenting complaint were neurological signs. In the other 14, neurological deficits were appreciated only after thorough expansion of the clinical history; neurological abnormalities had apparently been ignored due to intermittent occurrence, subtle manifestation, or misinterpretation of signs. In 27 of 29 cats retinal lesions were detected during fundoscopy. Of these 27 cats, 6 were presented because of visual deficits and/or mydriasis), and neurological signs were not a primary complaint. 13 cats were presented because of neurological abnormalities and fundic abnormalities were detected subsequently. All neurological signs resolved with appropriate antihypertensive treatment corroborating SHT causing encephalopathy in these cats.

This study is in line with previous reports implicating the brain as important target organ for SHT induced damage. Even mild behavioral changes, gait abnormalities, tic-like episodes should prompt clinicians to rule out SHT as potential cause, especially in at-risk populations, before performing advanced brain imaging.

**Disclosures**

No disclosures to report.

## ESVIM-O-19 - European Society of Veterinary Internal Medicine

### Pregabalin alleviates feline anxiety and fear during transport and veterinary visits - A clinical field study

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The objective of the study was to confirm clinical efficacy and safety of a novel feline specific formulation of pregabalin 50 mg/ml oral solution in cats with anxiety and fear associated with travel and veterinary visits.

This was a randomised, double-blind, placebo-controlled, parallel group study. A total of 209 client-owned cats with a history of anxiety and fear associated with travelling and veterinary visits received either a single dose of pregabalin oral solution of 5 mg/kg or placebo approximately 90 min before placing the cat into the carrier. Cats were transported for at least 20 min to a veterinary clinic. Treatment effect during transport was evaluated by the owner and during clinical examination by the investigator. An external expert evaluated the treatment effect during transport using video recordings. Usability of the product was assessed by the owner.

Treatment effect during transport ($p < 0.01$) and during clinical examination ($p < 0.01$) significantly favored pregabalin. The external observer assessment confirmed the owner's assessment. Clinical safety was good, with few cats showing signs of mild and transient incoordination (4.6%) and tiredness (2.8%). No serious adverse events were reported. The majority (79%) of the cat owners assessed administration of the new pregabalin oral solution formulation as very easy or easy.

In conclusion, the feline specific pregabalin oral solution at the dose 5 mg/kg is effective for alleviation of acute anxiety and fear associated with transport and veterinary visit in cats. Clinical safety and usability of the product were good.

**Disclosures**

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## ESVIM-O-20- European Society of Veterinary Internal Medicine

### Prevalence and causes of fasting hypertriglyceridaemia in Australian Miniature Schnauzers

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Fasting hypertriglyceridaemia can occur as a primary disorder or secondary to underlying diseases such as hyperadrenocorticism, diabetes mellitus and hypothyroidism. Primary hypertriglyceridaemia in Miniature Schnauzers has a reported prevalence of approximately 30% in North America, increasing with age. Data on the prevalence of primary and secondary hypertriglyceridaemia in other countries are lacking. The aim of this study was to investigate the prevalence and causes of fasting hypertriglyceridaemia in Australian Miniature Schnauzers.

Two hundred and fifteen healthy Miniature Schnauzers were recruited into a cross-sectional study. Each dog was assessed with an owner questionnaire and a physical examination. Triglyceride concentrations were measured after a 15-hour fast. If fasting hypertriglyceridemia was identified, haematology, serum biochemistry, urinalysis (including urine protein:creatinine ratio), total thyroxine (including thyroid stimulating hormone if total thyroxine was low) and a low-dose dexamethasone suppression test were performed. Dogs with no underlying cause for the fasting hypertriglyceridemia had an adrenocorticotrophic hormone stimulation test. Thirty dogs with normal triglyceride concentrations underwent the same testing to act as controls.

Forty of 215 dogs (18.6%; 95% CI: 14.0%-24.3%) had fasting hypertriglyceridemia. The prevalence of fasting hypertriglyceridaemia increased with age; 0%, 9%, 11%, 26% and 64% in dogs <1 year,1-3 years, 4-6 years, 7-9 years and >9 years, respectively. Thirty-nine hypertriglyceridaemic dogs underwent further testing and 31 (79.4%; 95% CI: 63.5%-90.7%) had hyperadrenocorticism, one (2.5%; 95% CI: 0%-13.4%) had hypothyroidism and seven (17.9%; 95% CI: 7.5%-33.5%) had primary hypertriglyceridemia. Ten of the 30 control dogs (33.3%; 95% CI: 17.2%-52.8%) were diagnosed with hyperadrenocorticism. No other diseases were identified in the control group. Fasting hypertriglyceridaemia was significantly associated with secondary disease (P<0.001) with a relative risk of 2.4 (CI 1.4-4.1).

The estimated prevalence of fasting hypertriglyceridaemia in Australian Miniature Schnauzers was lower than in North America. Primary hypertriglyceridaemia was uncommon in this study, with the majority of affected dogs being diagnosed with hyperadrenocorticism. Thorough investigation of hyperadrenocorticism and other secondary causes should be undertaken when fasting hypertriglyceridaemia is identified in Miniature Schnauzers.

**Disclosures**

Sue Foster is an Adjunct Associate Professor in Small Animal medicine at Murdoch University and provides consultancy services to Vetnostics, the laboratory which performed the majority of the laboratory tests. Sue Foster has no other disclosures. Doug Hayward is employed by Vetnostics, the laboratory that performed the majority of the laboratory tests.

## ESVNU-O-1 - European Society of Veterinary Nephrology and Urology

### Urinary liver-type fatty acid-binding protein in cats with International Renal Interest Society (IRIS) stage 1 chronic kidney disease within a healthy elderly cohort

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Urinary liver-type fatty acid-binding protein (uL-FABP) is a tubular renal biomarker that holds promise for the detection of early feline chronic kidney disease (CKD). Also, uL-FABP is detected in 7% of healthy cats, but the reason for this presence remains unknown. Evaluation of uL-FABP in IRIS stage 1 CKD cats and healthy elderly cats without evidence of kidney dysfunction may give more insight. The aim of this study was to evaluate uL-FABP in a large population of healthy middle-aged to aged cats including cats with CKD IRIS stage 1.

Urine samples collected by cystocentesis from 196 healthy client-owned cats ≥7 years old undergoing health screening were measured for uL-FABP concentrations using validated commercial feline L-FABP ELISA. Routine blood and urine results were used to divide the cats from our cohort into two subgroups: either IRIS stage 1 CKD or no evidence of early CKD.

Only twenty-five cats had IRIS stage 1 CKD, while the remaining 171 cats did not have any evidence of early CKD. These 25 cats with CKD IRIS stage 1 had serum creatinine (sCr) <140 μmol/L and one of the following criteria: USG <1.035 without an identifiable non-renal cause (n=22), persistent renal proteinuria (urinary protein: creatinine ratio (UPC) >0.4 at two consecutive time points without evidence of pre- or postrenal proteinuria) (n=1), or serum symmetric dimethylarginine >14 μg/dL at two consecutive time points (n=2). Apart from the persistent proteinuric cat, 47 cats had borderline proteinuria (UPC 0.2-0.4), and 147 cats were non-proteinuric (UPC <0.2). In one cat with macroscopic hematuria, a reliable UPC value could not be determined.

All cats with IRIS stage 1 CKD had uL-FABP concentrations below the detection limit (0.21 ng/ml), whereas 6 cats without evidence of early CKD had detectable uL-FABP concentrations (median 1.83 ng/ml, range 0.88-3.66 ng/ml). Of these 6 cats, only one cat was borderline proteinuric and none were proteinuric.

In conclusion, uL-FABP is detectable in 3% of our cohort of healthy middle-aged to aged cats independent of the presence of IRIS stage 1 CKD or borderline proteinuria. Secondly, uLFABP is not able to identify cats with IRIS stage 1 CKD according to the current IRIS criteria.

**Disclosures**

No disclosures to report.

## ESVNU-O-2 - European Society of Veterinary Nephrology and Urology

### Risk factors and implications associated with renal mineralisation in feline chronic kidney disease (CKD)

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Nephrocalcinosis is of uncertain importance in feline CKD. Nephrocalcinosis was investigated to determine: (i) associated baseline risk factors (RFs); (ii) its influence on CKD progression and mortality.

Cats with CKD undergoing post-mortem examination after longitudinal study, were identified. Histopathological sections were graded for nephrocalcinosis (von Kossa). Baseline variables were compared (Kruskal-Wallis and Chi-squared tests) and nephrocalcinosis RFs were explored by ordinal logistic regression. Association of nephrocalcinosis with CKD progression and risk of mortality (ROM) were assessed (linear mixed model and Cox regression).

Fifty-one CKD cats (IRIS stages 2 [n=34] and 3 [n=17]) were grouped by nephrocalcinosis severity: Grade 0 (G0; n=11); Grade 1 (G1; n=14) and Grade 2 (G2; n=26). G2 cases had higher baseline plasma total calcium [tCa] than G0 (2.57 [2.46, 2.67] vs. 2.37 [2.33, 2.49] mmol/L; $P$=0.015). Proportionately more G1 and G2 cats were fed a phosphate-restricted diet (PRD) than G0 cats (86% and 85% vs. 45%; $P$=0.006). Baseline [tCa] (OR=1.54 [95% CI: 1.09–2.25] per 0.1 mmol/L; $P$=0.019) and feeding a PRD (OR=5.30 [95% CI: 1.41–22.15]; $P$=0.016) remained independent nephrocalcinosis RFs. Plasma [creatinine], [urea] and [phosphate] increased over time in G0 cats, which had shorter median survival times (189 [119–258] days) vs. G1 (607 [337–714] days; $P$=0.008) and G2 cats (368 [236–891] days; $P$=0.019). Cats fed a PRD had reduced ROM (HR=0.44 [0.22–0.86]; $P$=0.017) vs. cats that were not.

Higher plasma tCa at CKD diagnosis and PRD feeding are nephrocalcinosis RFs. However, nephrocalcinosis is not associated with progression or mortality.

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## ESVNU-O-3 - European Society of Veterinary Nephrology and Urology

### Erythrogram patterns in chronic kidney disease of dogs

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Anaemia is considered a common finding in dogs with chronic kidney disease (CKD), typically as normochromic, normocytic, and non-regenerative. Although anaemia could be occurring at any CKD IRIS stage, its severity has been related with the loss of kidney function.

The aim of the present study was to retrospectively evaluate quantitative and morphological abnormalities of the erythrogram in dogs at different CKD IRIS stages.

The study included erythrogram of azotaemic dogs with documented history, laboratory, and ultrasonographic findings of CKD. Exclusion criteria were represented by 1) rechecks of the same patient; 2) dogs with history, laboratory and/or ultrasonographic findings, consistent with acute kidney injury (AKI); 3) missing report of the blood smear evaluation; 4) use of alpha-darbepoetin or red blood cell transfusion prior to presentation. Median values of the CBC parameters were compared among the CKD groups (IRIS staging) through Kruskal Wallis test and Dunn's multiple comparisons test. Chi squared test was used to compare the frequency, and the degree of anaemia (mild, moderate, severe), regeneration rate (number of reticulocytes), and morphological abnormalities among different CKD groups.

A total of 482 dogs over 3,648 initially screened, were included in the study. Anaemia was present in 302/482 (63%) dogs, in the majority of which it was normochromic, normocytic, and non-regenerative (295/302; 98%). The number of reticulocytes was <60,000 /μL in the majority of dogs (248/295; 84%), with a strong correlation between poor regeneration and progression of CKD (p=0.0001; Phi=1.03). The frequency of anaemia significantly differed (p=0.0001) among the IRIS stages: 108/231 (47%) in IRIS 2, 77/109 (71%) in IRIS 3, and 117/142 (82%) in IRIS 4. Dogs at IRIS stage 3 and 4 were more likely to have moderate to severe anaemia, compared to dogs at IRIS stage 2 (p=0.0001). Anisocytosis (291/482; 60%) was the most frequent morphological abnormality of the erythrogram in the study population, although no association with the progression of CKD was noticed.

Anaemia was frequently found in CKD dogs, mostly associated with poor regeneration rate. Although anaemia may be present at any IRIS stage, its frequency and its degree of severity were associated with the loss of kidney function. Similarly to human medicine, advanced canine CKD IRIS stages are more frequently characterized by none to poor regeneration rate, compared to early stages. These findings may suggest a more severe condition of impaired bone marrow activity and erythropoietin deficiency.

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The Authors have no disclosures.

## ESVNU-O-4 - European Society of Veterinary Nephrology and Urology

### Evaluation of the clinical efficacy of benazepril in the treatment of renal proteinuria in dogs

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Persistent renal proteinuria is a hallmark of nephropathy and a predictor of progression in chronic kidney disease (CKD) in dogs. Furthermore, antiproteinuric therapy influences the progression of the disease, reducing the risk of mortality. The aim of the study was to determine the efficacy of benazepril in the treatment of renal proteinuria due to spontaneous CKD in dogs, using two different protocols.

For this multicentric prospective randomized trial 45 dogs diagnosed with idiopathic spontaneous proteinuria were enrolled, staged from IRIS-1 to IRIS-3. Other drugs affecting blood pressure (SBP) were not allowed. At enrollment (T0), all dogs underwent a physical examination and measurement of SBP, haematology and biochemical profile, urinalysis including urinary protein/creatinine ratio (UPC).

Dogs were fed a renal diet and randomized to two different benazepril dosage groups: group A (0.25 mg/kg/q24h; 22 dogs) and group B (0.25 mg/kg/q12h; 23 dogs). Dogs were monitored monthly for four months (T1, T2, T3, T4). The benazepril dose was doubled if UPC was not reduced by at least 50% one month later. Further modifications of dose were not allowed. Statistical analysis was performed.

The benazepril dose was doubled in 17 patients of group A (77.2%) and 14 patients of group B (66.6%). Two dogs in group B were withdrawn from the trial 15-20 days after the beginning of treatment. In group A, UPC decreased by >50% at least at one timepoint of the trial in 7/22 dogs (31.8%) and by >40% in 10/22 dogs (45.5%), while in group B UPC decreased by >50% in 10/21 dogs (47.6%) and by >40% in 11/21 dogs (52.4%). Kruskal–Wallis test didn't show any significant difference between UPC at the different timepoints, neither for groups A and B analyzed together nor when analyzed separately. Within group A, no difference was observed among timepoints with the paired samples Wilcoxon test. Conversely in group B, UPC obtained in T1 (p=0,0039) and T4 (p=0,0295) lowered significantly when compared to UPC in T0. No correlation was observed between UPC and SBP measurements.

In conclusion, the starting dose of benazepril at 0.25 mg/kg/q24h had clinically relevant efficacy in reducing proteinuria. Additionally, benazepril q12h proved to be superior to q24h. The lack of efficacy of benazepril in some dogs might be due to the need of additional treatments (i.e., immunosuppressive medications), as reported in the literature.

**Disclosures**

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## ESVNU-O-5 - European Society of Veterinary Nephrology and Urology

### Acute kidney injury in dogs: Etiology, clinical and clinicopathologic findings, prognostic markers, and outcome

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Acute kidney injury (AKI) is a common and potentially fatal condition. This retrospective study aimed to characterize the etiology, clinical, clinicopathologic findings and outcome of dogs diagnosed with AKI. A total of 230 dogs were enrolled, including 110 males and 120 females, with a median age of 81 months (range, 1-204) and a median body weight of 19.5kg (range, 1.2-75.8). The common etiologies were ischemic/inflammatory (59%), infectious (8%) and nephrotoxicity (5%). Median creatinine concentration (sCr) at presentation was 4mg/dL (range, 1.1-37.9), increasing to a peak of 4.6mg/dL (range, 1.1-43.2), which was used for classifying dogs to IRIS grades as follows: Grade-I, 6; 2.6%, Grade-II, 36; 15.7%, Grade-III, 81; 35.2%, Grade-IV, 68; 29.6%, Grade-V, 39; 17%. Overall mortality was 31%, increasing with IRIS AKI grade. Dogs with an infectious etiology had mortality rate of 11.1%, while dogs with toxic and ischemic/inflammatory etiologies had mortality rates of 16.7% and 29.6%, respectively. Forty-five dogs (19.5%) underwent hemodialysis, of these 67% survived. Activities of ALP, ALT, AST, concentration of bilirubin, phosphorus and peak creatinine were higher in non-survivors, whilst blood-pH, bicarbonate, albumin, hematocrit and platelet count were lower. Anuria and diarrhea were also more common in non-survivors. In a multivariable analysis platelet count ($P$=0.05), albumin ($P$=0.03) and blood-pH ($P$=0.001) remained associated with survival.

Survival was substantially higher compared with the last large scale retrospective study published 25 years ago, potentially due to earlier identification and advancement in therapy. Non-infectious etiologies, higher AKI IRIS Grade, acidemia, thrombocytopenia and hypoalbuminemia were associated with a worse prognosis.

**Disclosures**

No disclosures to report.

## ESVNU-O-6 - European Society of Veterinary Nephrology and Urology

### Effect of storage conditions and measurement device on serum symmetric dimethylarginine in cats and dogs

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Data concerning storage stability of symmetric dimethylarginine (SDMA) are scarce though desirable as time and temperature induced kinetics might compromise accurate assessment of kidney function. Recently an in-clinic test has become available to enable rapid SDMA evaluation, but comparison with the commercially available immunoassay is essential to support interchangeable use by veterinarians.

This prospective study aimed to evaluate short- and long-term stability of serum SDMA (sSDMA) at various pre-analytical conditions and determine accuracy and precision of the in-clinic device (i.e. IDEXX Catalyst SDMA Test) compared to sSDMA measured by an external IDEXX laboratory.

Serum left-overs of 35 healthy and renal diseased adult client-owned dogs and cats were collected. Baseline values (T0) were determined for the laboratory and in-clinic device as soon as possible after blood sampling. Duplicate measurements of T0 in-clinic were performed to assess (im)precision of the device. Short-term stability of sSDMA was tested after storage for 24 hours at room temperature (RT) and at 4°C with the in-clinic device; and after storage for 7 days at 4°C both in-clinic and in the external laboratory. Long-term stability was evaluated in the external laboratory after 10 months of storage at -20°C and -80°C. Statistical analysis was performed using Analysis of Variance (ANOVA) and Bland-Altman plots.

Based on a predefined equivalence interval of [-3 – 3 µg/dL] for the difference between T0 and the stored sample, short-term storage for 7 days at 4°C (95% CI: -2.3 – 2.3) and long-term storage for 10 months at -20°C (95% CI: -2.3 – 2.3) and -80°C (95% CI: -2.9 – 1.7) were found to be equivalent to T0 when sSDMA was quantified in the external laboratory. For in-clinic analysis, short-term stability of sSDMA could only be claimed after 24-hour storage at 4°C (95% CI: -2.5 – 2.1) highlighting a clinically important difference with T0 when serum was stored at RT for 24h (95% CI: -4.1 – 0.5) or at 4°C for 7 days (95% CI: -3.5 – 1.1). Repeated in-clinic measurements of sSDMA at T0 equally demonstrated equivalence (95% CI: -2.6 – 2.0). The in-clinic device assessment differed significantly from the assessment in the external laboratory both at T0 and at day 7 after blood sampling ($P$ <.001).

Although clinically important changes in sSDMA concentration may occur during storage, short-term refrigerated and long-term frozen storage will generally lead to acceptable results. Results of in-clinic and external laboratory sSDMA measurements are preferably not used interchangeably.

#### Disclosures

No disclosures to report.

## ESVNU-O-7 - European Society of Veterinary Nephrology and Urology

### Bacterial urinary tract infection and subclinical bacteriuria in dogs receiving chemotherapy: A prospective observational longitudinal clinical study

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An increased prevalence of positive urine culture (PUC) has been reported in dogs treated with corticosteroids or ciclosporin. The goal of this study was to investigate the prevalence of subclinical bacteriuria (SB) and urinary tract infection (UTI) in dogs with non-urogenital cancer treated with chemotherapy. Secondary goals were to identify risk factors for PUC and to compare survival time between dogs with and without PUC.

A total of 46 client-owned dogs were included into this prospective observational longitudinal clinical study. Urine culture was performed before the start of chemotherapy and at least once during chemotherapy.

Urine culture yielded significant bacterial growth in 21/185 urine samples in 8 dogs. Chemotherapy did not influence the prevalence of PUC, which was 10.9% (95% confidence interval [CI]: 4.7–23.0%) before the start of chemotherapy and 13.0% (CI: 6.1–25.7%) during chemotherapy. Eight dogs had 10 episodes of PUC; 8/10 episodes were classified as SB, and in 2/10 episodes UTI was diagnosed. One dog had a prostatic abscess and one dog an emphysematous cystitis. Hyperadrenocorticism and urine retention were identified as risk factors for PUC. There was no difference in survival time between dogs with and without PUC, and death or euthanasia was not related to UTI in any dog.

Chemotherapy seems not to be a major predisposing factor for development of PUC. Although most dogs with PUC have SB, complicated infections are also possible. An impact of PUC on survival time was not observed.

#### Disclosures

No disclosures to report.

## ESVNU-O-9 - European Society of Veterinary Nephrology and Urology

### Occurrence of cardio-vascular events in cats with acute urinary tract obstruction

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Cardiovascular dysfunction due to acute kidney injury have been recently described in veterinary medicine, but no information is available in cats with urinary tract obstruction (UTO). This retrospective study aimed to describe type, frequency, timeline and risk factors for cardio-vascular events (CVE) in cats treated for acute UTO.

Medical records of cats admitted in the ICU for UTO between 2016 and 2021 were reviewed. Cases with either ureteral (upper - UUTO) or urethral (lower - LUTO) UTO were included. Signalment, clinicopathological and imaging findings, treatments and occurrence of CVE were recorded. CVE were defined as arrhythmia, heart murmur development, gallop sound, signs consistent with fluid overload or decreased tissue perfusion. Age, obesity, prior obstructive episode, fluid administration before referral, cardiac murmur or galop sound at admission, presence of UUTO or LUTO, plasma creatinine concentration at admission time, administration of fluid boluses, fluid therapy duration, time to obstruction relief and to azotaemia resolution were all examined as predisposing factors for CVE. Statistical analysis was carried out using Pearson's Chi squared test and exact Fisher test (significance set at $p<0.05$).

Seventy-six cats with UTO were recruited (20 UUTO, 56 LUTO). Median age was 6 years (interquartile range (IQR: 0-3.6). At admission, median serum creatinine level was 307.5 umol/L (IQR: 167-1047), 17/76 cats were non-azotemic (all with LUTO) and 22 were hyperkaliaemic. CVE were reported in 82.8% of cats, including gallop rhythm (27.6%), cardiac murmur (19.8%), arrhythmias (23%), clinical signs of decreased tissue perfusion (2.8%). Fluid overload was diagnosed in 3 cats. Median number of CVE per cat was 1 (range: 0-3); median time to the first CVE was 24 hours (IQR: 0-36), 48 (IQR: 33-72) for the second. The percentage of cats with CVE was not significantly different between UUTO vs LUTO but was higher in azotemic cats compared with non-azotemic cats ($p=0.018$). Plasma creatinine concentration at admission ($p=0.008$) and number of days of fluids therapy ($p=0.001$) were associated with occurrence of CVE. All other factors were not statistically associated with onset of CVE..

This study highlights frequent CVE in cats treated for UTO, especially in azotemic cases, suggesting that cardiovascular disorders are commonly associated with acute renal dysfunction in the feline species. Based on our findings, further prospective studies looking at cardiovascular-renal axis disorder would be useful to adapt monitoring and fluids therapy and improve outcomes in cats with UTO.

**Disclosures**

No disclosures to report.

## ESVONC-O-1 - European Society of Veterinary Oncology

### Change of feline injection site sarcoma incidence and localization within the last 30 years

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Feline injection site sarcoma (FISS) was initially associated to an increased use of adjuvanted rabies and feline leukemia virus vaccines but in the meantime also has been linked to other vaccines and drugs. The Vaccine Associated Feline Sarcoma Task Force (VAFSTF) recommended in 1996 injection locations in which tumors are easier resectable. Aim of this study was to determine FISS incidence and localization within the last 30 years with focus on changes after introduction of adjuvant-free vaccines and publication of vaccination guidelines.

Data on all cutaneous and subcutaneous masses of cats submitted for histopathology (full tumour samples and/or biopsies) to a specialized pathology laboratory between 1987 until 2019 was included.

During the study period, 140999 full tumour samples or biopsies were submitted of which 30871 (21.9%) were FISS. There was an increase in FISS incidence from 1987 to 2001 and a steady decrease thereafter. A significant decrease of FISS in the neck, shoulder, back and thorax region ($p<0.001$ for all comparisons) occurred after publication of the VAFSTF and different vaccination guidelines, as compared to a significant increase in the mammary region ($p<0.001$).

Overall, there was a clear decrease in incidence of FISS since 2001 which can be explained by an increasing awareness of potential vaccination adverse effect and recommendation of restrictive vaccination (e.g., long intervals). Incidence of FISS decreased significantly in cranial body parts which could indicate compliance to VAFSTF guidelines.

**Disclosures**

W. von Bomhard runs the Specialty Practice for Veterinary Pathology where the samples were analyzed.

## ESVONC-O-2 - European Society of Veterinary Oncology

### An update on environmental risk factors for the development of feline oral squamous cell carcinoma

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Squamous cell carcinoma is the most common oral tumor in cats, accounting for 70-80% of oral neoplasms. Despite multimodal treatment, the prognosis for this fast-growing, invasive tumor remains grave. Identification of environmental risk factors and screening surveillance of the subjects at risk might contribute to feline oral squamous cell carcinoma (FOSCC) control and prevention.

Exposure to environmental tobacco smoke (ETS), use of flea collar, a tuna-based diet and consumption of canned petfood have been linked to the development of FOSCC. It can be hypothesized that a high intake of canned petfood may lead to poorer dental hygiene, favoring tartar buildup and oral inflammation, which in turn may result in neoplastic transformation. An association between oral inflammation and cancer has been confirmed in people and was recently suggested in cats by the observation of an altered methylation profile in feline oral stomatitis, although no causative role has been demonstrated.

In order to re-evaluate the previously proposed risk factors and to investigate novel potential risk factors, we performed an observational epidemiological case-control study on cats with histologically-confirmed FOSCC. Results were compared with an age-matched random population of client-owned cats and with two groups of cats with feline chronic gingivostomatitis (FCGS) and periodontal disease (PD).

Cats were prospectively enrolled in the study upon completion of an anonymous online questionnaire by the owner, including demographic, environmental and lifestyle information.

Eighty-five FOSCC, 59 FCGS, 59 PD and 500 controls were included. On univariable logistic regression, variables significantly associated with an increased risk of FOSCC were rural environment, outdoor access, ETS, consumption of canned petfood, low-cost petfood and petfood containing any chemical additive. Variables significantly associated with an increased risk of FCGS included rural environment, outdoor access, cohabitation with other cats, use of antiparasitic products, FIV and FeLV positive status. Consumption of canned petfood was the only factor significantly associated with PD.

On multivariable analysis, covariates retaining prognostic significance were outdoor access, ETS and consumption of canned petfood for FOSCC; rural environment, cohabitation with other cats, endectocide administration and positive FIV status for FCGS.

FOSCC is likely a multifactorial disease, with several risk factors contributing to its development. Although a history of previous inflammatory oral disease was not significantly more frequent in FOSCC compared with an age-matched random feline population, the sharing of different risk factors in two groups of cats with FCGS and PD leaves the hypothesis open of a possible link between these diseases.

#### Disclosures

No disclosures to report.

## ESVONC-O-3 - European Society of Veterinary Oncology

### Possible association between anesthesia and recurrence in dogs with medium/large B-cell lymphoma in complete remission after chemo - immunotherapy

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Surgery-induced stress and anesthesia-related immunosuppression are believed to play a critical role in human oncology patients. Immune response is partially regulated by the hypothalamic-pituitary-adrenal axis and sympathetic nervous system, and, theoretically, their activation following surgery and/or anesthesia may trigger tumor growth. Murine preclinical and human clinical studies have hypothesized that anesthesia influences carcinogenesis and, consequently, patients outcome, promoting tumor recurrence and metastasis. In veterinary medicine, a possible association between anesthesia and cancer recurrence has not been documented yet. Aim of this study was to investigate whether anesthesia promotes recurrence in dogs with medium/large B-cell lymphoma that were in complete remission (CR) after treatment.

Medical records were retrospectively searched for dogs with completely staged B-cell lymphoma, including diffuse large B-cell lymphoma, late-stage marginal zone lymphoma, and grade 3 follicular lymphoma, that received the same chemo-immunotherapy protocol and were in CR based on a negative minimal residual disease assessed by flow cytometry and/or PARR. A complete follow-up was mandatory. Two groups were defined: dogs undergoing anesthesia within 180 days after diagnosis (anesthesia group, AG) and dogs not undergoing anesthesia during follow-up (control group, CG). Time to recurrence (TTR) was compared between the two groups.

Seventeen (22.4%) dogs were included in AG and 59 (77.6%) in CG. In AG, 16 dogs had anesthesia for diagnostic lymphadenectomy, and 1 for neutering. The two groups were well-balanced for known prognostic factors, including histotype, clinical stage and substage. In AG, 14 (82.4%) dogs recurred after a median of 75 days (range, 9–151)

from the date of anesthesia, while 3 (17.6%) dogs did not recur, with a duration of the first remission of 237, 301, and 2155 days, respectively. TTR of dogs in CG was significantly longer than TTR of dogs in AG (332 days, range 108–1946; and 249 days, range 169–330, respectively, p=0.039). Anesthesia significantly increased recurrence risk (HR 1.89, 95% CI 1.022–3.53, p=0.042).

Better treatment modalities have prolonged survival in veterinary oncology patients. As a result, the likelihood that these same patients will undergo anesthetic procedures for other reasons throughout their lives has also increased. In our series, more than 80% of dogs with medium/large B-cell lymphoma treated with chemo-immunotherapy, that underwent anesthesia during CR, relapsed on average within 75 days. These preliminary findings suggest that anesthesia may play a role in recurrence in dogs with medium/large B-cell lymphoma. Prospective trials are needed to confirm these findings.

**Disclosures**

No disclosures to report.

## ESVONC-O-4 - European Society of Veterinary Oncology

### BRAF mutation status and its prognostic significance in 79 canine urothelial carcinomas: A retrospective study (2006-2019)

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Urothelial Carcinoma (UC) is the most common malignant bladder tumour in dogs. Recently BRAF mutation testing, e.g. on desquamated UC cells in free-catch urine, emerged as a promising and less invasive diagnostic alternative as BRAF mutations have been detected in 43-87% of canine UC. Presence of BRAF mutation is a negative prognostic factor in some human cancers, but in dogs with UC its prognostic significance is largely unknown. This study investigated the relationship between BRAF (variant V595E) mutation status and overall survival in dogs with urinary tract UC.

Patients histologically diagnosed with UC of the bladder and/or urethra between 2006 and 2019 were included in this retrospective single-center-study. Patients with evidence of metastases, advanced disease (e.g. hydronephrosis), significant clinical illness or those lost to follow-up were excluded.

Seventy-nine patients met the inclusion-criteria: Mean age at diagnosis was 10.7 years (SD: 1.9 years). BRAF mutation was identified in 51 tumours (=64.6%). Dogs were either treated with meloxicam alone (n=39, "Melox") or meloxicam combined with chemotherapy (maximum tolerated dose (MTD) IV mitoxantrone ± followed by metronomic oral chlorambucil) (n=23, "Chemo"). Seventeen dogs had partial cystectomy followed by meloxicam ± MTD IV mitoxantrone ("Sx"). At the time of data retrieval, five dogs were still alive with a median follow-up time of 512 days (range 388-958 days). Those patients were right censored. Overall median survival time (MST) was 232 days (range: 1-1918 days).

BRAF-mutation status had no statistically significant influence on overall survival despite a numerically longer MST in BRAF-negative dogs (359 days) compared to BRAF-positive dogs (214 days; p=0.055). Survival depended significantly on treatment choice in BRAF-positive dogs (p=0.006): MSTs for "Melox", "Chemo" and "Sx" were 151 days, 244 days, and 853 days, respectively; whereas this was not demonstrable in BRAF-negative patients (p=0.138; MSTs: 179 days, 370 days and 388 days for "Melox", "Chemo" and "Sx", respectively). Use of metronomic chlorambucil therapy after MTD IV mitoxantrone more than doubled MST in BRAF-positive "Chemo"-patients compared to patients receiving mitoxantrone alone (588 days vs. 216 days; p=0.030). However, there was no statistically significant survival benefit in dogs without the BRAF mutation treated with metronomic chlorambucil following MTD mitoxantrone (p=0.519; MST for mitoxantrone alone as compared to mitoxantrone + chlorambucil: 348 days vs. 407.5 days, respectively).

Based on the results of our study, BRAF mutation status can guide treatment decisions as dogs with BRAF-positive UC, benefit from metronomic chlorambucil treatment following MTD IV mitoxantrone therapy.

**Disclosures**

Alexandra Kehl and Dr. Heike Aupperle-Lellbach are employed by the laboratory Laboklin GmbH & Co KG (Bad Kissingen) which offers BRAF-mutational testing on a fee-for-service basis. The relationship does not have any impact of the data presented. BRAF-mutation testing was kindly provided for free by Laboklin GmbH & Co KG (Bad Kissingen).

## ESVONC-O-6 - European Society of Veterinary Oncology

### Outcomes of dogs with anal sac gland carcinoma treated with surgery and adjunctive radiotherapy in Ten 3.6Gy fractions

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Canine anal sac gland carcinoma (ASGC) is commonly treated with surgery however, around 50% of patients develop locoregional progression at a median of 247 days. Adjuvant radiotherapy should provide longer disease control and has been shown to prolong remissions

in a small case series. The aim of this study was to evaluate the outcomes and toxicity of dogs with ASGC undergoing adjunctive radiation therapy with ten Monday to Friday 3.6Gy fractions (total dose 36Gy).

Clinical records were retrospectively reviewed. Fifteen dogs were included, 5 each in stage one, 2 and 3 (4 dogs 3A and one 3B). All received surgery to the primary tumour and regional lymph nodes when affected followed by radiotherapy in all cases to the primary and secondary sites. Two dogs had second tumours (a high grade scrotal mast cell tumour (MCT) excised, and an adrenal mass).

Median follow up was 581 days. Five dogs had disease progression with a median TTP of 451 days. One had regional progression at 451 days, one had regional and systemic progression at 644 days, and three had systemic progression at 143, 294 and 674 days.

Two dogs received chemotherapy prior to or during radiation (melphalan in the dog which then had regional and systemic progression, carboplatin in the dog with the adrenal mass). Three dogs received therapy with toceranib phosphate after systemic progression.

Thirteen dogs were alive at the end of the study. One dog was euthanised at 164 days for tenesmus and hind limb paresis considered due to locoregional progression of either ASAC or MCT. The other was euthanised for hypercalcemia at 807 days. The three dogs receiving toceranib remain alive, two with stable disease and one with a complete resolution of pulmonary nodules.

All dogs developed acute radiation toxicity with VRTOG grade 1-2 cutaneous toxicity. Four dogs had grade 1-2 anal mucositis, three had reported colitis or diarrhoea and three dogs had tenesmus. All acute toxicities resolved within two weeks of completion of radiotherapy, except for one dog which had ongoing, improving tenesmus. Late radiation toxicity was reported in two dogs (focal alopecia and cutaneous hyperpigmentation).

Adjunctive radiation therapy with 36Gy in 3.6Gy fractions provided good long-term locoregional control for 12/15 dogs with anal sac gland carcinoma, with minimal morbidity for patients. Larger populations and longer term follow up is required to further assess outcome and late toxicity associated with this protocol.

**Disclosures**

No disclosures to report.

**ESVONC-O-7 - European Society of Veterinary Oncology**

## Evaluation of outcome and toxicity in dogs undergoing 'quad shot' radiation therapy for anal gland adenocarcinoma: A single center retrospective study of 17 cases

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Canine anal gland adenocarcinoma (AGAC) is a tumour arising from the apocrine glands of the anal sac. Surgical excision of the primary tumour, with or without lymphadenectomy, remains the main treatment for AGAC. Palliative external beam radiotherapy (RT) has been described in the gross disease setting for non-surgical candidates. The "quad shot" protocol is a cyclical palliative regimen of three 2-day cycles (4 BID fractions) given at 3-4 week intervals.

The aim of this retrospective study was to determine the outcome and toxicity in dogs with AGAC undergoing "quad shot" radiotherapy.

Seventeen dogs with AGAC were included in this single center retrospective study. Five dogs were stage 3a, six dogs were stage 3b and five dogs were stage 4 (Polton). One dog of non-classified stage, was included due to enlarged regional lymph nodes. Fourteen dogs received 12 fractions over 3 cycles to a mean of 43.8 Gy. One dog received 11 fractions due to machine failure (3 cycles to a total of 40 Gy), and two dogs received 8 fractions (2 cycles to a total of 28 Gy).

The median overall survival time (OST) was 485 days (range 107-1575). 13/17 (76%) dogs were restaged and the median time to progression (TTP) was 163 days (range 72-563). Toxicity was assessed according to the VRTOG system. Eight dogs (47%) had acute Grade I, and one dog (5.8%) acute Grade II, cutaneous toxicity. Three dogs (17.6%) had late Grade III toxicity. Two of the grade III toxicities were anal strictures and 1 anal necrosis. For four dogs (23.5%) no side effects were noticed and one dog (5.8%) had no record of side effects.

Regarding chemotherapy, prior to RT one dog received carboplatin, epirubicin, and toceranib phosphate, one received carboplatin and one toceranib phosphate. All stopped when RT started. Two dogs received chemotherapy continuously with toceranib phosphate and two dogs stopped during the RT, but received prior and post RT. One received mitoxantrone prior and toceranib phosphate, cyclophosphamide and melphalan post and the other received cyclophosphamide prior and toceranib phosphate post. Six dogs received chemotherapy after the end of their RT with toceranib phosphate and 5 dogs received no chemotherapy.

The "quad shot" protocol is well tolerated and results in similar outcomes as other palliative protocols. Larger studies are necessary to evaluate the best palliative radiotherapy protocol for canine AGAC.

**Disclosures**

No disclosures to report.

**ESVONC-O-8 - European Society of Veterinary Oncology**

## High-grade feline gastrointestinal lymphoma in 43 cases: One treatment does not fit them all

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Feline high-grade gastrointestinal lymphoma is associated with poor long-term survival rates and a consensus on the best approach to treatment is lacking. The aim of this retrospective study was to describe the signalment, anatomical distribution, clinical signs, clinicopathological findings, treatment and outcomes in 43 cats diagnosed with high-grade gastrointestinal lymphoma presented to a single referral institution between 2017-2020. Nine cats (20.9%) had gastric lymphoma (GL) and 34 cats (79.1%) had intestinal lymphoma (IL). The most common clinical signs were weight loss, hyporexia and vomiting, occurring more frequently in cats with GL compared with IL. For cats with GL the most frequent haematological abnormalities at presentation were anaemia in 55%, monocytosis in 88% and neutrophilia in 77% of cases respectively. For cats with IL, monocytosis was present in 73.5% of cases and neutrophilia in 67.6%. The most common anatomical location for IL was the jejunum in 13/34 cases (38.2%). Six cats with GL were treated with chemotherapy using a high-dose COP protocol, with a median survival time (MST) of 280 days,(range 60-425d). Two cats received a 25-week CHOP protocol with a MST of 90 days,(range 75-105d). No cats underwent surgery for their disease. Fifteen cats with IL received a high-dose COP protocol with a MST of 60 days,(range 21-425d). Eight cats received a 25-week CHOP protocol with a MST of 71 days,(range 3-141d). Six cats with IL were treated with surgery prior to chemotherapy. Four cases subsequently received high-dose COP therapy and had a MST time of 360 days,(range 30-2007d).One case underwent a 25-week CHOP protocol and survived 912 days. This cat remains alive. Five of 9 cats (55%) with GL achieved complete remission (CR) of which one developed progressive disease after one month and four cats remain alive. Eight of 34 (23.5%) cats with IL achieved CR of which 5 subsequently developed progressive disease, 3 remain alive to this date. Six patients developed either ocular or renal progression, or both. Seventeen from 43 cases received a variety of rescue protocols when progressive disease was observed. This study describes a notable presence of monocytosis and neutrophilia in cats with GL and IL. It shows a potentially superior response to chemotherapy in GL, compared to IL, when a standardised approach to treatment was carried out. In addition, surgery with adjuvant chemotherapy for IL may result in a prolonged survival time compared to medical therapy alone.

**Disclosures**

No disclosures to report.

**ESVONC-O-9 - European Society of Veterinary Oncology**

## A phase 2, single-arm, open-label clinical trial on adjuvant active immunotherapy in dogs with appendicular osteosarcoma undergoing amputation and chemotherapy

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Osteosarcoma is biologically aggressive and the most common bone cancer in dogs. Treatment of choice involves limb amputation followed by adjuvant chemotherapy. Unfortunately, most dogs die within one year due to metastasis. Thus, new anticancer strategies with greater selectivity and efficacy against metastatic cells are warranted.

Immunotherapy, currently considered the fourth strategy in the fight against cancer, alongside surgery, radiotherapy and chemotherapy, aims to instruct or reactivate the immune system to recognize tumor cells as foreign, ultimately eliminating them. It has been shown that Salmonella infection of tumor cells promotes the release of immunogenic peptides, that induce an antitumor response in several model systems. A previous study documented that a heterologous, peptide-based anticancer vaccine expanded tumor specific CD8+ T-cells in dogs with sarcoma, without any reported toxicity. Aim of this trial was to evaluate whether a peptide-based anticancer vaccine administered to dogs with appendicular osteosarcoma undergoing limb amputation and traditional chemotherapy prolonged survival time (ST) by delaying the onset of metastasis and was well-tolerated.

Dogs without distant metastases were eligible for recruitment. Following limb amputation and dose-intense carboplatin, dogs were vaccinated on a monthly basis for 6 times and followed-up with serial thoracic radiographs. Since outcome has not dramatically changed over the last decade, dogs undergoing the gold-standard treatment (amputation and carboplatin) over the same timeframe served as controls.

Twenty-one client-owned dogs were vaccinated, and 23 served as controls. The two groups were well-balanced with regard to known prognostic variables, including anatomic site, clinical stage and serum ALP level.

Among vaccinated dogs, 10 (47.6%) completed the chemo-immunotherapeutic protocol, while 11 did not due to cancer-related death (n=8) or cancer-unrelated death (n=3). All vaccinated dogs completed chemotherapy and received at least one vaccine dose. Vaccine-related toxicity did not occur. Among unvaccinated dogs, all completed chemotherapy (4-6 cycles).

Among vaccinated and unvaccinated dogs, the median time to metastasis (TTM) was 392 (95% CI, 87-697) and 252 days (95% CI, 83,421), respectively (p=0,017).

At the time of writing, 6 (28.6%) vaccinated dogs were still alive, with a median follow-up of 465 days (range, 135-785). Median ST was 392 days (95% CI, 0-995). Among unvaccinated dogs, all had died due to osteosarcoma, with a median ST of 278 days (95% CI, 142-414). The difference was significant (p=0,031).

The peptide-based anticancer vaccine was well-tolerated, and significantly increased TTM and ST. Active immunotherapy holds promise for changing and improving osteosarcoma management in dogs.

**Disclosures**

No disclosures to report.

## ESVONC-O-10 European Society of Veterinary Oncology

### Impact of a 10% dose reduction and length of treatment delays in the management of chemotherapy-induced neutropenia in dogs: A single-centre experience

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Neutropenia is a common chemotherapy-associated adverse event (AE) in veterinary oncology. Dose reductions (DRs) and treatment delays (TDs) are frequently applied to prevent further neutropenic events (NEs) and decrease the risk of AEs in neutropenic patients, respectively. These preventive measures are a significant cause of decreased relative dose intensity in chemotherapy protocols and their implementation vary amongst clinicians. A better understanding of the effect of DRs on absolute neutrophil count (ANC) and length of TDs on subsequent AEs could help optimise dose individualisation and improve treatment outcomes.

The primary objectives of this study were to 1) determine the failure rate of a 10% DR at preventing an inadequate nadir (< 0.75 x$10^9$/L) or pre-treatment ANC ( 1.5 x$10^9$/L) in dogs undergoing chemotherapy; 2) determine the effect of the length of TDs due to pre-treatment neutropenia on the following chemotherapy AEs. A secondary objective was to predict failure of a 10% DR. We hypothesized 1) a 10% DR would fail to prevent further NEs in < 50% of cases; 2) the length of TDs would not be associated with the following chemotherapy AEs.

In this institution, a 10% DR is systematically applied to prevent any NE, regardless of the category (inadequate nadir or pretreatment ANC) and severity of neutropenia; but the length of TDs is variable. Chemotherapy protocols prescribed between January 2013 and January 2019 were retrospectively reviewed. NEs and related information were collected. The 10% DR failure rate, the association of failure with several parameters, and the association between the length of TDs and subsequent AEs were determined.

Seven hundred and seventeen chemotherapy treatments were recorded for 64 dogs that developed at least one NE. Seventy-three 10% DRs were prescribed, of which 26 and 47 were due to inadequate nadir and pre-treatment ANC, respectively. A 10% DR failed to prevent a subsequent NE in 19/73 cases (26.0%, 95% CI: 16.4-37.6%). Failure was not associated with the NE category ($P = .897$) or severity of previous neutropenia ($P = .146$), but was associated with the drug prescribed ($P < .001$). Seventy-two TDs were prescribed (median: 5 days, interquartile range [IQR]: 4-7 days). The length of TDs was not associated with subsequent neutropenia ($P = .422$) or non-haematological toxicity ($P = .415$).

A 10% DR failed to avoid a subsequent NE in a minority of cases. The length of TDs was not associated with the tolerability of the following chemotherapy treatment.

**Disclosures**

No disclosures to report.

## ESVONC-O-11 - European Society of Veterinary Oncology

### Pharmacokinetic study of doxorubicin in cancer-bearing dogs: Validation of a simple high-performance liquid chromatography (HPLC) method in 10 dogs

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The anthracycline doxorubicin is widely used for the treatment of tumors, but its pharmacokinetics (PK) remains largely unknown in unhealthy dogs. The objective of this prospective pilot study was to validate a sensitive high-performance liquid chromatography-fluorescence detection (HPLC-FLD) method for the determination of doxorubicin concentration in dog plasma and to study the pharmacokinetics of doxorubicin in a cohort of cancer-bearing dogs. A HPLC-FLD with a gradient elusion method and using canine plasma was validated according the EMA guidelines. Thereafter, dogs were included if they received an intravenous infusion of doxorubicin over 30 minutes as part of their chemotherapeutic protocol. Plasma samples were collected at 5-, 15-, 30-, 60- and 120-minutes post-infusion. Adverse events were evaluated 7 to 10 days after infusion and were graded according to the VCOG-CTCAE grading system.

Method validation revealed a wide linearity (0.01-1.5 μg/ml) together with satisfactory intra- and inter-day precision, accuracy, recovery and stability after storage and freeze-thaw cycles. Ten dogs were included for varied tumors including 4 multicentric high-grade lymphoma, 2 splenic hemangiosarcoma, and one of each appendicular osteosarcoma, oral melanoma, pleural mesothelioma and dermal sarcoma. Seven dogs received doxorubicin at 30 mg/$m^2$, and three dogs weighing less than 10 kg, at 1 mg/kg. Great interpatient variability was observed in the area-under-the-curve (mean ± SD, 159 ± 56 nM hour) and peak plasma concentration (mean ± SD, 580 ± 221 nM), with a coefficient of variation of 35% and 38%, respectively. Half-life ranged from 2.7 to 10.5 minutes with a clearance varying between 112 and 393 ml/kg/min. Two dogs exhibited neutropenia, one grade 1 and one grade 3. Two dogs had grade 2 diarrhea and lethargy. No association between PK or the absolute dose (mg) and the occurrence of adverse events was observed, though this could not be statistically confirmed due to the low number of cases. Our method is one of the

few of its kind using canine plasma as calibration and validation matrix, with similar results concerning method validation. The wide variability of PK parameters and short half-life of doxorubicin suggest a fast uptake from the plasma. A larger prospective randomised study is warranted to determine the relationships between PK and adverse events, and to identify risk factors of higher toxicity.

**Disclosures**

No disclosures to report.

## ISCAID-O-1 - International Society for Companion Animal Infectious Diseases

### Results of a study about vaccination decision of dog owners

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The high priority of dogs as important partners of many people leads to differentiated considerations about the health care of the animals also in terms of vaccinations. In our study, vaccination motives of private dog owners and dog breeders were analyzed and compared with the statements of veterinarians. It was also investigated whether there are differences in the satisfaction of dog owners with regard to veterinary vaccination advice compared to other sources of information. A total of 1,480 private dog owners, 349 dog breeders and 365 veterinarians participated in this questionnaire conducted exclusively online in 2019 using LimeSurvey software. Absolute and percentage frequencies, means and standard deviations were analysed based on the information provided by dog owners and veterinarians.

The evaluation of the stated vaccination motives showed that fears and feelings of responsibility played a greater role for private dog owners and breeders than external circumstances. From the point of view of the participating veterinarians, financial aspects and requirements by third parties such as obedience schools or boarding kennels influenced the vaccination decision more than both groups of dog owners indicated. For the majority (97%) of private dog owners, veterinarians are the most important source of information on vaccination, but more than 70% search the internet for vaccination information. Although more than 80% of private dog owners rated the relationship with their vet as good or very good, only 68 % of the private dog owners indicated the veterinary advice as the decisive source of information for their vaccination decision. In contrast to 97% of the participants' dogs that had received a primary vaccination, only 59% were subsequently boosted annually.

The comparison between statements of the participating veterinarians and statements of the dog owners shows a clear need for communication about vaccinations in veterinary surgery. Strategies already established in the human sector can be used for this purpose but should also be developed for the veterinary sector. Veterinarians should be aware of their significant influence on dog owners' vaccination decisions. The availability of reliable information about vaccination on the internet, on homepages of veterinarians and in social media needs to be reconsidered by the veterinary profession in general.

**Disclosures**

No disclosures to report.

## ISCAID-O-2 - International Society for Companion Animal Infectious Diseases

### Detection of mutated and non-mutated feline coronaviruses in cats without feline infectious peritonitis

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Two pathotypes of feline coronavirus (FCoV) exist, a harmless variant and a pathotype causing feline infectious peritonitis (FIP). Although specific mutations in the FCoV spike (S) gene have been associated with FIP, it is unclear whether these mutations can also occur in cats without FIP. This study investigated the presence of FCoV with and without S gene mutations in cats that did not have FIP.

The study included 87 cats in which FIP was definitely excluded by necropsy, histopathology, and immunohistochemical (IHC) staining of FCoV antigen. Incisional biopsies and fine-needle aspirates of mesenteric and popliteal lymph nodes, liver, spleen, lung, omentum, kidney, intestine (duodenum, jejunum, ileum, colon), and brain, EDTA blood, cerebrospinal fluid, aqueous humour, faeces, and peritoneal lavage were obtained in all cats and 2 RT-PCRs were performed in all samples, detecting all FCoV as well as targeting S gene mutations.

Of the 87 cats without FIP, 21 (24.1%) were positive for FCoV RNA. S gene mutations were found in 14 cats (16.1%) in at least one tissue or fluid sample; in 8 of those cats, a mixed population of non-mutated

and mutated FCoV, and in 6 cats, only FCoV with S gene mutations were detected.

FCoV RNA without and even with S gene mutations resulting in amino acid differences at position 1058 and 1060 of the spike protein can also be found in cats without FIP. The detection of these mutations therefore does not seem to be specific for FIP.

**Disclosures**

Dr. Christian Leutenegger was the Head of Molecular Diagnostics at IDEXX Laboratories, Inc. This laboratory offers the FCoV and FIP virus real-time RT-PCR on a commercial basis and performed the testing in this study.

## ISCAID-O-3 - International Society for Companion Animal Infectious Diseases

### Report of one year surveillance of SARS-CoV-2 detection by PCR in dogs and cats with various exposure risk

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Since its emergence, SARS-CoV-2 has spread mainly through human-to-human transmission. Despite several cases of mild infection in dogs and cats and serological surveys showing that infection rate can exceed 40% in companion animals living with owners affected by COVID-19, their role in the outbreak seems irrelevant.

In order to confirm a negligible risk of contamination for owners and veterinarians handling dogs and cats, a prospective survey was conducted to evaluate SARS-CoV-2 isolation rate by qRT-PCR from oropharyngeal swabs in dogs and cats with different exposure risk. Groupe A (A) was recruited from animals owned by people with either suspected or confirmed (PCR positive) SARS-CoV-2 infection. Dogs and cats presented to our hospital, with an unknown household exposure, were sampled randomly to form group B (B). Animals were included from April 2020 to March 2021. Semi-quantitative RT-PCR reactions performed using the Bio-T kit Tristar Covid-19 were considered positive when cycle thresholds (Ct) were below 40 and samples were positive for both genes E and Orf-1.

Three hundred twenty-six samples obtained from 298 animals (16 in A, 282 in B) were tested, positive results (Ct 35-36) were obtained in 1 cat in A and 2 cats in B; all were asymptomatic. Doubtful results (positive for only 1 gene) were also observed in 2 animals in B. The A positive cat was first sampled 8 days after its owner started to show symptoms. Oropharyngeal swabs were collected twice daily during 13 days (feces were sampled every time they were passed) and further positive results were obtained after 1 and 5 days; serology performed after 20 days was negative. Repeated testing after 13 days was available for 1 of the 2 B positive cats, PCR and serology were negative. No members of this cat's household were suspected of SARS-CoV-2 infection but the cat was adopted recently.

The study showed a low detection rate of SARS-CoV-2 in dogs and cats, suggesting that handling of these species represents a negligible risk. Even if the virus load appeared to be low in positive cases and probably no sufficient to cause pet-to-human transmission, individual protective measure should be implemented as a precaution while handling an animal coming from a currently positive household. Given the emergence of new variants with enhance infectivity and transmissibility for humans, surveillance of domestic animals should be maintained to identify any modifications.

**Disclosures**

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## ISCAID-O-4 - International Society for Companion Animal Infectious Diseases

### Comparison of eight commercially available point-of-care tests to detect canine parvovirus in faeces of dogs

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Real-time polymerase chain reaction (PCR) is considered gold standard for diagnosis of canine parvovirus (CPV) infection, but can only be

performed in specialized laboratories. Several point-of-care tests (POCT), detecting CPV antigen in faeces of dogs within minutes, are commercially available. The aim of this study was to evaluate 8 POCT in comparison with the gold standard PCR.

Faecal samples of 150 dogs from 3 groups (H: 50 client-owned, healthy dogs, not vaccinated against CPV within the last 4 weeks; S: 50 shelter dogs, healthy, not vaccinated against CPV within the last 4 weeks; P: 50 dogs with suspicion of CPV infection) were tested with 8 POCT (Snap®Parvo, Fassisi®Parvo, Primagnost®ParvoH+K, FASTest®PARVOCard, Vetexpert RapidTestCPVAg®, AnigenRapid CPVAgTestKit®,ImmunoRun®ParvovirusAntigenDetectionKit, WITNESS®Parvo) and PCR. Practicability, sensitivity, specificity, positive (PPV), and negative predictive values (NPV), and overall accuracy were determined. To assess differences between and agreement among POCT, McNemar's test and Cohen's Kappa statistic were performed.

All POCT were easy to perform; questionable results occurred in up to 6.0%. Specificity and PPV were 100.0% in all POCT. Sensitivity varied between 22.9 and 34.3%. VetexpertRapidTestCPVAg® had the highest sensitivity (34.3%) and differed significantly from the 3 POCT with the lowest sensitivities (Fassisi®Parvo (27.7%), Primagnost®ParvoH+K (24.3%), FASTest®PARVOCard (22.9%)). CPV shedding in dogs of groups H and S could not be detected by POCT, only by PCR. Agreement among all POCT was at least substantial (kappa >0.80).

A positive POCT result confirms infection with CPV in unvaccinated dogs, whereas a negative POCT result does not exclude CPV infection.

**Disclosures**

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## ISCAID-O-5 - International Society for Companion Animal Infectious Diseases

## Modified-live FCV vaccination reduces viral RNA loads, duration of RNAemia and the severity of clinical signs after heterologous FCV challenge

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Feline Calicivirus (FCV) is a common cat virus causing clinical signs as oral ulcerations, fever, reduced general condition, pneumonia, limping and systemic disease with inner organ involvement. Efficacious FCV vaccines protect from severe disease but not from infection. FCV is a highly mutagenic RNA virus and the resulting high genetic diversity of FCV poses a challenge in vaccine design. The use of predominantly one modified-live FCV strain over several decades might have driven the viral evolution towards more vaccine-resistant variants. The present study investigated the clinical signs, duration and extent of FCV shedding, RNA presence in blood (RNAemia), haematological changes and acute phase protein reaction (serum-amyloid-A) in ten specified pathogen-free (SPF) cats after FCV vaccinations and two subsequent heterologous FCV experimental infections. Five cats were vaccinated with a commercially available subcutaneous modified-live single strain FCV vaccination containing FCV F9 and five cats received a placebo injection at 15, 18 and 94 weeks of age. All cats were oronasally infected with recently isolated field strains at 46 and 99 weeks of age. Clinical scores were assessed with a clinical score sheet and oropharyngeal cytobrushes and blood were collected to investigate FCV shedding, RNAemia and serum-amyloid-A. After vaccinations, neither clinical signs, FCV shedding from the oropharyngeal region, nor FCV RNAemia were detected. After the first experimental infection, vaccinated cats showed significantly lower clinical scores (total scores $median_{Vacc} = 20$ and $median_{Control} = 78$; maximum scores $median_{Vacc} = 3$ and $median_{Control} = 9$), less increased body temperature and lower serum-amyloid-A levels than control cats. The viral RNA loads from the oropharynx and duration and amount of RNAemia were significantly lower in the vaccinated animals. All cats showed a transient increase in leukocytes and 5/10 cats exceeded the upper reference range shortly after the first experimental infection. A transient decrease below the reference range in lymphocytes was observed in 6/10 cats. No clinical signs and no RNAemia were observed in any of the cats after the second experimental infection. A transient significant increase above the reference range in serum-amyloid A was observed in 9/10 cats after the second experimental infection whereas all haematological parameters stayed within reference ranges. In conclusion, the currently available FCV F9 vaccination is beneficial in protecting SPF cats from severe clinical signs, reducing viral loads and inflammation after challenge with recent FCV field strains. The first experimental infection induced a cross-immunity towards the second experimental infection in all cats.

**Disclosures**

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## ISCAID-O-6 - International Society for Companion Animal Infectious Diseases

### High prevalence of antibodies against feline morbillivirus type 1 and 2 and association with FLUTD and increased blood creatinine concentrations in domestic cats

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Feline morbilliviruses (FeMV) are fairly newly discovered paramyxoviruses found in cats. The first description indicated an association with chronic kidney disease (CKD) in this host species. In various studies, a global prevalence and a different genotype, designated FeMV-2, and the involvement of other organ systems in infected individuals were shown. In this study, surplus serum samples collected between 2013 and 2017 from feline patients during routine clinical diagnostic investigations and/or follow-up evaluations during treatment, all unrelated to this study, were used to determine the seroprevalence against feline morbilliviruses. This data set was also used to investigate a possible association between the prevalence of FeMV-antibodies and disease parameters, including clinical and biochemical data, in these cats. Using an immunofluorescence assay (IFA), we detected an overall seroprevalence of FeMV in almost half of the cats investigated (n=380), with a significantly increased proportion in younger animals. In comparison to European Shorthair cats, the rate of seropositivity was higher in pedigree cats. Regardless of the breed, FeMV infection was associated with increased blood creatinine concentrations, suggesting an association with CKD. Further analysis indicated that this association was the strongest in animals having high IFA titers against FeMV-2. In addition, a significant association between FeMV-positive status and the diagnosis of feline lower urinary tract disease (FLUTD) was elucidated. This association was dominated by cats having antibodies against FeMV-1 only. To further evaluate the positive correlation between FeMV seroprevalence and CKD as well as FLUTD, consideration of additional clinical characteristics and laboratory parameters is warranted, and controlled infection studies with both FeMV genotypes are necessary. The high seroprevalence against feline morbillivirus genotypes 1 and 2 in this study correspond to previously published results from other parts of the world. Clinicians should be aware of a possible link between renal and/or lower urinary tract disease and FeMV infections in cats.

#### Disclosures

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## ISCAID-O-7 - International Society for Companion Animal Infectious Diseases

### Emerging canine leptospirosis in NSW, Australia

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Leptospirosis is an important global zoonotic and neglected disease. Canine leptospirosis has not been reported in the Sydney dog population since 1976. Therefore, dogs are not routinely vaccinated against leptospirosis. However, in 2017 cases with an unusually high fatality rate began to emerge in urban Sydney dogs.

Aims of the study were to determine the clinicopathologic and serological characteristics of the recent outbreak of canine leptospirosis in Sydney, investigate the geospatial distribution of cases, facilitate an epidemiological investigation of case risk factors and create evidence-based guidelines to promote awareness of canine leptospirosis in Sydney.

Medical records of confirmed cases presenting between December 2017 and June 2019 were retrospectively reviewed. From July 2019, cases were enrolled prospectively. Cases were identified following referral or direct contact from referring veterinarians after a leptospirosis alert was issued across Sydney. Dogs were included if clinical and clinicopathological findings confirmed leptospirosis.

Between 2017 and 2021, leptospirosis has been confirmed in 19 urban Sydney dogs. Dogs infected between 2017 and 2019 all lived within a 3km radius in the Inner City of Sydney. In 2020 and 2021 the radius of identified cases extended up to 280km from Sydney. Six dogs were known to hunt rodents and one was used on a farm to herd sheep, but otherwise no risk factors were identified. Diagnosis was confirmed by positive PCR on whole blood (n=1), kidney (n=1), urine (n=6), whole blood and urine (n=11) or by seroconversion (n=3). Microagglutination antibody titres (MAT) to *Leptospira* serovars were measured in 15 dogs: seven were positive for serovar Copenhageni, one was positive for serovar Hardjo, one was positive for serovar Australis; five were negative for all serovars, likely due to insufficient time for seroconversion; and one had a low positive titre (1:50) for serovars Australis and Robinsoni. The disease was characterized by severe hepatorenal involvement resulting in an unusually high case fatality rate (90%).

This sudden emergence of a highly fatal disease in pet dogs in Sydney has led to the introduction of *Leptospira* vaccination protocols for dogs at risk (location, rat contact) using a monovalent vaccine containing serovar Copenhageni. The success of this vaccination program will require ongoing research to determine the epidemiology of leptospirosis in this region. Initial observations indicate that although most cases are due to serovar Copenhageni, the apparent emergence of new serovars in dogs in Sydney highlights the potential need for multivalent vaccines.

**Disclosures**

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## ISCAID-O-8 - International Society for Companion Animal Infectious Diseases

### Experimental infection by leptospira Australis in cats

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In cats, *Leptospira spp* infection rarely causes disease, and therefore is not always well diagnosed and prevalence is most probably underestimated. Moreover, the number of experimental infections using *Leptospira spp* reported in cats are limited. To better understand the potential role of cats in the prevalence of this worldwide bacterial zoonosis and investigate the link between *Leptospira spp* infection and Chronic Kidney Disease (CKD) in cats, we performed the exploration of leptospirosis pathogenesis in an experimental infection.

Fifteen cats aged from 18 to 28 weeks were infected by intraperitoneal route with 5 ml of an inoculum of *Leptospira* Australis (titre: $2.6.10^8$ *Leptopirae*/ml). After infection, cats were monitored for clinical signs, *Leptospira* detection in blood and urine (by qPCR), blood cell counts, biochemical markers of renal and liver failure in blood (PAL, ALAT, ASAT, urea, creatinine, SDMA) and in urine (urine creatinine/protein ratio (UCPR), albuminuria, N-Acetyl-Glucosaminidase (NAG)). On different timepoints post infection (22 days, 54 days or 84 days), kidneys were sampled to determine the quantity of bacteria (qPCR), and kidneys, spleen, liver and lungs were submitted to histopathological analysis.

After infection, no clinical sign was observed in the cats within the monitoring period. Renal failure markers increased in urine (Proteinuria, RCPU, NAG and microalbuminuria) in 5 cats out of 15 within 3 weeks following infection. A transient leptospiraemia was observed in 2 cats with detectable bacteria in the blood 4 days after infection. From Day4 post infection, *Leptospirae* were detected in urine from all cats, at least once. Eighty percent of infected cats showed persistent shedding of bacteria in urine with bacteria still detected in the kidneys at the end of the study (up to 84 days post infection). Moreover, the mean quantity of bacteria increased between Day 22 and Day 84 in kidney samples.

The high percentage of infected cats showing persistent shedding of *Leptospira* in urine combined to the absence of clinical sign leads to the question of the potential role of cats as reservoir of *Leptospira*.

**Disclosures**

No disclosures to report.

## ISCAID-O-9 - International Society for Companion Animal Infectious Diseases

### Exploration of the role of *Leptospira* spp. in cats with chronic kidney disease (CKD)

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Chronic kidney disease (CKD) is a commonly diagnosed disease of older cats. Despite its high prevalence, the underlying aetiology is to-date unknown. Infection with *Leptospira* spp. has been suggested to act as a potential mechanism in the development of CKD. Clinical leptospirosis in cats is rarely reported and cats have historically been believed to be resistant to leptospiral infections. However, since cats are frequently exposed to rodents, an important leptospirosis reservoir, it has been postulated that prior infection with leptospirosis can precede development of CKD later in life.

This study aimed to explore leptospirosis as a potential aetiology of CKD through evaluation of reactivity of CKD and non-CKD cat sera to two leptospiral outer membrane proteins, LipL32 and LipL41. Both proteins are expressed in pathogenic leptospires and LipL41 is reportedly highly upregulated in infection. Serum reactivity towards LipL32 indicates exposure to leptospira organisms whereas reactivity towards LipL41 indicates leptospiral infection and renal colonisation. Serum from cats diagnosed with chronic kidney disease (n=38) was made available from the archives of a feline research group, with ethical approval and owner consent. Residual serum from non-azotaemic cats (n=20) presenting to a referral hospital for conditions unrelated to CKD were used as controls. Serum reactivity towards LipL32 and 41 was evaluated through a serological assay: the luciferase immunoprecipitation systems (LIPS) assay. Two constructs for each recombinant protein were made, C and N terminal versions, to allow for variation in epitope recognition. Differences between study groups' reactivity towards recombinant antigens were explored through Mann-Whitney U tests after a normality assessment with the Kolmogorov Smirnov test.

There was no significant difference in reactivity towards either recombinant antigen between CKD and non-CKD cats ($p=0.52$ and 0.73). Cat serum reactivity towards the LipL32 antigen was significantly higher than towards the LipL41 antigen ($p=<0.0001$).

Although only a small-scale study, there was evidence of exposure and a subsequent serological response to leptospires in cats. The study suggests that cats with CKD do not have evidence of increased leptospiral exposure or infection with leptospirosis. Therefore, the causative agent of feline CKD remains unknown.

**Disclosures**

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## ISCAID-O-10 - International Society for Companion Animal Infectious Diseases

### Epidemiological, clinical and biological impact of hemoplasmas in cancer-bearing dogs: A case-control study on 324 cases

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Dogs infected with hemoplasmas generally show absent to mild clinical disease, unless they are co-infected with another vector-borne pathogen, or if they present predisposing factors including immunodepression, splenectomy or a neoplasia. Severe cases can present with fever and fatal immune-mediated hemolytic anemia, especially if the infection is due to *Mycoplasma haemocanis*. Recent data suggested a high frequency of PCR-positivity for *Mycoplasma* in anemic cancer-bearing dogs, which all harbored the same species: *Mycoplasma haemocanis*. This observation raises questions about the relationship between cancer and hemoplasma infection in dogs.

The objectives were to report the frequencies of canine hemoplasma infection, detail the risk factors and survival in a global cohort of sick dogs, then to compare 2 subgroups: cancer-bearing and non-cancer-bearing dogs.

Three hundred and twenty-four client-owned dogs were included between January 1st 2016 and December 31st 2017, in a mixed (retrospective and prospective) case-control study. Dogs presented to our Veterinary Teaching Hospital were included if surplus-discarded EDTA-anticoagulated blood (sampled for diagnostic purpose) and medical data were available for screening and follow-up. The respective frequencies and their 95% Confidence Intervals (CI) were estimated using binomial test. Univariate (Fisher's and Mann-Whitney Wilcoxon tests) then multivariate (generalized linear model with subsequent estimation of Akaike Information Criterion: AIC) analyses were performed to identify risk factors for canine hemoplasma infection.

There were 127/324 (39.20%) cancer-bearing and 197/324 (60.80%) non-cancer-bearing dogs. Canine hemoplasma infection was found in 29/324 cases (8.95%), including 21/29 (72.4%) cancer-bearing and 8/29 (27.6%) non-cancer-bearing dogs. Cancer-bearing dogs were significantly older than non-cancer-bearing dogs ($p < 0.005$) but did not differ with regard to other variables. Cancer-bearing dogs had a 4.66-increased odd of being infected compared with non-cancer-bearing dogs (95% CI, [1.90–12.6], $p < 0.005$). Dogs receiving chemotherapy had a 6.71-increased odd of being infected compared with the dogs naïve of chemotherapy (95% CI, [1.90–12.6], $p < 0.005$). Upon multivariate analysis, chemotherapy alone remained the most influential variable to explain canine hemoplasma infection (AIC = 173.61, OR: 7.70, 95% CI, [3.47–18.09]). Median survival times did not differ between cancer-bearing dogs with (104 days) and without (144 days) hemoplasma infection ($p = 0.26$).

Canine hemoplasma infection was not infrequent in this cohort of sick dogs. Cancer, but mainly chemotherapy both appear to be important risk factors for the infection. This should prompt oncologists to look for the infection during the follow-up of cancer-bearing dogs, particularly in case of refractory and unexplained cytopenias.

#### Disclosures

No disclosures to report.

## ISCAID-O-11 - International Society for Companion Animal Infectious Diseases

### Dissemination of *bla*$_{OXA-48}$ carbapenemase- and extended-spectrum beta-lactamase-producing Enterobacteriaceae in a Swiss companion animal clinic

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Human health-care institutions play an important role in the spread of antimicrobial-resistant microorganisms (ARM). The role of companion animal clinics in ARM dissemination is less clear. As part of a study to assess infection prevention and control (IPC) standards in companion animal clinics in Switzerland, extensive contamination with *bla*$_{OXA-48}$ carbapenemase- and extended-spectrum beta-lactamase-producing Enterobacteriaceae (CPE, ESBL-E) was identified and characterized. A total of 200 swabs from high-touch surfaces and 20 hand swabs from veterinary employees were collected over four days in a medium-sized veterinary clinic at the end of 2020 and analyzed for ESBL-E, CPE, vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococci (MRS). A total of 19 (9.5%) environmental specimen tested positive for ESBL-E, 23 (11.5%) for CPE, and 7 (3.5%) for MRS. MRS were isolated from two (10%) hand swab specimen. ESBL-E and CPE comprised *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Citrobacter braakii* and *Serratia marcescens*. All CPE genes belonged to *bla*$_{OXA-48}$, the MRS strains harbored the *mec*A gene. The strains were isolated from all areas within the clinic, including those where no animals are permitted, such as the staff kitchen, toilet, and laboratory. An audit revealed major IPC deficits regarding surface disinfection and hand hygiene infrastructure. Our results indicate that CPE dissemination in large companion animal clinics pose a worrisome threat to public health as this is the second report of extensive CPE contamination in such a facility in Switzerland. The study highlights the need to develop and implement evidence-based IPC concepts in veterinary clinics to prevent the spread of ARM into the community.

**Disclosures**

No disclosures to report.

## ISCAID-O-12 - International Society for Companion Animal Infectious Diseases

### Methylprednisolone induces neutrophil extracellular trap formation and enhances bactericidal effect of canine neutrophils

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Neutrophils can mediate antimicrobial defense not only by phagocytosis but also by neutrophil extracellular trap (NET) formation. NETs are formed by a DNA backbone, containing antimicrobial components and can entrap, disarm and kill pathogens. The formation of NETs is described in many species including dogs and can be induced by different stimuli. In this study the influence of methylprednisolone on canine NET formation and neutrophil killing efficiency of gram-positive and gram-negative bacteria was analyzed.

In total seven adult client-owned dogs (female and male) with a mean age of 3.9 years were included in this study after an unremarkable physical examination and blood count analysis. Neutrophils were isolated from EDTA blood by density gradient with Pancoll and Histopaque and incubated with *Staphylococcus pseudintermedius*, *Streptococcus canis* or *Escherichia coli*. Furthermore, methylprednisolone in three different concentrations (625 μg/mL;62.5 μg/mL;12.5 μg/mL) was added and co-incubated with neutrophils and bacteria for one and three hours, respectively. Serial dilutions were plated on blood agar plates and the colony-forming units were determined by counting on the next day to calculate the survival factor of bacteria. Additionally, free DNA as an indicator for NET release in the supernatant of all obtained samples were quantified by Pico Green assay. NET induction by methylprednisolone on canine neutrophils was analyzed in absence of bacteria by confocal immunofluorescence microscopy and determination of free DNA by Pico Green.

The gram-positive strains *Staphylococcus pseudintermedius* and *Streptococcus canis* showed in presence of methylprednisolone stimulated neutrophils at concentrations of 62.5 and 625 μg/mL time dependent significant lower survival factors. No significant influence was detected for the gram-negative *Escherichia coli*. Significantly higher amounts of free DNA were detected under methylprednisolone stimulation (62.5 and 625 μg/mL) in presence of *Streptococcus canis* and *Escherichia coli* compared to neutrophils without methylprednisolone stimulation. No significant changes were detected for *Staphylococcus pseudintermedius*. Immunofluorescence microscopy and detection of free DNA after neutrophil stimulation with methylprednisolone in absence of bacteria revealed a significant higher number of NET-releasing cells, and higher amount of free DNA at a concentration of 62.5 μg/mL.

Our findings suggest that methylprednisolone serves as a potential NET inducer and enhances time and concentration dependent the neutrophil killing of gram-positive bacteria by NET formation.

**Disclosures**

No disclosures to report.

## ISCAID-O-13 - International Society for Companion Animal Infectious Diseases

### Evaluation of serum 25-hydroxyvitamin D and C-reactive protein as biomarkers in dogs with coccidioidomycosis

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Coccidioidomycosis in dogs is a difficult fungal infection to diagnose and determine extent of disease (i.e. pulmonary versus disseminated). Serum vitamin D and c-reactive protein (CRP) have established roles in veterinary medicine as biomarkers for disease severity and prognosis but their role in coccidioidomycosis has not been investigated. Therefore, we aimed to determine if serum 25-hydroxyvitamin (OH)D and CRP concentrations were associated with canine coccidioidomycosis, extent of disease, and serologic test results at diagnosis. Dogs were included in this prospective study if they demonstrated compatible clinical signs and had ≥ 1 of positive AGID IgM or IgG, culture positivity, or if organisms were identified on cytological/histopathological examination. Dogs with coccidioidomycosis were categorized as pulmonary (PC): respiratory signs and thoracic imaging abnormalities; disseminated (DC): suspected/confirmed extrapulmonary disease; or uncharacterized (UC): insufficient evidence to determine PC or DC. Healthy nonimmune control dogs were also included. Serum 25(OH)D concentrations and CRP concentrations were measured with modified-HPLC and a commercial ELISA kit, respectively. Mann-Whitney Rank Sum tests were performed for group comparisons. Association analyses were performed using linear and logistic regression tests. $P < 0.05$ was significant. Sixty dogs were enrolled with a distribution of 35 with coccidioidomycosis (PC, $n = 11$; DC, $n = 15$, UC, $n = 9$) and 25 controls. Coccidioidomycosis dogs had a median

IgG titer of 1:16 (range, 1:2 to 1:128). Serum 25(OH)D concentrations did not differ between coccidioidomycosis or control dogs, nor was there a difference between PC and DC dogs. As expected, CRP concentration was higher in dogs with coccidioidomycosis compared to controls ($P < 0.001$), but no difference was found between PC and DC dogs. Serum 25(OH)D and CRP concentrations were not associated. AGID IgM positivity was not associated with serum 25(OH)D or CRP concentrations. Dogs with IgG ≥ 1:32 had lower serum 25(OH)D concentrations than dogs with IgG < 1:32 ($P = 0.03$). Serum CRP concentrations were greater in dogs with IgG ≥ 1:16 than dogs with IgG < 1:16 ($P = 0.02$). These data indicate a potential link between serum 25(OH)D concentrations, CRP concentrations, and disease severity in dogs with coccidioidomycosis. Future studies with larger populations are warranted.

**Disclosures**

No disclosures to report.

## ISCAID-O-14 - International Society for Companion Animal Infectious Diseases

### Expanded geographic occurrence of *Cytauxzoon* sp. infection in domestic cats in Switzerland and detection of the infection in felid samples collected two decades ago

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*Cytauxzoon* sp. infection is believed to be a newly emerging tick-borne disease in domestic and wild felids in Europe. In Switzerland, only rare infections have been documented in domestic cats in the west and northwest, the first of which was in 2014. The aims of the present study were to (A) describe new *Cytauxzoon* sp. cases, (B) elucidate the geographic distribution of *Cytauxzoon* sp. in Switzerland by a countrywide survey, (C) assess suspected high-risk populations, such as stray cats in a hotspot region and anaemic cats, and (D) investigate the newly emerging nature of the infection by analysing felid samples collected more than two decades ago.

The study included samples from A) 13 cats from two *Cytauxzoon* sp. affected households from central Switzerland, B) 881 cats from all regions of Switzerland, C) 91 stray cats from the northwest of Switzerland and 501 anaemic cats from across Switzerland, and D) 65 Swiss domestic cats sampled in 2003 and 34 European wildcats from neighbouring eastern France sampled in 1995–1996. The samples were analysed for *Cytauxzoon* sp. using real-time TaqMan qPCR, positive results were confirmed by sequencing. All samples used in this study were leftover material from samples submitted for routine diagnostic purposes.

A) 6/13 cats from two neighbouring households in central Switzerland tested *Cytauxzoon* sp. positive; two infected cats died from bacterial infections. B–C) Only 1/881 cats (0.1%, 95% CI 0–0.3%) in the countrywide survey and 1/501 anaemic cats (0.2%, 95% CI 0–0.6%), but 8/91 stray cats in the hotspot region (8.8%, 95% CI 3.0–14.6%) tested positive for *Cytauxzoon* sp. D) *Cytauxzoon* sp. was detected in 1/65 domestic cat samples from 2003 (1.5%, 95% CI 0–4.5%) and in 10/34 European wildcat samples from 1995–1996 (29%, 95% CI 14.2–44.7%). The isolates showed 99.3–100% sequence identity in the 18S rRNA gene. Phylogenetic analyses revealed that the isolates clustered together with other European *Cytauxzoon* sp. isolates from domestic and wild felids and with *Cytauxzoon manul* from a Pallas' cat.

The study adds to the knowledge of *Cytauxzoon* sp. infections in domestic cats, expands the geographic range of *Cytauxzoon* sp. in Switzerland and challenges its newly emerging nature in Central Europe.

**Disclosures**

No disclosures to report.

## ISCAID-O-15 - International Society for Companion Animal Infectious Diseases

### Prevalence of Taeniidae eggs and *Echinococcus multilocularis* in faecal samples of dogs in Europe

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*Echinococcus (E.) multilocularis* is a member of the family Taeniidae and a parasite of special zoonotic importance. While an infection of the definitive host is usually asymptomatic, in intermediate and aberrant hosts a life-threatening disease, called alveolar echinococcosis, occurs. Foxes play a key role as definitive hosts. But also dogs, especially those who hunt and eat small mammals (intermediate hosts), can serve as definitive hosts. *E. multilocularis* eggs cannot be differentiated from eggs of other Taeniidae (e.g. *Taenia spp.*) by light microscopy but via PCR tests. The aim of the study was to assess the prevalence of

Taeniidae infections in dogs in Europe with a special focus on *E. multilocularis*.

Between January 2020 and February 2021 60615 canine faecal samples were submitted to a commercial laboratory for routine coproscopic testing (flotation and sedimentation technique). Most of the samples originated from Germany (n=52478). Other European countries were represented as follows: Austria (n=3625), Bulgaria (n=54), Czechia (n=226), Denmark (n=637), Estonia (n=260), Finland (n=79), France (n=78), Great Britain (n=31), Greece (n=2), Hungary (n=158), Italy (n=218), Latvia (n=2), Lithuania (n=21), Luxembourg (n=323), Netherlands (n= 282), Norway (n=481), Poland (n=56), Romania (n=33), Slovakia (n=204), Slovenia (n=77), Spain (n=256), Sweden (n=294), Switzerland (n=740). Some of the samples which contained Taeniidae eggs where further analyzed via TaqMan realtime PCR for the presence of *E. multilocularis*.

In 96 samples Taeniidae eggs were identified by light microscopy. This reveals a prevalence of 0,16%. 35 of these Taeniidae positive samples were available as surplus material for further PCR testing: In 62,9% (n=22) *E. multilocularis*-DNA was detected in the faeces via PCR. The *E. multilocularis*-positive samples came from Southern Germany (n=18), Austria (n=3) and Switzerland (n=1), which are known as endemic areas.

Even if the total prevalence of Taeniidae infections in dogs in Europe is low, the high proportion of *E. multilocularis* infections is of clinical relevance. Every Taeniidae positive dog should be considered as potentially infected with *E. multilocularis* until proven otherwise. These dogs pose a zoonotic risk to owners as well as to veterinary staff who are or were in contact with the dog. Treatment has to be carried out in compliance with strict hygiene measures according to the ESCCAP guidelines.

**Disclosures**

MG, AH and EM are employed by the laboratory offering the diagnostic tests mentioned.

## SCH-O-1 - Society of Comparative Hepatology

### Prevalence of portal vein thrombosis in 153 dogs with chronic hepatitis: 2009-2019

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Alterations in hemostasis have been described in dogs and humans with chronic hepatitis (CH). Portal vein thrombosis (PVT) is a recognized complication of CH in humans. The prevalence of PVT in dogs with CH has not previously been reported. The aim of this study was to estimate the prevalence of PVT in dogs with CH and to assess the factors associated with PVT in these patients.

Medical records of dogs admitted to a veterinary teaching hospital between 2009 and 2019 were reviewed. Cases were included if CH was histopathologically confirmed by liver biopsy or necropsy. The presence of PVT was determined with abdominal ultrasound or computed tomography angiography. Clinical and laboratory data (i.e., hematology, biochemistry, and coagulation panels) were recorded. Categorical variables were compared between dogs with and without PVT using Pearson's Chi square tests. Continuous data were compared between dogs with and without PVT using Mann-Whitney U-test or t-tests as appropriate.

Records from one-hundred-fifty-three dogs with CH were identified. Four of these dogs (2.6%; 95% CI: 1.0 – 6.5%) were diagnosed with PVT. All four dogs with PVT were females. Additionally, five cases with CH showed thrombosis in other locations, one of each in the splenic vein, caudal vena cava, pulmonary vessel, intra-abdominal vessels, and combined aorta and splenic vein. In total 9 dogs with CH (5.9%; 95% CI: 3.1 – 10.8%) had some form of thrombosis. Mean ± SD age at the time of PVT diagnosis was 9.0 years ± 4.2. Two of four dogs with PVT were euthanized concurrent to PVT diagnosis due to end stage liver failure (peritoneal effusion, portal hypertension), one was lost to follow-up, and one euthanized due to pheochromocytoma 4 years later. Serum GGT activity and total bilirubin concentration were both significantly higher in dogs with PVT ($p = 0.0225$ and $p = 0.0076$, respectively) than in those without PVT. Serum phosphate was significantly lower in dogs with PVT ($p = 0.0303$) than in those without PVT. Other clinical and laboratory variables were not significantly different between dogs with and without PVT.

The prevalence of PVT in dogs with histologically confirmed CH was 2.6%. Also, 5.9% of dogs with CH had evidence of some form of thrombosis. PVT and thrombosis of other parts of the circulation are potentially serious complications of CH in dogs. Prospective studies in dogs with CH are needed to determine the associations between PVT and clinical and laboratory variables.

**Disclosures**

At the time of the study, all authors were/are affiliated with the Gastrointestinal Laboratory, which offers laboratory testing, including histopathological evaluation of hepatic biopsies on a fee-for-service basis.

## SCH-O-2 - SCH - Society of Comparative Hepatology

### Immunohistochemical expression of caspase-3 and malondialdehyde in archived liver specimens from dogs with chronic hepatitis

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Chronic hepatitis (CH) in dogs is histologically characterized by hepatocellular apoptosis or necrosis, a variable mononuclear or mixed inflammatory infiltrate, regeneration and fibrosis. Various hepatic diseases in dogs, including chronic hepatitis, are associated with decreased hepatic glutathione concentrations and oxidative stress. Caspase-3 (Casp3) is a key enzyme in apoptosis. Malondialdehyde (MDA) is a product of lipid peroxidation. Our study aimed to assess the immunohistochemical expression of Casp3 and MDA in liver tissue from dogs with CH.

Thirty-five archived surplus formalin-fixed paraffin-embedded liver specimens from dogs were used. The specimens were classified into 3 groups based on the histopathological reports, and clinical data: a control group with no significant hepatic changes (n = 10), copper-associated chronic hepatitis (n = 20), and idiopathic chronic hepatitis (n = 5). A qualitative copper score was assigned based on rhodanine-stained sections from each case. For 29 dogs, hepatic copper concentrations were quantified using flame atomic absorption spectroscopy. Polyclonal rabbit anti-Casp3 and anti-MDA antibodies were used for immunohistochemical staining. Hepatocytes staining for Casp3 were counted in 10 random fields (at 20x). MDA immunoreactivity for each section was scored from 0 (absent) to 4 (extensive). Counts of Casp3 positive hepatocytes and MDA scores were compared between groups using Kruskal-Wallis tests. Correlations between Casp3 and MDA immunoreactivity and copper scores and concentrations were assessed using Spearman's rank correlation. Statistical significance was set as $p < 0.05$.

There was no significant difference in the number of positive Casp3 hepatocytes between groups ($p = 0.1015$). However, a positive correlation between Casp3 immunoreactivity and copper score as well as copper concentration was found ($rs = 0.44$, $p = 0.0077$ and $rs = 0.52$, $p = 0.0037$, respectively). Dogs without significant hepatic histological changes had lower MDA scores than dogs with copper-associated or idiopathic CH ($p = 0.0003$ and $p = 0.0018$, respectively). MDA immunoreactivity was positively correlated with copper score as well as copper concentration ($rs = 0.53$, $p = 0.0011$ and $rs = 0.68$, $p < 0.0001$, respectively).

Both Casp3 and MDA are expressed in dog liver. Our results support the utility of these immunohistochemical markers in liver tissue from dogs for the assessment of hepatic apoptosis and oxidative stress, respectively.

**Disclosures**

No disclosures to report.

## SCH-O-3 - Society of Comparative Hepatology

## Investigation of risk factors for gallbladder mucocoele development in Border Terriers; a UK-based, online owner survey

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A predisposition to gallbladder mucocoele (GBM) has previously been documented in UK Border Terriers (BTs). This retrospective survey of UK BT owners evaluated recognised risk factors for GBM development. Questions investigated the influence of previously described co-morbidities including endocrinopathies, the use of imidacloprid (and other ectoparasiticides), dietary factors and breed-specific conditions including paroxysmal gluten sensitive dyskinesia (PGSD) and gluten intolerance.

A questionnaire created using an online survey platform (SurveyMonkey®) was distributed via breed societies, veterinary associations, and social media groups. Twenty-seven questions were designed to gather information including signalment, whether the BT had suffered a GBM and the presence of relevant comorbidities.

BTs were grouped according to the presence or absence of a GBM (as reported by the owner). Only BTs >10 years of age were included in the non-GBM group. The prevalence of hyperadrenocorticism, hypothyroidism, diabetes mellitus, PGSD and exposure to imidacloprid or a gluten-free diet, were compared using a Fisher's exact test. Significance was set at $p<0.05$.

Between October 2018 and October 2019, 1887 people accessed the survey; 615 questionnaires were completed and considered for evaluation.; 269 dogs met the inclusion criteria (completeness of the survey, age category), 141 BTs with GBM and 128 BTs >10 years without GBM.

Of the 141 BTs with GBM, there were 11 entire and 68 neutered males and 5 entire and 57 neutered females. The median age at diagnosis was 10 years (interquartile range 8-12). 72/141 (51.0%) BTs had undergone cholecystectomy, 47 (33.3%) were treated medically, 9 (6.3%) received no treatment, 5 (3.5%) were euthanised at the time of diagnosis and no details were provided for 8 BTs. Sadly, 39/141 (27.6%) BTs were reported to have died due to GBM.

14/141 GBM BTs were diagnosed with hyperadrenocorticism which was significantly greater than 3/128 non GBM BTs (p=0.01). In contrast to previous reports in Shetland Sheepdogs, exposure to imidacloprid was significantly (p=0.003) higher in the non GBM BTs (52%) than the GBM BTs (34%). The prevalence of hypothyroidism (p=0.232), PGSD (p=0.7353), diabetes mellitus (p=0.2168) or a gluten free diet (p=0.3266) were not significantly different between the groups.

This study supports an association between hyperadrenocorticism and GBM in BTs. However, in this population of BT an association was not detected between GBM development and feeding a gluten free diet, hypothyroidism, imidacloprid or diabetes mellitus.

**Disclosures**

No disclosures to report.

## SCH-O-4 - Society of Comparative Hepatology

### Clinical findings and prognostic variables in dogs from Asia with gallbladder mucocele

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Gallbladder mucocele (GBM) is a common biliary disorder in dogs that can be readily diagnosed and categorized into one of six different types based on ultrasonographic appearance. There is scant literature describing clinical associations of this ultrasonographic classification scheme. This study had two objectives 1) determine if GBM type was associated with several relevant clinical variables and 2) to investigate prognostic variables in dogs with GBM that have a cholecystectomy.

A retrospective, multi-center, case series was performed. Dogs with a GBM diagnosis made either via ultrasonogram or gross/histopathological evaluation between 2014-2019 were eligible for inclusion. Static images from ultrasonograms were reviewed by a single radiologist and a GBM type (i.e., I-VI) was recorded. Multivariable logistic regression clustered on institution was performed for each type of GBM to determine association with clinical factors and validated using link test and Hosmer–Lemeshow goodness-of-fit. Multilevel mixed-effects logistic regression clustered on institution was performed to determine predictors of mortality following cholecystectomy, competing models evaluated using AIC/BIC criterion, and the best model validated using likelihood ratio and Wald tests.

Two hundred and sixteen dogs from six veterinary hospitals in Japan, South Korea, Taiwan, and Hong Kong were included in this study. One hundred and eighty-five (86%) dogs had sufficient images to designate a GBM type of which type II (55/185, 30%), type IV (44, 23.7%), and type I (32, 17.3%) were most common. Dogs with GBM type I had lower likelihood for gallbladder rupture (OR, 0.9; 95% CI, 0.03-0.28) than other types. Gallbladder rupture (OR, 2.65; 95% CI, 1.98-3.54) and clinical signs (OR, 3.32; 95% CI, 1.54-6.75) were more likely in dogs with GBM type V than other type.

Cholecystectomy was performed in 39% (85/216) of dogs with 84% (71/85) and 16% (14/85) of them being clinical or subclinical, respectively. Of the dogs undergoing cholecystectomy, 11% (9/85) of dogs did not survive to discharge. In a multivariable model, predictors of mortality following cholecystectomy included age (OR, 2.74; 95% CI, 1.34-5.61), gallbladder rupture (OR, 21.70; 95% CI, 1.07-441.94), and lowest intraoperative systolic blood pressure (OR, 0.91; 95% CI, 0.82-0.99).

These data suggest that the ultrasonographic classification scheme could have clinical value in dogs with GBM, but additional studies with larger populations are needed. In addition, these results underscore the importance of intraoperative hemodynamic stability in dogs that have a cholecystectomy as well as reaffirms the prognostic value of gallbladder rupture.

#### Disclosures

No disclosures to report.

## SCH-O-5 - Society of Comparative Hepatology

### Dogs with congenital portosystemic shunts have altered amino acid profiles compared to healthy dogs

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Alterations of circulating amino acid (AA) concentrations have previously been reported in dogs with advanced liver disease. Given the liver's importance in protein metabolism, such alterations would also be expected in dogs with congenital portosystemic shunts (CPSS). AA profiling might provide insight into the pathogenesis of hepatic disorders as well as providing valuable information for the diagnosis and management of these conditions. The purpose of this study was to compare serum AA profiles between dogs with CPSS and healthy control dogs.

Serum samples were collected from 50 dogs with extrahepatic congenital portosystemic shunts (eCPSS), 10 dogs with intrahepatic congenital portosystemic shunts (iCPSS), and from 21 healthy control dogs. Serum samples were collected at the time of intervention in all dogs with CPSS. Serum AA were measured with a Biochrom 30+ (Biochrom Ltd., Cambridge, UK) amino acid analyzer. The concentration of each AA was compared between groups using a Kruskal-Wallis test followed by Dunn's multiple comparisons tests, as appropriate, while using the Benjamini-Hochberg procedure to control for false discovery. Significance was set at $q < 0.05$.

Twenty-four AA were measurable in the serum samples. Serum concentrations of 7 AA were significantly increased in dogs with CPSS

compared to healthy controls: phenylalanine, tyrosine, glutamic acid, asparagine, histidine, serine, and ornithine. The serum concentrations of 4 AA were significantly lower in dogs with CPSS compared to healthy controls: threonine, valine, leucine, and isoleucine. Finally, serum concentrations of the following AA were not significantly different among groups: aspartic acid, tryptophan, α-aminoadipic acid, proline, glycine, citrulline, hydroxyproline, methionine, taurine, lysine, arginine, alanine, and glutamine. There were no significant differences in serum AA concentrations between dogs with eCPSS and dogs with iCPSS.

Dogs with CPSS had altered serum AA concentrations compared to healthy control dogs. Notably, serum concentrations of branched-chain AA (BCAA) were decreased while aromatic AA (AAA) were generally increased in dogs with CPSS. A reduced BCAA/AA ratio has been previously reported in human patients with hepatic encephalopathy. Serum AA profiles have the potential to differentiate dogs with CPSS from healthy dogs but not dogs with eCPSS from dogs with iCPSS.

**Disclosures**

No disclosures to report.

## SCH-O-6 - Society of Comparative Hepatology

### Plasma amino acid profiles in dogs with closed extrahepatic portosystemic shunts improve but remain abnormal three months after successful gradual attenuation

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The liver is involved in many essential functions, including protein metabolism. Liver perfusion is reduced in dogs with a portosystemic shunt (PSS), an aberrant blood vessel causing blood to bypass the liver. This results in an underdeveloped liver with impaired function. Dogs with PSS have an abnormal blood amino acid profile, with a decreased branched-chained amino acid (BCAA) to aromatic amino acid (AAA) ratio being the most obvious and important aberrance. This study aimed to assess the evolution of plasma amino acid profiles in dogs with extrahepatic PSS (EHPSS) from diagnosis to complete closure.

A prospective cohort study was performed in dogs diagnosed with EHPSS. After at least 4 weeks of medical therapy, gradual surgical attenuation was performed. Three months after the surgery, EHPSS closure was confirmed by transsplenic portal scintigraphy. Clinical signs were scored and blood was taken prior to institution of medical therapy, at time of surgery, and three months postoperatively. At the end of the study, plasma amino acid profiles of these different time points were analyzed in batch.

Ten client-owned dogs were included with EHPSS closed after surgical attenuation. The median BCAA to AAA ratio was extremely low (0.6) at time of diagnosis and remained low (0.5) at time of surgery, despite the fact that the median neurological score significantly improved after starting medical therapy ($p=0.041$). Three months after surgical attenuation, a significantly higher BCAA to AAA ratio (1.5) was observed ($p<0.001$).

Normal BCAA to AAA ratios in dogs are reported to be 3.0-4.0, whereas dogs with severe hepatic insufficiencies have ratios of less than 1.5. In this cohort of dogs, medical therapy did not improve the BCAA to AAA ratio in dogs with EHPSS, despite significant clinical improvement. Although the ratio significantly increased after EHPSS closure, it was still indicative of moderate to severe hepatic dysfunction in all dogs.

**Disclosures**

No disclosures to report.

## SCH-O-7 - Society of Comparative Hepatology

### Serum vitamin concentrations suggest incomplete restoration of liver function three months after successful gradual attenuation of extrahepatic shunts in dogs

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Deficiencies in vitamin A and D are often present in people with chronic liver diseases. In experimental animals in which portocaval shunts are created, blood concentrations of these vitamins decrease significantly. In people with chronic liver dysfunction, disorders in the vitamin B complex are found, which entails most commonly hypocobalaminaemia, although hypercobalaminaemia is also observed in patients with hepatopathy. This study aimed to assess serum concentrations of vitamin A, 25-hydroxyvitamin D, and molecules associated with the vitamin B complex in dogs with congenital extrahepatic portosystemic shunts (EHPSS) from diagnosis to successful EHPSS closure.

A prospective cohort study was performed. Serum concentrations of vitamin A, 25-hydroxyvitamin D, folic acid, cobalamin and methylmalonic acid (MMA) were measured at diagnosis prior to institution of medical therapy, at time of surgery which was performed after four weeks of medical therapy, and three months after gradual attenuation and complete closure of the EHPSS in ten dogs.

At diagnosis, median serum concentrations of vitamin A, 25-hydroxyvitamin D and folic acid were 18.2 μg/dL (8.8-79.5 μg/dL), 51.8 ng/mL (19.4-109.0 ng/mL), and 8.1 μg/L (5.2-14.5 μg/L), respectively, which increased significantly postoperatively (88.3 μg/dL (51.6-182.2 μg/dL, p=0.005), 89.6 ng/mL (49.3->150.0 ng/mL, p=0.005), and 14.8 μg/L (11.5-17.7 μg/L, p<0.001), respectively). At diagnosis, serum MMA concentrations were very variable, but after starting medical therapy, concentrations were below the lower reference value in most dogs. Median serum cobalamin concentrations were 735.5 ng/L (470-1388 ng/L) at diagnosis and did not significantly decrease postoperatively (715 ng/L (394-999 ng/L), p=0.122). Both at diagnosis and three months postoperatively 7/10 dogs had hypercobalaminemia.

In conclusion, serum concentrations of vitamin A, 25-hydroxyvitamin D and folic acid suggest improvement of liver function over time. Many dogs with EHPSS had hypercobalaminaemia, which persisted despite successful surgical attenuation of the EHPSS. Long-term follow-up would demonstrate whether and when hypercobalaminaemia normalizes after successful EHPSS closure.

**Disclosures**

No disclosures to report.

**Research reports**

Feline diabetes mellitus – Can we blame the genes?

*K. Hazuchova*

Diabetes mellitus (DM) is a common feline endocrinopathy that resembles human type 2 DM. In both species, genetic and environmental factors likely play a role in disease susceptibility.

This project was divided into two parts: a clinical study investigating exenatide extended-release (EER), a GLP-1 analogue, as a potential therapeutic for feline DM, and a genetics study to identify genetic risk factors for DM in domestic shorthair (DSH) and Burmese cats (a highly susceptible breed).

Twenty-four cats (EER n=11; placebo n=13) were enrolled onto a randomised, double-blinded, placebo-controlled clinical trial examining the effect of monthly injections of EER on β-cell function (BCF) and glycaemic control. EER treatment did not have an effect, but both glycaemic control (assessed by serum fructosamine) and BCF (assessed by glucagon stimulation testing) improved over the 6-month trial period.

In the first part of the genetics study, results from a recent genome-wide association study (GWAS) were used to identify potential candidate DM susceptibility genes. Based on their location near GWAS 'hits', the coding sequences and predicted regulatory regions of *TMEM18*, *ACP1* and *DPP9* were sequenced in diabetic and control cats. DM-associated SNPs were identified in the promoter region of *ACP1*, which encodes an enzyme involved in insulin signalling, and their functional impact was confirmed using a luciferase reporter assay in a myoblast cell line. Further combined GWAS data analysis, using genotypes of both DSH and Burmese cats was performed using various statistical models. Here, candidate diabetes susceptibility genes located near the GWAS SNPs showing the strongest association with DM or Burmese breed were *DPP9*, *ACP1*, *CDK6* (associated with DM in both breeds), *DPP10*, *KLF13* (associated with DM in DSH) and *AGBL4* (associated with Burmese breed).

Finally, whole genome sequencing (WGS) was performed in four diabetic and four non-diabetic Burmese and two DSH cats to identify novel diabetes-associated variants. Data were analysed using a bespoke GATK-based pipeline, and prioritized by various criteria including potential impact, gene function and allele frequency in diabetic or Burmese cats. Allele frequencies of variants in the 99Lives database of 282 cats were also used to assist with prioritization. In a follow up study, 130 candidate diabetes susceptibility variants were genotyped in 100 diabetic and non-diabetic Burmese and DSH cats. This revealed several novel diabetes-associated variants, some of which were exclusive to Burmese cats. Identification of novel genetic associations in feline diabetes has the potential to reveal novel therapeutic targets in this common condition.

**Research reports**

Unravelling the pathogenesis of feline hyperthyroidism

*J. Aguiar*

Feline hyperthyroidism remains a major area of veterinary interest given its high prevalence and that causal factors leading to this disease are not fully understood.

The practice of neutering cats became commonplace in the 1960s, pre-dating the widespread occurrence of hyperthyroidism by approximately a decade. Therefore, a link between neutering and development of hyperthyroidism is possible. Gonadotropin hormones such as luteinizing hormone (LH) and follicle stimulating hormone (FSH) are structurally related to other glycoproteins including thyroid stimulating hormone. It was hypothesized that increased concentration of gonadotropins in neutered cats, lacking negative feedback from sex hormones, could be implicated in the hyperthyroidism pathogenesis due to receptor cross-reactivity.

Using an immunoassay, a long-term effect of neutering on circulating feline LH concentration was identified, with neutered cats having a significantly higher plasma LH concentration than age-matched entire cats. Stimulation with human chorionic gonadotrophin (hCG), an LH analogue, led to concentration-dependent cAMP production. In the presence of TSH, an antagonistic effect of FSH and hCG on cell proliferation was also observed at higher concentrations, suggesting a cross-reactivity between these hormones and the TSH receptor. Conventional polymerase chain reaction did not demonstrate transcripts for the FSH and LH receptors' genes in thyroids of hyperthyroid cats. Therefore, should raised concentrations of gonadotropins due to neutering alter signalling pathways leading to increased thyroid

hormone synthesis and/ or thyrocyte proliferation in cats, this must occur via the TSH receptor.

Feline thyroid organoids were developed and characterised for the first time, using thyroid stem cells from euthyroid and hyperthyroid cats. Organoids were successfully developed from 7 of the 8 collected thyroids tissues. Organoid growth was evaluated under the effect of various concentrations of TSH and characterisation was performed by immunofluorescent cytochemical expression analysis of tissue markers. These constitute a novel *in vitro* model for feline hyperthyroidism research which will be an invaluable resource to advance understanding of the disease pathogenesis.

Finally, hypothesis-generating, unbiased transcriptomic analysis of archived feline hyperthyroid and euthyroid thyroid tissue was successfully performed using RNA-sequencing. Pooled barcoded RNA-seq libraries were sequenced with Illumina 150 bp paired-end sequencing. Reads were mapped to the feline reference genome and differential gene expression analysis was performed, providing an exciting opportunity to unravel the pathogenesis of feline hyperthyroidism. In addition, differentially expressed genes associated with treated and untreated hyperthyroidism were also identified. Work is ongoing to use these data to identify potential novel treatment and prevention targets for this common disease.

## Research reports

### Phosphate homeostasis in early feline chronic kidney disease

*H. J. Sargent, R. E. Jepson, Y. M. Chang, J. Elliott*

Chronic kidney disease mineral and bone disorder (CKD-MBD) is a systemic disorder of mineral metabolism. The phosphaturic hormone, fibroblast growth factor-23 (FGF23), predicts onset of azotaemia in senior cats indicating early onset CKD-MBD. Phosphorus-restricted renal diets prolong survival in azotaemic CKD; greater understanding of mineral imbalance in early CKD is needed to inform evidence-based management.

Symmetric dimethylarginine (SDMA) may be more sensitive than creatinine in detecting an early decline in glomerular filtration rate. A cross-sectional study of non-azotaemic senior cats demonstrated higher plasma FGF23 (PIFGF23) in cats with elevated SDMA (>14 µg/dL) than in those with SDMA within reference interval (271 [176, 523] pg/mL vs 155 [127, 243] pg/mL), supporting early occurrence of CKD-MBD.

A pilot study at two 1st opinion practices, in which 11 non-azotaemic (creatinine < 177 µmol/L) cats with SDMA >14 µg/dL and/or creatinine>140µmol/L with USG<1.040, were fed a renal diet (phosphorus 0.8-1.1 g/Mcal), demonstrated stability of PIFGF23 in 7 cats (median PIFGF23: baseline 344 [255,392] pg/mL; 2-month follow-up 370 [262, 413] pg/mL; <20% change from baseline). Compared to retrospective data from a cohort of cats on a standard diet, a higher proportion of renal-fed cats demonstrated PIFGF23 stability (65% vs 34%). Stability of PIFGF23 is proposed to be indicative of improved outcome. This pilot informed the design of a prospective randomised controlled clinical trial evaluating the effect of a phosphorus-restricted diet in early CKD.

To explore serum phosphorus (SrP) regulation further, a conceptual model of the interacting systems influencing SrP was created and evaluated by path analysis. Data were analysed from a study feeding high phosphorus (4.8g/Mcal; n=24) or control diets (1.2g/Mcal; n=24) to non-azotaemic cats for 4-weeks. Blood and urine were collected at baseline and 4 weeks and directed dependencies among hormonal and biochemical variables were evaluated. At baseline no interdependencies of any variable on SrP were observed. At follow-up a significant negative effect of test diet on SrP and a positive effect of test diet on logPIFGF23 and of logPIFGF23 on total urinary phosphate excretion was observed. On test diet, median SrP at follow-up was lower than baseline (0.91 [0.86, 1.01] mmol/L vs 1.31 [1.19, 1.46] mmol/L).

The results suggest the high phosphorus diet induced FGF23 secretion, which increased urinary excretion, lowering srP. Lower SrP in cats on test diet at follow-up indicates overcompensation for increased dietary phosphorus, suggesting hysteresis in PIFGF23 regulation. These models will be applied to data in cats on diets of differing phosphorus content, where cats exhibiting kidney dysfunction will be compared with those that do not.

## ESCG-P-1 - European Society of Comparative Gastroenterology

### Electrolyte imbalances in dogs with chronic inflammatory enteropathies

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Chronic intestinal inflammation is associated with electrolyte shifts due to decreased absorption ($Na^+$, $Cl^-$) or increased secretion ($Cl^-$, $K^+$). Besides the high luminal osmole content resulting from malabsorption, $Na^+/Cl^-$ transport abnormalities are major contributors to diarrhea with inflammatory bowel disease (IBD) in humans. The resulting electrolyte and acid-base changes vary with the severity, extent, and location of the disease and may be detrimental to the patient. Chronic inflammatory enteropathies (CIE) in dogs share characteristics of human IBD. However, inflammatory lesions are usually located more proximally in the gastrointestinal tract, and the prevalence of electrolyte imbalances in dogs with CIE is unknown.

Serum electrolyte ($Na^+$, $Cl^-$, corrected $Cl^-$, and $K^+$) concentrations were retrospectively evaluated in 37 treatment-naïve dogs diagnosed with CIE (14 with food-responsive enteropathy [FRE] and 23 with immunosuppressant-responsive enteropathy [IRE]). These measurements were compared to those in 40 healthy control dogs and 40 dogs

with acute hemorrhagic diarrhea syndrome (AHDS). Concentrations of serum electrolytes in dogs with CIE were further assessed for a correlation with the clinical disease activity, histologic lesion severity, and clinicopathologic variables. Statistical significance was set at $P<0.05$.

Severe electrolyte abnormalities were not seen in any dog with CIE. In CIE dogs, the concentration of at least one serum electrolyte was decreased in 13 (35%) of the dogs and was increased in 5 (14%) dogs. Hypokalemia was the most obvious electrolyte abnormality in dogs with CIE, affecting 7 (19%) dogs and with no difference between IRE vs. FRE, but was less frequent than with AHDS (55%). Hyponatremia was less common with CIE (14%) and was seen predominantly in dogs with IRE. $Na^+/K^+$ ratios were significantly higher with CIE (median: 35.0) than in health (median: 31.7, $P=0.001$), with no difference between FRE and IRE, but were lower than in dogs with AHDS (median: 38.2, $P=0.005$). Serum $Na^+$ concentration was moderately inversely correlated with the severity of histologic lesions in the duodenum. Group comparisons showed no significant differences in measured and corrected serum $Cl^-$ concentrations. Hypo- (5%) and hyperchloridemia (11%) were seen in dogs with CIE.

Electrolyte imbalances occur with equal frequency in canine CIE and human IBD. Increased $K^+$ secretion might exceed compromised $Na^+/Cl^-$ absorption and/or $K^+$ shifts (e.g., acid-base imbalances or aldosterone-induced) might be more pronounced in dogs with CIE. Thus, the mechanisms underlying CIE-associated diarrhea in dogs warrant further exploration. Compared to CIE, severe electrolyte abnormalities are more common in dogs with acute gastrointestinal disease.

**Disclosures**

N/A.

**ESCG-P-2 - European Society of Comparative Gastroenterology**

## Fecal S100A12 (calgranulin C) concentrations in cats with chronic enteropathies

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Chronic enteropathies occur commonly in cats, especially in those that are middle-aged to older. Non-invasive biomarkers for chronic enteropathy diagnosis, disease monitoring, and response to therapy have been extensively evaluated in humans and dogs but studies in cats are lacking. The aim of this study was to measure and evaluate the fecal calgranulin C (S100A12) concentrations in cats diagnosed with chronic enteropathy.

Cats were prospectively enrolled. All diseased cats showed gastrointestinal signs for >3 weeks and had a complete diagnostic work-up, including bloodwork, abdominal ultrasound, endoscopic biopsies with histopathology and when necessary PCR and/or immunohistochemistry. All cats were assigned a feline chronic enteropathy clinical index (FeCEAI) score. Cats were either diagnosed with small cell lymphoma (LSA) n=30 or inflammatory bowel disease (IBD) n=19. Control cats n=19 were enrolled for comparison. One free catch fecal sample was collected from each enrolled cat and S100A12 concentrations were quantified by an analytically validated in-house ELISA. Descriptive statistics were performed using a commercial software package. A Wilcoxon test was performed to compare the fecal S100A12 concentrations between groups. Statistical significance was set at $P<0.05$.

Results showed a significant difference of fecal S100A12 between cats with LSA and control cats ($P<0.02$) and also between IBD and control cats ($P<0.03$). However, we were unable to identify a significant difference between LSA and IBD cats ($P=0.9$).

S100A12 was quantified in all study cats and was elevated in those with chronic enteropathy (either LSA or IBD). S100A12 may be a reasonable biomarker to identify cats with chronic enteropathy, but does not differentiate LSA versus IBD. Further studies are needed to determine whether this biomarker might be useful to monitor for impending disease or response to therapy.

**Disclosures**

Collaboration with the Texas A&M Gastrointestinal Laboratory, who performed an in-house ELISA to quantify fecal S100A12 concentrations. The authors have no conflicts of interest to report.

**ESCG-P-3 - European Society of Comparative Gastroenterology**

## Alterations of fecal unconjugated primary bile acids and correlation with abundance of *C. hiranonis* in dogs with chronic enteropathy

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Previous studies have demonstrated alterations in intestinal bile acid (BA) metabolism in a subset of dogs with chronic enteropathy (CE). *Clostridium hiranonis* is the main converter of primary to secondary BAs in dogs, and was shown to have a reduced abundance in a proportion of dogs with CE. This study aimed to investigate fecal concentrations of unconjugated BAs in dogs with CE compared to healthy controls. Additionally, the correlation between fecal abundance of *C. hiranonis* and the ratios of fecal primary to secondary BAs were evaluated.

Seventeen dogs with chronic enteropathy (CE) and 17 healthy dogs (H) were enrolled. Unconjugated primary (cholic-, chenodeoxycholic

acid) and secondary (lithocholic-, deoxycholic-, ursodeoxycholic acid) BAs were quantified by gas chromatography/mass spectrometry. Abundance of *C. hiranonis* was determined by qPCR. A Mann-Whitney test was used for comparison of BAs between groups. To assess the relationship between the abundance of *C. hiranonis* and proportion of primary BAs Spearman's correlation coefficient was used. P<0.05 was considered statistically significant.

Changes in BA metabolism were detected in 10/17 dogs with CE, with primary BAs above the reference interval. Compared to H, the concentration of primary BAs (median [range]: CE, 2,057 [45–13,374] μg/mg; H, 130 [32–1,142] μg/mg; P=0.002) and percentage of primary BAs (median [range]: CE, 50.4 [0.6–95.0] %; H, 4.0 [0.9–13.0] %; P=0.02) were significantly increased in dogs with CE. The abundance of *C. hiranonis* and percentage of primary BAs showed a strong negative correlation (r=-0.68, P<0.001). Of note, 76% of dogs with CE responded to dietary changes alone.

BA metabolism and the abundance of *C. hiranonis* are altered in a subset of dogs with CE. Regardless, a large proportion of the dogs of our study population were considered diet-responsive.

**Disclosures**

Drs. Sung, Lidbury, Steiner, and Suchodolski are employed by the Gastrointestinal Laboratory at Texas A&M University, which offers gastrointestinal function testing on a fee-for-service basis.

## ESCG-P-4 - European Society of Comparative Gastroenterology

### Correlation of intestinal histopathologic findings with mucosa-attached bacteria, clinical disease activity, and clinical outcome in dogs with chronic enteropathies

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While the pathogenesis of canine chronic enteropathies (CE) is not fully understood, an aberrant immune response to antigens derived from endogenous microbiota and/or diet is likely to play an important role. In this study, we assessed the relationship between histopathologic findings and mucosa-attached bacteria (as determined by routine histopathology), canine inflammatory bowel disease activity index (CIBDAI) and clinical outcome (food-responsive enteropathy [FRE], antibiotic-responsive enteropathy [ARE], steroid-responsive enteropathy [SRE]).

Endoscopic biopsies of intestinal mucosa from 65 dogs with CE (duodenum [n=43], ileum [n=34], and colon [n=35]) were collected from three study centers. Published scoring systems were used to assess clinical and histological severity of disease. *Spearman rank* correlation *tests were used to assess the relationship between* histopathologic findings, mucosa-attached bacteria, and CIBDAI. *Kruskal-Wallis tests were used to* assess the association of histopathologic findings with clinical outcome groups (FRE, ARE, and SRE).

Histopathologic findings only correlated significantly with mucosal bacterial attachment in the colon. The correlation was moderate for lamina propria neutrophils ($r = 0.427$, $p = 0.010$; 95% confidence interval [CI] = 0.010 to 0.671) and weak for surface epithelial injury ($r = 0.371$; $p = 0.028$; 95% CI = 0.033 to 0.632). There were no significant correlations between mucosal bacterial attachment in duodenum or ileum and histopathologic findings or clinical outcome groups. Among different clinical outcome groups, there were statistically significant differences in mean rank scores of the duodenum for summative histopathologic score (SRE 27.9 vs. ARE 16.6, $p = 0.028$), villus stunting (SRE 26.6 vs. FRE 18.5, $p = 0.033$), and lamina propria lymphocytes and plasma cells (FRE 27 vs. ARE 15.6, $p = 0.025$ and SRE 26.5 vs. ARE 15.6, $p = 0.028$). In the colon, the score of goblet cells differed between FRE and ARE (mean rank scores: FRE 23.0 vs. ARE 14.6, $p = 0.036$). There was no significant correlation between histopathologic findings and CIBDAI.

This study demonstrated that mucosa-attached bacteria show mild to moderate positive correlation with surface epithelial injury and lamina propria neutrophils in colon of dogs with CE. In addition, dogs with SRE had the highest summative histopathologic and villus stunting scores in the duodenum and dogs with FRE had the most severe infiltration with lymphocytes and plasma cells in duodenum, as well as decreased colonic goblet cells.

**Disclosures**

No disclosures to report.

## ESCG-P-5 - European Society of Comparative Gastroenterology

### Evaluation of serum biochemical and urinary parameters suggesting renal involvement in a population of dogs with primary chronic enteropathy

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Approximately a half of IBD human patients show extra-intestinal manifestations, in which 4-23% may develop renal and urinary involvement. These findings may be linked to several conditions, such as the immune-system response of the primary chronic enteropathy (CE), reduction in short-chain fatty acids, or endotoxemia. No specific studies have been conducted in dogs, except for those describing familiar protein-losing nephropathy and enteropathy in soft-coated wheaten terriers.

The aim of this study was to describe alterations of selected serum biochemical and urinary parameters suggesting renal injury in dogs with CE.

Retrospective bi-centric study including dogs with CE. CE diagnosis was made after the exclusion of intestinal diseases of other etiologies and extra-intestinal diseases. Dogs with history of previous kidney or low urinary tract diseases (previous clinicopathological finding and/or imaging alterations) and with severe proteinuria (urine protein-to-creatinine ratio >2, [UPC]) were excluded. Canine Chronic Enteropathy Activity Index Score (CCECAI), muscular condition score (MCS; 3-point scale), serum albumin, urea, creatinine, presence of glycosuria, proteinuria (UPC>0.5) and urinary casts were recorded for each dog. Dogs with albumin <2.7 mg/dL were classified as protein-losing enteropathy (PLE). Dogs with glycosuria, proteinuria and/or urinary casts were classified as having kidney injury. Mann-Whitney u-test was used to compare CCECAI of dogs with and without kidney injury. Chi-square test was used to evaluate the association of PLE and presence of kidney injury, and proteinuria.

One-hundred-six dogs with CE were included. Fifty-two dogs (49%) had mild-to-severe reduction in MCS. Only 6/106 dogs (6%) had azotemia (median creatinine 1.6 mg/dL; range 1.5-2.4 mg/dL), whereas 40/106 dogs (38%) showed kidney injury. In particular, 2 dogs had glycosuria, 23 dogs had proteinuria, and 23 dogs had urinary casts. CCECAI was not different between dogs with, and without kidney injury (both median=4; p=0.9). Forty-four dogs were classified as having PLE. The prevalence of kidney injury was not different between PLE, and non-PLE (p=0.3) dogs, whereas PLE dogs showed a higher frequency (61%) of proteinuria, than non-PLE dogs (p=0.03 OR 2.8 95%CI 1-6.8). Serum markers of kidney injury should be interpreted with caution in CE dogs, since approximately half of our dogs showed a reduction in muscular mass. On the other hand, assessment of urinary markers of kidney injury may be useful and advisable, especially due to the high risk of proteinuria in PLE dogs.

**Disclosures**

No disclosures to report.

## ESCG-P-6 - European Society of Comparative Gastroenterology

### First attempt at using Narrow Band Imaging-like endoscopy to differentiate chronic enteropathy and alimentary lymphoma in dogs

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In dogs, inflammatory conditions of the gastrointestinal tract (GIT) like chronic enteropathy (CE) and alimentary lymphoma (AL) have a near identical clinical presentation and diagnostic workup, while prognosis and treatment are distinctly different. Diagnosis is often based on histology from endoscopic mucosal biopsies. Studies in people suggest that Narrow-Band Imaging (NBI) endoscopy can aid in diagnosing GIT cancer due to differences in microvessel density. However, this technique has so far only been applied in dogs with CE, not in AL.

The aims of this study were firstly to assess if Storz Professional Image Enhancement (SPIES), a form of NBI-like endoscopy, has a superior capacity for microvessel detection in dogs with different GIT conditions; and secondly to determine if SPIES would allow differentiation between CE and AL in dogs. For this, dogs undergoing traditional White Light (WL) GIT endoscopy were simultaneously evaluated with SPIES. Standard histopathology ± immunohistochemistry determined the final diagnosis. Side-by-side WL and SPIES images of the duodenum and colon were obtained. For analysis, a published pipeline to assess "vesselness" based on quantification of microvessel pixel area (MPA) was used on all images (MeVisLab; ImageJ/Fiji) by an observer blinded to the final diagnoses. WL and SPIES MPA were compared using Bland-Altmann diagrams. Student t-test was used to compare SPIES parameters between CE and AL cases.

A negative bias for WL-SPIES differences was detected for both the colon and duodenum, based on analysing image pairs from 45 dogs with a variety of GIT conditions, suggesting that SPIES facilitates an increased detection of microvessels. A total of 37 images from dogs with CE (n=28) and 6 images from dogs with AL (n=3) were available to compare SPIES data. No differences of SPIES MPA parameters were detected between dogs with CE and AL in neither duodenum (p=0.643) or colon (p=0.766).

In summary, even though more microvessels were detected from images taken with SPIES compared to WL GIT endoscopy, in this small patient sample SPIES could not be used to differentiate AL from CE. This should be verified in future studies with larger sample sizes.

**Disclosures**

No disclosures to report.

## ESCG-P-8 - European Society of Comparative Gastroenterology

### Evaluation of the efficacy of an ultra-hydrolyzed diet in the management of chronic diarrhea in dogs

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Food responsive enteropathy (FRE) represents the most important cause for chronic diarrhea (CD) in dogs, with an estimated prevalence of 50-60%. Elimination diets and rechallenge are considered the gold standard to diagnose FRE. Treatment trials with commercial hydrolyzed diets

are frequently performed in dogs with CD, but few studies have evaluated their efficacy. Thus, the aim of this study was to assess the efficacy of exclusively feeding an ultra-hydrolyzed diet as a treatment for CD.

Thirty-nine dogs with chronic diarrhea for at least 3 weeks were enrolled retrospectively. Of these dogs, 50% had already finished one or more diet trials and 46% have been on medical treatment (immunosuppressants, antibiotics, probiotics) for longer than 3 weeks before presentation.

They underwent standard diagnostic evaluation and in all dogs an ultra-hydrolyzed diet for at least 2 weeks was started. Additional treatment changes during the diet trial were not allowed. For evaluation of the treatment success the CCECAI indices at presentation and at recheck after at least two weeks were compared by using the Wilcoxon matched-pairs signed rank test.

The acceptance of the diet was high in 62%, moderate in 15% and poor in 23% of dogs. In 11 dogs (28%) the diet trial was aborted, because of the refusal to eat the diet (9) or due to vomiting (2). In the remaining 28 dogs (72%), the median CCECAI score decreased significantly during the study period (at presentation 7 (range 3 – 11); at recheck 1.5 (range 0 – 10), $p<0.0001$).

Newly developed clinical signs after the initiation of the diet were obstipation (n=3), vomiting (n=2), licking (n=2) and worsening of diarrhea (n=1).

In conclusion, an ultra-hydrolyzed diet improved clinical signs in most dogs with CD and should be recommended as a therapeutic trial for FRE, although few dogs develop unwanted side effects.

**Disclosures**

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## ESCG-P-9 - European Society of Comparative Gastroenterology

### The microbiome composition in dogs with suspected antibiotic-responsive diarrhea and the effect of tylosin on it: A multicentric-perspective case-control study

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Despite tylosin has been used in the control of chronic diarrhea, studies evaluating its effect on microbiome in dogs with antibiotic-responsive diarrhea (ARE) are lacking.

The aim of this prospective case-control study was to characterize the gut microbiome in young-adult dogs with suspected ARE and comparing it with the ones of healthy controls. A second aim was to evaluate the variation of microbiome before (T0), 30 days after tylosin (T30), and 30 days after its discontinuation (T60).

We included 15 dogs showing chronic diarrhea (Group A) selected on basis of inadequate response to food trials and a clinical response to previous antibiotic therapy with subsequent relapse. Dogs were treated with Tylosin (10mg/kg q12h) for 30 days. No antibiotic or probiotic was used in the 45 preceding days. Feces were collected at T0, T30 and T60. A clinical follow-up was collected at T120. A control population (Group B) of 15 healthy dogs was included and feces were collected at T0 and T30.

Microbiome DNA was extracted using Cador Pathogen 96 QIAcube HT Kit protocol and sequenced using the V3 kit-300PE strategy. Qiime2 version 2020.2 was used to perform bioinformatic analyses.

Alpha- and Beta-diversity were calculated.

Group A was composed of 7 German shepherds and 8 dogs from other breeds while Group B consisted in 8 German shepherds and 7 dogs from other breeds.

T0 Alpha-diversity showed no difference between groups while a significance was reached in Beta-diversity (increased Lactobacillaceae in Group B compared to Group A).

Significant changes between T0 and T30 were observed in Alpha-diversity and Beta-diversity in Group A ($p<0.001$). 14/15 dogs from group A showed no diarrhea at T30 while 5/15 dogs form the same group relapsed between T30 and T60.

T60 Alpha-diversity and Beta-diversity revealed no significant difference to T0 from Group A.

At T120 the diarrhea was reported in 8 dogs. A treatment with tylosin was restored and the resolution of diarrhea was recorded, however it reoccurred when the drug was tapered. No statistical difference was noted from these 8 dogs, classifiable as patients with ARE, to the ones of the same group at any timepoint.

Tylosin showed relevant effect on microbiome however, as previously reported in healthy dogs, almost complete resilience was noted a T60.

In our opinion, 8 dogs from the study displayed the typical clinical setting of ARE. Nevertheless, we failed to find any difference in the microbiome to dogs that didn't relapse.

**Disclosures**

No disclosures to report..

## ESCG-P-10 - European Society of Comparative Gastroenterology

### Primary chronic enteropathy in dogs: What cardiovascular effects?

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Endocarditis, myocarditis, pericarditis, and different types of arrhythmias have been described in people with inflammatory bowel disease. In the veterinary literature, very limited information is available on cardiovascular effects of primary chronic enteropathy (CE) in dogs. Therefore, this study aimed to investigate possible structural heart diseases and cardiac arrhythmias in dogs with CE.

This prospective observational study (n°46/2019) included dogs with primary CE and an age- and body weight-matched control group composed of healthy dogs. Primary CE was diagnosed after the exclusion of extra-intestinal disease and other intestinal disease based on diet trials, clinicopathological findings and abdominal ultrasound. All CE dogs were assigned a Chronic Canine Enteropathy Clinical Activity Index (CCECAI) score. All dogs underwent measurement of serum cardiac troponin I, standard transthoracic echocardiography, and 6-lead electrocardiography (ECG) at the time of study enrolment. Heart rate, P wave, PQ interval, QRS complex, QT interval, and the vasovagal tonus index was evaluated as an indicator of vagal tone.

The study included 21 dogs with primary CE, of which 7 (33%) with protein losing enteropathy, and 20 control dogs. The median CCECAI score was 4.5 (range, 1-9). In dogs with CE, no echocardiographic signs of endocarditis, myocarditis or pericarditis were found, and all dogs showed normal levels of serum cardiac troponin I (<0.1 ng/mL). All dogs showed sinus rhythm, and no significant differences were found in ECG parameters between CE dogs and the control group, except for the vasovagal tonus index that was significantly increased in the CE group (9.0 ± 1.4 versus 8.0 ± 1.5; P=0.043). In the CE group, serum total proteins were positively correlated with heart rate (r=0.53; P=0.01), and dogs with sinus bradycardia (<70 bpm) showed a significantly lower total proteins in comparison to dogs with normal heart rate (>70 bpm; 3.7 ± 1.0 versus 5.9 ± 0.8 mg/dL; P=0.028).

In our study no clinical evidence of inflammatory or infective structural heart disease was found in dogs with CE. Similarly, no pathologic arrhythmias were found in our sample population of canine CE. However, our results suggest the presence of sinus bradycardia and increased vagal tone in dogs with CE, especially those with lower levels of protein.

**Disclosures**

No disclosures to report.

**ESCG-P-11 - European Society of Comparative Gastroenterology**

## Serum amino acid concentrations in dogs with chronic enteropathies prior to treatment, and correlation with clinical activity index and gastrointestinal histopathology

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Alterations in serum and plasma amino acids (AA) have previously been reported in dogs with chronic enteropathy (CE) that did not respond to dietary or antibiotic therapy. While many dogs with CE will respond to a change in diet and are categorized as having food responsive enteropathy (FRE), others respond to treatment with antibiotics or steroids and are categorized as having ARE or SRE, respectively. The aim of this study was to compare serum AA profiles between dogs with FRE, dogs with ARE or SRE, and healthy control dogs.

Serum samples were collected from 23 healthy dogs and 15 dogs with CE prior to treatment, which were ultimately categorized as having FRE (n = 7) or as having ARE or SRE (n = 8) based on response to treatment. Gastrointestinal histopathology was obtained for 6 dogs with CE and 5 healthy control dogs. Serum AA concentrations were measured with ninhydrin derivatization on an amino acid analyzer and compared between groups using Kruskal-Wallis testing followed by Dunn's post hoc multiple comparisons tests. Correlation analysis between serum AA, clinical activity index (CCECAI), and WSAVA histopathological scores for gastric, small intestinal, and colonic tissues was performed using Spearman's rank correlation and correction for multiple comparisons with Benjamini & Hochberg FDR.

Serum concentrations of four amino acids were significantly increased in dogs with ARE/SRE compared to healthy dogs: aspartate (p = 0.047), alanine (p = 0.033), valine (p = 0.007), and isoleucine (p = 0.011). Serum methionine concentrations were significantly increased in dogs with FRE when compared to healthy dogs (p = 0.027). Serum concentrations were not significantly different between the combined ARE/SRE group and the FRE group for any of the AA. Isoleucine concentration was significantly positively correlated with CCECAI. Serum citrulline, proline, and methionine positively correlated with WSAVA severity scoring of gastric tissue, while phenylalanine, lysine, and leucine corelated negatively. Serum methionine correlated positively and serine and lysine concentrations correlated negatively with small intestinal severity scores. Citrulline, proline, and methionine concentrations correlated positively and glutamine negatively with colonic severity scores.

When compared to healthy dogs, dogs with ARE or SRE showed increased serum concentrations of four AA, and dogs with FRE showed increased concentrations of one AA. While serum AA profiles were not able to differentiate dogs with FRE from dogs with ARE/SRE, some AA correlated with gastrointestinal histopathological scores or clinical activity index.

**Disclosures**

No disclosures to report.

**ESCG-P-12 - European Society of Comparative Gastroenterology**

## Serum concentrations of fPLI, fTLI, cobalamin, and folate in growing kittens

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Serum concentrations of feline pancreatic lipase immunoreactivity (fPLI), feline trypsin-like immunoreactivity (fTLI), cobalamin, and folate are commonly used for the diagnostic investigation of cats with gastrointestinal signs. No information on these parameters in cats less than 1 year of age is available. We aimed to evaluate serum concentrations of fPLI, fTLI, cobalamin, and folate in cats at different time-points during their first 12 months of life.

Fourteen healthy 2-month-old kittens were included in the study. All kittens received standard antiparasitic treatment monthly and consumed the same balanced diet during the study. Blood was collected at 2, 3, 4, 6 and 12 months of age and serum concentrations of fPLI, fTLI, cobalamin, and folate were measured. Data were assessed for normality and non-parametric parameters were evaluated using Friedman test followed by Tukey's post hoc test with Bonferroni correction. Statistical significance was set at $p<0.05$.

While there was a statistically significant difference in serum fPLI concentrations over time ($p=0.0168$), there was no statistically significant difference between individual time-points. Also, there was no clinically significant shift over time. There was no significant difference in serum fTLI concentrations over the time-period evaluated ($p=0.4914$), though 1 kitten had an increased concentration between 2 and 6 months that could not be explained. Serum cobalamin concentrations were below the lower limit of the reference interval in 3/11 at 2 months (median, 518 ng/L; range, <150-1704 ng/L) and were significantly decreased by 3 months (10/11 had hypocobalaminemia; median, 178 ng/L; range, <150-611 ng/L, $p=0.0121$). By 12 months, cobalamin concentrations had significantly increased (median, 404 ng/L; range, 217-938 ng/L, $p=0.0007$), yet 3/11 cats still had hypocobalaminemia. Folate was increased in 2/7 and decreased in 1/7 at 2 months of age. Folate significantly decreased from 2 (median, 16.5 μg/L; range, 6.9-43.4 μg/L) to 6 months (median, 2.6 μg/; range, 2-4.3 μg/L, $p=0.0235$) and 12 months of age (median, 2.6 μg/L; range, 1.4-4.7 μg/L, $p=0.001$) with 7/7 cats having hypofolatemia at 12 months of age.

Serum fPLI and fTLI concentrations did not show any statistically or clinically significant changes in young kittens compared to adult cats. Serum cobalamin concentrations in kittens were overall low at 2 months, then dropped even more at 3 months, and only slowly increased over time with approximately 25% of cats remaining hypocobalaminemic at 12 months of age. Many of these kittens also had hypofolatemia. Further studies are needed to evaluate the clinical significance of these findings.

**Disclosures**

Some of the authors work at a lab that offers these assays for profit.

**ESCG-P-13 - European Society of Comparative Gastroenterology**

## Influence of canine-specific lactic-acid bacteria on the fecal microbiota and inflammatory markers in dogs receiving nonsteroidal anti-inflammatory treatment - A prospective, randomized, double-blinded placebo-controlled trial

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Nonsteroidal anti-inflammatory drug (NSAID) treatment may cause diarrhea, but the pathogenetic mechanisms, and possible prevention strategies remain unknown. This study aimed to determine whether canine-specific lactic acid bacteria (LAB) affect the fecal microbiota and gastrointestinal inflammation in dogs receiving NSAIDs.

The LAB product consisted of *Limosilactobacillus fermentum*, *Lacticaseibacillus rhamnosus*, and *Lactiplantibacillus plantarum* fermented in milk and containing a minimum of $3\times10^{11}$ CFU/kg. The placebo product was powdered micro-crystallized cellulose.

Dogs treated with NSAIDs for varying clinical reasons were enrolled in this 7 day randomized, double-blinded placebo-controlled interventional trial.

Fecal samples were collected on days 1 and 7. Where diarrhea required a discontinuation of NSAID treatment, the second fecal sample was collected on the last day of the treatment. Dog-owners were instructed to score the fecal quality on a daily basis. The fecal microbiota was evaluated using the dysbiosis index (DI), and gastrointestinal inflammation was evaluated by measurement of fecal calprotectin (CP) and S100A12 concentrations.

A multiple regression analysis (R software) was conducted using S100A12, CP, and DI as response variables. The model was adjusted for individual baseline values. The test product, diarrhea, and the interaction between these, were explanatory variables.

Statistical significance was set at $p<0.05$.

A total of 22 dogs were enrolled in the study, of which 10 dogs received LAB, and 12 received placebo.

Orthopedic diseases were the most common reason (7/22 dogs, 32%) for NSAID prescription. A total of 15/22 dogs (68%) underwent a surgical procedure, with 9/12 dogs (75%) in the placebo group and 6/10 dogs (60%) in the LAB group.

Diarrhea occurred in 5 dogs (23%), including 4 dogs in the placebo group and 1 dog in the LAB-group, but this difference was not significant (chi square, $p=0.19$). The diarrhea was severe enough to discontinue treatment in 3 dogs (2 dogs in the placebo group on days 3 and 4, and 1 dog in the LAB group on day 3).

There was a significant interaction between diarrhea and placebo product on S100A12 ($p=0.027$, $R^2=66.5\%$). Fecal S100A12 and CP were significantly correlated (Spearman test, $p<0.001$). The effect of

the placebo on CP was significant without this interaction (p=0.04, $R^2$ =0.53). Neither treatment nor diarrhea had any significant effect on DI (p>0.05).

Fewer dogs treated with NSAIDs that were also treated with LAB developed diarrhea than those treated with a placebo. Dogs receiving placebo also demonstrated higher fecal S100A12 concentrations than those in the LAB-group.

**Disclosures**

In this study, we used a product of lactic acid bacteria, licenced as Canius, which is commercially available. However, the company of this product did not contribute in this study.

**ESCG-P-14 - European Society of Comparative Gastroenterology**

## Fecal bile acids and microbial amino acid metabolites in serum are correlated with fecal zonulin in dogs with exocrine pancreatic insufficiency

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Dogs with exocrine pancreatic insufficiency (EPI) are treated with pancreatic enzyme replacement therapy (PERT), but up to 40% of dogs have persistent weight loss and diarrhea. EPI is associated with enteric dysbiosis which is known to cause mucosal abnormalities, including increased intestinal permeability, in dogs and other mammals. Mucosal barrier dysfunction could contribute to the persistence of clinical signs in dogs with EPI after PERT. The goal of this study was to detect host-microbiome interactions by correlating serum and fecal concentrations of microbial metabolites with fecal concentrations of zonulin. Zonulin is a biomarker for mucosal barrier integrity and increased fecal zonulin is associated with increased mucosal permeability.

Serum and feces from 20 dogs with EPI and 10 healthy controls were sampled. Dogs with EPI had received PERT for at least 30 days before sampling. To control for the effect of PERT, healthy controls were fed pancreatic enzymes for 14 days before sampling. Serum metabolomes were generated by liquid chromatography-mass spectrometry. Fecal concentrations of unconjugated bile acids were measured by gas chromatography-mass spectrometry. Fecal concentrations of zonulin were measured by ELISA. The Wilcoxon rank-sum test was used to compare fecal metabolites and zonulin between groups. Pearson correlation coefficients were used to detect correlations among serum/fecal metabolites and fecal zonulin.

Fecal zonulin was higher in dogs with EPI compared with healthy controls, but the difference was not statistically significant (p=0.11). Total fecal secondary bile acids were lower in dogs with EPI compared with healthy controls (p=0.03) and inversely correlated with fecal zonulin (r=-0.63, p=0.0006). Total fecal primary bile acids were not significantly different between groups (p=0.430) but were positively correlated with fecal zonulin (r=0.52; P=0.006). Serum taurohyodeoxycholic acid, a secondary bile acid, was inversely correlated with fecal zonulin (r=-0.47, p=0.016). Serum indolepropionate, a microbial tryptophan metabolite, was inversely correlated with fecal zonulin (r=-0.52; P=0.006). Serum N-trimethyl 5-aminovalerate (r=-0.54, p=0.005) and 5-aminovalerate (r=0.47, p= 0.015), microbial lysine metabolites, were positively correlated with fecal zonulin. A microbial phenylalanine metabolite, 4-hydroxyphenylacetate, was also positively correlated with fecal zonulin (r=0.39, p=0.047).

Our findings are consistent with previous studies showing altered bile acid metabolism in dogs with EPI. Furthermore, these results indicate a significant effect of microbial bile acid and amino acid metabolism on mucosal barrier function of dogs with EPI. Limitations of this study include the small number of dogs and high false discovery rates for correlations among serum metabolites and fecal zonulin.

**Disclosures**

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**ESCG-P-15 - European Society of Comparative Gastroenterology**

## Modification of fecal microbiota and disease activity index with only nutritional intervention in dogs with inflammatory bowel disease: A retrospective study in 17 dogs

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Inflammatory bowel disease (IBD) is a common cause of idiopathic, chronic and relapsing gastrointestinal (GI) signs in dogs. The etiology remains unknown but an interplay between genetic factors, dietary/environmental factors and enteric bacteria is considered crucial for disease development.

Several studies have demonstrated that canine IBD is associated with alterations in fecal microbial communities

The aim of this study is to evaluate the effects of diet and symbiotic therapy on clinical score indices and fecal microbial communities in dogs with IBD.

Inclusion criteria were history of persistent or intermittent GI signs of at least 3 weeks duration, histopathologic evidence of intestinal inflammatory cellular infiltration on endoscopy biopsies, availability of the clinical activity index (CCECAI) and of microbiota profile at presentation and at least after 8 weeks of dietary and symbiotic treatment.

Exclusion criteria included treatment with anti-inflammatory drugs, antimicrobials or both within 4 weeks prior to presentation.

Histopathological diagnoses and grading of IBD were performed according to WSAVA guidelines. Sequencing of the hypervariable regions V2-V3-V6-V7-V8-V9 of 16SrRNA, from DNA samples, extracted from each fecal sample, was performed. The sequences were processed to separate the regions and then analyzed with Mothur to find Operational Taxonomic Units (OTUs), and their abundances and taxonomies by using RDP database as a reference.

A dysbiosis index (Shannon index) was utilized to determine bacterial taxonomic diversity within the samples.

A total of 17 dogs diagnosed with IBD were included in the study.

Each dog was switched to a home-made diet containing a moderate amount of a selected protein source and carbohydrates with low glycemic index. According to the specific alterations in each fecal microbiota, each dog received different symbiotic and/or soluble fiber in addition to the diet.

Analysis of fecal microbiota before treatment revealed increased abundance of *Proteobacteria and Bacteroidetes* compared with that performed after dietary modulation in which an increase of *Firmicutes* and a decrease of *Proteobacteria* was observed.

The Shannon index was significantly lower in dogs at presentation than after diet modulation ($p=.02$).

Median CCECAI score was 6.17 (range = 4–10) and 1.35 (range = 1–2) before and after treatment respectively.

Our study suggests that diet modulation with adjuvant symbiotic administration improves richness and diversity of microbiota, facilitating clinical remission in dogs with IBD.

Further studies are needed to better evaluate the benefits of a personalized nutrition on the microbiome in dogs with IBD.

**Disclosures**

No disclosures to report.

## ESCG-P-16 - European Society of Comparative Gastroenterology

### Endoscopic resection of benign gastroduodenal polyp in six cats: Procedure, complications and outcome

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Gastroduodenal polyps are an uncommon condition, poorly documented in cats. These lesions are characterized by an inflammatory, hyperplasic or adenomatous growth from the gastric or duodenal mucosa.

The aim of this retrospective study was to describe the management, complications and outcome of cats treated with endoscopic polypectomy. Inclusion criteria were histological diagnosis of benign polyp removed with endoscopic procedure in cats previously submitted to abdominal ultrasonography and gastroduodenal endoscopy examinations. Three male and two female domestic shorthair cats and one male Chartreux were included. Median age was 12 years [10-15]. Clinical signs included vomiting (n=6), dysorexia (n=2), weight loss (n=2) and abdominal pain (n=1). Ultrasonographic examination revealed a gastric or duodenal mass in four out of six cats. Endoscopic examination revealed a single pedunculated pyloric (n=4), duodenal (n=1) and pyloro-duodenal mass (n=1). Comorbidities were reported in four cats, including bacterial cholecystitis (n=1), acute pancreatitis (n=1), acquired pyloric stenosis (n=2), chronic enteropathy (n=2), alimentary lymphoma (n=1). Per-endoscopic resection was performed in four cats, using an electrosurgical snare through the working channel, without complete resection of peduncle. Due to an acquired pyloric stenosis, one gastro-duodenal polyp was reduced by debulking with biopsy forceps as the peduncle was not reachable (n=1), another lesion was almost totally reduced with forceps during biopsies, so the use of electrosurgical snare was judged unnecessary (n=1). Histological analysis revealed 3 adenomatous, 2 hyperplasic and 1 inflammatory polyps. Complications were met with electrosurgical resection; with asymptomatic superficial ulceration (n=1) and a mild ulceration leading to anorexia and vomiting treated with symptomatic treatment (n=1). One cat developed a septic peritonitis, leading to death, secondary to gastric perforation 24 hours after the electrosurgical resection. For the five surviving cats treated by endoscopic polypectomy, a complete (n=3/5) and partial (n=1/5) resolution of vomiting episodes was observed. One cat had an endoscopy 2 months after polypectomy showing no sign of recurrence. For the three cats with complete vomiting resolution, two presented vomiting, 17- and 81-months post-polypectomy, without ultrasonographic signs of polyp recurrence. One cat showed no clinical relapse after 11 months.

Our results suggest that endoscopic removal of gastro-duodenal polyps can be associated with good prognosis. Endoscopic polypectomy by electrosurgery is generally associated with resolution of vomiting. Complications included gastric or duodenal mucosal

ulceration and septic peritonitis. Resurgence of polyp seemed unfrequent even without full resection. A careful evaluation of the pedicle size is needed to avoid gastric perforation following endoscopic removal.

**Disclosures**

No disclosures to report.

## ESCG-P-17 - European Society of Comparative Gastroenterology

### Specific Operational Taxonomic Units can be used as potential diagnostic markers for inflammatory bowel disease in dogs? A preliminary metagenomic analysis study

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The intestinal microbiome composition and its interactions with the host are important elements in the pathogenesis of gastrointestinal disorders. In small animals, several studies have demonstrated that acute and chronic gastrointestinal diseases are associated with alterations in fecal microbial communities. The aim of this study was to evaluate possible taxonomic biomarkers of inflammatory bowel disease (IBD) using 16S rDNA amplicon sequencing in dogs. Inclusion criteria were histological evidence of intestinal inflammatory cellular infiltration on endoscopy biopsies according to the WSAVA parameters, availability of the clinical activity index (CCECAI) and the microbiota profile. Exclusion criteria included treatment with anti-inflammatory or antimicrobials drugs within 2 months before presentation. We included in the study 360 samples: 326 from dogs with IBD and 34 from control dogs that were clinically healthy and had no gastrointestinal disease in the last year. Microbiome DNA of aliquots were extracted, six hypervariable regions of the 16S genes (V2, V3, V6+V7, V8, V9) were amplified and sequenced on Ion Torrent. The sequences were processed to separate the regions and then analyzed with Mothur to find Operational Taxonomic Units (OTUs), their abundances and taxonomies by using relational database (RDP) as a reference. Alpha and beta diversity for V4 was calculated by using the Phyloseq R. OTUs were significantly different in the two groups of dogs by DESeq2 analysis. There was no significant difference in alpha diversity between IBD and healthy dogs, and it was not possible to discern them in ordination plots. DESeq2 analysis resulted in 34 differentially abundant OTUs belonging to phyla Bacteroidetes, Firmicutes, Proteobacteria. Bacteroidetes were significantly more abundant in IBD dogs with Bacteroides and Prevotella as the most represented Genera. Among Proteobacteria Phylum, larger abundances of Enterobacteriacee, Anaerobiumspirillum, Sutterella and Helicobacter were observed in IBD dogs, while Burkholderia was more abundant in healthy dogs. Taxa within the Firmicutes phylum, including Clostridium-XIVb, Streptococcus and Enterococcus were also more abundant in IBD dogs compared to healthy dogs. Lachnospiranacee data were conflicting as OTUs were significantly abundant in both healthy and IBD dogs. As previously reported, Collinsella was found more abundant in IBD dogs. Our data are consistent with previous studies suggesting intestinal microbial dysbiosis in dogs with IBD. As new finding, we identified further gut microbial taxa significantly increased in dogs with IBD that might be useful to better understand the etiology of chronic gastrointestinal diseases and might represent diagnostic markers for IBD.

**Disclosures**

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## ESVC-P-1 - European Society of Veterinary Cardiology

### Relationship between weight and aortic annulus in male and female Boxer dogs

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A relationship between aortic annulus diameter, body weight (BW) and gender in Boxer dogs has been already documented by our group (poster 29TH ECVIM-CA Congress). It has been shown that aortic annulus dimension increases with increasing of BW and we can state that BW based 95% confidence interval may help in screening dogs for heart disease, discriminating normal aortic annulus dimension.

The objective of this study is to analyse the model of the relationship between BW and aortic annulus dimension in male and female Boxer dogs.

Cardiovascular screening conducted between November 1999 and March 2018 were reviewed. Four thousand two hundred one Boxer dogs free from cardiovascular diseases were included. The dogs for which gender data was missing ($n = 16$) and the outliers in terms of weight ($n = 2$ over 45 kg) or annulus ($n = 119$; annulus less than 15 mm) were excluded from the statistical analysis, performed on a total of 4064 dogs.

The starting hypothesis was that the relationship between weight and annulus is linear: $y = \beta x + \varepsilon$ (model 1), where $y$ = annulus; $x$ = weight; $\beta$ = weight coefficient; $\varepsilon$ = error. Since we assumed that the relationship could be not linear, we also tested the possibility of entering the

powers of x. To do this, we estimated the coefficients of the following models: $y = \beta x + \gamma x^2 + \varepsilon$ (model 2) and $y = \beta x + \gamma x^2 + \delta x^3 + \varepsilon$ (model 3). The results obtained were then compared to verify which model is the most suitable by the evaluation of the following indicators: $R^2$, Bayesian information criterion (BIC), Akaike information criterion (AIC), Vairance Inflation Factors (VIF), Ramsey RESET test. Once the best model was identified, this was further verified by the stepwise procedure.

The results show that for female dogs, model 2 best explains the relationship, which is increasing proportional up to about 27 kg, while for male dogs, model 1 provides a statistically significant coefficient. Consequently, the relationship between weight and aortic annulus diameter for male Boxer is linear and for each kg more of the dog, the annulus increases by 0.118 mm.

This study suggests that there are two different models of relationship between body weight and aortic annulus diameter between male and female dogs, however knowing the weight and gender of the dog is not enough to predict exactly the size of the annulus.

**Disclosures**

No disclosures to report.

## ESVC-P-2 - European Society of Veterinary Cardiology

### Diagnostic accuracy of the precordial lead system for the detection of right ventricular enlargement in dogs

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Electrocardiography (ECG) is the *gold standard* method for the diagnosis of cardiac arrhythmias at rest. However, 12-lead ECG can also help in the detection of cardiac chamber enlargement. Different precordial lead systems have been described in dogs. The most recent method proposed by Kraus et al. 2002 and modified by Santilli et al. 2019 has not been evaluated for the assessment of right ventricular enlargement (RVE). The aim of this study was to evaluate the diagnostic accuracy of this precordial lead system for the diagnosis of RVE in dogs.

This was a prospective observational study, including dogs with RVE and a control group of healthy dogs. All dogs underwent 12-lead ECG and standard echocardiography. The Q wave amplitude (mV), the R wave amplitude (mV), the S wave amplitude (mV), and the R/S wave ratio were assessed in limb leads (I, II, III, aVR, aVL, aVF) and precordial leads (V1, V2, V3, V4, V5, V6). The mean electrical axis of the QRS complex on the frontal plane was calculated. The diagnostic accuracy was assessed using the ROC curve analysis, and an area under the curve (AUC) > 0.7 defined good diagnostic accuracy. The best cut-offs for detection of RVE were calculated using the Youden index.

A total of 84 dogs were enrolled, 27 with RVE and 57 controls. Among dogs with RVE, 14 had pulmonic stenosis, 11 pre-capillary pulmonary hypertension and 2 tricuspid valve dysplasia. Considering limb leads, only Q wave amplitude in aVR showed good diagnostic accuracy for RVE [cutoff >0.10 mV, sensitivity (Se) 53%, specificity (Sp) 95%, AUC=0.727]. Right shift of the mean electrical axis was evident in 9/27 dogs with RVE (Se 33%, Sp 95%). Combining Q wave in aVR and presence of right shift, 12/27 (44%) dogs with RVE were correctly identified. Considering precordial leads, S wave in V6 (cutoff >0.70 mV, Se 52%, Sp 92%, AUC=0.703), R/S ratio in V4 (cutoff <1.15, Se 63%, Sp 96%, AUC=0.842), and R/S ratio in V5 (cutoff <1.95, Se 69%, Sp 92%, AUC=0.839) showed good diagnostic accuracy for detecting RVE. Among dogs with RVE, 19/27 (70%) showed at least one of these precordial lead findings.

Using this precordial lead system, S wave amplitude in V6 and R/S ratio in V4-V5 showed the best diagnostic accuracy for the detection of RVE. Adding these precordial criteria to standard 6-lead ECG evaluation, it increases the diagnostic accuracy for RVE detection in dogs.

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No disclosures to report.

## ESVC-P-3 - European Society of Veterinary Cardiology

### Laryngeal hemiplegia following surgical ligation of a patent ductus arteriosus in 4 dogs

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Patent ductus arteriosus is one of the more common congenital cardiac anomalies in dogs. Currently its treatment modalities consist of surgical ligation by thoracotomy (/thoracoscopy) or interventional embolization by coils or Amplatz™ devices. When the ductus is fully closed, life expectancy and exercise tolerance is normal in nearly most cases. In children left vocal cord paralysis is a significant side-effect of PDA closure in preterm neonates and has been associated with low weight at time of surgery. Unilateral laryngeal paralysis post-surgical PDA closure has been described in the feline breed.

In our referral center where dogs are routinely followed up to several years after successful closure, we experienced permanent voice changes and reduced exercise tolerance associated with exertional stridor in 4 young dogs. All dogs underwent traditional surgical ligation of the ductus, via 4$^{th}$ intercostal left-sided thoracotomy.

Four different breeds were represented with dogs weighing from 5-25 kg at the time of surgery. The first clinical signs were observed one month post-closure with voice changes occurring first, and panting and reduced exercise intolerance appearing later, but always within one year of closure. All dogs also had intermittent signs of mild

dysphagia. Cough was present in none. Laryngeal auscultation revealed increased laryngeal sounds in all 4, however no stridor was observed at rest, only after exercise in all dogs. Thoracic radiographs were within normal limits for all dogs. Doppler echocardiography revealed full closure of the PDA in all dogs.

Endoscopy together with a doxapram challenge confirmed the stridor being secondary to left arytenoid immobility. The cause of the laryngeal hemiplegia is thought to be iatrogenic damage to the left recurrent nerve during surgery. In our center none of the embolized dogs have experienced left-sided hemiplegia, despite this also being described in children.

So far (4-8 y post) none of the dogs needed lateralization of their paralyzed left vocal cord. However, the warned owners are very careful with exercising the dog in hot weather. All dogs are still alive and none experienced aspiration pneumonia.

In conclusion, the presence of vocal changes and exercise intolerance post-PDA closure should alarm the veterinary surgeon to perform respiratory endoscopy. Handling of the vagal nerve and its recurrent branch during surgery should be done very cautiously. Size and weight of the dogs do not seem to play a role.

**Disclosures**

No disclosures to report.

## ESVC-P-5 - European Society of Veterinary Cardiology

### Effects of levomepromazine on left ventricular systolic function and blood pressure in propofol and isoflurane-anesthetized dogs

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Phenothiazines are commonly used in anesthetic premedication. These drugs produce transitory hypotension and have a negative inotropic effect. The objective of this study was to evaluate and compare the effects of two different doses of levomepromazine on systolic function and blood pressure in propofol and isoflurane-anesthetized dogs. Eighteen 1-5 years old, 10-30 kg healthy bitches derived for ovariohysterectomy were included in this study. In all the animals, primary cardiac diseases were ruled out. The bitches were randomly assigned to one of three groups according to the premedication used: levomepromazine at 0.25 mg/kg IV (L25, n=6), levomepromazine at 0.5 mg/kg IV (L50, n=6) and acepromazine at 0.05 mg/kg IV as control group (ACE, n=6). All the bitches received tramadol at 3 mg/kg IV. Anesthesia was induced by administering propofol (6 mg/kg IV) followed by a continuous infusion at 25 mg/kg/h, then maintained with isoflurane (2,5%) in oxygen (2 L/min) administered through a circle rebreathing system. Bitches were evaluated before premedication administration (T0) and 10 (T1) and 45 minutes after (T2) premedication administration; 3 minutes after propofol administration (T3) and 10 minutes after isoflurane administration (T4). Shortening fraction (SF), ejection fraction (EF), systolic volume (SV) and cardiac output (CO) were echocardiographically assessed while systolic (SBP), diastolic (DBP) and mean (MBP) blood pressures were evaluated with an oscillometric monitor. A standardized sedation score based on vocalization, posture, appearance, interactive behaviors, restrainability, noise response and analgesia was assessed at T0, T1 and T2. All variables were converted to percentage change ([Final value-initial value/initial value]*100), and analyzed by ANOVA for repeated-measure followed by Tukey test, while sedation score was analyzed by Kruskal-Wallis test. All variables were similar among the groups at the beginning of the study (P>0.1). No differences were found in sedation scores at each evaluation point (P>0.1). Interactions between time and group were found for SF (P<0.01), EF (P<0.01) and SV (P<0.01). These variables decrease throughout the study, however ACE showed a higher fall than L25 and L50 from T1 to T4. CO (P<0.01), SBP (P<0.01), DBP (P<0.01) and MBP (P<0.01) decreased in all bitches during the study, but no differences were found among groups (P>0.1). This is concluded that premedication with levomepromazine exerts a decrease of left ventricular systolic function and blood pressure. However, for the same level of sedation, it showed a lower impact on left ventricular systolic function than acepromazine.

**Disclosures**

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## ESVC-P-6 - European Society of Veterinary Cardiology

### Thin and akinetic left ventricular myocardial segments in cats

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Thin and akinetic myocardial segments (TAMS) represent a form of adverse left ventricular (LV) remodeling in humans with hypertrophic cardiomyopathy. We aimed to describe the echocardiographic features and outcome of cats with TAMS and to describe the cardiac phenotype before development of TAMS (pre-TAMS).

Clinical records of multiple referral centres were searched for cats with TAMS, defined as LV segment(s) with end-diastolic wall thickness

(LVWT) <4mm and decreased wall motion in the presence of ≥1 LV segment with LVWT >4mm and normal wall motion.; 64 cats with TAMS were identified. Thickest LV segment (MaxLVWT) was 6.1mm (5.8-6.4) and thinnest (MinLVWT) 1.6mm [0.6-3.7]. MaxLVWT-systolic excursion was 4.0mm (3.7-4.3) and MinLVWT-systolic excursion 1.4mm (1.2-1.5). Mean left atrium/aorta was 2.4 (2.0-2.9) with poor atrial (LA) and LV systolic function. TAMS affected the LV free wall in 77%, LV apex in 12.5% and interventricular septum in 5%. Median troponin-I was 1.5ng/ml [0.07-180]. 13/64 cats had an echocardiogram 2.5 years (1.3-3.7) pre-TAMS. LV segments undergoing thinning measured 6.7mm (5.8-7.7) pre-TAMS versus 1.9mm (1.5-2.4) at last echo (p<0.0001). Left atrial size increased (LA/Ao 1.7 (1.5-1.9) versus 2.1 (1.8-2.4), p=0.04). LA and LV systolic function decreased. 9/13 cats had systolic anterior motion (SAM) pre-TAMS, and 7/13 cats lost SAM over time (p=0.02). Median survival time was 46 days (9.1-83.8). Cardiac micro-CT and histopathology in 1 cat revealed that TAMS was caused by severe transmural scarring with replacement fibrosis.

TAMS is associated with transmural fibrosis suggesting a severe myocardial insult (e.g. infarction) and has a poor prognosis.

**Disclosures**

No disclosures to report.

## ESVC-P-7 - European Society of Veterinary Cardiology

### Breed and sex affect serum cardiac troponin I concentration in healthy cats

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Cardiac troponin I (cTnI) is a sensitive biomarker for myocardial damage. High sensitivity cTnI assays allow detection of low concentrations in serum. In people, sex-specific reference intervals are used. Breed differences for cTnI have been found in healthy dogs. In cats, cTnI concentrations have, hitherto, not been associated with sex nor breed.

The objective was to investigate associations between serum concentration of cTnI and breed, sex, age, bodyweight, and body condition score in healthy cats.

Ninety-six healthy Norwegian Forest (NF, N=33), Birman (N=33), and domestic shorthair (DSH, N=30) cats were prospectively included. The health examination included blood pressure measurement, physical examination, echocardiography, hematology, and biochemistry. Concentrations of cTnI are presented as median and interquartile range (IQR).

A chemiluminescent microparticle immunoassay for human cTnI was validated for cats using surplus serum. Within run, between run, and within lab assay coefficients of variations were <9 %. Cardiac troponin I was detectable in 89/96 cats. Using multiple regression analysis, cTnI was associated with breed and sex. Norwegian Forest cats had lower median cTnI concentration (4.0 (IQR 2.6-7.7) ng/l) than Birman (7.6 (IQR 3.3-14.4) ng/l, $P$ =.005) and DSH (7.7 (IQR 3.0-16.1) ng/l, $P$ =.023) cats. Intact female cats had lower cTnI concentration (3.7 (IQR 2.1-7.5) ng/l) than neutered female (6.3 (IQR 3.3-11.5) ng/l, $P$ =.045) and neutered male (7.5 (IQR 3.0-16.0) ng/l, $P$ =.0067) cats.

In summary, serum concentrations were lower in NF cats than in Birman and DSH cats, as in intact female cats than in neutered females and neutered males.

**Disclosures**

No disclosures to report.

## ESVC-P-8 - European Society of Veterinary Cardiology

### Clinical, echo-Doppler features and prognosis of dogs with chordae tendineae rupture related to degenerative mitral valve disease (500 cases, 2009-2019)

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Degenerative mitral valve disease (DMVD) is the most common heart disease in dogs, characterized by myxomatous degeneration of the mitral valve involving both leaflets and chordae tendineae. Chordae tendineae rupture (CTR) is a well-recognized DMVD complication, leading to mitral regurgitation worsening and, potentially, congestive heart failure (CHF). Different types of CTR are described, i.e., ruptures of CT attached to the anterior (AML) or posterior mitral valve leaflet (PML) and, in both cases, ruptures of first-order CT (inserted on the free edges of the leaflets) or second-order CT (inserted on the undersurface of the leaflets). Clinical and prognostic data about CTR is currently lacking and the impact of CTR type on survival remains unknown. The aims of this retrospective study were therefore: 1) to describe the epidemiological, clinical, and echo-Doppler characteristics of a large population of dogs with CTR related to DMVD, 2) to compare dogs according to the CTR type, and 3) to identify potential variables associated with survival. A total of 500 small-breed dogs with DMVD and CTR diagnosed by echocardiography were included

in the study (median [interquartile ratio, IQR] age =11.2 years [9.0-13.0], male-to-female ratio =2.0, weight =7.0 kg [4.9-9.1]). Most dogs had clinical signs, including cough (61%) and dyspnea (25%), with CHF (pulmonary edema) in 35% cases (174/500). Ruptured CT were more frequently observed on the AML (300/403, 74%) than the PML (121/403, 30%) and were more often second-order (276/417, 66%) than first-order CT (153/417, 37%). The left atrium (LA) and the left ventricle were dilated in respectively 59% (295/500) and 63% (316/500) dogs, and these dilatations were more frequently observed in dogs with first-order than second-order CTR (p<0.01 and p=0.03, respectively). The median (IQR) regurgitation fraction (RF) assessed by the PISA method was 64% [52-76], with pulmonary arterial hypertension identified in 454/484 (94%) dogs. Follow up was available for 400 dogs: median survival time for cardiac-related death was 26.8 (9.1-54.2) months. Univariate analysis identified a significant association between cardiac death and presence at inclusion of CHF, thrilling murmur, cardiovascular clinical signs, diuretic pre-treatment, arrhythmias, LA dilation, LV dilation, RF ≥50%, systolic pulmonary arterial pressure ≥50 mmHg. Survival times were not significantly different according to CTR types. More advanced ACVIM stages were associated with shorter survival times. In conclusion, this study demonstrates that LA and LV dilations are more frequently observed with first-order CTR, but survival time was not significantly affected by CTR types.

**Disclosures**

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## ESVC-P-9 - European Society of Veterinary Cardiology

### Survival analysis and predictive value of global and regional right ventricular function variables assessed by conventional echocardiography, speckle tracking imaging and two-dimensional color tissue Doppler imaging, in dogs with congenital pulmonic stenosis: a prospective study of 75 cases (2013-2020)

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Pulmonic stenosis (PS) is one of the most common congenital canine heart diseases. A previous study demonstrated that dogs with PS show various systolic and diastolic right ventricular (RV) alterations associated with mechanical intra-RV dyssynchrony, as confirmed by conventional echocardiography (CE), speckle tracking echocardiography (STE) and two-dimensional color tissue Doppler imaging (TDI). However, to the best of our knowledge, no study has investigated the predictive value of global and regional RV function variables derived from CE, STE, and TDI in dogs with PS.

The aim of this prospective observational study was therefore to document survival times of dogs with PS and identify risk factors for cardiac death (CD) among CE, STE, and TDI variables of systolic and diastolic RV function. Tested imaging variables included tricuspid regurgitation severity, right atrial dilation, RV dilation, RV free wall (RVFW) hypertrophy, maximal trans-stenotic pressure gradient (MTSPG), right-sided congestive heart failure (CHF), RV fractional area change (RFAC), indexed tricuspid annular plane systolic excursion (iTAPSE), RVFW longitudinal systolic strain (StS), StS base:apex ratio, systolic and diastolic STE strain rate (SR), post-systolic strain percentage (PSS%), early pre-stretch index (early pre-stretch/[early pre-stretch+systolic StS]), synchrony time index (difference in timing of peak RVFW StS from the earliest to latest RVFW segment), TITMD (time interval from end of T wave to maximal deformation), and segmental RVFW TDI systolic and diastolic velocities.

The study sample consisted of 75 dogs with PS, 18 dogs were excluded from the analysis due to lack of follow-up (n=4) or balloon valvuloplasty procedure (n=14). Among the 57/75 dogs for which a follow-up was available 15/57 (26%) died during the study period, with CD in 12/15 (80%) dogs. As more than 50% of PS dogs survived during the study period, median survival time was not determined, with 58% dogs living more than 6 years. Univariate analysis revealed that only tricuspid regurgitation severity (p <0.0001), right atrial dilation (p =0.037), RFAC (p <0.0001), presence of CHF (p <0.0001), early pre-stretch index (p=0.015) and PSS% (p =0.047) were significantly associated with shorter median survival times to CD. Interestingly, the MTSPG showed no statistically significant association with survival time to CD, considering that most dogs had a high MTSPG.

In conclusion, these results confirm that in addition to tricuspid regurgitation severity, right atrial dilation, and CHF, decreased RFAC, early pre-stretch, and PSS% are also associated with shorter times to CD in dogs with PS.

**Disclosures**

No disclosures to report.

## ESVC-P-10 - European Society of Veterinary Cardiology

### Two-dimensional echocardiographic estimates of left atrial volumes obtained from two different views in dogs are similar but not interchangeable

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Echocardiographic left atrial (LA) volume estimates can help clinicians to quantify LA dimension and function in dogs. Little information

currently exists regarding the interchangeability of LA volume estimates using a monoplane Simpson's Method of Discs (SMOD) on images obtained from the left apical four-chamber (LA4C) and right parasternal long axis four-chamber (RPLA) views. Therefore, we sought to examine the agreement between the two methods of obtaining LA volumes in a heterogenous population of healthy dogs and dogs with various cardiac diseases affecting the left heart. Additionally, we compared the LA volumes obtained by SMOD with estimates obtained from cube or sphere volume formulas using linear dimensions.

Archived echocardiographic examinations were retrieved and, where both RPLA and LA4C views were recorded, included in the study. We obtained measurements from 130 dogs that were either apparently healthy (n=32) or had various left-sided cardiac chambers diseases (n=98). The LA volume of each dog was measured using a monoplane SMOD, from both views, in systole and diastole. Estimates of LA volume based on the RPLA-derived LA diameters (cube or sphere volume) were also calculated. We then used Limits of Agreement analysis to determine agreement between the estimates obtained with each view, and those calculated from linear dimensions.

The two methods obtained by SMOD provided similar estimates for both systolic and diastolic volumes but did not agree sufficiently to be interchangeable (absolute differences were mostly <10ml). The LA4C method tended to slightly underestimate (small LA sizes) and overestimate (large LA sizes) LA volume compared to RPLA method, with increasing disagreement as the LA size increased, for both systolic and diastolic volumes. Estimates based on cube method overestimated volumes compared to both SMOD methods, while the unidimensional volume estimates using the sphere method showed agreement with the SMOD estimates similar to that obtained when comparing the two SMOD estimates.

Our study suggests that SMOD estimates of the LA volume from the two echocardiographic views are similar but not interchangeable. Clinicians might consider using LA4C-derived LA diameters to estimate LA volume by sphere volume formula.

**Disclosures**

No disclosures to report.

**ESVCN-P-1 - European society of Veterinary & Comparative Nutrition**

## Assessment of a proactive approach to pet obesity prevention by Portuguese veterinary nurses and technicians

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Obesity is a disease of increasing prevalence in human and pet populations. Although veterinarians have a strong influence in educating owners on the importance of this disease, veterinary nurses and technicians (VNT) also have an active role on it. This study aims to evaluate the behaviour of veterinary nurses and technicians from Portugal in managing obesity in cats and dogs.

An observational cross-sectional survey-based study was carried out and spread through VNT groups on social media. The study covered 31 questions about the importance given to obesity, the existence of a weight management programme in their practices, if they would recommend it in overweight patients and what should be done to enhance awareness of this disease.

A total of 126 responses from veterinary nurses (61%) and technicians (39%) were collected. The majority of the respondents (94%) consider obesity an important disease and 90% defend that a weight management programme is crucial, whilst only 48% of VNT refer the patient for it. Of the remaining participants, 30% reveal that besides not running a weight loss programme, they do not know where to refer the animal; and 21% address that it is not their responsibility to make such recommendation. A total of 58% of VNT admitted never or rarely assessing the body condition score (BCS), which is mainly due to the fact that it is not part of their normal routine (47%) or because they see it is as a veterinary surgeon's task (22%). These are also the most frequent reasons why 36% of VNT do not provide a nutritional assessment to pets. About 71% of the respondents classify obesity as an easy preventable disease, but only 39% find it easy to manage. According to the participants' responses, obesity management should be more often promoted by the pet food industry (67%) or through specific lectures (58%).

This study supports that Portuguese VNT are aware of obesity epidemic. Nevertheless, BCS is not assessed by a high percentage of them and more than half of the respondents do not recommend a weight loss programme, either by in-clinic unavailability or because it is considered a veterinarian's responsibility. Although almost three-quarters consider obesity easy to prevent, only a few find it effortlessly treatable, stressing the need for further knowledge in this subject. Eventually, only by promoting a taskforce between doctors and VNT, will we be able to fight obesity in a more foreseeing perspective.

**Disclosures**

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**ESVCN-P-2 - European society of Veterinary & Comparative Nutrition**

## Are Portuguese veterinarians taking the initiative on preventing small animal obesity? - A survey-based study

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Obesity is an emerging disease in companion animals. Several studies have been published associating owner's behaviour with the risk of developing this disease in cats and dogs, however, less is known about the veterinary

staff conduct. This study aims to assess veterinary surgeon's attitudes as well as the risk factors that can impact pet obesity in Portugal.

An observational cross-sectional survey-based study was conducted, consisting on 31 questions, shared online through private network groups for Portuguese veterinarians. Questions focused on the importance given to obesity in clinical practice, in which medical situations veterinarians would emphasize this problem to the owner, if a weight management programme was set in their clinical practice and if they would recommend it in overweight pets.

A total of 395 answers were obtained. Among the respondents 92% acknowledged consulting overweight pets on a daily-basis or several times a week and 67% confessed that always alert owners about it. Pets are routinely weighted by 99% of the veterinarians, though 53% never or rarely assess body condition score. Although recommended by WSAVA Nutrition Guidelines, only 17% of the participants evaluate the animal nutritional status in every visit. Facing an overweight patient, participants admitted they recommend owners a weight loss programme (81%), a change in diet (76%), a bloodwork panel (37%) or an endocrine screening profile (35%). The majority of the clinicians highlight the risk of obesity when consulting a predisposed breed (86%), a puppy/kitten (81%), patients with osteoarthritis (99%) or with any endocrinopathy (92%). This topic is also addressed before spay/neuter (94%), recommending an alimentary change at surgery discharge (83%). About 54% of the participants reveal that obesity was an unimportant subject throughout their university training. A weight management programme is judged essential by 89% of the respondents; however, about 57% of clinicians do not run it and 78% of these do not know where to refer the patient.

This study supports that Portuguese veterinarians are overall aware about obesity in pets. Nonetheless, body condition score and nutritional assessment are not routinely performed by most of them. The majority of the participants do not have an established weight management programme, which reflects the need for its existence in Portuguese veterinary practices. Finally, obesity was considered an irrelevant topic during veterinary degree by more than half of the respondents, highlighting the possibility that the seriousness of this disease is not being given due importance.

**Disclosures**

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**ESVCN-P-3 - European society of Veterinary & Comparative Nutrition**

## A cohort study to examine associations between initial weight loss outcomes and overall success of a controlled weight loss programme in dogs with obesity

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There has been little research on whether success at the start of a controlled weight loss programme (i.e. the first month) is important for overall success. Therefore, the current study aimed to assess initial weight loss outcomes and then to determine associations between such early outcomes and overall success.

This cohort study involved 272 dogs referred to a specialist clinic for investigation and management of obesity. After initial enrolment, each dog underwent a tailored controlled weight loss plan, using either wet or dry (or combinations) therapeutic weight management diets, and had to have completed at least 28 days of their programme. Dogs were then followed until they had either reached their target weight or did not complete (for various reasons). Differences in continuous variables, between dogs reaching target or not completing, were compared with either T-tests or Mann-Whitney tests (as appropriate), whilst proportions were compared with Chi squared tests. Variables associated with total percentage weight loss were assessed with multiple linear regression, with the model refined by forwards and backwards stepwise regression. Data are expressed as median (interquartile range).

For the whole programme, total percentage weight loss was 20% (12-28%) over a period of 240 (140-373) days. Average metabolisable intake during this time was 60 (56-64) Kcal per $kg^{0.75}$ starting weight (SW) per day. In all, 150 dogs (55%) reached their target weight, whilst the remaining 122 dogs (45%) did not complete. Reasons for not completing included inability to contact owner, refusal to comply with the weight loss programme, development of another illness (including dogs that were euthanased). The median period of initial weight loss was 32 (29-42) days in dogs that ultimately reached target and 34 (29-40) in those not completing ($P$=0.206). During this time, dogs reaching target lost 6.2% (4.7-8.4%) SW, whilst dogs not completing lost 4.5% (2.5-6.6%) SW ($P$<0.001). Therefore, initial rate of weight loss was 1.3% (1.0-1.7%) SW/week and 0.9% (0.4-1.6) SW/week in dogs that reached target and stopped for other reasons, respectively ($P$<0.001). Using multiple linear regression, starting body fat percentage ($P$<0.001), reaching target weight ($P$<0.001) and initial rate of weight loss ($P$<0.001) were all positively associated with the total percentage of weight loss.

Initial rate of weight loss is faster in dogs that ultimately reach target weight, than those not completing. Therefore, it is suggested that veterinary professionals should focus on ensuring success during the early stages of any weight management plan.

**Disclosures**

The study was funded by a grant from Royal Canin, a division of Mars Petcare, and this company manufactured the diets fed in this study. Vincent Biourge and John Flanagan are employees of Royal Canin. Alexander J. German and Georgiana R.T. Woods are employees of the

University of Liverpool but their positions are funded by Royal Canin. Both have received financial remuneration and gifts for providing educational material, speaking at conferences, and consultancy work.

## ESVCN-P-4 - European society of Veterinary & Comparative Nutrition

### Outcome and factors affecting success of a controlled weight loss program for dogs

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Canine obesity is a major problem in veterinary practice, increasing the risk of several diseases and significantly shortening life expectancy. Even though most dog owners are familiar with the consequences of obesity, many are still reluctant to engage in a controlled weight loss program with their dog. The aim of the current study was to identify factors associated with success in a controlled weight loss program.

Dogs that had been enrolled in a controlled weight loss program at the veterinary hospital from August 2015 to September 2019 were identified in the electronic medical record system. In case of acceptance to participate, owners were asked to fill out a questionnaire. Data relating to the weight loss process was collected from the medical records. To identify factors associated with weight loss success, a multivariate analysis was conducted.

Owners of 24 dogs (12 male (all neutered) and 12 female (7 neutered, 5 intact)) accepted to participate. Median age was 5.8 (1.6-12.4) years, median body weight (BW) was 12.5 (3-56.6) kg and 12/24 dogs had a concurrent chronic disease (7 orthopedic, 3 respiratory, 1 neurologic and 1 gastrointestinal). At initiation dogs were evaluated to have a median body condition score of 7 (5-9) corresponding to an average of 25% (8-40%) overweight. Average duration of weight loss intervention was 244 (0-854) days. Fifteen dogs completed the program and nine dogs stopped prematurely. Average weight loss for the dogs completing the program was 17.2% (7.2-26.6%) of initial BW, with a weekly weight loss rate of 0.7% (0.2-1.4%). Dogs stopping prematurely lost an average of 6.6% (-3.9-32%) with a weekly weight loss rate of 0.3% (-0.2-0.8%). Only 30% of owners experienced an increase in food seeking behavior. Despite significant encouragement to increase the dogs' physical activity, only 4.3% succeeded with increasing time spent walking the dog. Factors associated with achieved weight loss was a faster weight loss rate, higher owner BMI, owner's attachment to the dog and owner's ability to change type of treat.

Weight loss is achievable in pet dogs but this study underscores the difficulty in changing habits such as increasing duration of dog walks. Changing as few habits as possible such as exchanging treats to less energy dense alternatives seems to increase the chance of success compared with expecting owners to significantly alter treat and activity level. Still, the number of dogs stopping prematurely underscores that prevention of obesity is better than the cure.

### Disclosures

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## ESVCN-P-5 - European society of Veterinary & Comparative Nutrition

### Experiences with feeding a hydrolyzed protein diet to dogs with acute diarrhea: A prospective pilot study

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Severe acute gastroenteritis has been associated with an increased risk of developing chronic enteropathies. A compromised gut barrier function and increased food allergen exposure to a reactive immune system might delay recovery and predispose to chronic enteropathy. This prospective, block-randomized, open-label, single-center study aimed at investigating whether feeding a hydrolyzed protein diet to dogs hospitalized with acute diarrhea lead to a faster recovery compared with a commercial gastrointestinal diet (GI-diet)

Dogs ≥12 months old, hospitalized with acute diarrhea and without specific dietary requirements were allocated to either a hydrolyzed test diet (95% proteins<1kDa; Royal Canin Anallergenic (RCA)) or a GI-diet. Dogs undergoing surgery or receiving pre- or probiotics during hospitalization were excluded.

Daily recordings on clinical and laboratory findings, AHDS index and treatment were collected. Following initial antiemetic treatment and stabilization, food was offered and syringe-feeding initiated if they did not eat voluntarily. RCA was dissolved in water to make a gruel appropriate for syringe feeding and for dogs preferring wet diet. Dogs not accepting the assigned diet where offered an alternative diet and excluded if they accepted the alternative. All owners were recommended to continue the allocated diet following discharge. Data are presented as (median, IQR).

Twenty-six dogs were enrolled (RCA N=12; GI-diet N=14). One dog in each group was excluded due to pancreatitis. The AHDS index was similar between groups at admission. After enrollment 45% of RCA

dogs refused to eat the allocated diet but accepted the offered alternative (5/11 dogs: GI canned N=4; cooked chicken N=1) and were excluded. These dogs had a higher AHDS index (14, 13-15) on admission compared to dogs remaining in the RCA group (11.5, 11-12).

One dog in the RCA group was euthanized on day 3 due to development of acute kidney injury and intravascular hemolysis. The median duration of hospitalization was one day for the remaining five dogs, with no significant difference compared to excluded RCA dogs. One GI-diet dog was excluded on day two because the owner elected probiotic treatment. Median hospitalization duration was two days for remaining GI-diet dogs.

The high number of exclusions in the RCA group due to preference for alternative diets precluded further analyses. A highly hydrolyzed diet, usually well accepted by dogs with chronic enteropathies was elected. However, the gruel preparation may have adversely impacted palatability and therefore diets offering both canned and dry versions should be preferred for dogs with severe acute gastrointestinal disease.

**Disclosures**

No disclosures to report.

## ESVCN-P-6 - European society of Veterinary & Comparative Nutrition

### Outcome related to acquired Fanconi syndrome associated with ingestion of jerky treats in 30 dogs

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Acquired canine proximal renal tubulopathy (Fanconi syndrome) related to excessive ingestion of jerky treats has been recognized since 2007. Apart from a retrospective study of 108 dogs, current literature mainly consist of case reports/case series. This retrospective observational study aimed to improve the knowledge about disease characteristics, recovery and long-term prognosis specifically related to quantity of jerky ingestion and progression to chronic kidney disease (CKD).

Dogs suspected of previous or current acquired Fanconi was recruited from May 2019 to June 2020 by reaching out to veterinarians and owners through social media and national veterinary magazines. Medical records were reviewed and owners interviewed. Data was analysed using linear mixed models and t-test (R Core Team, 2020, $p<0.05$ was considered significant). Results are stated as median (Range).

Thirty dogs (5 entire, 4 neutered females; 15 entire, 6 neutered males), body weight 6.75 (1.2-59) kg and age 6.5 (0.5-14) years were enrolled. Preceding/concurrent medical conditions were recorded in 21/30 dogs – none of which were renal disease. Clinical signs included polydipsia (23/30), polyuria (21/30), lethargy (19/30), weight loss (15/30), hyporexia (11/30), vomiting (7/30) and diarrhoea (7/30). Two dogs showed no clinical signs. All 30 dogs presented with normoglycemic/hypoglycemic glycosuria. Additional paraclinical findings included azotemia (6/28), hypophosphatemia (9/25), metabolic acidosis (3/8), hypokalemia (6/20), proteinuria (13/26), aminoaciduria (4/4), haematuria (22/29) and ketonuria (7/27).

Clinical signs resumed completely in 22/28 dogs before study ending. Owners reported ongoing clinical signs for 5/30 dogs despite resolution of glycosuria in 4/5 on latest recheck (weeks 8, 9, 35, 68 and 72 respectively). Two of the five dogs were subsequently diagnosed with CKD. One dog died for unknown reasons 2-3 months following clinical improvement. For 12/30 dogs, only refraining from giving jerky treats was necessary, 11/30 received supportive medical treatment as outpatients, while 7/30 were hospitalised for 4 (1-9) days. Time to owner estimated clinical resolution was 11 (0.3-52) weeks, while glycosuria resolved in 6.5 (1-31) weeks. Medical treatment (as outpatient or hospitalised) did not affect time to clinical resolution ($p=0.55$). There was no association between indicators of glomerular disease (creatinine, urea, symmetric dimethylarginine) and the amount/duration of jerky ingestion.

Apart from a larger percentage of dogs achieving complete clinical resolution the current findings are in agreement with previous reporting. The low incidence of azotaemia as well as lack of association between glomerular damage and owner quantitation of jerky feeding support the conception that jerky primarily induces tubular renal disease.

**Disclosures**

No disclosures to report.

## ESVCN-P-7 - European society of Veterinary & Comparative Nutrition

### Exploration of body weight in 115 000 young adult dogs of 72 breeds

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High body weight (BW) in dogs, resulting from large size or excess body fat, has been associated with developmental and degenerative diseases, negatively affecting quality of life. The BW of young adult dogs of different breeds is not well explored and, especially, it is unknown whether breed BW has changed over time. The study objectives were to determine mean BW in young adult dogs of different breeds, including changes in breed BW over a ten-year period.

Mandatorily recorded BW data from the official hip dysplasia screening program of the Swedish kennel club were used. Data from dogs screened at 1-2.5 years of age, in breeds with at least 15 individual BW observations per year during 2007-2016, were included. General linear models were used to evaluate mean BW and changes in BW for each breed separately, with BW considered a continuous variable.

Mean BW per breed and sex was established from 114 568 dogs representing 72 breeds. A significant difference in mean BW between sexes was present in 54 (75%) of the breeds. Estimates of breed BW showed a significant change in 33 breeds over the ten-year period, with an increase in five breeds and a decrease in 26 breeds. The increase in the five breeds ranged from 0.8 to 3.1 kg, representing 1.9 to 14% change in BW, respectively. Of the 26 breeds with a decrease in BW, 22 breeds did not show a sex difference in estimates, with BW changes ranging from -0.4 to -2.2 kg, (1.4 to 4.0%), respectively, while in four breeds, estimates differed between male and female dogs. In two of the 33 breeds, the male dogs had increased and the female dogs had decreased in BW.

In conclusion, this study provides extensive data of BW on young adult dogs of different breeds in Sweden. A change in breed BW was noted in around 45% of the breeds with a change greater than 10% in some. Lacking details about body condition, the cause of these changes are difficult to establish; it could be a consequence of either changes in breed-related size or in body fat mass. The change in BW over time in certain breeds might have an impact on the overall health. Thus, monitoring of BW over time can be an important tool in breed health evaluation.

**Disclosures**

Linda Andersson receives salary from the Swedish Kennel Club, SKK. Åke Hedhammar is a consultant for SKK.

**ESVE-P-1 - European Society of Veterinary Endocrinology**

## Use of a mobile phone application to evaluate quality of life of diabetic pets and their owners

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In recent years, impact of diabetes mellitus (DM) and its treatment on quality of life (QoL) of pets and caretakers has been acknowledged as an important factor in treatment decisions. Development of a validated psychometric tool, the DIAQoL-pet, has facilitated scientific quantification and study of this impact.

The current study describes the use of a mobile phone application (app) to provide an extensive up-to-date picture of perceived QoL of diabetic pets and their owners.

An app was designed to support diabetic pet owners in the daily care of their pet, as well as progress scientific understanding of diabetic pet ownership through voluntary anonymous data sharing, including DIAQoL-pet data. The app was made freely available to diabetic pet owners using iPhones and Android smart phones. User-entered data was periodically downloaded between 2016 and 2021. Item-weighted impact scores (IWIS) and average-weighted impact scores (AWIS) were calculated to document QoL-impact characteristics.

Data of 886 diabetic animals (333 dogs; 553 cats) in 26 countries were harvested. The most common dog breeds included West Highland White Terrier (6.3%), Labrador Retriever (5.9%) and Miniature Schnauzer (4.3%); the most common cat breed was Domestic Short Hair (34.3%). DIAQoL-pet data were provided for 191 dogs and 294 cats. The most negative QoL impact proved 'worry about pet's DM' (dog IWIS±SD: -7.4±4.0; cat -8.1±3.5), followed by 'owner's social life' (dog -6.5±4.7; cat -6.3±4.6), 'leaving with friends and family' (dog -5.9±4.4; cat -6.8±4.3) and 'worry hypo' (dog -5.9±4.1; cat -6.0±4.1). Highest positive QoL-impact scores were documented for 'having a more special bond with their pets because of DM' (dogs +5.9±4.9; cats +5.7±4.9). AWIS for dogs was -3.0±2.9; for cats -3.0 ±3.0 while 38.7% of dog owners and 36.1% of cat owners reported their pet's life would be "much better" without DM.

This study reports the first use of an app to study diabetic pet ownership by documenting and monitoring the QoL of diabetic pets and their owners. The data suggests improvements in pet DM management should focus on both the impact of the disease and treatment on the owner as well as pet-owner bond.

**Disclosures**

No disclosures to report.

**ESVE-P-2 - European Society of Veterinary Endocrinology**

## Glycemic variability in non-diabetic, healthy beagle dogs

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The therapeutic aim for diabetes management is to alleviate clinical signs while avoiding acute complications including iatrogenic hypoglycaemia and diabetic ketoacidosis. Although guidelines state that fluctuations and mild hyperglycemic periods are acceptable, current recommendations may be insufficient to avoid long-term sequela such as cataract formation. To define more stringent treatment targets for future studies, knowledge on physiological glycemic variability and diurnal variations are essential. This study aimed to investigate measures of variability (coefficient of variation [CV%], mean amplitude of glycemic excursions [MAGE]) and diurnal variations (% deviations from the individual mean) in a cohort of healthy beagles using a validated flash glucose monitoring system.

Ten male, neutered, clinic-owned beagles were enrolled in a prospective study from August to November 2020. The dogs were in mean (±SD) 3.2 (±1.2) years old and had a BCS of 5 (±0.8) of 9. The sensors were placed on the dorsolateral neck and additionally fixed with an adhesive patch. During the study period which included two 24h glucose profiles, the dogs were kept separately, perceived natural as well as electronic light (6.a.m. to 9 p.m.), were fed their regular dry diet twice daily (7:30 a.m. and p.m.), and had unlimited access to fresh water. In addition to short walks, they were taken on extended walks lasting more than one hour twice daily.

All glucose measurements were transferred from the reader to the manufacturers' server and then to a local Excel data sheet. The 24h-MAGEs were calculated by dividing the sum of all glucose excursions exceeding one standard deviation by the number of excursions. To visualise diurnal glucose variations, glucose measurements were depicted as percent deviations from the mean individual glucose concentrations. Statistical analyses were performed using SPSS version 24 and a P-value <0.05 was considered significant.

Mean, minimum, and maximum glucose concentrations ranged from 5.3-6.4 mmol/L, 3.7-5 mmol/L and 6.8-8.5 mmol/L, respectively. The mean (±SD) CV% was 5.3 (±1.09) on day 1 and 5.6 (±1.72) on day 2 ($p = 0.393$). The mean (±SD) MAGE was 0.86 mmol/L (± 0.19) on day 1 and 0.83 mmol/L (±0.18) on day 2 ($p = 0.549$). Significant diurnal variations were observed with lowest glucose concentrations at 2 p.m. (- 5.3%, p<0.001) and 4 a.m. (-5.6%, p<0.001).

This study suggests that glucose concentrations are kept constant within narrow limits in non-diabetic, healthy dogs and that in line with earlier studies daily rhythmicity must be considered.

**Disclosures**

No disclosures to report.

**ESVE-P-3 - European Society of Veterinary Endocrinology**

## Phagocytic function, inflammatory phenotype, and serum 25-hydroxyvitamin D in dogs with diabetes mellitus

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Type-1 diabetes mellitus (T1DM) in humans is associated with increased risk for infections related to immune dysregulation and is correlated with glycemic control. Vitamin D deficiency, commonly identified in T1DM humans is contributory to aberrant immune function. Dogs with T1DM could share an increased risk for infections, but little is known about immune function, inflammatory phenotype, or a possible interplay with vitamin D. Therefore, we aimed to interrogate granulocyte/monocyte (GM) phagocytic function, inflammatory phenotype, and serum 25-hydroxyvitamin (OH)D concentrations in dogs with T1DM and subanalyses based on glycemic control. Otherwise healthy dogs with T1DM as well as healthy controls were eligible for inclusion. Dogs with T1DM were categorized as either controlled (T1DM-C) or uncontrolled (T1DM-U) based on clinical signs and serum fructosamine concentrations. Serum 25(OH)D concentrations and c-reactive protein (CRP) concentrations were measured with modified-HPLC and a commercial ELISA kit, respectively. Phagocytosis of opsonized-*E. coli* were evaluated via flow cytometry and constitutive plasma concentrations of TNF-α, IL-6, IL-10, and IL-8 were measured using a canine-specific multiplex assay. Normally distributed or transformed data were compared using paired T-tests while non-normal data were analyzed using paired sign tests. Analysis of T1DM-C, T1DM-U, and controls was performed using linear regression (normal data) or Kruskal-Wallis equality of populations rank tests followed by Dunn's test. Forty dogs were enrolled, 20 with T1DM (T1DM-C, n=10; T1DM-U, n=10) and 20 age, sex, and breed matched controls. T1DM dogs had a lower percentage of GM that phagocytized *E.coli* compared to controls ($P = 0.03$) and T1DM-U dogs were lower than controls ($P = 0.025$). T1DM dogs had a higher number of phagocytized *E.coli* per cell compared to controls ($P = 0.003$). Both subgroups, T1DM-C ($P = 0.003$) and T1DM-U ($P < 0.0001$) had a higher number of bacteria phagocytized per cell compared to controls. T1DM dogs had higher CRP concentrations than controls ($P = 0.011$). Constitutive plasma cytokine concentrations and serum 25(OH)D were not different between T1DM and control dogs. These data indicate that dogs with T1DM have altered phagocytic function *in vitro* and a proinflammatory phenotype that could be affected by glycemic control. Lastly, no differences in serum 25(OH)D or constitutive plasma cytokine concentrations were found in this small cohort. Future studies with larger populations are warranted to further investigate immune function, inflammatory phenotype, and vitamin D in dogs with T1DM.

**Disclosures**

No disclosures to report

## ESVE-P-4 - European Society of Veterinary Endocrinology

### The ones that did not make it: Owners' perceptions and reasons for why cats with diabetes mellitus were euthanized within four weeks of diagnosis

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Caring for the diabetic cat entails an unneglectable effort for the owner and a potentially great influence on the lifestyle of both cat and owner. Although treatment options have evolved over the last decades, mortality rates up to 17 % have been reported within the first four weeks of diagnosis. Though owners' decisions hold a significant impact on the consequence for the cat, there is little information on owners' perceptions and concerns upon their cat's diagnosis of diabetes mellitus (DM) and if these perceptions influence the outcome for the cat. The aim of this study was to investigate reasons for why cats did not survive more than four weeks after diabetes diagnosis, alongside owners' perceptions and worries.

A questionnaire was distributed to owners of cats diagnosed with DM (n = 477). The questions concerned factors associated with the cat (e.g. age and outdoor confinement), the owner and household (e.g. place of residence), the impact of DM on the quality of life of the cat and owners' perceptions and concerns regarding DM and treatment. Data were analyzed using multiple regression analysis and survival for less than four weeks was set as outcome. The final regression model was decided with a backwards elimination process combined with a lowered Akaike information criterion, and variables with p >0.05 were excluded. Odds ratio (OR) was calculated with a 95 % confidence interval (CI).

Within four weeks of DM diagnosis, 15 % of the cats (n = 72) were euthanized. The most common reason for the cat not surviving was the owner wanting to limit the suffering for the cat (53 %), followed by poor prognosis (32 %) and failure to respond to treatment (26 %). One of eight owners (12.5 %) stated that the treatment was too difficult, but only 4 % experienced poor support from the veterinarian. Cats belonging to owners who expressed concern over costs were less likely to survive (OR 2544, CI 5.7-7.5 x $10^5$, p = 0.007). Increasing age of the cat at diagnosis in combination with owner experiencing expectations from surroundings to euthanize the cat, resulted in decreased survival (OR 5.4, CI 1.8 – 30, p = 0.02).

This study highlights the significance of owners' perceptions on the outcome for cats newly diagnosed with DM and emphasizes the importance of informative owner – veterinarian contact, recognizing the owners' concerns to facilitate the right decision for the cats and the owners.

**Disclosures**

No disclosures to report.

## ESVE-P-5

### Assessment of euthanasia rates of dogs diagnosed with diabetes mellitus in primary care practice in Australia

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Euthanasia of dogs with diabetes mellitus can be driven by both client and patient related factors. Recently, the prevalence of diabetes mellitus among dogs attending primary care practice in Australia was estimated to be 0.36%. To our knowledge, an in-depth assessment of euthanasia rates in this specific population has not yet been performed nor an evaluation of factors that might influence this. The identification of factors that influence owner's decisions to euthanise dogs with diabetes mellitus may aid in the management and treatment of this condition and promptly identify cases where additional owner support may be warranted. The aims of this study were to: (1) describe the timing of euthanasia in dogs diagnosed with diabetes mellitus relative to the date of diagnosis; and (2) identify risk factors at diagnosis that increased the probability of euthanasia.

This was a retrospective cohort study of dogs diagnosed with diabetes mellitus over a ten-year period (2008-2017). Cases were identified utilising an electronic database of primary care practices across Australia. Time to euthanasia was described using Kaplan-Meier survival curves. Factors associated with the interval from the date of diagnosis to the date of euthanasia were quantified using a Cox proportional hazards regression model. A piece-wise Cox model with time-dependent covariates was developed to account for violation of the proportional hazards' assumption.

A total of 1901 dogs met the criteria to be included in the study. Of this group, 1482 dogs were euthanised during the study period, at a median time post-diagnosis of 39 days. Euthanasia occurred at diagnosis in 17% (313/1901) of cases. The proportion of dogs euthanised within one week, one month, three months and six months post-diagnosis were 30% (576/1901), 37% (700/1901), 45% (849/1901) and 51% (963/1901), respectively. Dogs that were entire, greater than 10 years of age, comorbidity positive and those that had diabetic ketoacidosis at diagnosis had an increased daily hazard of euthanasia during the first 12 months post-diagnosis (hazard ratios 1.8 [95% CI 1.5 to 2.1], 2.8 [95% CI 2.4 to 3.1], 1.7 [95% CI 1.5 to 1.9], and 2.0 [95% CI 1.7 to 2.3], respectively). Beyond 12 months post-diagnosis, only age at diagnosis was statistically significantly associated with the daily hazard of euthanasia.

This study documents euthanasia rates and associated risk factors for dogs diagnosed with diabetes mellitus in primary care practice in

Australia. Findings from this study can help identify cases where additional owner support may be warranted.

**Disclosures**

For Caroline Mansfield: 1) On the member advisory board for Purina and Boehringer-Ingelheim 2) Receives speaking honoraria from Blackmores, Boehringer-Ingelheim and Hills Pet Nutrition 3) Receives research funding from Mars.

## ESVE-P-6 - European Society of Veterinary Endocrinology

### Feline hypersomatotropism: The owners' point of view

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Feline hypersomatotropism (FHS) has been recognized more frequently in recent years. FHS can affect the quality of life (QoL) of cats and owners though little is known about their QoL and life expectancy. The aim of this observational study was to collect epidemiological data and describe the owners' perception of the disease, its treatment, its impact on cat QoL, and investigate the life expectancy.
An online survey focusing on diagnosis, treatment, QoL, and follow-up of cats with FHS was developed and translated into Italian, English, Portuguese, Spanish, and German. Respondents were recruited through social networks, internet forums, and direct contact by e-mail. The owners were asked to define their cats' QoL using a score ranging from 1 (poor) to 5 (excellent), and the improvement following treatment (IFT) using a score ranging from 1 (absent) to 5 (obvious). The QoL scores at diagnosis and after treatment were compared using the Wilcoxon test.
One-hundred-one cats from >10 countries were included. Most represented countries were Italy (n=23), USA (n=20), Austria (n=15), Argentina (n=14), and UK (n=5). The median age at diagnosis was 11 years (range:6-16). Male (70/101,[70%]) and indoor (69/101,[69%]) cats were overrepresented. Diabetes mellitus was present in 94/101 (93%) cats, and 86/94 (91%) were already receiving insulin treatment when FHS was diagnosed. The median daily insulin dose at diagnosis was 14U (3-60) and the median interval between start of insulin treatment and FHS diagnosis was 5 months (0.5-60). The maximum (median) daily insulin dose administered during the course of the disease was 24U (4-110). Symptomatic hypoglycemia occurred at least once in 22/96 (23%) cats, with 11 (12%) experiencing >1 episode. Diabetic cats were treated with insulin therapy only (51/94, [54%]), insulin therapy plus cabergoline (20/94,[21%]), radiotherapy (12/94,[13%]), and hypophysectomy (8/94, [9%]). The median QoL score at diagnosis (3,[1-5]) was lower than after treatment (4,[1-5]; P<0.0001). The median IFT score was 4 (1-5). Hypophysectomized cats had the highest IFT score (5,[5-5]). Forty-six cats (46%) developed co-morbidities after FHS diagnosis, with chronic kidney disease (CKD) (16/46,[35%]), pancreatitis (14/46,[30%]), and left ventricular hypertrophy (7/46,[15%]) being the most common. Thirty-one (31%) cats were deceased at time of questionnaire completion, with the most common causes of death/euthanasia being CKD (10/31, [31%]), uncontrolled diabetes mellitus (7/31,[22%]), and heart failure (5/31,[16%]). Median survival time was 28 months (0-69).
This study confirms the most common presentation of FHS and suggests treatment usually improves QoL; hypophysectomy was the most effective. FHS co-morbidities were common.

**Disclosures**

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## ESVE-P-7 - European Society of Veterinary Endocrinology

### Feline hypersomatotropism: What is the veterinarians' approach to the disease?

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Fundac diagnostic and therapeutic approaches exist, depending on the preferences of veterinarians and the availability of technical equipment.
The aim of this observational study was to collect epidemiological data and describe the veterinarians' experience with FHS, as well as their approach to the disease.
An online survey was developed and translated into Italian, English, Portuguese, Spanish, and German. The survey focused on

veterinarians' choices of diagnostics, treatment, and follow-up of FHS, with improvement following treatment (IFT) described using a score ranging from 1 (absent) to 5 (obvious). Respondents were recruited through social networks, internet forums, and direct contact by e-mail. The veterinarians' responses were reviewed; FHS diagnosis was considered correct if the clinical suspicion was confirmed by elevated insulin-like growth factor-1 (IGF-1) and/or visualization of a pituitary mass.

One-hundred-twelve veterinarians from 14 countries were included. Among them, 81 (72%) never diagnosed FHS. Thirty-one out of 114 (28%) veterinarians diagnosed FHS at least once. Among them, 15 (48%) diagnosed FHS only once, 12 (39%) between 2 and 5 times, and 4 (13%) more than 5 times. Eighteen out of 31 (58%) worked in first opinion practice, while 13/31 (32%) worked in University or private referral practice. Overall, data regarding 60 cats with FHS were collected. Among them, 53 (88%) had concurrent diabetes mellitus, while 7 (12%) had not. All non-diabetic cats were diagnosed by 3 veterinarians. The most common clinical signs of non-diabetic cats were external physical changes (5/7,[71%]) and weight gain (4/7,[57%]). Overall, most common clinical signs were polyuria/polydipsia (49/60,[82%]), polyphagia (29/60,[48%]), external physical changes (27/60,[45%]), weight gain (17/60,[28%]), and stertor/stridor (13/60,[22%]). Serum IGF-1 measurement (59/60,[98%]), which was reported >1000 ng/mL in 50/53 (94%) cases, and advanced diagnostic imaging (12/60,[20%]) were the most commonly performed diagnostic tests. The most common treatments used in diabetic cats were insulin therapy only (32/53,[60%]) and insulin therapy plus cabergoline (15/53,[28%]). Cabergoline was used as monotherapy in 6/7 (85%) non-diabetic cats. Hypophysectomy and radiotherapy were performed in 2/60 (3%) and 3/60 (5%) cats, respectively. The median overall IFT score was 3 (interquartile range [IQR]:2-4).The survival time was available for 17/24 deceased cats, with a median of 18 months (IQR:7-26).

This study showed that the majority of veterinarians' never diagnosed FHS; non-diabetic FHS should be considered and medical treatment (insulin and/or cabergoline) remains the most common treatment.

**Disclosures**

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## ESVE-P-8 - European Society of Veterinary Endocrinology

### Feline adrenomegaly in clinical practice - A retrospective study

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Literature concerning adrenomegaly in cats is scarce. In daily practice, feline adrenomegaly seems rare and can be an incidental finding at the abdominal ultrasound (AUS).

This study aims to assess the prevalence of adrenomegaly in cats submitted to AUS, associating it with the final established diagnosis, and estimating its clinical relevance.

Feline AUS reports, performed in a Veterinary Teaching Hospital between October 2018 and February 2021, were retrospectively reviewed and cats with adrenomegaly (dorsoventral axis greater than 4.8mm in longitudinal section, at least in one of the adrenal glands) were selected. Data concerning breed, gender, age, weight, sex, environment, reason for performing AUS, if adrenomegaly was uni- or bilateral, and final established diagnosis was reviewed. Adrenomegaly was judged relevant if specific endocrine tests were performed or suggested by clinicians, after the AUS. Descriptive and analytical statistics (chi-square) were performed ($p<0.05$).

Adrenomegaly was identified in 68 (6.9%) out of 983 reports, being bilateral in 36/68 (52.9%) cats, and unilateral with non-atrophied contralateral gland in the remainders. The percentage of males (44/68; 64.7%) was significantly higher ($p=0.015$). European cats (62/68; 91.2%) and indoor ones (50/68; 73.5%) were overrepresented. Mean age and weight were 11,6±4,3(2-22) years and 4,0±1,7(1,6-8,5) kg, respectively. The two main reasons for performing AUS were chronic kidney disease (CKD) exploration (57.3%;39/68) and a clinical suspicion of pancreatitis (14.7%;10/68). In 6 cats (8,8%), AUS was specifically performed in the context of exploration an endocrine disease namely: ketoacidosis (4,4%;3/68), suspected primary hyperaldosteronism (2,9%;2/68) and insulin resistance (1,5%;1/68). In 48/68 (70.6%) cats, the established or presumed final diagnosis was set as a non-endocrine disease and adrenomegaly was considered a secondary problem or an incidental finding. In 20/68 (29.4%) cats, the established final diagnosis was an endocrine disease, detailing: hyperthyroidism (13.2%;9/68), diabetes mellitus (10.3%;7/68), primary hyperaldosteronism (2.9%;2/68), hypersomatotropism (1.5%;1/68) and pituitary-dependent hyperadrenocorticism (1.5%;1/68). After AUS assessment, medical exploration of adrenomegaly was suggested in 12/68 (17.6%) cases but only performed in 6 (8.8%) of them, due to financial restrictions.

This study shows that feline adrenomegaly is not a prevalent finding in cats submitted to AUS, but when present it seems more common in geriatric male cats. Adrenomegaly was more frequently identified on the exploration of non-endocrine diseases, namely CKD, stressing the role of chronic illness or concurrent unexplored endocrine cause. After its identification, adrenomegaly was only explored in a minor percentage of cases, highlighting that it seems to be undervalued in clinical practice.

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## ESVE-P-9 - European Society of Veterinary Endocrinology

### Evaluation of the ACTH stimulation test using a low-dose of a depot formulation in healthy dogs and in dogs with naturally occurring Cushing's syndrome

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The adrenocorticotropic hormone (ACTH) stimulation test has been widely used to confirm the diagnosis of Cushing's syndrome (CS) and to monitor trilostane and mitotane treatment in dogs with CS. Previous studies have shown that the use of a depot formulation of tetracosactide may represent an alternative to the non-adsorbed synthetic ACTH products in dogs. Nonetheless, its sensitivity to diagnose CS requires further evaluation.

The aims of this study were to propose reference intervals for cortisol values 1 hour after administration of a low-dose of depot ACTH in healthy dogs and to evaluate the sensitivity of this test to detect CS. The sensitivity was also evaluated among different types of CS based on an ultrasonographic classification.

Forty-one healthy dogs (20 males, 21 females) with a median age of 9 years were prospectively included. Additionally, 91 dogs with CS (31 males, 60 females) with a median age of 11 years were retrospectively included. Dogs with CS were ultrasonographically classified as follows: 45 (49.4%) dogs with symmetrical adrenomegaly consistent with pituitary-dependent hypercortisolism (PDH), 8 (8.8%) dogs with unilateral adrenomegaly and atrophy of the contralateral adrenal gland or unilateral or bilateral adrenomegaly with malignancy features consistent with adrenal-dependent hypercortisolism (ADH), 34 (37.4%) dogs with equivocal adrenal asymmetry (EAA) and 4 (4.4%) dogs with normal adrenal thickness. A low-dose (5 μg/kg) of depot ACTH was intramuscularly administered to all dogs and cortisol was measured before and 1 hour post-ACTH.

In healthy dogs, lower and upper limit of the 95% reference interval for post-ACTH cortisol concentration and their confidence intervals, were 4.4 (2.7-5.8) μg/dl and 18.4 (16.5-20.0) μg/dl, respectively. Post-ACTH cortisol concentration was above the reference interval in 81 out of 91 dogs (89.0%) with CS. Forty two of the 45 (93.3%) dogs with PDH, 5 of the 8 (62.5%) of dogs with ADH and 30 of the 34 (88.2%) of dogs with EAA had an elevated post-ACTH cortisol concentration consistent with CS. Therefore, using a low-dose of a depot ACTH formulation, the 1-hour post-ACTH cortisol concentration had good sensitivity to detect CS in dogs with PDH and EAA but low sensitivity to detect CS in dogs with ADH. In dogs with suspected CS and with ultrasound findings consistent with ADH, a normal ACTH stimulation test does not rule out CS and, in such cases, performing a different diagnostic test (ie, low-dose dexamethasone suppression test) is recommended.

**Disclosures**

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## ESVE-P-10 - European Society of Veterinary Endocrinology

### Myotonia associated with naturally occurring canine hypercortisolism: 30 cases (1984-2020)

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Myotonia, a disorder characterized by delayed muscle relaxation after voluntary contraction or percussion, has been rarely associated with canine hypercortisolism (HC). This retrospective multicenter study aimed to describe the clinical findings and outcome of myotonia cases associated with HC.

Cases (n=30) of naturally occurring canine HC and myotonia were retrospectively (1984-2020) included in the study. Twenty-two females (17 neutered) and 8 males (4 neutered) dogs were included. The most represented types were mixed breed (11/30), Poodle (7/30), and Dachshund (4/30). The median (range) body weight was 7.75 (3.6-21) Kg. All dogs had pituitary-dependent HC. The mean (±standard deviation) age at the time of HC and myotonia diagnosis was 10.7 years (±2.8) and 11.4 years (±2.8), respectively. In 10 dogs, HC was diagnosed first; in 19 cases, myotonia was diagnosed first, and in one dog, they were diagnosed simultaneously. Myotonic signs (stiffness, stilted gait, and hyperextension) developed first on the hindlimb in 18 dogs, first on the forelimbs in 5 dogs, and simultaneously in all the limbs in 7 dogs. Pain was not reported in any case by the referring veterinarian or the owner. Electromyography, performed in 11 dogs, showed the presence of myotonic discharges. Muscle/nerve biopsies, performed in 4 dogs, showed variation in muscle fiber size, moderate fiber fibrosis, and hypomyelination or demyelination. Twenty-two dogs were treated with trilostane, 5 with mitotane, 2 with trilostane and then mitotane, and one with

melatonin. In 27/30 dogs HC signs improved after treatment. Myotonia treatment consisted of combined therapies including benzodiazepines (6/30), physiotherapy (4/30), cyclobenzaprine (3/30), mexiletine (2/30), dantrolene (2/30), nonsteroidal anti-inflammatory drugs (2/30), botulinum toxin (1/30), gabapentin (1/30), and methocarbamol (1/30). No dogs showed complete resolution of myotonic signs. However, a mild improvement was noted in one dog treated with diazepam, one dog with mexiletine, and one dog with physiotherapy and diazepam. Six dogs were lost to follow up, 10 dogs are still alive at the time of writing, and 14 dogs died. Euthanasia was performed in 5/14 dogs because of myotonic signs, in one because of HC signs, and in 8 dogs the cause of death was unknown. The median survival time from the diagnosis of myotonia was 583 (125-4402) days.

In conclusion, in most dogs, myotonic signs persisted despite the resolution of HC signs. However, our results suggest that the survival time of dogs with HC-associated myotonia does not differ from that previously reported for canine HC without myotonia.

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## ESVE-P-11 - European Society of Veterinary Endocrinology

### Evaluation of serum electrophoresis in dogs with pituitary-dependent hypercortisolism

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Changes in serum electrophoresis (SE) of dogs with naturally occurring hypercortisolism (HC) have been rarely described. This retrospective study aimed to compare SE of dogs with HC at diagnosis and healthy dogs (HDs).

Agarose gel electrophoresis of HDs and dogs with pituitary-dependent hypercortisolism (PDH), diagnosed at a referral veterinary hospital, were retrospectively analyzed. PDH dogs and HDs were defined based on clinical findings, CBC, serum biochemistry, urinalysis, and endocrine testing (PDH dogs only). SE fractions absolute values (AVs) were reported as mean± standard deviation (SD) or median (range) and compared between the two groups using unpaired T-Test or Mann-Whitney U Test, based on data distribution. Receiver operating characteristic (ROC) curve analysis with the area under the ROC curve (AUC) calculation was performed. $P < 0.05$ was considered significant.

Forty-nine PDH dogs and 34 HDs were included in the study. In PDH dogs the following SE fractions AVs resulted significantly lower compared to the HDs: albumin (3.18 ± 0.46 vs 3.42 ± 0.39 g/dL; P=0.0144), alfa-1 globulins (0.3 ± 0.07 vs. 0.33 ± 0.05 g/dL; P=0.0120), beta-2 globulins (0.66 ± 0.13 vs. 0.76 g/dL ± 0.18, P=0.0076) and gamma globulins (0.2 ± 0.12 vs. 0.62 ± 0.18 g/dL; P<0.0001). In PDH dogs alfa-2 globulins AVs were significantly higher in comparison to HDs (1.47 ± 0.26 vs. 0.92 ± 0.18 g/dL; P<0.0001). Most PDH dogs had the following SE fractions AVs within the laboratory reference interval (RI): albumin (33/49), alfa-1 globulins, (41/49), beta-2 globulins (43/49). Most PDH dogs had gamma globulins below the RI (34/49) and all PDH dogs had alfa-2 globulins above the RI (49/49). The gamma:alfa-2 globulin (gamma:alfa-2) ratio was able to differentiate PDH dogs from HDs. In particular, PDH dogs had significantly lower gamma:alfa-2 ratio in comparison to HDs (0.02 ± 0.01 vs. 0.05 ± 0.01; P<0.0001). ROC curve analysis for gamma:alfa-2 showed AUC of 0.97 and identified a cutoff of 0.025 as the best value to discriminate between PDH dogs and HDs, with a sensitivity of 97% and a specificity of 86%.

In conclusion, at SE, increased alfa-2 globulins, and decreased gamma-globulins and gamma:alfa-2ratio seemed to characterize dogs with PDH. The gamma:alfa-2 ratio showed promising results in differentiating PDH dogs from HDs with a high sensitivity and good specificity. However, the absence of a control group of dogs with other diseases (i.e., inflammatory or neoplastic diseases) represents a limitation of the present study.

**Disclosures**

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## ESVE-P-12 - European Society of Veterinary Endocrinology

### Treatment and monitoring of naturally-occurring hypercortisolism by primary care veterinarians: A Western European survey

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Treatment of naturally-occurring hypercortisolism (HC) includes surgery or medical management. This study aimed to determine how Western European primary care veterinarians (WEPCV) treat and monitor dogs with HC.

An online survey translated into four different languages (Portuguese, Spanish, French and Italian) was developed using an electronic platform. Questions focused on therapeutic management and monitoring. Respondents were recruited through social network veterinary groups, mailing lists and seminars.

Overall, 2021 responses from 8 European countries (Italy [n=1297], Portugal [n=261], France [n=222], Spain [n=192], Belgium [n=41], Switzerland [n=4], Luxembourg [n=3] and Netherlands [n=1]) were collected. Overall, 1579 (78.1%) respondents attempted to differentiate functional adrenal tumour (FAT) and pituitary-dependent HC (PDH); 68.8% recommended adrenalectomy and 31.2% recommended hypophysectomy in dogs with FAT and PDH, respectively. Reasons for not recommending adrenalectomy included high perceived risk (60.8%), low perceived benefit over medical therapy (33.2%) and lack of surgical expertise in the area (31.0%). Reasons for not recommending hypophysectomy included high perceived risk (67.8%) and lack of surgical expertise in the area (50.2%). When surgery (hypophysectomy or adrenalectomy) was not contemplated, 75.8% always recommended medical treatment while 24.2% did not. Main reasons for not treating included expected poor owner compliance (54.9%), concurrent conditions that may benefit from glucocorticoid excess (39.1%) and mild clinical signs (30.1%). Of 1757 (87%) respondents who use trilostane, 58.6% and 41.4% prescribed it once or twice daily, respectively. Median (range) starting dosages were 2 (0.5-3.0) mg/kg once daily and 0.5 (0.5-6.0) mg/kg twice daily. Time of first follow-up was <10, 10-14 and 15-30 days after starting trilostane for 5.9%, 59.5% and 31.4% of respondents, respectively. Long-term follow-up was every 3-4 and 5-6 months for 41.4% and 38% respondents, respectively. Monitoring was most commonly performed with ACTH stimulation test (ACTHst) (53.9%), pre-pill cortisol (18.8%) and both pre- and post-pill cortisol (13.5%). When ACTHst was performed, 63.9% of respondents did it 2-4 hours post-pill.

This study shows that approximately one-third of WEPCV do not recommend adrenalectomy for FAT and approximately two-thirds do not recommend hypophysectomy in PDH. Despite demonstrated benefits of medical treatment, approximately one-quarter of respondents did not always recommend it, which may contribute to reduced life expectancy and quality of life in dogs with HC. Starting dosage, follow-up and monitoring strategies vary among WEPCV. These results suggest that there is room for further education of WEPCV and highlight the lack of surgical expertise for some procedures in several Western European regions.

**Disclosures**

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**ESVE-P-13 - European Society of Veterinary Endocrinology**

## Changes in systolic blood pressure in dogs with adrenal dependent hyperadrenocorticism during trilostane treatment or after adrenalectomy

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In people with Cushing's syndrome systemic hypertension (SH) can persist after successful treatment of hypercortisolemia, and antihypertensive multidrug therapy is frequently necessary to manage hypertension. This has also been recently reported in dogs with pituitary-dependent-hyperadrenocorticism. However, in dogs with adrenal-dependent-hyperadrenocorticism (ADH) changes in SBP during trilostane treatment, or after adrenalectomy, have not been reported.

The aims of this study are to describe the changes in SBP and in the prevalence of SH (SBP≥160 mmHg) in dogs with ADH during the first year of treatment (adrenalectomy group [AG] vs trilostane group [TG]), its relation with clinical control of the disease and ACTH-stimulation test (ACTH-st) results, and their response to antihypertensive treatment.

Nine dogs (3/9 AG and 6/9 TG) were prospectively included and evaluated at diagnosis (T0), before surgery (T0'), and 1, 3, 6 and 12 months after initiation of trilostane or after surgery (T1, T3, T6, T12). Parameters recorded were: clinical control of the disease, SBP measurement (Doppler ultrasonography), and results of the ACTH-st (TG). Systemic hypertension was treated with benazepril alone or combined with amlodipine if control of SH was not achieved. Four dogs died before T12.

Median age at diagnosis was 13 years (range, 10-17 years). Prevalence of SH (T0) was 88.9% (8/9) and median SBP was 180 mmHg (range, 140-255 mmHg). In the TG prevalence of SH at T0 was 100% (6/6) and median SBP was 181 mmHg (range, 170-255 mmHg) and decreased to 33.3% (1/3) and 156 mmHg (range, 133-165 mmHg) at T12. In the AG prevalence of SH at T0 was 66.7% (2/3) and median SBP was 180 mmHg (range, 140-210 mmHg) and decreased to 0% (0/2) and 151 mmHg (range, 148-155 mmHg) at T12 respectively. Changes in SBP or prevalence of SH were not statistically significant. Control of the disease and results of the ACTH-st were not associated with blood pressure at any time point. Antihypertensive treatment could not be reduced in any dog in the TG. In 1 dog in the AG who was treated with multidrug therapy at T0', only benazepril was necessary at a lower dose after adrenalectomy. All dogs hypertensive at T0 in the AG normalized their SBP at T3, but benazepril could not be suspended.

Blood pressure should be routinely evaluated in dogs with ADH during treatment and, especially in those surgically treated, it should be

closely monitored as adjustments in the antihypertensive treatment might be necessary to avoid hypotension.

**Disclosures**

No disclosures to report.

**ESVE-P-14 - European Society of Veterinary Endocrinology**

## $^{1}$H NMR metabolomics identifies multiple metabolites associated with persistently elevated cortisol

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Cortisol is a corticosteroid hormone that exerts complex regulatory effects on metabolism. However, global metabolomics studies on its effects in dogs have not yet been conducted. This study aimed to evaluate the metabolic changes associated with persistently elevated cortisol concentrations, unresponsive to the negative feedback on endogenous production caused by low-dose dexamethasone administration.

The study was conducted using leftovers of serum samples submitted for laboratory analysis. The control group included 25 samples with normal clinical chemistry and the case group consisted of 21 samples with inadequately suppressed cortisol (>10 ng/ml) in the low-dose dexamethasone suppression test. The samples were analysed by a canine-specific $^{1}$H NMR metabolomics platform quantifying 123 measurands. Differences between case and control groups were evaluated using Firth logistic regression.

Multiple metabolites were associated ($p < 0.0045$) with high cortisol levels after suppression. Dogs having elevated concentrations of the novel inflammatory marker glycoprotein acetyls as well as the energy metabolites citrate and lactate were more likely to belong to the case group. Higher concentrations of amino acids phenylalanine and tyrosine but lower concentrations of glutamine and ratio of glycine to branched-chain amino acids were associated with higher likelihood of the dog belonging to the case group. Increased levels of multiple lipid measures, including cholesterol, triglycerides, fatty acids, HDL triglycerides, and LDL and VLDL particle measures were also associated with increased likelihood of the dog belonging to the case group.

These results increase our understanding of the metabolic effects of persistently elevated cortisol concentrations and pave the way for identifying novel diagnostic markers and therapeutic targets. Further studies in dogs suffering from hyperadrenocorticism are warranted to elucidate the aetiology of these changes, how they reflect the patient's clinical state and whether routine treatments correct them.

**Disclosures**

Claudia Ottka and Jenni Puurunen are current employees, and Hannes Lohi the board director of PetBiomics Ltd. - a company developing NMR metabolomics testing for dogs. Elisabeth Müller is the managing director, Corinna Weber, and Ruth Klein heads of department of LABOKLIN - an animal diagnostic laboratory.

**ESVE-P-15 - European Society of Veterinary Endocrinology**

## Evaluation of ACTH-stimulation test, urinary cortisol to creatinine ratio and urinary specific gravity as monitoring tools in dogs with hyperadrenocorticism

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The monitoring method to evaluate trilostane treatment response is under continuous review. The ACTH-stimulation test (ACTH-st) has been used as a monitoring tool for years but as described, it poorly correlates with clinical signs. The urinary cortisol to creatinine ratio (UCCR) hasńt been previously found to be useful as a monitoring tool; however, in most studies it was evaluated using the ACTH-st as the gold standard what might have altered the results. Lastly, the urinary specific gravity (USG) has been reported as unable to discriminate between dogs underdosed and those adequately dosed.

The aims of this study were to reevaluate the sensitivity and specificity of the ACTH-st, UCCR, and USG to assess clinical control of dogs with pituitary-dependent-HAC (PDH) treated with trilostane twice-daily.

Dogs were classified based on clinical signs perceived by the owner using the questionnaire proposed by MacFarlane et. al (2016) as dogs having excellent control (EC), reasonable control (RC) and poor control (PC). Thirty-five visits of 28 dogs were prospectively included. Eight visits were excluded as the dogs were classified as “unwell”. Thus, twenty-seven visits from 21 dogs were finally included. Ten dogs were classified as having EC, 10 as RC, and 7 as PC. Urine, collected and brought in by the owner on the day of evaluation was used to assess the UCCR and USG. The ACTH-st was performed 2-4 hrs post trilostane administration.

Median UCCR was significantly higher in dogs with PC (405 $x10^{-6}$, range 64-983 $x10^{-6}$) compared to the rest of the dogs (94 $x10^{-6}$, range 35-348 $x10^{-6}$; $p$=0.009). A UCCR ≥217 $x10^{-6}$ was 90% specific and 71% sensitive to detect dogs with PC.

Median USG was significantly higher in dogs with EC (1.033, range 1.019-1.057) than in dogs with RC or PC (1.014, range 1.008-1.036; $p$=0.028). A USG ≥1.017 was 62.5% specific but 100% sensitive to detect dogs with an EC.

The median post-ACTH cortisol concentrations were significantly lower in dogs with EC (4.85 μg/dL, range 2.39-6.37 μg/dL) compared to the rest of the dogs (6.45 μg/dL, range 1.34-9.39 μg/dL; $p$=0.040). A cut-off point of ≤5.07 μg/dL was 83% specific and 60% sensitive to detect dogs with EC, similar than described for pre-trilostane cortisol concentrations.

Owner's opinion remains a cornerstone when monitoring trilostane treatment. The UCCR and USG in combination with either the post-ACTH cortisol concentrations or the pre-trilostane cortisol concentrations might be useful tools when deciding whether a dose change is indicated.

**Disclosures**

No disclosures to report.

**ESVE-P-16 - European Society of Veterinary Endocrinology**

## The impact of low-dose dexamethasone suppression test patterns in clinical practice - A retrospective study

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Low-dose dexamethasone suppression test (LDDST) patterns have been recently studied but literature is scarce about its value in clinical practice.

This study aims to assess the prevalence and impact of LDDST patterns on the confirmation or exclusion of hypercortisolism (HC) in dogs with compatible clinical and/or laboratorial signs.

A retrospective study was performed. Medical records of dogs submitted to an LDDST between January 2014 and December 2020 in a Veterinary Teaching Hospital were reviewed. Those with compatible clinical signs (namely polyuria/polydipsia (PU/PD), polyphagia) and/or laboratorial findings (e.g., increased alkaline phosphatase (ALP) or inappropriate urine specific gravity (USG)) were included. When available, adrenal ultrasound findings were also detailed. LDDST patterns were reviewed and appropriately classified according to the recent literature in lack of suppression, partial suppression, complete suppression, escape or inverse pattern. The impact of LDDST patterns was assessed by evaluating whether HC was confirmed or excluded based on these respective profiles. Descriptive and analytical statistics (Fisher's Exact test) were used ($p<0.05$).

A total of 128 dogs met the inclusion criteria. From these, 39.1% (50/128) showed a complete suppression, 31.2% (40/128) a lack of suppression, 14.1% (18/128) a partial suppression, 10.1% (13/128) escape and 5.5% (7/128) an inverse pattern. Abdominal ultrasound was performed in 92.2% (118/128) of the cases. There was no association among clinical signs ($p$=0.11), ALP increase ($p$=0.32), USG ($p$=0.33), adrenal ultrasound findings ($p$=0.19) and the different LDDST patterns. The LDDST patterns were interpreted as compatible with HC in 50% (64/128) of the cases, of which 57.8% (37/64) had a lack of suppression, 26.6% (17/64) a partial suppression and 15.6% (10/64) an escape pattern. In practice, HC was excluded in all dogs showing a complete suppression and in those with an inverse pattern. The disease was also excluded without further exploration in 23.1% (3/13), 7.5% (3/40) and 5.6% (1/18) of dogs showing an escape, lack of suppression and partial suppression patterns, respectively.

This study reinforces that there is no association among clinical, laboratorial, adrenal ultrasound findings and LDDST patterns. A lack of suppression, partial suppression and escape patterns were overrepresented among LDDST profiles that were found compatible with HC. The disease was excluded without further exploration in all dogs presenting an inverse pattern and in almost one quarter of those with an escape pattern, highlighting that clinicians are not aware of these specific LDDST patterns in daily practice.

**Disclosures**

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**ESVE-P-17 - European Society of Veterinary Endocrinology**

## Prediction of azotemia in medically-treated hyperthyroid cats: an attempt to identify risk factors and to develop a scoring system

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Chronic kidney disease and hyperthyroidism frequently occur in association in elderly cats. Identification of compromised renal function is of prime importance in hyperthyroid cats, as some patients may show clinical deterioration once euthyroidism is achieved. No reliable predictor for the occurrence of azotemia after treatment has yet been identified.

The aim of our study was to identify independent factors predicting development of azotemia after restoration of euthyroidism, and to

develop a scoring system as an easily applicable tool to predict azotemia in hyperthyroid cats.

Medical files of cats from three referral centers were retrospectively reviewed. Non-azotemic hyperthyroid cats were included. Clinical signs, physical examination, biochemical and electrolytic variables were recorded. Azotemia at follow-up was defined as a creatinine concentration (Cr) ≥ 16 mg/L with concomitant total thyroxine concentration (T4) < 40 nmol/L at the first visit (1-3 months) after treatment initiation.

Fourteen variables were considered for statistical analysis: age, weight, T4, Cr; concentrations of urea, potassium, phosphorus, urine specific gravity (USG), presence of polyuro-polydipsia, vomiting, diarrhea, lethargy, dysorexia, and systemic hypertension. Quantitative variables were converted to binary ones using medians as cut-offs. Our approach being exploratory, other cut-offs ($25^{th}$ and $75^{th}$ percentiles) were also tested if the use of median as a cut off did not lead to statistically significant results. All statistical analyses were performed using a commercially available software (SAS University Edition). Significance was set at $p<0.05$.

Ninety-one cats were included. Median age was 13.2 years old [11.6-15.5]. Median time between inclusion and follow-up visit was 42 days [31-56]. Twenty-four cats (26%) were azotemic at follow-up (Cr: 17.3 mg/L [16.3-20]). Median variation of Cr was +22% [+10; +61]. Median follow-up Cr among non-azotemic cats was 11 mg/L [9.1-13.2].

Variables statistically associated with the occurrence of azotemia in univariate analysis were urea concentration > 0.6 g/L (OR = 3.3; p = 0.046) and Cr > 10 mg/L (OR = 6.6; p = 0.002). T4 < 104 nmol/L and USG < 1.024 approached significance.

These results are in accordance with previous studies. A larger cohort may improve both univariate and multivariate models and lead to the development of a robust and clinically relevant scoring system to predict azotemia in hyperthyroid cats.

**Disclosures**

No disclosures to report.

## ESVE-P-18 - European Society of Veterinary Endocrinology

### Investigation to detect of traces of methimazole in the urine of owners of hyperthyroid cats

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Hyperthyroid cats are most frequently treated with oral or transdermal antithyroid drugs. Antithyroid drugs are potential human teratogens and although careful handling by the owners of hyperthyroid cats treated medically is mandatory, this seems not to be always respected. Furthermore, as cats often live in close contact with their owners, exposure of the owners to antithyroid drugs should be assessed. The objective of this study was to evaluate the presence of traces of methimazole in the urine of owners of hyperthyroid cats treated with antithyroid drugs.

Urine of owners of hyperthyroid cats, of human patients treated with methimazole and of healthy humans without any contact with antithyroid drugs was collected. All owners of hyperthyroid cats were asked to fill out a questionnaire about daily handling of the medication and contact with their cat. Urine of hyperthyroid cats was collected by spontaneous micturition. All urine samples were stored at – 20°C until analysis by ultra-high performance liquid chromatography coupled to high-resolution Orbitrap mass spectrometry (Q-Exactive).

Twenty-four owners (for a total of 23 cats), 5 healthy humans and 5 humans treated with antithyroid drugs were included. Twelve cats were receiving sirop (Apelka®) and 11 receiving tablets (Felimazole®, Thiafeline®). The majority of owners (22/24) mentioned having close contact to their cat. Two out of 24 were splitting the tablets without using gloves. One person used gloves to give the tablets. High concentrations of methimazole were detected in all feline samples (mean concentration 4952.18 ng/ml; range 103.73 – 15143.00 ng/ml) and in the urine of all human patients treated with antithyroid drugs (mean concentration 3665.78 ng/ml: range 1827.74 - 5178.45 ng/ml). No traces of methimazole were detected in the urine of owners of hyperthyroid cats (LOD = 1 ng/mL).

The results concerning the potential exposure of owners of hyperthyroid cats to antithyroid drugs are rather reassuring. Prudence is still warranted. Whether these results can be extrapolated to the use of transdermal application, is not known.

**Disclosures**

No disclosures to report.

## ESVE-P-19 - European Society of Veterinary Endocrinology

### Retrospective comparison of different vitamin D analogues in the management of canine primary hypoparathyroidism

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Canine primary hypoparathyroidism (PH) is treated with oral vitamin D analogues, possibly combined with oral calcium supplementation. Studies comparing different treatment strategies in canine PH are currently lacking.

The aim of this study was to examine if the choice of vitamin D analogue or the addition of calcium supplementation, affected the duration of hospitalisation in dogs with newly diagnosed PH. Case records were retrospectively reviewed and entered into a data-capture platform (CastorEDC). Associations between oral calcium and vitamin D analogue, and hospitalisation time were determined using Mann Whitney U test, and Kruskal Wallis test, respectively.

In total, 23 veterinary centres made 122 entries with 12 cases excluded due to incomplete data entry (n=5), unclear diagnosis (n=3) or the prescription of vitamin D before referral (n=3) or after discharge (n=1). A median (and range) of 3 (1-15) cases per centre were recorded. Vitamin D analogues administered included calcitriol (Rocaltrol, n=49, 45%), alfacalcidol (One-Alpha, n=31, 28%), dihydrotachysterol (AT10, n=16, 14%), other calcitriol preparation (n=11, 10%), other vitamin D analogues (n=2, Calcifediol, Cholecalciferol) and one dog received an unknown vitamin D supplement. For purpose of analysis treatments were grouped as; calcitriol (Rocaltrol, other calcitriol), alfacalcidol (One-Alpha) or dihydrotachysterol (AT10) therapy. Eight-six (78%) dogs received oral calcium therapy, 13 (12%) did not and 11 (10%) had incomplete records. Eight dogs were discharged the day of starting vitamin D administration, two dogs suffered extensive skin slough after receiving continuous-rate-infusion of calcium gluconate prompting prolonged hospitalisation and two dogs were euthanised prior to discharge due to financial limitations (n=1) and the development of azotaemia (n=1). These 12 cases, as well as the dog with unknown vitamin D type, were censored from descriptive and comparative analyses.

Median hospitalisation time was 5 (1–21) days from starting vitamin D administration. There was a significant difference between vitamin D treatment groups ($H(2) = 9.12$, $p=0.010$) and hospitalisation time. Dihydrotachysterol therapy was associated with shorter hospitalisation time (median 3.5 days) compared to alfacalcidiol (median 7 days), but not calcitriol therapy (median 5 days). Treatment with oral calcium did not significantly affect hospitalisation time, however, there were few dogs not receiving this therapy.

Several forms of vitamin D therapy are used to effectively manage canine PH across Europe. While dihydrotachysterol is no longer available, duration of hospitalisation was similar for both alfacalcidiol and calcitriol therapy. Individual case factors, as well as clinician decision may have affected hospitalisation times.

**Disclosures**

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## ESVIM-P-2 - European Society of Veterinary Internal Medicine

### Use of tranexamic acid in dogs with primary ITP. A feasibility study

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Immune-mediated thrombocytopenia (ITP) is a common cause of severe thrombocytopenia in dogs. Until therapy is effective, spontaneous haemorrhage can occur, particularly where platelet concentration <30,000 cells/μL, contributing to morbidity, mortality and expense of management. Antifibrinolytic agents, such as tranexamic acid (TXA), have demonstrated efficacy in reducing post-operative bleeding in dogs, however, little is known about their effect in managing ITP.

We conducted a multi-centre, prospective, randomised study, aiming to evaluate whether TXA, would reduce the risk of spontaneous bleeding, when given in addition to standard therapy in dogs with primary ITP. We hypothesized that this would reduce blood transfusion requirements and hospitalisation times.

Ten dogs with a diagnosis of primary ITP were included. All dogs received standard therapy for ITP including corticosteroids, a single dose of vincristine, and omeprazole. Additional therapies (e.g., fluid therapy, blood products) were administered at the discretion of the attending clinician. Patients in the treatment group (n=4) received TXA (20mg/kg intravenously q8h). Daily monitoring was performed using a clinical bleeding score, PCV/TS and platelet count. Remission was defined as platelet count ≥ 40,000/μl.

The median time from the start of treatment until remission was 5 days in the TXA group and 5.2 days in the control group (p=1.0). Two dogs, one in each group, did not achieve remission. Clinical bleeding scores were not significantly different between groups (p=0.75) over the hospitalisation times and the median blood volume transfused was 37.5 ml/kg for the TXA group and 9.72 ml/kg for the control group (p=0.084). Three out of four dogs receiving TXA at 20mg/kg IV developed vomiting within 15 minutes of administration and required dose reductions.

Tranexamic acid given intravenously to dogs with ITP did not confer a large clinical benefit in this small cohort, and was associated with a high incidence of vomiting. Future studies with larger patient populations and refined dosing protocols are needed in order to draw more robust conclusions about the use of antifibrinolytics in dogs with ITP.

**Disclosures**

No disclosures to report.

## ESVIM-P-3 - European Society of Veterinary Internal Medicine

### Usefulness of chlorambucil for treatment of canine Steroid-Responsive Meningitis-Arteritis

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Steroid-Responsive Meningitis Arteritis (SRMA) is a systemic immune-mediated disease that mainly affects large-breed, young adult dogs. Diagnosis relies on the combination of epidemiologic, phenotypic and clinocopathological findings. Treatment involves long-term immunosuppression, primarily with corticosteroids. Second-line immunosuppressors can improve treatment efficacy and reduce the adverse effects of steroids. Chlorambucil is an alkylating agent used for many immune-mediated diseases in companion animals. To our knowledge, no study reports the efficacy and safety of chlorambucil in dogs with SRMA.

We aimed at comparing the findings in dogs with SRMA prescribed chlorambucil or other immunosuppressors.

Client-owned dogs presented between January 1st 2018 and December 31st 2020, were retrospectively included if complete data was available. Diagnosis was based on associations of the following: predisposed breed, fever, neck pain, leukocytosis/neutrophilia, increased CRP (>30mg/L), absent CT lesions, CSF pleocytosis and/or proteinorrachia, negative PCR-screening and bacterial culture, obvious and sustained improvement with immunosuppression. Categorical and continuous variables were estimated with percentages and medians (min–max) then compared between dogs receiving chlorambucil and other immunosuppressors using Fisher and Mann-Whitney-Wilcoxon tests, respectively.

Thirty-six dogs were diagnosed with SRMA (20 females, 16 males). Concurrent immune-mediated polyarthritis and myocarditis were diagnosed in 3/36 and 1/36 cases, respectively. Median age was 17 (7–55) months. Median weight was 22.7 (6.3–58.3) kg. There were 4 Bernese Mountain Dogs, 3 of each (Belgian and German Shepherds, Beagle, Boxer, Weimaraner), 2 Retrievers and other breeds (15). Chlorambucil was prescribed to 22/36 dogs with prednisolone (20/22) or alone (2/22), as first-line (13/22) or second-line treatment (9/22 of which, 4/9 were refractory to prednisolone and azathioprine, 3/9 had severe hepatopathy with azathioprine, and 2/9 displayed iatrogenic hyperadrenocorticism). Another treatment was prescribed to 14/36 dogs, including prednisolone alone (7/14), with azathioprine (5/14), with cytarabine (1/14), or with cyclosporine (1/14). The populations were similar with regard to epidemiological and clinicopathological findings. Median follow-up was 19 (8–44) weeks for dogs treated with chlorambucil and 22 (10–57) weeks for the other group. Response (both 100%) and relapse rates (5/22 *versus* 5/14, p=0.46) were similar between subgroups. Adverse effects were less frequent (p=0.008) in dogs prescribed chlorambucil (1/22 with pancytopenia after 20 weeks of treatment) than for the other group (6/14 including 3/6 under prednisolone with iatrogenic hyperadrenocorticism, 1/6 with cranial-cruciate ligament rupture and 2/6 under azathioprine with severe hepatopathy, all requiring treatment interruption).

Chlorambucil appeared effective and safe for dogs with SRMA, though few cases received this agent alone.

**Disclosures**

No disclosures to report.

## ESVIM-P-4 - European Society of Veterinary Internal Medicine

### Serologic testing and radiographic measurements of the tracheobronchial lymphoid soft tissue in dogs with pulmonary coccidioidomycosis

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Diagnosis of pulmonary coccidioidomycosis is based upon a combination of clinical signs, positive serology test results, and thoracic

radiographic abnormalities, but this triumvirate of criteria is nonspecific. Tracheobronchial lymphadenopathy (TBL), a common radiographic feature of coccidioidomycosis, can also be found in other infiltrative diseases, and serology results often cannot differentiate active infection from prior exposure. Associations between serologic test results and TBL presence/severity could ameliorate this diagnostic challenge.

We aimed to determine if there was an association between serologic test results and TBL presence or severity in dogs with pulmonary coccidioidomycosis. Dogs were included in this prospective study if they demonstrated respiratory signs and had ≥ 1 positive AGID [IgM or IgG] or EIA IgG test result, in addition to either radiographic abnormalities or clinical/serologic improvement after antifungal therapy. When present, soft tissue opacity in the tracheobronchial lymph node (TBLN) region was measured for length and height in a lateral image; area was calculated using (length/2) x (height/2) x 3.14 . Ratios were generated by comparing TBLN area to the length of manubrium (LM) and length of T4 (LT4). Severity was categorized as mild, moderate, or severe. Fisher Exact or logistic regression tests were used to assess associations between TBLN presence/severity and serologic test results. Analysis of EIA IgG and TBLN area ratios were performed using Spearman rank correlation tests. $P < 0.05$ was significant.

Thirty-two dogs were included. Tracheobronchial lymphadenopathy was identified in 81% (26/32) of dogs. Positive AGID IgM, AGID IgG, and EIA IgG results were found in 31% (8/26), 92% (24/26), and 62% (16/26) of dogs with TBL, respectively. Dogs with TBL had AGID IgG titers that ranged from negative to 1:128 and EIA IgG values ranged from 0- ≥ 80 EU (positive, 10- ≥ 80 EU). Neither AGID IgM/IgG positivity nor EIA IgG values were associated with TBL presence. When present, TBL was most commonly mild (50%, 13/26) or moderate (38%, 10/26). Associations between serologic test results and TBL severity were not found. The median (range) TBLN ratio for area:LM and area:LT4 were 19.7 (4.3-579.9) and 41.3 (9.5-1623.8), respectively. EIA IgG values were not associated with either TBLN area ratios. These data highlight the complexity of diagnosing pulmonary coccidioidomycosis using thoracic radiographs and serologic testing. Each diagnostic test used in isolation demonstrates an inability to identify all positive patients and it will be a collaboration of tests to best diagnose and monitor patients with coccidioidomycosis.

**Disclosures**

Dr. Andrew Hanzlicek is employed by MiraVista Diagnostic Laboratory. MiraVista Laboratory partially funded this study but did not influence data analysis or reporting of results.

## ESVIM-P-5 - European Society of Veterinary Internal Medicine

### T-cell dependent immune responses in cats treated with frunevetmab

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Frunevetmab is a monoclonal antibody (mAb) that targets and inhibits nerve growth factor (NGF). Inhibition of NGF signaling provides relief from pain associated with osteoarthritis. The objective of this laboratory safety study was to evaluate the effect of frunevetmab on the functional immune response in naïve cats. Keyhole limpet hemocyanin (KLH; 0.1 mg in 0.5 mL), unadjuvanted and adjuvanted, was the model antigen. This study, along with evaluations of immune tissue morphology and clinical monitoring from other studies, formed an integrated screening for immunomodulation.

Thirty-two (32) domestic shorthair cats (16 males, 16 females) were enrolled in this prospective, randomized, placebo-controlled, parallel-group study (protocol approved by the Kalamazoo Institutional Animal Care and Use Committee). Cats were randomly allocated to 4 treatment groups (n=8 per group; 4 males, 4 females): group T01, placebo plus unadjuvanted KLH; group T02, placebo plus adjuvanted KLH; group T03, frunevetmab plus unadjuvanted KLH; or group T04, frunevetmab plus adjuvanted KLH. Placebo (0.9% saline) and frunevetmab (2.8 mg/kg) were administered subcutaneously (SC) on study days 0, 21, and 42, and KLH SC on days 26 and 47. In-life assessments included general health and clinical observations, body weight, and body temperature. Blood samples were obtained at various times from study day -9 to day 63 to assess clinical pathology, anti-KLH IgG titers, frunevetmab levels, and anti-frunevetmab antibodies (ADA).

All cats had low anti-KLH titers (≤900) on study days -2 and 26. After the day 26 priming KLH immunization, anti-KLH titers increased in 3/8 cats in group T02 and in 8/8 cats in group T04. After the day 47 boosting KLH immunization, titers increased markedly in all cats in both T02 and T04. Mean anti-KLH titers in group T04 (frunevetmab) were somewhat higher ($p<0.0001$) compared with group T02 (placebo) on days 40 and 63. Unadjuvanted KLH failed to induce meaningful anti-KLH titers in either T01 or T03. Frunevetmab ADAs were not observed in any cat. Frunevetmab serum concentrations on study day 63 ranged from 6.35 to 34.9 μg/mL.

This study demonstrated that frunevetmab at 2.8 mg/kg SC 3 times, at 3-week intervals, was clinically well-tolerated and had no adverse effects on the functional immune response of cats against the model antigen KLH, as determined by T-cell dependent seroconversion. These functional results align well with morphologic and clinical evaluations in cats, and with literature from other species, in showing no adverse immunomodulation via inhibition of NGF signaling.

**Disclosures**

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## ESVIM-P-6 - European Society of Veterinary Internal Medicine

### Autologous blood-patch pleurodesis treatment for persistent pneumothorax in 5 dogs

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Autologous blood-patch pleurodesis (ABP) has been previously described for treating persistent pneumothorax in the dog and in humans. Despite exploratory thoracotomy and pulmonary lobectomy being the gold standard for treatment of this condition, it cannot always be performed due to medical or financial constraints. The aim of this retrospective case series was to describe the clinical course, etiology and outcome of 5 dogs with persistent pneumothorax treated with ABP.

The medical records of 5 client-owned dogs treated with ABP from 2016 to 2021 were reviewed. Persistent pneumothorax was defined as a continuous leakage of air into the pleural cavity that did not respond to conservative management after three or more days. This included rest and air drainage via repeated percutaneous thoracocentesis. Two dogs had pneumothorax due to congenital pulmonary bullae, one due to thoracic trauma, another due to lungworm infection and a fifth of unknown cause in the context of a relapsing subcutaneous haemangiosarcoma. Diagnosis of pneumothorax and its cause was performed via CT scan in 4 out of 5 dogs and thoracic x-rays in the remainder.

The ABP procedure was standardized for all dogs. Animals were anaesthetized and one or two chest tubes were placed aseptically depending on the localisation of the pneumothorax. All dogs had the air removed from the chest prior to the procedure via the chest tube. Around 5 mL/kg of non-coagulated blood was aseptically collected from the jugular vein and injected via thoracotomy tube immediately into the pleural cavity. Control thoracic x-rays were obtained 24 hours and 1 week after the procedures which did not show any signs of relapse in 4 out of 5 dogs. A repeat ABP was attempted in the fifth dog 12 hours after the first injection due to the recollection of the pneumothorax, but the dog died during induction of anaesthesia in preparation for the pleurodesis. The procedure was successful in 4 of 5 dogs after one procedure, therefore a success rate of 80%. No complications that could be directly linked to ABP occurred.

This case series suggests that ABP is a simple, rapid, inexpensive, effective and safe procedure that can be useful for the treatment of persistent pneumothorax that does not respond to conservative treatment and where surgical exploration cannot be performed due to medical or financial concerns.

#### Disclosures

No disclosures to report.

## ESVIM-P-7 - European Society of Veterinary Internal Medicine

### Quantifiable features of the tidal breathing pattern in dogs with severe bronchomalacia

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Lower airway obstruction has been conventionally described by concave or flat expiratory curves on flow-volume loops, but little is known about tidal breathing patterns in dogs with bronchomalacia. The aim of this study was to quantify characteristics of tidal breathing in dogs with expiratory effort due to bronchomalacia.

Spirometry was performed in 6 dogs with expiratory effort ascribed to bronchomalacia using pneumotachography (RSS100 and SmartLab, Hans Rudolph). Similar testing was performed in 7 non-respiratory or healthy dogs that served as controls. Respiratory parameters and spirometric values for expiratory flow measures (peak flow and flow at mid-tidal volume or later expiration) were compared between groups using the Mann Whitney U test for nonparametric data.

Dogs with bronchomalacia had a significantly lower respiratory rate (20 vs 32/min, $p=0.005$), lower minute volume (198 vs 425mL, $p=0.035$), and longer expiratory time (1.92 vs 1.01s, $p=0.005$) compared to control dogs. Conventional features such as a concave or flat expiratory curve were not observed in dogs with bronchomalacia examined here. Compared to control dogs, dogs with bronchomalacia had an exponential rise feature of the expiratory curve on volume-time plots ($p=0.001$), and significantly prolonged low-flow expiratory phase (defined as the time from flow <25% of peak expiratory flow to end expiration) on flow-time plots ($p=0.001$).

In conclusion, the tidal breathing phenotype in dogs with severe bronchomalacia was characterized by lower respiratory rate and minute volume, exponential rise of the expiratory curve on volume-time plots, and prolonged low-flow expiratory phase on flow-time plots, suggesting abnormal ventilatory mechanics.

#### Disclosures

No disclosures to report.

## ESVIM-P-8 - European Society of Veterinary Internal Medicine

### Meta-analysis of complication rates of tracheal stenting in dogs

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Tracheal collapse is common in dogs, especially in Yorkshire terriers. Associated clinical signs range from mild cough or mild exercise intolerance to severe raspy breathing, permanent cough, respiratory distress, and syncope. Although tracheal stenting has become increasingly popular to treat tracheal collapse in dogs, complications might arise from this treatment and negatively impact quality of life.

This study aimed to determine the overall complication rates of tracheal stenting in dogs from the available literature.

A meta analysis was performed on studies reporting rates of complications after tracheal stenting in dogs. Studies were identified from seven databases and ECVIM/ACVIM conference proceedings. Eight complications were a-priori considered: stent fracture, stent migration, relapsing collapse possibly due to stent shortening, granulation tissue ingrowth, tracheobronchial infection, early cough (ie, cough reported within 6 weeks of stenting), late cough (ie, cough still reported more than 6 weeks after stenting), and clinically relevant late cough (ie, late cough reported at rest, requiring treatments, and/or negatively impacting quality of life according to the owners). Complication rates were extracted for each included study and random-effects models were used to estimate pooled complication rates. Stratified analyses were performed in attempt to solve heterogeneity when present.

Of the 1197 studies retrieved initially, 14 met inclusion criteria. All pooled complication rates were significantly different from 0%. No significant heterogeneity was identified regarding pooled complication rates of stent migration (5%; 95% confidence interval [CI]: 1–9%), relapsing collapse possibly due to stent shortening (11%; 95% CI: 6–16%), and early cough (99%; 95% CI: 95–100%). Pooled complication rates of stent fracture (12%; 95% CI: 5–20%), granulation tissue ingrowth (20%; 95% CI: 11–30%), tracheobronchial infection (24%; 95% CI: 14–35%), late cough (72%; 95% CI: 60–84%), and clinically relevant late cough (44%; 95% CI: 32–56%) were also estimated but significant heterogeneity was identified. Stratified analyses by types of stent used (ie, Vet Stent, Duality Stent, or other stents), institutions/teams, and study types (ie, abstracts versus articles) failed to resolve heterogeneity among studies.

This study confirms a very high prevalence of coughing within the first six weeks of stenting. The risks of granulation tissue ingrowth, infection, and late cough might be considered clinically relevant (pooled estimate ≥ 20%), although the rates of these complications significantly varied among studies. Further studies are warranted to understand the variation of these complication rates, and more carefully select appropriate candidates for tracheal stenting.

**Disclosures**

No disclosures to report.

**ESVIM-P-9 - European Society of Veterinary Internal Medicine**

## Evaluation of canine fresh frozen plasma CRI: Risk of contamination and effects on albumin and coagulation factors

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Throughout the past decades, increasing concern has been raised regarding the risks of synthetic colloids administration in critically ill patients. The alternative use of human albumin concentrate has been associated to life threatening hypersensitivity reactions. Constant rate infusion (CRI) of plasma derivatives has been suggested as a viable substitute. Furthermore, the use of plasma CRI could mitigate bleeding or attenuate vascular permeability, edema and inflammation in patients with coagulopathy or systemic inflammatory response syndrome, respectively. Plasma CRI is being used in veterinary medicine, despite the uncertainty of plasma proteins stability during prolonged CRI administration at room temperature, and the potential risk of bacterial contamination/overgrowth. The objectives of this study were to assess the albumin concentration and activity of coagulation factors V, VII, VIII and IX, and to determine the risk of bacterial overgrowth during a 12h plasma CRI.

Twenty canine fresh frozen plasma units (mean storage time of 6 months SD±2), were thawed using a water bath at 35°C for 20 minutes. An infusion system was connected to the plasma bag on one end and to a 22G catheter on the other. Simulating the skin of a patient, the catheter was connected to an empty collecting bag through a rubber pinch device. These procedures were performed under regular veterinary hospital sterility conditions. A second rubber pinch device was attached to the empty bag to collect samples for analysis. A CRI was simulated during 12h at 24°C. Samples were collected at the beginning of the CRI (T0), after 4h (T1), and after 12h (T2). Plasma culture was performed and specific clotting times for factors V, VII, VIII and IX were measured at the three time points. Albumin levels were assessed at T0 and T2.

All culture results were negative. There was no significant difference between the activity for factors VIII (106.55±18.59% at T0, 109.93±22.58% at T1 and 115.46±23.73% at T2) and IX (122.33 ±22.84% at T0, 125.29±26.07% at T1 and 120.99±22.59% at T2) and for albumin levels (2.05±0.15g/dl at T0 vs 2.06±0.18g/dl at T2) at any time point. A slight but significant increase in factor V activity was observed when comparing T0 (108.92±27.14%) to T1 (121.97±27.31% p=0.002) or T2 (123.26±31.63% p=0.001), and for factor VII when comparing T0 (117.34±31.95%) to T1 (125.63±33.01% p=0.005), and T1 to T2 (134.70±32.51% p=0.002).

We may conclude that plasma CRI at room temperature during 12h is safe regarding bacterial growth, and effectively provides albumin and clotting factors.

**Disclosures**

No disclosures to report.

## ESVIM-P-10 - European Society of Veterinary Internal Medicine

### SARS-CoV-2 and the veterinary profession: A longitudinal-study in Italy (February 23-July 1, 2020)

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Veterinarians have frequent contacts with companion animals and/or livestock as well as with their owners, which possibly can affect the risk of SARS-CoV-2. Moreover, exposure to animal coronaviruses and other pathogens could induce cross-reacting antibodies against SARS-CoV-2 that may mitigate or exacerbate outcome in affected veterinarians. The study aimed to assess if there is any professional risk in relation to SARS-CoV-2 in veterinarians.

Between February 23 to July 1, 2020, information on the occurrence of SARS-CoV-2 in veterinarians and on the number of dogs and livestock in Italy was collected from the National Veterinary Welfare and Assistance Body database and public animal registries. Data of veterinarians with SARS-CoV-2 were analyzed to identify possible differences with the general population and associations with the number of animals.

The incidence of SARS-CoV-2 in veterinarians was 2.60/1,000 (76 out of 29,247 veterinarians, 0.26%) with the incidence in the general population being 3.96/1,000 (P=0.002). Among the former, the majority (68, 89.5%) worked with companion animals. Similar to the general population, of 76 veterinarians diagnosed with SARS-CoV-2 infection, 57 (75%) were quarantined at home with mild symptoms, 15 (19.7%) required hospitalization and 4 (5.3%) died. Two (13.3%) of veterinarians that had been hospitalized were subsequently transferred into an intensive care unit. From univariate analyses the following variables were retained: number of cases and the incidence of SARS-CoV-2 in the general population, and the number of cattle, pigs and dogs. In the multivariate analysis, a positive correlation between the number of SARS-CoV-2 affected veterinarians and cases in the general population (ri=$3.37\times10^{-3}$; 95%CI=$2.30\times10^{-3}$-$4.44\times10^{-3}$; P<0.001) as well as the number of dogs (ri=$5.45\times10^{-6}$; 95%CI=$0.23\times10^{-6}$-$10.7\times10^{-6}$; P=0.042) was documented. When the incidence of SARS-CoV-2 was compared, the correlation was only observed between veterinarians and general population (ri=0.376; 95%CI=0.090-0.662; P=0.013).

In conclusion, it would appear that the risk of SARS-CoV-2 in veterinarians is lower compared to the general population. The underlying reason(s), however, need to be clarified.

#### Disclosures

No disclosures to report.

## ESVIM-P-11 - European Society of Veterinary Internal Medicine

### Alloimmunization in dogs following transfusion: A serial crossmatch study

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Crossmatching is performed to determine the serological compatibility of donor and recipient blood. In dogs, crossmatching is performed when the initial transfusion was performed more than 4 days ago or when the transfusion history is unknown. To the authors' knowledge, to date, this 4-day rule has not been evaluated in dogs. The aim of this prospective study was to determine at what time point alloantibodies are formed in dogs after transfusions.

The study population included dogs that were transfused and monitored for at least 4 subsequent days. Exclusion criteria consisted of persistent red blood cell (RBC) agglutination and previous/multiple transfusions. Crossmatching was performed before the initial DEA 1-compatible transfusion and on days 1, 2, 3, and 4 and preferably between days 5-14 and 15-28, respectively, using the tube agglutination procedure (major crossmatch, recipient controls). Hemolysis and degree of agglutination ([AG]: 0 - 4+) were evaluated macroscopically and microscopically. Recipients were monitored for transfusion reactions. Statistical analysis was performed via IBM SPSS Statistics 27 using logistic regression ($p \leq 0.05$ was considered significant).

Between 10/2019 - 12/2020, 99 dogs received 163 RBC transfusions; 21 dogs were enrolled in the study. A total of 130 crossmatches were performed. Macroscopic agglutination or hemolysis did not occur. 37 crossmatches (16 dogs) were weakly positive (microscopic AG 1+ - 2+). The first microscopic positive crossmatches were seen in 2 dogs on day 1 (AG 1+), 1 on day 2 (AG 2+), 3 on day 3 (AG 1+), 6 on day 4 (AG 1+ - 2+), and in 4 dogs between day 6 and 13 (AG 1+ - 2+). In 7 dogs, a decrease in agglutination was observed within the study period. No association was detected between RBC storage time (p=0.402) or immunosuppressive treatment (p=0.410) and the occurrence of positive crossmatches within 4 days after transfusion.

In total, 16/21 dogs showed alloimmunization. Since formation of alloantibodies was detected in 12 dogs within 4 days after transfusion, the 4-day rule should be reconsidered and crossmatching should be performed before every subsequent transfusion.

#### Disclosures

LH declares that she is supported by a grant from the Ernst Reuter Foundation and that she has ongoing research collaborations with companies that offer commercial crossmatching test kits. CW declares that she has held lectures for veterinary pharmaceutical and

diagnostic companies. RM, NM, NB declare that they have no conflict of interest. BK declares that she repeatedly has lectured for and acted as consultant for veterinary pharmaceutical, nutritional and diagnostic companies and has previous and ongoing research collaborations with various veterinary pharmaceutical, nutritional and diagnostic companies.

## ESVIM-P-12 - European Society of Veterinary Internal Medicine

### Hypertension in apparently healthy elderly dogs: Prevalence and comparison of in clinic versus at home measurement with Doppler ultrasonic technique

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Prevalence of hypertension in healthy dogs is reported to be low. However, a previous study found frequent hypertension in apparently healthy elderly dogs, with a prevalence of 53%. Situational hypertension, previously called white coat hypertension, was suggested as possible explanation of these findings. In nervous or stressed dogs, blood pressure might be lower at home. This study aimed to measure systolic blood pressure (SBP) in apparently healthy elderly dogs and to repeat the measurements at home in dogs with hypertension in clinic.

Senior and geriatric dogs that were healthy according to the owner were prospectively recruited. SBP was measured by Doppler ultrasonic technique according to the ACVIM guidelines, by the same experienced individual and following a standardized protocol. Indirect fundoscopy was performed in all dogs in order to detect ocular target lesions secondary to hypertension. Hypertension was defined as SBP ≥160 mmHg and in those dogs SBP was remeasured at home using the same methodology.

In total, 114 dogs were recruited. In clinic blood pressure measurement was not successful in 3 dogs due to highly nervous behavior. Measurement of blood pressure was obtained at home for 2 of them (respective values of 161 and 160 mmHg). In the remaining 111 dogs mean (± SD) SBP in clinic was 148.6 (± 24.2 mmHg). None of these dogs had evidence of hypertensive retinopathy or choroidopathy. In clinic, 31 (27.9%) had SBP ≥160 mmHg with severe hypertension (≥180 mmHg) in 9 dogs (8.1%). Repeated measurement at home was performed in 16/31 dogs. On average, SBP was 20.4 mmHg higher at home than in clinic. The highest SBP value was measured in clinic in 8 dogs and at home in 7 dogs. One dog had exactly the same value in clinic and at home. Most dogs (11/16; 68.8%) still showed hypertension at home, but SBP normalized in 5/16 (31.2%). Unfortunately, repeated measurements at home were not possible in the other 15 dogs due to COVID restrictions.

Hypertension is frequent in apparently healthy elderly dogs. Blood pressure values measured at home often differ from values measured in clinic and it was unexpected to find higher mean SBP at home than in clinic. However, blood pressure measurement in the home environment of the dog might be useful to differentiate true hypertension from situational hypertension in approximately one-third of dogs.

#### Disclosures

This study is part of a larger longitudinal prospective study for which partial funding from Hill's and CEVA was received.

## ESVIM-P-13 - European Society of Veterinary Internal Medicine

### Assessment of circulating inflammatory mediators in dogs with tracheal collapse

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Tracheal collapse (TC) is an obstructive respiratory disorder that can significantly impact the quality and longevity of life in dogs. Systemic inflammation has been noted in other obstructive airway conditions in dogs and humans. Our aim was to investigate whether a systemic pro-inflammatory state exists in dogs with TC.

Dogs with TC (n=15) and healthy, non-affected controls (C, n=15), were prospectively enrolled. Signalment and body condition score (BCS) were recorded for all dogs. For TC dogs, cough duration, pharyngeal collapse, bronchial collapse, and previous stent placement were recorded. Plasma samples were banked at -80C and batch analyzed for TNF-É‘, IL-6, IL-8, IL-10, and IL-17A. Differences between groups were evaluated via Mann-Whitney Rank Sum test, and correlations between IL-8 and clinical data were assessed via Spearman Rank Order Correlation with a $p<0.05$ significance level.

No difference between groups was found for sex or BCS. Yorkshire terriers were overrepresented in the TC group. The TC group was older than the C group (mean 9.2±3.2 vs. 4.9±3.4 years, respectively, p=0.006). IL-8 (P=0.008), but not TNF-É‘ (P= 0.576), IL-6 (P=0.089), IL-10 (P=0.372), or IL-17A (P=0.110), was significantly greater in the TC group compared to controls. IL-8 was not correlated with age (P=0.257), sex (P= 0.104), BCS (P=0.219), breed (P=0.506), cough duration (P=0.240), pharyngeal collapse (P=0.323), bronchial collapse (P=0.186), or stent placement (P=0.240).

Increased plasma IL-8 supports the presence of systemic inflammation in canine TC. Dogs with TC were older than controls, but this did not account for the increased IL-8 concentrations.

#### Disclosures

This study was funded with a grant from the AKC Canine Health Foundation. Dr. Yanke has received funding as a collaborator from the NIH for translational studies investigating canine gliomas as part of a

multi-institutional study started at the University of Alabama-Birmingham (UAB).

## ESVIM-P-14 - European Society of Veterinary Internal Medicine

### Stopping leaks in the chamber - Differences in valve performance on inhalation and exhalation leakage for pet inhaler therapy

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Feline asthma is treated with inhalers and holding chambers with valves that allow animals to inhale their medication over several tidal breathing cycles. These valves should be sensitive enough to open on weak inhalations for cats in respiratory distress yet remain closed on strong exhalations to ensure that the aerosol inside the chamber remains undisturbed from the previous breath. There are a variety of valves currently used in different chambers and all must work across a wide range of flow rates.

Four chambers were evaluated for valve performance: AeroKat* Chamber, PawGreet, Canack (AKA Cat Aerosol Chamber) and Aeroflow FAC. Inspiration flow rates were simulated by connecting the chambers to a vacuum source set to draw 10, 20, and 30 L/minute. To measure inhalation leakage a TSI Flowmeter was attached and sealed to the opposite end to record the flow pulled through the chamber. To measure exhalation leakage a positive airflow of 10, 20, and 30 L/minute was created and the TSI Flowmeter measured the flow passing back into the chamber.

Inhalation leakage can be a proxy for valve sensitivity because air flows preferentially from paths of lower resistance. A less sensitive valve can lead to air entrainment on inhalation. Significant inhalation leakage would dilute the aerosol bolus the patient receives reducing the dose per breath and increasing the time and breaths needed to evacuate the chamber of medication. Considerable inhalation leakage was found with the PawGreet (≤48% of simulated flow), Canack (≤30%) and Aeroflow FAC (≤60%). AeroKat chamber demonstrated higher sensitivity with inhalation leakage of ca. ≤13%, possibly coming from chamber air flowing through the Flow-Vu Indicator.

Exhaled breath leaking into the chamber could dilute the aerosol reducing the dose efficiency of the chamber for the next inhalation, or it could blow medication out of the chamber reducing the total dose available. Exhalation leakage was significant for the PawGreet (≤48%), Canack (≤73%) and AeroFlow (≤19%) chambers suggesting that they might be inefficient at delivering the full amount of medication. The AeroKat chamber showed no exhalation leakage (0%) suggesting that the valve effectively diverted flow from the chamber and would be appropriate for a wide variety of patients.

A properly designed valve must be sensitive and able to both open and close efficiently at a wide range of flow conditions to prevent drug loss, optimize dose per breath, and limit administration time.

**Disclosures**

All authors are employees of one of the device manufacturers mentioned in the abstract.

## ESVIM-P-15 - European Society of Veterinary Internal Medicine

### Evaluation of serum C-reactive protein, neutrophil-lymphocytic ratio and abdominal fluid cell count in dogs with septic and non-septic abdominal exudates

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Serum C-reactive protein concentration (CRP) and blood neutrophil-to-lymphocyte ratio (NLR) can be used in combination to aid the diagnosis of spontaneous bacterial peritonitis in people. In veterinary medicine the diagnosis of septic peritonitis can be challenging in many instances. The primary aim of this study was to describe the CRP, NLR and fluid cell count in dogs with abdominal exudates. We hypothesised that dogs with septic exudates would have higher serum CRP and NLR compared with exudates of other causes. A secondary aim was to evaluate if CRP was associated with prognosis.

This retrospective study included any dog with an abdominal exudate diagnosed (nucleated cell count >5.0x10$^9$/L), for which there was a contemporaneous haematology and serum CRP measurement on the same day. Fluid cytology, and bacterial culture results whenever available, assessed by a board-certified clinical pathologist, were used to classify exudates as septic or non-septic. Survival at discharge, 1-month and 3-months post diagnosis was also evaluated.

Seventy-three dogs met the inclusion criteria. Thirty dogs were diagnosed with septic exudates and forty-three dogs with non-septic exudates. CRP was higher in patients with septic exudates in comparison to those with non-septic exudates [$p<0.01$, Area Under Receiver Operating Curve (AUC)=0.68]. NLR was not significantly different between groups. The fluid cell count was also significantly higher in the septic group ($p<0.01$, AUC=0.81). Serum CRP at diagnosis was not predictive of outcome for any group studied.

In this study, serum CRP and fluid cell counts, but not blood NLR, are higher in dogs with septic abdominal exudates compared to non-septic ones, although further studies are required to validate this data and determine the clinical utility of these findings. Serum CRP does not appear prognostic in dogs with abdominal exudates.

**Disclosures**

No disclosures to report.

## ESVNU-P-1 - European Society of Veterinary Nephrology and Urology

### Study of 4970 uroliths in cats over a five year period

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We aimed to evaluate the prevalence and nature of feline uroliths. The study involved 4970 cats: 4535 from Europe (E) and 435 cats from China (C) during 2014 – 2018. Analyses of urolith composition were performed using infrared spectroscopy.

The study population of E:C cats consisted of 3789:151 cats of 26:12 known breeds and 746:284 unknown breeds. The general statistics of E:C cats were: male 8.8:28.3(%), neutered male 40:18.2(%), female 8.2:27.1(%) spayed female 32.6:14.7(%) and unknown gender 10.3:11.7(%).

The uroliths from E:C cats comprised calcium oxalate (CaOx) 57.2:15.6(%), struvite 35.4:78.6(%) ammonium urate 2.2:1.6(%), calcium phosphate 1.9:2.1(%) cystine 0.8:1.1(%), xanthine 0.4:0(%) and others.

The E:C cats had median ages (years): struvite 6:3, CaOx 7:4, cystine 5:2, ammonium urate 4:4.5, calcium phosphate 7:6 and xanthine 2 (none).

CaOx was the most prevalent urolith in E-cats and accounted for neutered 60.7%> spayed 57.2%> intact female 53.6%> intact male 50.5%, while struvite uroliths were for male 40%> female 37.5%> spayed 37.4% and neutered 31.2%.

In contrast, struvite was the prevailing urolith in C-cats: spayed 87.5% > female 87.3%> male 74%> neutered 67.1% while CaOx uroliths accounted for neutered 24%> male 20.3%> spayed 10.9%> female 8.5%.

In E:C-cohorts, numerous breeds were subjected to urolith formation: for ex. domestic shorthair (DS), British shorthair (BS), Persian, Maine Coon, Chartreux, Scottish fold, Norwegian Forest- and Siamese cats. Differences in propensity for urolith formation were observed in E:C-cohorts. The nature of urolith was breed-dependent on one hand and cohort-dependent on the other hand.

In the E-cohort, BS:DS cats showed the prevalence of 86:50(%) CaOx and 12:42(%) struvite, whereas their C-counterparts (BS:DS) showed 15:16(%) CaOx and 79:82(%) struvite. Overall, CaOx uroliths were ~4 times more prevalent in E-cats, whereas struvite were ~2 times more in C-cats. In both cohorts (E:C) Siamese cats had the highest percentage of cystine uroliths and they occurred at a similar rate 25.5:20(%), whereas widely different prevalence was observed for CaOx 43.6:13.3(%) and struvite 21.8:53.3(%). DS cats of the E-cohort accounted for 71% (12/17) of all xanthines, while none of the C-cohort had xanthines.

Our study showed that the nature of feline uroliths and their prevalence were dependent on sex, neutering status and breed. Remarkably, cats of a breed could present completely different patterns of uroliths depending on habitat (E or C), suggesting a significant role of feline diet regimen.

#### Disclosures

Disclosures The authors Breu D and Rossnagel I are employed at the Laboklin GmbH & Co KG, Bad Kissingen, Germany. Müller, E is owner/manager of the Laboklin GmbH & Co KG, Germany.

## ESVNU-P-3 - European Society of Veterinary Nephrology and Urology

### Xanthinuria secondary to allopurinol treatment in dogs with leishmaniosis: Current perspectives of the Iberian veterinary community

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Xanthinuria is an important adverse urinary effect in dogs with leishmaniosis on therapy with allopurinol.

This study aimed to investigate current medical approach of the Iberian veterinary community (IVC) on prevention and management of xanthinuria secondary to allopurinol therapy in canine leishmaniosis (CanLeish).

An online anonymous survey including 4 to 26 questions (depending on the answering pathway chosen) was conducted. The content was divided into five sections focusing on: general information about the respondents, allopurinol prescription regimen, therapeutic monitoring, causes for allopurinol withdrawal, adverse effects, xanthinuria diagnosis, treatment, and preventive measures. After internal validation, the survey was uploaded through an online platform and diffused via Iberic social network veterinary groups. Only answers regarding xanthinuria diagnosis and preventive measures were finally selected.

A total of 230 answers were obtained: 131 from Portugal and 99 from Spain. About 99.6% (229/230) of the clinicians use allopurinol as part of CanLeish treatment. A total of 71.6% (164/229) have identified xanthinuria in dogs with leishmaniosis; 78.7% (129/164) generally diagnose xanthinuria based on identification of crystalluria, 12.2% (20/164) by post-removal urolith analysis, and 5.5% (9/164) based on detection of urolithiasis on abdominal ultrasound. Regarding complications associated with xanthinuria, urinary clinical signs was reported

by 68.8% (110/160) of clinicians, non-obstructive urolithiasis by 59.4% (95/160), renal mineralization by 37.5% (60/160), bacterial cystitis by 31.3% (50/160), urethral obstruction by 29.4% (47/160), and ureteral obstruction by 17.5% (28/160).

Regarding xanthinuria prevention, 75.1% (172/229) of clinicians commonly inform the clients of the adverse effects of allopurinol treatment although only 28.4% (65/229) consider a change to a low purine diet. Regarding monitoring of urinary adverse effects, urinalysis and diagnostic imaging are prioritized by 71.2% (163/229) and 31% (71/229), respectively. Abdominal ultrasonography is preferred (94.4%; 67/71), followed by abdominal radiographs (5.6%; 4/71).

When facing xanthinuria, 43.2% (99/229) of clinicians stop allopurinol treatment, 24% (55/229) switch for active hexose correlated compound (nucleotides), 17.9% (41/229) change the frequency or dosage of allopurinol administration, and 5.2% (12/229) keeps the same therapy. Dietary modification (59.4%; 98/165), stimulation of water intake (15.2%; 25/165), increase in wet food consumption (4.9%; 8/165), and increased frequency of clinical monitoring (15.2%; 25/165), were also implemented.

The IVC is aware of the high prevalence of xanthinuria as a common complication in CanLeish. Although preventive measures are often neglected, clinicians seem to be conscious about the different options to manage xanthinuria in dogs with leishmaniosis, under allopurinol treatment.

**Disclosures**

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## ESVNU-P-4 - European Society of Veterinary Nephrology and Urology

### Effect of storage time and temperature on feline urinary protein: Creatinine ratio

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Proteinuria is an important prognostic factor and therapeutic target in cats with chronic kidney disease. Immediate laboratory analysis on the day of sampling is not always possible in practice and for research purposes, long-term storage of urine is sometimes needed. However, studies on the effect of short-term and long-term storage of urine on feline urinary protein: creatinine ratio (fUPC) are lacking.

The current prospective study aimed to determine the effect of storage time and temperature on fUPC and more specifically whether storage leads to clinically relevant differences in fUPC (i.e. a change in the proteinuria substage for cats as defined by the International Renal Interest Society (IRIS)).

Urine samples were collected from 23 cats (11 healthy, 12 diseased) and aliquoted. Samples were analysed after preservation at room temperature as soon as possible (D0) and one day later (D1) and after being refrigerated during one day (D1) and one week (D7) to assess short-term storage. The effect of long-term storage was evaluated in frozen samples (-24°C and -80°C) after six months (D180) and twelve months (D365), as well as after an additional freeze-thaw cycle (D366).

At D0, mean ± SD UPC was 0.32 ± 0.35 with 13 cats being non-proteinuric (UPC <0.2), 6 borderline proteinuric (UPC 0.2-0.4) and 4 proteinuric (UPC >0.4). After short-term storage (D1 and D7), mean differences were not statistically different from 0 (95% confidence interval (CI) -0.006 – 0.025 respectively -0.005 – 0.026) and the same applied to urine stored for 180 days at -80°C (95% CI -0.004 – 0.027). A statistically significant difference was seen at D180 and D365 with storage at -24°C (95% CI 0.015-0.047 respectively 0.024 – 0.056), and at D365 for urine stored at -80°C (95% CI 0.008 – 0.039). There was no additional effect of an extra freeze-thaw cycle (95% CI -0.009 – 0.023). After long-term storage of urine, a change in IRIS substage for fUPC occurred at ≥ 1 time point in 6/22 (27.3%) cats, whereas only in 1/23 cats (4.3%) a shift took place during short-term storage.

This study suggests that short-term storage of feline urine at room temperature for 1 day or in the refrigerator for up to 7 days does not cause clinically relevant changes in fUPC. However, long-term storage can lead to small changes in fUPC, affecting the IRIS substage in almost 30% of cases.

**Disclosures**

This study is part of a PhD project that is financially supported by IDEXX Laboratories Inc. However, for the study described in this abstract, we used another laboratory (a local one, since the samples had to be analyzed as soon as possible).

## ESVNU-P-6 - European Society of Veterinary Nephrology and Urology

### Canine and Feline Urolithiasis in Portugal: New trends and findings - a retrospective study of 13 years (2007 - 2020)

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The chemical composition and prevalence of canine and feline uroliths are variable among countries and over time. In Portugal, the last epidemiological survey concerning canine and feline urolithiasis dates from 2007. In dogs, this report showed that struvite was overrepresented (44.2%) and two times greater than calcium oxalate (CaOx) (22.1%) while in cats, these mineral types were equally represented (44.6%). Updated information is lacking.

This study aims to revise the current epidemiological data about canine and feline urolithiasis in Portugal over the last thirteen years.

A retrospective study was conducted. Medical data concerning Portuguese canine and feline uroliths submitted for analysis between January 2007 and January 2020, were reviewed. The uroliths were evaluated with optical, infrared, and energy dispersive X-ray spectroscopy in a Urolith Research Laboratory. Data regarding sex, age, breed, mineral composition, and location were assessed. Descriptive and analytical statistics (Chi-square) were used ($p<0,05$).

A total of 1707 uroliths (1174 canine and 533 feline) were included. In dogs, 61.5% (691/1124) were from males, of which 81.8% (565/691) were intact. The median age was 7 years-old and Yorkshire Terriers were overrepresented (13.1%; 154/1174). About mineral composition, the more prevalent in dogs were: struvite (37.2%; 437/1174), CaOx (30.0%; 352/1174), purines (11.4%; 134/1174) and compound (11.3%; 133/1174). CaOx was more common in male dogs and struvite in female dogs ($p<0.01$). In cats, 56.9% (291/511) were males of which 72.5% (211/291) were castrated. The median age was 7 years old and European Shorthairs were overrepresented (31.7%; 169/533). The most common uroliths in cats were: CaOx (51.4%; 274/533); struvite (38.6%; 206/533); compound (3.9%; 21/533) and purines (3.0%; 16/533). There was no difference on urolith composition between males and female cats ($p=0.24$). Cystoliths were overrepresented in both species (82.5%-969/1174 for dogs and 75.4%-402/533 for cats).

This study supports that uroliths were overrepresented in adult dogs and cats, being more commonly located in the urinary bladder. Correlating these results with previous epidemiological data, it was observed that CaOx prevalence increased in both species. This is particularly relevant in cats, in which CaOx uroliths are overrepresented, stressing the need of clarifying and monitoring predisposing factors in this species. The prevalence of struvite uroliths decreased in both species, possibly due to its accurate identification and the awareness for prescription of therapeutic diets. This study updates the prevalence of urolithiasis, highlighting the variability of urolith composition in Portuguese canine and feline population over the last thirteen years.

**Disclosures**

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## ESVNU-P-7 - European Society of Veterinary Nephrology and Urology

### May urinary neutrophilic degeneration and intracellular bacteria predict urine culture outcome in canine suspected urinary tract infections?

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In veterinary medicine, stained urinary sediment evaluation has shown higher diagnostic performance than wet-mount evaluation in diagnosis of urinary tract infections (UTIs). For bronchoalveolar lavage and body cavity fluids, it is reported that degenerative neutrophil changes, and intracellular bacterial localization have known diagnostic relevance in evaluating the role of bacteria in inflammation. The aim of this prospective study is to evaluate associations between urine culture and the detection of neutrophil morphological alterations or intracellular bacteria in stained urinary sediment in dogs with clinically suspected UTI.

UTIs were suspected based on clinical history and clinical signs (such as pollakiuria, dysuria, and hematuria). Each dog had cystocentesis and underwent routine urinalysis with wet-mount sediment evaluation, urine culture, bacterial cell count and antibiotic-sensitivity test for their routine care for suspected UTI. Diff-Quik-stained cytospin samples were microscopically evaluated for the presence/absence of neutrophils and bacteria, neutrophil morphological alterations and bacterial localization (intra/extracellular). Urine culture with <100 colony-forming units were classified as negative. Presence/absence of degenerated neutrophils, bacteria and bacterial localization were compared between positive/negative urine culture using Fisher's exact test. Odds ratio (OR) was calculated.

Forty-five dogs were prospectively included. Seventeen dogs (38%) had positive urine culture. Sixteen dogs showed bacteria at cytospin evaluation, of which 14 dogs had intracellular bacteria. Dogs showing cytologically-evident bacteria had a 19-fold chance to have positive urine culture (OR 19.5 95%CI 4.2-83-5;$p<0.0001$), which increases if intracellular bacteria were highlighted (OR 27 95%CI 5-108; $p<0.0001$). Twenty-two dogs showed neutrophils, in particular 19 dogs had degenerated neutrophils. Dogs having neutrophils had a 6-fold probability to have positive urine culture (95%CI 19-22; $p=0.006$), whereas if degenerated changes were present the OR=12 (95%CI 3-40; $p=0.0005$). Finally, the combination of degenerated neutrophils with intracellular bacteria was present in 16 dogs, (OR 27 95%CI 5-108; $p<0.0001$). Interestingly, 3 dogs (6.5%) with degenerated neutrophils and intracellular bacteria had negative urine culture.

Based on our results, the probability to have urine culture positivity increases if intracellular bacteria and degenerated neutrophils are

seen. Most importantly, in some cases it allows to identify an active infection even with negative urine culture. Their presence in stained-mount samples may help the clinician in the diagnostic process of dogs with suspected UTIs.

**Disclosures**

No disclosures to report.

## ESVNU-P-8 - European Society of Veterinary Nephrology and Urology

### Sodium bicarbonate 8.4% as a potential alternative to unfractionated heparin as a dialysis catheter locking solution: A pilot study

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In human medicine, catheter-related thrombosis is one of the leading causes of premature dialysis catheter dysfunction. This complication results in reduced blood flow and poor dialysis adequacy, often requiring catheter exchange. To prevent thrombus formation and maintain patency during interdialytic periods, the lumens are locked with an anticoagulant solution, typically unfractionated heparin (UFH) or trisodium citrate. Due to the small size of veterinary dialysis patients, secondary hemorrhage or coagulopathy are significant risks associated with the use of these locking solutions. More recently, sodium bicarbonate ($NaHCO_3$) has been proposed as an alternative locking solution in human medicine. Its antibacterial and anticoagulation properties are making it a possible safer alternative in our small animal population.

The goal of this pilot study was to investigate the use of $NaHCO_3$ 8.4% and its potential to inhibit intraluminal clot formation for an interdialytic period of 48 hours.

This was a randomized cross-over pilot study. Four healthy beagles were sedated for placement of a jugular dialysis catheter. Each dog had a baseline activated clotting time (ACT) measured. Two dogs were assigned to have their catheter locked with $NaHCO_3$ 8.4% and the other two with UFH 1000 iu/mL. After 48 hours, 3 mLs was aspirated from each lumen, which represented more than twice the intraluminal fill volume (1.2 and 1.1 mLs). An ACT was performed on the mixture of locking solution and venous blood. Catheters were then removed and visually assessed. The same process was repeated three weeks later. At this time, each dog had the dialysis catheter placed in the contralateral jugular vein and locked with the opposite solution.

All catheters remained patent during the study period. Only one catheter, locked with UFH 1000 iu/mL developed a macroscopic clot, which was not the case in the same dog when the catheter was locked with $NaHCO_3$ 8.4%. The ACTs of all mixtures from the lumens locked with UFH 1000 iu/mL were markedly prolonged (>999 seconds). In the mixtures from the lumens locked with $NaHCO_3$ 8.4%, the ACTs were prolonged at least 4 times above baseline, ranging from 435 to >999 seconds.

This pilot study suggest that $NaHCO_3$ 8.4% could be a safe and efficacious alternative in maintaining patency of dialysis catheters during interdialytic periods.

**Disclosures**

Financial support was through an in-house Staff Development Grant provided by the Dean of the Cummings Veterinary School at Tufts University The dialysis catheters were donated to this study by Medcomp.

## ESVNU-P-9 - European Society of Veterinary Nephrology and Urology

### Comparison of Doppler ultrasonic technique and High Definition Oscillometric systolic blood pressure measurements in conscious apparently healthy elderly dogs

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Blood pressure measurement is essential in dogs with diseases associated with systemic hypertension, as well as part of routine health screening in elderly dogs. Non-invasive blood pressure (NIBP) devices are easy-to-use and routinely available, however data on agreement of systolic blood pressure measurements (SBP) between NIBP devices in conscious dogs is scarce.

This study aimed to compare SBP obtained through Doppler ultrasonic (Doppler) and High Definition Oscillometric (HDO) technique in conscious apparently healthy elderly dogs.

Senior and geriatric dogs, defined based on an Age Analogy Chart, that were healthy according to the owner were prospectively recruited for health screening. Systolic blood pressure was obtained consecutively through Doppler and HDO technique according to the ACVIM guidelines in a randomized order per dog, by the same experienced individual and following a standardized protocol. Position and site of measurement were chosen based on the preference of the dog (i.e. least stressful measurement). Dogs were considered hypertensive when SBP ≥160 mmHg was measured with either one of the methods.

In total, 118 dogs were recruited. In 1 dog SBP could not be obtained with neither of the devices due to severe stress. In 18 remaining dogs SBP could only be obtained with 1 NIBP device (Doppler n=3, HDO

n=15). Dual measurements were available in 99 dogs. Doppler SBP was measured in standing (n=38), lying (n=26) or sitting (n=35) position, on the left (n=55) or right (n=44) radial artery. In 98 dogs HDO SBP was measured at the coccygeal artery, in standing (n=75), lying (n=19) or sitting (n=4) position. In the remaining dog HDO SBP was measured at the left radial artery in sitting position. Median SBP was 147 (105-239) and 152.8 (113-221) mmHg for Doppler and HDO technique respectively. A SBP ≥160 mmHg was measured in 26 (median 173.5; 160.6-239 mmHg) and 34 (median 168; 160.2-221 mmHg) dogs using Doppler and HDO technique respectively, in which 7 and 8 measurements were ≥180 mmHg, respectively. Eleven dogs were hypertensive on both methods, no dog had a consistent SBP ≥180 mmHg.

No significant difference ($p = 0.174$) was found between the two devices using a Wilcoxon signed rank test in the global study population, neither within the group of hypertensive dogs ($p = 0.255$).

Although Doppler and HDO technique did not result in significant different SBP values, an increased SBP was not consistently detected by the two NIBP devices.

**Disclosures**

For the presented study no specific funding was received. This study is part of a larger longitudinal prospective study for which partial financial support was received by Hills and Ceva. The author provides consulting to veterinary practices working with a veterinary commercial lab (Velab nv), however this lab is not linked (direct or indirect) with neither this study or the longitudinal prospective study.

## ESVNU-P-10 - European Society of Veterinary Nephrology and Urology

### Evaluation of the safety of alogenic adipose tissue derived mesenchymal stem cells (aAMSC) therapy in dogs diagnosed with glomerular disease: Short- and medium- term

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Currently, regenerative medicine has opened new alternatives in the treatment of inmunomediated renal diseases in human and veterinary medicine due to its immunomodulatory properties. However, the development of these therapies is limited by the possible adverse acute reactions such as fever, phlebitis, cardiovascular or respiratory disorders and thromboembolism; besides, medium- and long-term side effects such as systemic infections and tumorigenesis have also been described. In this study, our objective was to demonstrate the safety of aAMSC administration in patients affected with glomerulonephritis. In this study, a total of 13 dogs were included after being diagnosed with glomerular disease secondary to leishmaniosis (LeishVet 3 and 4). Each of them was administered a dose of 1-1.3 x $10^6$ aAMSC per kilogram intravenously over 45 minutes. During the administration, the vital signs of patients were checked every 5 minutes and adverse reactions were recorded. In addition, blood and urine tests, echocardiography and abdominal ultrasound were performed at the beginning (13/13), after one month (10/13) and three months after aAMSC administration; in the last checkup, X-rays were performed as well (9/13). Four of the patients could not be followed up as their renal condition worsened and they were euthanized.

The results showed that a very small number of patients showed adverse effects (2/13). Both patients presented gastrointestinal signs; specifically, one of the patients showed two vomits and the other had nausea. Vomits responded to a single dose of intravenous maropitant at 1 mg/kg; concomitantly, administration rate of aAMSC was reduced. The rest of the patients did not show any adverse reaction during or after the administration of aAMSC therapy. None of them showed medium-term alterations.

Hence, administration of aAMSC in patients with glomerular disease is safe in the short- and medium-terms. However, studies including a larger canine population for longer intervals are necessary.

**Disclosures**

No disclosures to report.

## ESVNU-P-11 - European Society of Veterinary Nephrology and Urology

### Urolithiasis in dogs: A retrospective study of 6,700 canine urinary stones collected from 2016 to 2020 in France and analyzed by infrared spectrometry

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The objective of this retrospective study is to measure the evolution of various types of stones and risk factors of urolithiasis in France.

The breed, age, sexual status of the dog and the location of stones are filled in using a form. The various layers of the stones are analysed by infrared spectrophotometry. A urolith without a nidus of different composition which contains 70% or more of one type of mineral is identified by that mineral. Statistical analysis was performed on Rstudio software Version 1.1.456 - © 2009-2018 RStudio, Inc.; 6700 stones were analysed. Struvite (48%) is more common than calcium

oxalate (36%), cystine (8%) and purines (6%). Only 2% of stones originate from the upper urinary tract. Small dogs (67%), Yorkshire Terriers (18%), Shih Tzu (10%), Dalmatians (3%) and males (60%) are overrepresented. Struvite is more common in females (82%) and in Shih Tzu (70%). Calcium oxalate is more frequent in mature dogs (8.5 years on average), small dogs (82%), males (55%) and in Yorkshire Terriers (63%). The monohydrate form of calcium oxalate is in the majority (80%) but in a different proportion in Shih Tzu (73%) and in neutered dogs (86%). Cystine is more frequent in Chihuahua (20%), Teckel (22%), but rare in neutered dogs (2%) and in females (<1%). Purines are more frequent in Dalmatians (99%).

Surprisingly, the proportion of struvite is high, which may indicate an increase in the frequency of urinary tract infections or a failure of calculolytic treatment or lack of knowledge of medical approaches. The proportion of calcium oxalate has decreased and could be the consequence of the decrease of predisposed breeds. Monohydrate form is less frequent in Shih Tzu, intact and younger males, which may be related to a different etiology compared dihydrate calcium oxalate. In humans, the monohydrate form suggests a search for conditions associated with hypercalciuria whereas the dihydrate form suggests a condition related to hyperoxaluria. However, this distinction is not validated in veterinary medicine but this difference between race needs further investigation. The proportion of cystine has increased probably in association with the popularity of predisposed breeds. Our results confirm the predisposition of intact males. The frequency of purines is stable.

The very high proportion of struvite emphasizes the need to inform practitioners of the appropriate medical approach and to evaluate failures. The respective etiology of calcium oxalate monohydrate or dihydrate might require to be investigated.

**Disclosures**

No disclosures to report.

## ESVNU-P-12 - European Society of Veterinary Nephrology and Urology

### Longitudinal follow-up of renal function, hydration and electrolytic status of dogs affected by immune-mediated diseases and treated with oral prednisolone

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Immune-mediated diseases (IMD), known risk factors for renal disease, are usually treated with steroids. Immune-suppressive doses of prednisolone induce renal hyperfiltration and proteinuria in healthy dogs. Objectives were to document the evolution of renal biomarkers in dogs spontaneously affected by IMD. Dogs suffering from primary IMD which did not received steroids within the previous month were eligible for this longitudinal prospective study. Glomerular filtration rate (GFR) using plasma exogenous creatinine clearance, biochemistry and complete urinalysis, including urine protein-to-creatinine ratio (UPC) and protein electrophoresis, were obtained before and after 7, 30 and 90 days of treatment. General linear model and logistic regressions were used to evaluate differences from baseline. P values<0.05 were considered significant. Nine client-owned dogs with various IMD were included; maximal dose (mean=2.8 mg/kg/day) of administered prednisolone was reached at D7. Each mg/kg of prednisolone was associated with significant decreases in mean body weight (-0.63kg), heart rate (-7bpm), creatinine (-6μmol/L), chlorides (-2.6mmol/L), ionized calcium (-0.03mmol/L) and urine specific gravity (-0.007) and increases in systolic blood pressure (+5mmHg), GFR (+0.17mL/kg/min), potassium (+0.14mmol/L), bicarbonates (+1.0mmol/L), magnesium (+0.03mmol/L) and albumin (+1.36g/L). UPC did not change significantly. Although SDS-AGE patterns were significantly associated with treatment and GFR, no simple relationship was identified. GFR and creatinine were weakly correlated (r=-0.42). In 2 dogs, GFR decreased during treatment. Moderate renal hyperfiltration and electrolytic disorders were observed in most dogs in association with prednisolone treatment. Creatinine did not accurately predict GFR. Kidney function should be monitored by other biomarkers than classical ones in dogs with IMD.

**Disclosures**

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## ESVNU-P-13 - European Society of Veterinary Nephrology and Urology

### Urinary clusterin as a potential biomarker for the early diagnosis of chronic kidney disease in cats

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Chronic kidney disease (CKD) is characterized by a permanent reduction in functioning nephrons, being the most commonly diagnosed kidney disease in older cats. Biomarkers such as the recently validated symmetric dimethylarginine (SDMA) are able to provide an earlier diagnosis of CKD; however, the disease is still frequently diagnosed quite late. Urinary clusterin is a glycoprotein whose levels were shown

to become altered in the context of kidney injury and has been suggested as a potential biomarker of CKD.

The aim of this study was to investigate differences in urinary clusterin levels between healthy cats and cats with CKD and whether there is a correlation between the levels of SDMA and urinary clusterin: urinary creatinine ratio in healthy cats.

Twenty-seven client-owned cats allocated to three groups were included in this prospective study: 15 healthy cats under seven years of age (group 1); 5 healthy cats seven years of age and over (group 2) and 7 cats diagnosed with CKD and allocated to International Renal Interest Society (IRIS) stages 1 and 2, seven years of age and over (group 3). To be considered healthy, cats were required to have no signs of disease at history collection, unremarkable physical examination and kidney ultrasound, and serum creatinine under 1,6 mg/dL. Diagnosis of CKD was made based on evidence of persistent azotemia, increased SDMA levels or decreased urinary specific gravity. Blood and urine were collected for assessment of SDMA and urinary clusterin levels, respectively. Urinary clusterin levels were quantified through Enzyme-Linked Immunosorbent Assay and expressed as urinary clusterin: urinary creatinine ratio, to account for discrepancies in urine density between animals.

Median values for urinary clusterin: urinary creatinine ratio were increased in group 3 (18,73 ± 13,43 ng/mg) in comparison to healthy groups 1 (11,86 ± 8,63 ng/mg) and 2 (7,68 ± 7,02 ng/mg), although differences between groups did not show statistical significance. No statistical significance was found between SDMA levels and urinary clusterin: urinary creatinine ratio (P=0,07) for group 1. This study suggests there is a difference in the median values for urinary clusterin: urinary creatinine ratio between healthy cats and cats with CKD; however, the lack of overall statistical significance between groups and between the levels of SDMA and urinary clusterin: urinary creatinine ratio shown in this study does not allow to confirm urinary clusterin as a reliable marker for early CKD diagnosis in cats.

**Disclosures**

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## ESVNU-P-14 - European Society of Veterinary Nephrology and Urology

### Plasma concentration of the activated and non-activated forms of the extrahepatic vitamins K-dependent protein Matrix Gla Protein in healthy dogs and dogs with chronic kidney diseases

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Besides coagulation, Vitamins K (Vit-K) regulate the process of calcification through the activation of extra-hepatic Vit-K dependent proteins such as Matrix Gla Protein (MGP). Partial Vit-K deficiency is associated with an increase of undercarboxylated MGP (ucMGP) plasma concentration and decreased tissue content of carboxylated MGP (cMGP), participating to spontaneous soft-tissue calcification in humans with chronic kidney diseases (CKD).

The aims of this prospective observational multicentric study were to measure ucMGP and cMGP concentrations in plasma obtained from healthy dogs and compare their concentrations with those of dogs suffering from CKD.

Healthy dogs (n=119, median age 30 months, range 12-204) were recruited in one hospital, healthy status was confirmed based on history, physical examination and blood work. Forty-one dogs with CKD were recruited from 3 hospitals. ucMGP and cMGP plasma concentrations were measured by commercial canine ELISA kits. Reference intervals (RI) were determined in healthy dogs using the non-parametric method. Wilcoxon signed-rank test was used to compare the results between groups.

Plasma concentration of ucMGP was significantly (p=0.009) higher in MRC dogs (median 2.6ng/mL, interquartile range [2.1-3.6]) compared to healthy dogs (2.2ng/mL, [1.7-2.9]) but only 1 MRC dog had value above the determined RI (0.5-4.2). Plasma concentration of cMGP was not significantly different between groups but concentrations were below the lowest standard concentration in most dogs.

Reference intervals were determined for plasma concentration of ucMGP in dogs. Plasma ucMGP is significantly elevated in MRC dogs, suggesting that some of them might suffer from partial Vit-K deficiency as in humans.

**Disclosures**

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## ESVNU-P-15 - European Society of Veterinary Nephrology and Urology

### Long-term follow-up of Norwegian dogs with acquired Fanconi syndrome

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Acquired Fanconi syndrome (AFS) is defined as an acquired generalized proximal renal tubulopathy, characterized by inadequate reabsorption of substances such as glucose, amino acids, bicarbonate, potassium, calcium, sodium, chloride, phosphate, magnesium, ketones and lactate. AFS is associated with various causes, including infections, diseases, drugs and intoxications. The severity of the syndrome varies greatly and the literature concerning long-term prognosis is limited.

Between September 2017 and February 2019, an outbreak of AFS was registered in Norway, and data from 50 dogs diagnosed with AFS were collected.

The aim of the present study was to establish the survival rate, recovery rate and current status regarding renal disease in these dogs 1-2 years after diagnosis.

Forty-five of the 50 dogs were available for follow-up through structured telephone interviews and patient record reviews, 5 owners were unavailable or had refrained from participation in follow-up studies.

Twenty female (10 intact, 10 castrated) and 25 intact male dogs of 24 different breeds were included. Age ranged from 1-16 years (mean and median 8 years).

More than half of the dogs, 26/45 (58 %), had recovered from AFS. Of these 26 dogs, 24 were still alive, while 2 dogs were euthanized of reasons unrelated to AFS after recovery. Nineteen dogs (42 %) had, at the time of the study, not recovered from AFS. Of these 19 dogs, 7 were alive, but still symptomatic, 8 were euthanized due to AFS and 4 were euthanized due to co-morbidities while still showing clinical signs of AFS. Recovery rates were 65 % (13/20) for female dogs and 52 % (13/25) for males.

The majority of the dogs that recovered from AFS (21/26, 81%) did so within 6 months, while 1 dog recovered after 7-12 months and 4 dogs after more than 12 months.

Of the 19 dogs that did not recovered from AFS, 6/19 dogs (32 %) were azotemic. Of these, 5 dogs were diagnosed with chronic kidney disease (CKD) (1 still alive) and 1 dog was euthanized due to acute kidney injury (AKI).

In the present study there was a recovery rate of 58 % and an overall survival rate of 69 %, 1-2 years after diagnosis. Complications such as CKD and AKI were identified in 1/3 of the dogs that did not recover from AFS. Based on these results, the long-term prognosis may be defined as guarded to fair.

**Disclosures**

No disclosures to report.

## ESVONC-P-1 - European Society of Veterinary Oncology

### Urinary bladder rhabdomyosarcoma in seven dogs

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Non-laryngeal, non-cardiac rhabdomyosarcoma occurs rarely in dogs. Juvenile urinary bladder rhabdomyosarcoma (ubRMS) is a known entity, however literature regarding its clinical behaviour and endoscopic features is scarce.

The aim of this study was to describe clinical, endoscopic features and outcome of urinary bladder ubRMS in dogs.

Seven dogs were included in this retrospective, multicenter and descriptive study. Clinical data were searched for dogs underwent transurethral cystoscopy and with histopathological diagnosis of ubRMS. Age, breed, sex and sexual status, body weight, history, imaging and endoscopic features and outcome were reviewed.

Median age and weight were 18 months (range 6-32 months) and 17.5 kg (6.5-33 kg), respectively. Breeds included English Setter (2), German Shepherd (1), French Bouledouge (1), Jack Russell Terrier (1), Cane Corso (1) and one was mixed-breed. There were 3 females and 4 males and all dogs were intact. Dogs presented dysuria (6/7), hematuria (5/7), urgency (2/7), and vesical tenesmus (2/7).

Ultrasonography was performed in all cases and computed tomography in five cases. Median tumor size was 5.8 cm (range 3-6.5 cm), and tumor location was trigone (5), and body (2). Two dogs had monolateral ureteral obstruction, one of them had also prostate involvement. Two dogs presented regional lymphadenopathy and one dog had imaging suspicious of lung metastases.

Transurethral cystoscopy revealed a mass in the trigone (3), trigone and urethra (1) and in the body (2). In one case, urinary bladder was not evaluable due to an obstructive urethral mass. A cauliflower-like mass was reported in 5 cases (not reported in 1) with variable consistency (friable 2, tough 2, not reported 2).

Six dogs had mass cytology evaluation, and only in two a diagnosis of mesenchymal tumor was obtained.

Two dogs received radical surgery, one radical surgery plus adjuvant doxorubicin, one dog cytoreductive surgery and three dogs only palliative therapy.

All dogs died from tumor-related causes. Overall median survival time was 45 days (range 20-120 days). Survival times ranged from 20 to

45 days in dogs treated with cytoreductive surgery or palliative therapy alone, and from 70 to 120 days in dogs treated with radical surgery alone or combined with chemotherapy.

UbRMS should be considered as a differential diagnosis in young dogs presenting cauliflower-like masses in the bladder. In this study, UbRMS showed an aggressive clinical behaviour and a poor prognosis. However, radical surgery and chemotherapy seemed to increase survival times.

**Disclosures**

No disclosures to report.

## ESVONC-P-2 - European Society of Veterinary Oncology

### Adenosine deaminase and uric acid in feline spontaneous malignant mammary tumors

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Adenosine deaminase (ADA) has been used as a marker of the immune status of the organism as it correlates with T lymphocytes activity. ADA is also implicated in inflammatory processes and in the metabolism of purines, being responsible for catabolism of adenosine into uric acid. Uric acid is an important antioxidant, and is used as a biomarker of the oxidative status of the organism. ADA and uric acid activity has been reported to change in inflammatory, immune-mediated and neoplastic diseases, including in human breast cancer and canine mammary tumors. However, information related with feline mammary cancer is lacking. Thus, this study aimed to investigate the serum activity of ADA and uric acid in queens with spontaneous malignant mammary tumors.

Serum concentrations of ADA and uric acid were determined in serum samples of 15 queens with malignant mammary tumors, and in 15 healthy control cats presented for elective surgical procedures or geriatric health checkups. Serum concentrations of ADA and uric acid were determined in surplus serum samples that were collected for clinical purposes in all diseased and healthy queens included in the study. The mammary tumor group was composed mainly by domestic short-hair (DSH) cats (n=14), with ages ranging from 6 to 16 years (mean 11.3 years; SD 3.0 years) and with disease in different clinical stages (including 9 queens in clinical stages I to III and 6 queens in clinical stage IV). Cats from the control group were also mainly DSH (n=12), with ages ranging from 2 to 12 years (mean 8.0 years; SD 2.8 years).

Cats with mammary tumors presented serum concentrations of ADA (median 23.12 U/L, IQR 16.28-31.81 U/L) and uric acid (median 0.15 mg/dL, IQR 0.08-0.28 mg/dL) significantly higher ($P<0.05$ in both cases) than healthy controls (ADA median 13.51 U/L, IQR 8.46-23.14 U/L; uric acid median 0.10 mg/dL, IQR 0.08-0.12 mg/dL).

The results obtained in our study revealed increased serum concentrations of ADA and uric acid in queens with malignant mammary tumors, suggesting that these analytes might be useful clinical biomarkers of this disease.

**Disclosures**

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## ISCAID-P-1 - International Society for Companion Animal Infectious Diseases

### Prevalence of vector borne pathogens in owned dogs from mediterranean area with cranial cruciate ligament rupture

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Cranial cruciate ligament rupture (CCLR) results from a multifactorial degenerative process. Canine vector-borne pathogens (CVBP) can induce joint disease but their role in CCLR has not been previously investigated. The aim of the present work is to evaluate the prevalence of CVBP in dogs with CCLR.

Forty-six dogs presented for CCLR surgical treatment and 16 control dogs euthanized for diseases unrelated to the joints were prospectively included. Pathogen testing consisted of serology for *Leishmania infantum* (Li) (quantitative ELISA), *Ehrlichia* (Eh), *Borrelia* (Bo), *Anaplasma* (Ap) and *Dirofilaria immitis* (Di) (4DX IDEXX Test), and PCR for Li, Eh/Ap sp., *Bartonella* sp., *Babesia/Theileria* sp. and filariae (*D. immitis*, *D. repens*, *A. dracunculoides*, *A. reconditum and Cercopithifilaria* spp.) both on EDTA-blood (EB) and synovial fluid (SF) samples. SF cytology and histopathological evaluation of synovial membrane were also performed. CVBP were detected in 9 dogs with CCLR: Li in 6 (3 seropositive, 1 SF PCR positive and 2 both serology

and SF PCR positive), Eh in 3 (3 seropositive, one also with DNA of *Ehrlichia canis* in EB), and *Theileria equi* in 1 (SF PCR positive). Among control dogs, Li was detected in 3 (1 seropositive, 1 EB PCR positive, and 1 seropositive, EB and SF PCR positive). SF cytology revealed inflammation in 30 dogs (including 5 controls) but CVBP were not detected. Histopathology yielded inflammatory changes in 21/46 dogs with CCLR (lymphoplasmacytic -17-, neutrophilic -1-, granulomatous -1-, and mixed lymphoplasmacytic and granulomatous -1-) and in 7/16 control dogs (all lymphoplasmacytic). It's worth noting that, one year after surgery, 2/3 dogs Li SF PCR positive developed clinical signs of leishmaniosis, and the third dog (also seropositive for Eh) was diagnosed with proteinuric renal disease (Li seronegative at that moment).

The prevalence of CVBP and presence of inflammation were not statistically significant between CCLR (19.6% / 45.6%) and control dogs (18.8%/43.7%), respectively. Limitations were the small number of dogs with CCLR included and heterogeneous population of control dogs. Furthermore, only dogs that received CCLR surgical treatment were included, excluding dogs initially diagnosed with CVBP and CCLR that ultimately didn't undergo surgery. This study was not able to demonstrate a role of CVBP in CCLR nor a presence or different pattern of joint inflammation in pathogen positive dogs. However, it is interesting that Li was the most frequent CVBP detected and that PCR SF for Li was more sensitive and earlier marker of disease than SF cytology.

**Disclosures**

No disclosures to report.

## ISCAID-P-2 - International Society for Companion Animal Infectious Diseases

### Assessment of both IDEXX Angio Detect on bronchoalveolar lavage fluid and Angiostrongylus vasorum quantitative polymerase chain reaction on EDTA blood in dogs that tested negative for IDEXX Angio Detect on EDTA blood

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Angiostrongylosis due to *Angiostrongylus vasorum* (*A. vasorum)* infection is a commonly encountered parasitic lung disease in dogs. On some occasions, the traditionally used in-clinic antigen test IDEXX Angio Detect performed on EDTA blood could lead to false negative results. Due to intermittent shedding of *A. vasorum* larvae in faeces, the sensitivity of faecal analysis by Baermann method is also imperfect. Accordingly, definitive diagnosis of angiostrongylosis sometimes requires polymerase chain reaction (PCR) for *A. vasorum* on a bronchoalveolar lavage fluid (BALF) sample obtained by bronchoscopy under general anaesthesia. The aims of this study were to assess the diagnostic utility of both (1) in-clinic IDEXX Angio Detect on BALF samples and (2) laboratory quantitative PCR for *A. vasorum* on EDTA blood sample in dogs that tested negative on blood IDEXX Angio Detect. Stored frozen leftover BALF and EDTA blood from 6 dogs (median age 4 years, range: 1-9) that tested negative on blood IDEXX Angio Detect, but were diagnosed with angiostrongylosis based on positive quantitative PCR on BALF were retrospectively used for the purpose of this study. All IDEXX Angio Detect performed on BALF (n=5, leftover BALF from dog n3 unavailable) were negative, whereas duplicate quantitative PCR on EDTA blood were positive in 2 out of 5 dogs (dogs n1 and n3, leftover EDTA blood from dog n5 unavailable). When positive, blood PCR results had high cycle threshold values (mean 37.0 ± 0.9) suggesting initial low template DNA in the sample. Results of this study demonstrated that IDEXX Angio Detect on BALF samples is of no added diagnostic value in dogs that tested negative on EDTA blood. However, quantitative PCR for *A. vasorum* on peripheral EDTA blood could be used for angiostrongylosis diagnosis in a subset of dogs that tested negative on blood IDEXX Angio Detect. This is of particular relevance in dogs that are clinically unstable and at risk of general anaesthesia for bronchoscopy and BALF sampling. Further larger study is advised to provide specific diagnostic accuracy characteristics for *A. vasorum* quantitative PCR on EDTA blood.

**Disclosures**

No disclosures to report.

## ISCAID-P-3 - International Society for Companion Animal Infectious Diseases

### Honney-coomb spleen pattern in dogs and cats with leishmaniosis

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The honey-coomb or mottled spleen pattern is highly suggestive of lymphoma in animals, especially in dogs, although it has been also associated with other malignant or benign medical conditions. Moreover, it has only been previously reported linked to leishmaniosis in 2 dogs. The aim of this retrospective observational study was to investigate the prevalence of honey-coomb pattern, as well as other splenic alterations, in dogs and cats diagnosed with leishmaniosis from an endemic area. Medical records of 505 animals with leishmaniosis were reviewed and 84 animals (79 dogs and 5 cats) with ultrasound performed were included.

The honey-coomb pattern was observed in 1/5 cats (20%) and alone or in conjunction with other splenic alterations in 19/79 dogs (24%). Splenomegaly was seen in 3/5 (60%) cats and alone or together with other abnormalities in 30/79 (38%) dogs. Heterogenicity of the parenchyma and masses were reported isolated or in conjunction with other patterns in 2/79 (2.5%) and 6/79 (7.6%) dogs respectively. No significant differences were observed regarding age, sex, breed, clinical signs, or analytical findings between dogs with and without honeycoomb spleen pattern.

An ultrasound-guided cytology was performed without any incidence in 24 animals (3 cats and 21 dogs) and *Leishmania* sp. amastigotes were detected in 21 (87.5%). Cytologies of honey-coomb spleen were positive in the unique cat and 9/10 dogs (90%); 4/5 with only mottled spleen, 3/3 together with splenomegaly and 2/2 with masses. The prevalence of positive results for *Leishmania* sp. was similar among dogs with different spleen ultrasound findings.

Ultrasound follow-up was performed between 1 and 10 months in 4 animals with normalization of the splenic size and resolution of the honey-coomb pattern with a mass in two of them. In the third dog mottled spleen persisted but follow-up was performed just one month later, and in the remaining dog the mottled pattern improved as well as the size of a mass.

This study suggests that leishmaniosis should be included in the differential diagnosis of dogs and cats from endemic areas with spleen honey-coomb pattern. Other diseases that cause splenitis and extramedullary hematopoiesis had been previously associated with this ultrasound finding, and it could also be the reason in animals with leishmaniosis. Spleen cytology in these cases seems to be a fast, safe and useful test for the diagnosis of the disease.

**Disclosures**

No disclosures to report.

## ISCAID-P-4 - International Society for Companion Animal Infectious Diseases

### Identification of prognostic factors in dogs hospitalized due to acute *Leptospira* spp. infection

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Leptospirosis is a zoonotic disease caused by spirochetes of the genus *Leptospira* spp., responsible for a systemic infection. In dogs, leptospirosis is caused by two pathogenic species, *Leptospira interrogans* and *Leptospira kirscheneri*.

The aim of this study was to identify prognostic factors that influence the course of hospitalization and the outcome of acutely infected dogs admitted at the Teaching Hospital.

Data from 165 dogs with clinical presentations compatible with leptospirosis, from December 2013 to December 2019, were retrieved from the hospital information system. Antibody detection-based IIF (IgM/IgG) and PCR were used to confirm suspected cases. Fifty-five (33.3%) dogs tested positive, 95 (57.6%) negative, 15 (9.1%) remained suspect.

Then a retrospective study was conducted including 55 dogs with a definitive leptospirosis diagnosis.

The most frequent clinical signs were vomiting (72.7%), anorexia (50.9%), diarrhoea (40.0%) and haematuria (40.0%). Main biochemistry changes were an increase in AST (100%), total bilirubin (81.8%), urea (70.6%) and creatinine (69.2%). Leading hemogram abnormalities were leucocytosis with neutrophilia (68.0%) and thrombocytopenia (51.3%). The main changes observed in electrolytes imbalance and urinalysis were proteinuria (94.7%), haematuria (73.7%), hypochloremia (63.2%) and hyponatremia (52.6%). Abdominal ultrasound showed hepatomegaly (47.2%), hepatic hypoechogenicity (38.9%), renal cortex hyperechogenicity (25.0%), splenomegaly (25.0%), biliary sludge (22.2%) and loss of corticomedullary differentiation (16.7%).

The median hospitalization stay was 6.0 days for surviving dogs and 3.0 days for non-survivors (p=0.001). The presence of neutrophilia significantly increased the hospital stay (p=0.023). The survival rate was 50.9%.

Ampicillin (69.1%) and doxycycline (65.5%) were the most given antibiotics. Their use was significantly associated to survival, ampicillin (p=0.009; OR=0.18) and doxycycline (p=0.0002; OR=0.02). On the contrary the use of furosemide was associated to an increase of 9.5 times in the probability of death (p=0.043). A worse prognosis was also associated to senior dogs (p=0.035), and to the presence of hypothermia (p=0.010), oligoanuria (p=0.0003), increased urea (p=0.010) and increased creatinine (p=0.016). Hypothermia increased 5.7 times the probability of death. High creatinine levels augmented 4.7 times the probability of death.

This study found several host determinants, clinical and laboratory abnormalities, associated with a negative outcome that should be assessed and taken into consideration, namely dog's age, rectal temperature, degree of azotemia and urine output. Moreover, treatment with doxycycline or ampicillin should start as soon as possible, to improve the prognosis.

**Disclosures**

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## ISCAID-P-5 - International Society for Companion Animal Infectious Diseases

### Canine parvovirosis versus canine distemper: Risk factors, hospitalization course, and outcome

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Parvovirosis and canine distemper are major causes of morbidity and mortality among dog populations, caused respectively by canine parvovirus (CPV-2) and canine distemper virus (CDV).

The aim of this study was to identify host-related risk factors leading to the hospitalization of infected dogs at the Teaching Hospital, and to compare the course of hospitalization and outcome between diseases.

Data from 130 dogs with confirmed parvovirosis and 26 dogs with confirmed distemper were retrieved from the hospital information system over a 7-year period (2013-2020). The influence of age, gender, neuter status, vaccination, breed, and presence of concomitant disorders was investigated through a logistic regression model. A descriptive analysis assessed the length of stay, outcome, readmissions and survival rate. A two-step approach was used to evaluate the hospitalization length and range, considering first all patients and then only discharged patients.

Concerning parvovirosis, age ≤1 year old ($p<0.001$; OR=71.8; $CI_{95\%}$=27.7-227.6) and absence of vaccination ($p<0.001$; OR=73.8; $CI_{95\%}$=12.9-1410.9) were risk factors for hospitalization. The overall median hospitalization stay was 4.5 days (1.0-18.0) and 5.0 days for discharged dogs, within the same range. The discharge rate (83.8%) and the survival rate (82.3%) were very close, when considering readmissions.

Regarding canine distemper, age ≤1 year old ($p=0.0013$; OR=43.8 $CI_{95\%}$=6.4-930.9) and absence of vaccination ($p=0.032$; OR=14.9, $CI_{95\%}$=1.8-352.3) were risk factors and being neutered was a protection factor ($p=0.036$; OR=0.04, $CI_{95\%}$=0.001-0.51). The overall median hospitalization stay was 3.5 days (1.0-20.0) and 4.0 days for discharged dogs within the same range. The discharge rate was 50.0%, decreasing to 34.6% survival rate, when considering readmissions.

This study found similar patterns of host-related risk factors between both diseases, as young unvaccinated dogs were high-risk animals, even though the courses of disease were different. In parvovirosis, longer hospitalization was need but full recovery was achieved in most cases with high survival rates. In opposition, distemper patients recovered faster from clinical episodes, but lower survival rates were observed. After an initial hospitalization, distemper patients tend to be readmitted with recurrent clinical episodes leading in many cases to euthanasia months after their first hospitalization.

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## ISCAID-P-6 - International Society for Companion Animal Infectious Diseases

### Autochthonous *Babesia canis* infections in 25 dogs in Berlin/Brandenburg (Germany)

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Autochthonous cases of canine babesiosis have been described in different German regions. Despite the high abundance of the vector *Dermacentor reticulatus*, this was until a few years ago only very rarely the case in Berlin/Brandenburg. More recently, i.e. between 4/2015 and 1/2021, babesiosis was diagnosed in 25 dogs located in the Berlin/Brandenburg region (5 in 2015-2016; 20 in 2019-2021); in 22 dogs a complete clinical record was available.

The aim was to describe history, clinical/laboratory findings, and course of disease in these 22 dogs with autochthonous acute *Babesia* spp. infection.

Dogs presented (April to September n=8, October to January n=14) for lethargy (n=22), fever (13), and "red urine" (9). Dogs had never (18) left the Berlin/Brandenburg area or not in the 6 weeks before onset of clinical signs (4). Most common clinical findings were pale mucous membranes (13) and fever (11). All dogs had thrombocytopenia (platelet count 0-139 G/l, median 15.5), 19 anemia (hematocrit 0.17-0.38 l/l, median 0.32), and 12 intravascular hemolysis at presentation; 10 dogs pancytopenia and 12 bicytopenia. Further initial laboratory abnormalities included hyperbilirubinemia (18), hypoalbuminemia (14), azotemia (8), and elevated liver enzyme activities (18). Spleno- and hepatomegaly were detected in 21 and 4 dogs, respectively; pancreatitis, uveitis, and myocarditis were occasionally diagnosed. *Babesia* were detected in the blood smear in 18 cases. PCR for detection of the 18S rRNA gene of Piroplasmida was positive in all dogs, and sequencing showed a complete match with *Babesia canis* sequences in GenBank. All dogs were treated with imidocarb (2.4-6.3

mg/kg, median 4.4) (2 dogs received 1, 19 received 2 and 1 dog each received 3 and 4 injections). PCR-based follow-up examinations in 17 dogs revealed that 4 were still PCR positive 15-32 days after the 1st, 3 dogs 22-57 days after the 2nd and 1 dog 22 days after the 3rd injection. Two dogs were euthanized on days 2 and 18, respectively.

This case series demonstrates the autochthonous occurrence and major clinical relevance of *B. canis* infections in dogs in Berlin/Brandenburg. This may be due to the spread of the vector, import of dogs infected with *B. canis* to Berlin/Brandenburg and infection of ticks with *B. canis*, although this has not yet been shown. PCR monitoring during and after treatment is advisable. Further investigations concerning the potential *B. canis* endemization are urgently needed. A year-round tick protection is strongly recommended.

**Disclosures**

BK declares that she repeatedly has lectured for and acted as consultant for veterinary pharmaceutical, nutritional and diagnostic companies and has previous and ongoing research collaborations with various veterinary pharmaceutical, nutritional and diagnostic companies. GvSH declares that he repeatedly has lectured for and acted as consultant for veterinary pharmaceutical and diagnostic companies and has previous and ongoing research collaborations with various veterinary pharmaceutical and diagnostic companies. JK declares that he has previous and ongoing research collaborations with various veterinary pharmaceutical companies. CH declares that she has previous and ongoing research collaborations with various veterinary pharmaceutical companies. CW declares that she has held lectures for veterinary pharmaceutical and diagnostic companies. SP: Elanco markets animal health pharmaceutical products including anti-parasitic drugs. IS and EM are employed by the laboratory offering the diagnostic tests mentioned.

## ISCAID-P-7 - International Society for Companion Animal Infectious Diseases

### Infections with Babesia spp. in dogs living in Germany (2007-2020)

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*Babesia* (*B.*) spp. are protozoal agents transmitted by ticks. Aims of the study were to assess the incidence of *Babesia* spp. infections in a large cohort of German dogs and to evaluate associations with possible stays abroad, tick infestation and ectoparasitic prophylaxis by sending questionnaires to the veterinarians. The database of a laboratory was screened for *Babesia* spp.-PCR tests in dogs between 01/2007 and 12/2020. From 2018 on, sequencing of PCR-products was performed and data on hematology/clinical chemistry was collected from positive tested dogs.; 659/20914 dogs (3.2%) were tested PCR-positive for *Babesia* spp. with the highest proportion identified as *B. canis* (199/205 dogs). Highest incidences were detected in the years 2010, 2013, 2015 and 2019 and the months April and October. Questionnaires were available from 1857/20914 dogs (8.9%). In 692/1857 dogs (37.2%) no stays abroad were reported, 54/692 (7.8%) tested positive. Seasonal distribution, tick infestation and ectoparasitic prophylaxis had a statistically significant impact.

In 139 dogs, data on hematology and/or clinical chemistry was available: pancytopenia existed in 55/135 dogs (40.7%), anemia in 114/139 dogs (82%), thrombocytopenia in 120/135 (88.9%) and leukopenia in 68/136 (50%). Hyperbilirubinemia was observed in 81/109 dogs (74.3%), hypoproteinemia in 48/111 (43.2%) and low serum iron concentrations in 55/99 (55.6%).

Infections with *Babesia* spp. occur throughout the year, high incidence is correlated with highest activity of *D. reticulatus* in Germany. Travel and import are most often stated as prominent source of infection in Germany. Our data indicate autochthonous infections with *B. canis* occurring in a considerable number.

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IS and EM are employed by the laboratory offering the diagnostic tests mentioned. CM and GH are emplyoyed by the company AVIA GIS, supporting the statistical analysis. BK declares that she repeatedly has lectured for and acted as consultant for veterinary pharmaceutical, nutritional and diagnostic companies and has previous and ongoing research collaborations with various veterinary pharmaceutical, nutritional and diagnostic companies. GvSH declares that he repeatedly has lectured for and acted as consultant for veterinary pharmaceutical and diagnostic companies and has previous and ongoing research collaborations with various veterinary pharmaceutical and diagnostic companies. JK declares that he has previous and ongoing research collaborations with various veterinary pharmaceutical companies. CH declares that she has previous and ongoing research collaborations with various veterinary pharmaceutical companies.

## ISCAID-P-8 - International Society for Companion Animal Infectious Diseases

### Relationship between novel biomarkers of glomerular function and circulating antibody levels in advanced leishmaniosis stages in dogs

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Frequently, patients classified in advanced stages of canine leishmaniasis (CL) may suffer from glomerular disease resulting from the deposition of immune complexes. Circulating antibody (CAb) levels have been positively correlated with greater intensification of clinicopathological abnormalities and kidney damage. The objectives of this study were: 1) to evaluate the relationship between antibody levels and glomerular biomarkers namely: urinary protein/creatinine (uP/C), plasma cystatin C (CysC), symmetric dimethylarginine (SDMA), urinary ferritin (uFerr) and urinary C-reactive protein (uCRP); 2) to determine the correlation between the rest of glomerular biomarkers.

Ten dogs classified in advanced stages of CL (Leishvet 3 and 4) were included. Hematology, biochemistry, urinalysis and urine protein/creatinine ratio (uP/C) were performed. CL was diagnosed using an ELISA technique for the determination of antibodies against recombinant protein (RP) and against total soluble antigen (SLA) of *Leishmania infantum*.

CysC was measured using a turbidimetric latex assay (Spinreact®, Spain) and SDMA was analyzed using a commercial kit (IDDEX®, U.S.A.). uFerr was determined using a commercial immunoturbidimetric assay (Tina-quantt Ferritin, cobas®, Germany) and expressed as urine ferritin/creatinine ratio (uFerr/c). uCRP was analyzed by turbidimetric latex assay (Spinreact®, Spain) and expressed as urine CRP/creatinine ratio (uCRP/c).

The data were examined using a Pearson correlation and a p-value $< 0.05$ was considered as statistically significant. Significant correlations were observed between the levels of CAb against SLA and uCRP/c with a correlation coefficient (CC) of 0.67 and a P-value of 0.03. When comparing the biomarkers against each other, significant correlations were obtained between CysC and uFerr/c (CC: 0.77; P-value: 0.009), as well as between SDMA and uP/C (CC: 0.76; P-value: 0.01).

In conclusion, this study demonstrates that an increase in uCRP/c correlates with an increase inCAb against SLA. Furthermore, an increase in the plasma concentration of CysC and SDMA are related to an increase in the levels of uFerr/c and uP/C respectively, suggesting that these of glomerular filtration rate also correlate with enhanced glomerular damage and increased proteinuria, respectively.

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**ISCAID-P-9 - International Society for Companion Animal Infectious Diseases**

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**Cytokine modulation of the innate immune response in natural canine leishmaniosis**

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Canine leishmaniosis (CanL) is caused by obligate intracellular parasites of the genus *Leishmania* that live within myeloid lineage cells. Immunotherapy strategies are being considered as novel approaches to prevent progression of the disease. However, immune control of *Leishmania* infection requires a delicate balance between innate and adaptive immunity, which is modulated by cytokine and immune cell interactions. To get a deeper understanding of the molecular mechanisms behind these interactions, we evaluate the impact of *Leishmania infantum* natural infection in dogs on the host immune response at whole transcriptome level.

Total RNA-seq was performed on lymph node aspirates collected for diagnostic purposes, including 10 sick dogs and 8 healthy non-infected dogs which served as controls. Diagnosis was based on serology results for *L. infantum* by immunofluorescence antibody assay (IFA) plus cytology and a PCR results obtained from lymph node aspirates, as well as considering the presence of clinical signs and/or clinicopathological abnormalities associated with CanL, according to LeishVet guidelines. Differential expression analysis was performed after pseudo-mapping the raw reads to the canine reference transcriptome (CanFam 3.1). Then, weighted gene co-expression network analysis was performed on the most variable transcripts (SD < 0.25). This analysis allowed us to identified clusters of co-expressed transcripts, defined as modules, that significantly correlates with CanL (correlation > 0.6 and a p-value ≤ 0.05), and thus could play a role in the disease. To further characterize these modules, we performed functional enrichment analysis for Gene Ontology (GO) and pathways identification, using the web tools Enrichr and Gene Set Enrichment Analysis (GSEA), respectively.

These analyses resulted in the identification of cytokine signaling and regulation of innate immunity as key pathways in CanL. There were no variations in the expression of IFN-γ or IL-12, two critical Th1 cytokines for control of macrophage infection by *Leishmania*; neither in the expression of TGF-β nor IL-10, which are Th2 cytokines related to parasite persistence. In contrast, the expression of IL-15, interferon regulatory factor 1 and other key factors for IFN-γ production were significantly increased; while MAPK and ERK cascades, as well as NCAM and calcium signaling pathways, which are essential for high-level expression of IFN-γ, were downregulated.

All this suggests that the upstream signaling events leading to the production of IFN-γ are stimulated in the lymph nodes of dogs with CanL, but the parasite may be hijacking some downstream pathways to suppress IFN-γ expression and subvert the host immune response.

**Disclosures**

No disclosures to report.

**ISCAID-P-10 - International Society for Companion Animal Infectious Diseases**

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**Use of serological screening tools to detect canine antibodies against *Leishmania infantum* in Spain**

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There are several serological screening tools used to detect *Leishmania infantum* infection in dogs. Some studies have compared their sensitivity and specificity, but none have tried to investigate their actual use in Spain. The aim of this study was to investigate which screening diagnostic tests to detect canine antibodies against *L. infantum* are employed in Spain nowadays and how their use changed from 2012 to 2017.

A database of clinical records was created using information sent from several veterinary centres. The veterinarians completed a set of electronic datasheets (about dog characteristics, screening tools and other relevant information) for each presented case. The serological tests were classified in five groups: 1) Direct agglutination test (DAT), 2) Enzyme-linked immunosorbent assay (ELISA), 3) Indirect immunofluorescence (IFI), 4) Rapid tests and 5) Other assays.

Information from 3721 dog cases was gathered. The most used screening tools were rapid tests (61.4%) and ELISA tests (28.5%), followed by IFI (7.7%), DAT (2.2%) and other tests (0.2%). Regarding trends of assays used over time, a simple linear regression was calculated to predict the proportion of use for each type of test based on time (from 2012 to 2017). ELISA tests and DAT were used similarly through the years but a significant change was found in the use of rapid tests ($p = 0.0274$), IFI ($p = 0.0139$) and other assays ($p = 0.0344$), with an $R^2$ of 0.74, 0.81 and 0.71, respectively. Thus, the predicted use of rapid tests increased 5.20% for each year while IFI tests and other tests decreased 1.13% and 0.12% for each year, respectively.

In conclusion, there seems to be a clinical preference in Spain for the use of rapid tests in the clinical setting to detect specific *L. infantum* antibodies, probably due to their fast results, low price and easy performance, while other types of tests such IFI are employed less due to increased time to performance and mainly because they need to be conducted in laboratories by trained personnel.

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**ISCAID-P-11 - International Society for Companion Animal Infectious Diseases**

## Rising trends on ESBL-producing Enterobacterales clinical strains from companion animals

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The level of resistance to the 3rd/4th generation cephalosporins in Enterobacterales is a well-established problem in Human Medicine. These highest priority critical important antimicrobials (HPCIAs) for humans according to WHO are also categorized in the EU as B "Restrict" regarding their use in Veterinary Medicine. The aim of this retrospective study (January 2017-December 2020) was to assess the frequency and trends of ESBL-producing Enterobacterales (ESBL-E) causing infections in companion animals (CAs).

Each sample was plated on standard media plates and on ESBL selective plates. Susceptibility testing was done according to CLSI. A total of 13338 samples submitted for culture, of which 6795 (51%) were positive. Sample origin was from soft skin and tissue infection (SSTI) (42.8%), urinary tract infection (UTI) (28.6%), otitis externa (OE) (24.7%) and upper-respiratory tract infections (URTI) (1.3%).

Eight-hundred and sixty-five ESBL-E (26.8%) were isolated out of 3229 clinical Enterobacterales strains (49.3% of all isolated bacteria). Approximately 16.4% of CAs with UTI infections had an ESBL -E as a bacterial pathogen, followed by 14.1% from SSTI and 5% from URTI. In 2017, 10.8% of all isolates were ESBL-E, rising to 15.7% in 2018. In 2019, a slight decrease to 14.6% was observed and in 2020 fell down to 10.3% of all isolated bacteria. *Escherichia coli* was the most prevalent species found among bacterial pathogens (58%), however just 21.2% were ESBL producers. On the other hand, *Klebsiella* spp. was only isolated on 10% of samples, but 52.3% were ESBL producers.

The rising numbers of ESBL-producing Enterobacterales in CAs pose daily therapeutic dilemmas in the use of HPCIAs/A and B antibiotics, limiting treatment efficacy. These percentages emphasize the microbiological hazard CAs pose to both animal and human health. Nevertheless, the small decrease in 2020 shows that change is possible.

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**ISCAID-P-12 - International Society for Companion Animal Infectious Diseases**

## Serosurvey of *Coxiella burnetii* in companion animals from Portugal

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Q fever is a zoonotic infection that regained worldwide interest from health authorities following the large-scale human outbreak of Q fever in The Netherlands from 2007 to 2011. Ruminants are considered the main reservoirs of human infection. *Coxiella burnetii* infection has also been reported in companion animals, however, their role in the epidemiology of this bacterium is still unclear. This study aimed to perform a serosurvey of *Coxiella burnetii*, the causative agent of Q fever, in companion animals from Portugal.

A cross-sectional study was conducted in dogs and cats presented to veterinary medical centres from the North and Centre regions of Portugal between October 2020 and March 2021, that required blood sampling as part of their diagnostic plan. Only surplus serum samples were used in this research. Sera were tested for the presence of specific antibodies anti-*C. burnetii* using a commercial ELISA adapted for multi-species detection (ID Screen Q Fever Indirect Multispecies®, IDVet). Laboratory results were expressed in S/P values (optic density of the sample / optic density of the positive control sample). Samples with an S/P value between 40% and 50% were considered suspicious, and samples with SP values >50% were classified as positive.

A total of 107 animals were sampled (dogs n=60; cats n=47). The canine population was composed by 25 pure-breed and 35 crossbreed dogs, with ages ranging from 5 months to 15 years old. Cats were mainly of the Domestic Short-Hair breed (n=45), with ages ranging between 6 months and 9 years old. The estimated exposure rate was of 1% (95% CI: 0.02-5.1%), meaning that only one positive result was obtained (1/107) with an S/P of 54.9%, corresponding to a six years old female dog living in a rural area. Another female dog with five years old living in a semi-rural area had a suspicious result (S/P=43%).

The rate of exposure found in pets was very low, and even inexistent in cats. This finding suggests that companion animals from the North and Center regions of Portugal are not often exposed to the pathogen. However, the monitoring of *C. burnetii* infection in companion animals is a major tool to prevent human outbreaks, considering the zoonotic potential for owners and veterinarians contacting with infected animals, mainly dogs and cats from rural areas which often contact with livestock.

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**SCH-P-1 - Society of Comparative Hepatology**

## Quantitative evaluation of gallbladder, cystic duct, and common bile duct size by magnetic resonance cholangiography in cats - A post mortem pilot study

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Conventional imaging methods have limitations in assessing the feline biliary tract. In human medicine, magnetic resonance cholangiography (MRC) is the non-invasive gold standard to evaluate biliary tract disorders. In veterinary medicine, first reports deemed MRC feasible in cats for qualitative assessment. However, MRC-based, quantitative measurement data of the feline biliary tract have been missing. With this post-mortem MRC pilot study, we aimed at determining the reference diameters of gallbladder, cystic duct, and common bile duct (CBD) in adult cats by comparing cats without and with biliary tract disorders.

Bodies of 10 adult pet cats donated for research purposes underwent MRC and necropsy. For MRC, a human extremity coil was used in a 1.5 Tesla MRI system (Ingenia, Philips, The Netherlands). T1 and T2 weighted turbo spin echo (TSE) sequences were used to localize the region of interest followed by three-dimensional (3D) T2w-TSE-MRC to visualize biliary tract anatomy. 3D-TSE-MRC images were saved in 2D and measurements of the biliary tract conducted using the image analysis software ImageJ 1.45 (Bethesda, Maryland, United States). Gross pathology and histology after MRC revealed no evidence of biliary tract disorders in six cats (control group). Reported disorders in the remaining four cats were mild to moderate multifocal lymphocytic cholangitis (two cats), bilobed gallbladder and alimentary lymphoma (one cat each). None of the four cats had histopathological evidence of ductal stricture or dilation. Diameters were measured for gallbladder (fundus, body, and neck), cystic duct (where leaving the gallbladder) and CBD (at duodenal papilla and extrahepatic duct junction).

Median (range) diameters in the control group were for gallbladder at fundus 7.39 mm (5.70-11.73 mm), body 11.52 mm (8.72-18.98 mm) and neck 2.67 mm (1.96-5.38 mm), for cystic duct 1.55 mm (1.39-2.01 mm), and for CBD at extrahepatic duct junction 2.04 mm (1.39-3.49 mm) and at duodenal papilla level 1.85 mm (1.30-2.28 mm). Respective diameters of the cats with lymphocytic cholangitis and alimentary lymphoma were within the same ranges. The cat with bilobed gallbladder had a wider CBD diameter at the duodenal papilla (2.50 mm).

This post mortem pilot study provided normative MRC measurements for the gallbladder, cystic duct, and CBD for adult cats. Quantitative measurements in cats with biliary tract disorders did not reveal major

differences probably due to missing formation of ductal strictures or dilations. Future studies in larger numbers of cats are warranted to assess the diagnostic value of quantifiable data in 3D-TSE-MRC images of cats.

**Disclosures**

No disclosures to report.

## SCH-P-2 - Society of Comparative Hepatology

### Serum protein electrophoresis in dogs with chronic hepatitis

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Data regarding serum protein electrophoresis (SPE) in dogs with chronic hepatitis (CH) are limited. CH may cause alterations on SPE of people and dogs, and polyclonal gammopathies with β" γ bridging has been suggested to be nearly pathognomonic.

The aim of this study was to describe the most common alterations on SPE in dogs with CH and evaluate SPE in relation with leukogram and histopathologic inflammatory activity and fibrosis score.

SPE of dogs with a histopathological-diagnosed CH were retrospectively reviewed. Serum proteins were separate on agarose gel into: albumin, alpha ($\alpha_1$ and $\alpha_2$), beta ($\beta_1$ and $\beta_2$) and gamma ($\gamma$) globulins; a computer produced a densitometer trace, showing protein fractions and reference ranges. We considered percentage values of each fraction. Dogs were included if a complete haemato-biochemical profile was also available. Based on the histopathologic evaluation, inflammatory activity score (A, from 0 to 5) and fibrosis score (F, from 0 to 4) were assigned to each sample. Dogs were divided into groups: A≤2 and A>2; F≤2 and F>2. Differences between groups were investigated using Unpaired t-test. Categorical data were analyzed using Fisher's exact test.

Twenty-six dogs were included. The most common alterations on SPE were decreased A/G ratio (76.9%) and hypoalbuminemia (57.7%), followed by increase α1-globulins% (34.6%) and α2-globulins% (34.6%); increase of γ-globulins% (26.9%); increase of β1-globulins% (23%) and β2-globulins% (19.2%). Only 1 dog showed the β-γ bridging. Dogs with hypergammaglobulinemia had leukocytosis and neutrophilia more frequently than dogs without (P= 0,014). Group A>2 showed higher β2%-globulins than group A≤2 (P=0.016) and group F>2 showed higher β2%-globulins (P=0.02) than group F≤2.

This is one of the few studies that evaluates SPE in dogs with CH. SPE in these patients showed signs of inflammation that can help clinicians to monitor the disease progression or remission. Increase of β2%-globulins seems to be influenced by a more severe grade of inflammation and fibrosis. The β" γ bridging described in literature do not seems to be a reliable indicator of CH.

**Disclosures**

No disclosures to report.

## SCH-P-3 - Society of Comparative Hepatology

### Serum bile acids in a referral population of dogs with liver diseases

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Serum bile acids (SBA) are a helpful diagnostic tool for the evaluation of the enterohepatic circulation. SBA can be influenced by cholestasis and are therefore not useful in differentiating most hepatobiliary diseases, but as they are highly concentrated in the portal blood, their systemic concentration increases significantly in diseases characterised by portosystemic shunting.

The aim of this study was to compare the SBA concentration in dogs with liver circulatory diseases, with biliary, liver parenchymal and liver neoplastic diseases.

All dogs with measured fasting and postprandial SBA and a final diagnosis of primary liver disease were included retrospectively from 2013-2020. Patients were included if total bilirubin had been measured concurrently and was normal, and if clinical history, physical examination findings, bloodwork and the appropriate diagnostic imaging and/or clinical pathology investigations were available for a review. Patients were divided into four groups according to the "WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases": circulatory disorders (1), biliary disorders (2), parenchymal disorders (3) and neoplastic disorders (4).

A total of 122 patients were included in the study. The median postprandial SBA in 68 dogs in group 1 was 121.4 (range 1 - 569 umol/L), in 4 dogs in group 2 - 45.2 (range 4.5 - 82.7 umol/L), in 45 dogs in group 3 - 26 (range 0.9 - 707 umol/L) and in 5 dogs in group 4 - 6.8 (range 2.2 - 38.1 umol/L). Dogs with circulatory liver disorder had significantly higher fasting and postprandial SBA values comparing to dogs with other hepatobiliary diseases ($p < 0.01$). The sensitivity and specificity of the post prandial SBA for liver circulatory disorders including acquired and congenital portosystemic vascular anomalies (CPVA) at a cut off value of greater than 100 umol/L was 69% and 91%, respectively; and 71% and 84% for dogs with the CPVA. Following the stringent inclusion criteria for this study, only 8% of dogs with CPVA had fasting and postprandial SBA values below 50 umol/L. Sixty percent of these dogs were diagnosed with a left gastrophrenic portosystemic shunt.

Based on the results of this retrospective study, we can conclude that most liver circulatory disorders are associated with significantly higher SBA values than any other hepatobiliary disease.

**Disclosures**

No disclosures to report.

**SCH-P-4 - Society of Comparative Hepatology**

## First description and preliminary characterization of a microfluidic canine liver-on-a-chip

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Organoids are miniature organs cultivated in a protein mixture simulating the physiological microenvironment of cells (Matrigel). Hepatic organoids can provide insight to the function, development, and maturation of different cell types (cholangiocytes and hepatocytes) affecting liver function in dogs and humans. The Organ-on-a-Chip is a clear, flexible polymer that contains hollow channels lined by living cells and tissues that mimic organ-level physiology. Combining hepatic organoid culture and Liver-on-a-chip technology can better simulate *in vivo* conditions. Next will be the addition of co-culture chambers (eg. vascular endothelial cells, or Kupffer cells) to simulate physiological blood flow and interaction of liver cells with phagocytic cells. The project aim was to develop and maintain a canine liver-on-a-chip culture system without the need for Matrigel.

The chip was formed by bonding a layer of micropores sandwiched between two layers of polydimethylsiloxane (PDMS) microfluidic channels. The organoid culture was derived from laparoscopic biopsy of the liver in a healthy adult beagle. Organoids were mechanically dissociated and micro-injected into the chip channel, which was devoid of Matrigel. Expansion media was administered intermittently for 8 days; then, a cultivation media was administered for 5 days by continuous infusion of 35 μL per hour. The resultant organoid culture was subsequently fixed in formalin-acetic acid-alcohol solution and paraffin embedded for tissue sectioning. Canine liver-specific mRNA probes targeting cell surface markers were designed for performance of RNA *in situ* hybridization (RNAscope). mRNA expression was evaluated semi-quantitatively for the following markers: stem cells (LGR5), cholangiocytes (SOX9, KRT-7, KRT-19, and AQP1), early hepatocytes (FOXA1), and mature hepatocytes (CYP3A12).

Cholangiocyte markers were expressed preferentially in comparison to other cellular markers in this culture system. The expression of KRT-7 was 10.29% (in signal area/total area of cells), KRT-19 5.36% (SA/TA), and SOX9 0.71% (SA/TA). FOXA1, CYP3A12, and AQP1 were not expressed significantly in tissue cultures. Stem cell marker (LGR5) expression was 2.10% (SA/TA).

We provide preliminary data describing the first successful canine Liver-on-a-chip microfluidic organoid culture. Our results show the presence of canine hepatic stem cells and cholangiocytes; however, markers of hepatocyte differentiation were not significantly expressed. This finding is not unexpected since hepatic progenitor cells preferentially differentiate into cholangiocytes. The decreased AQP1 expression observed in the culture was expected, given the overall low expression rate of this protein in canine hepatic tissues. These preliminary data suggest that 3D microfluidic Organ-on-a-Chip technology is a viable option for investigating the physiological responses of canine liver.

**Disclosures**

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