EUROPEAN COLLEGE OF VETERINARY INTERNAL MEDICINE – COMPANION ANIMALS

ORAL RESEARCH COMMUNICATIONS OF THE 15th ECVIM-CA CONGRESS Glasgow, Scotland, 1st to 3rd September 2005

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ESVNU—European Society of Veterinary Nephrology and Urology

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VBPS—Veterinary Blood Pressure society

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ESVONC—European Society of Veterinary Oncology

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ESVIM—European Society of Veterinary Internal Medicine

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ESCG—European Society of Comparative Gastroenterology

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ESFM—European Society of Feline Medicine

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1 EFFECT OF HYDRATION STATUS ON ECHOCARDIOGRAPHIC MEASURES OF THE LEFT HEART IN NORMAL CATS.

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The aim of this randomized crossover study was to determine the effects of hydration status on the echocardiographic measures of the left heart in normal cats (n = 10; median age 3 y, weight 3.8 ± 0.8 kg). This population was identified via physical examination, serum biochemistry, PCV, urinalysis, thoracic radiography and 2-D, color flow Doppler, and Doppler tissue imaging echocardiography. Three protocols were employed, including dehydration and intravenous fluid administration at two fluid rates, with a 6–7 day washout period between protocols. To create dehydration, water was withheld and furosemide administered (2–4 mg/kg IV q1–2hr to a total dose of 14 mg/kg) over 7hr. Normal saline was administered at a standard maintenance rate (2.5–3 mL/kg/hr IV for 24 hr) and at a rate typically used during surgery (10 mL/kg/hr IV for 7 hr). Prior to and at the completion of each protocol, cats were sedated, the urinary bladder emptied, and body weight, PCV, and TP determined, followed by an echocardiographic examination that was performed prior to randomization and at the completion of each protocol. From the right parasternal short-axis views, the left ventricular (LV) interventricular septum and LV free wall thicknesses in diastole (IVSd and LVFWd), LV internal diameter in diastole (LVIDd) and systole, largest left atrial (LA) diameter and aortic root diameter in diastole (Ao) were determined. Left ventricular chamber area (LVCAd) and left atrial area (LAAd) were determined by tracing the endocardial borders of the LV and LA in diastole. All measures were taken from 4 to 5 consecutive cardiac cycles and averaged. Data were analyzed using one-way repeated measures ANOVA with Bonferroni post hoc test.

The dehydration protocol resulted in a mean (±SD) 5.6% (±0.3%) reduction in body weight ($P < 0.001$) and a 24% (±7%) increase in PCV and TP ($P < 0.0001$). Significant and similar increases occurred in IVSd (4.5 ± 0.4 to 5.8 ± 0.6 mm; $P < 0.001$) and LVFWd while LVIDd (12.6 ± 1.8 to 9.3 ± 1.7 mm; $P < 0.001$), LVCAd (1.4 ± 0.4 to 0.7 ± 0.3 cm^2; $P < 0.001$), LA:Ao ratio (1.4 ± 0.2 to 1.2 ± 0.1; $P < 0.05$) and LAAd (1.4 ± 0.2 to 1.0 ± 0.2 cm^2; $P < 0.05$) decreased. Body weight was not altered by either fluid protocol and at the lower fluid rate only LAAd increased (to 1.8 ± 0.5 cm^2; $P < 0.05$). The higher fluid administration rate increased LVIDd compared to baseline (to 14.4 ± 2.0 mm; $P < 0.01$) and increased LVCAd (to 1.6 ± 0.3 cm^2; $P < 0.01$), LA:Ao ratio (to 1.7 ± 0.1; $P < 0.01$), and LAAd (to 2.3 ± 0.6 cm^2; $P < 0.001$) relative to the lower fluid administration rate and to baseline. In conclusion, hydration status alters the echocardiographic measures of the left heart in normal cats. LV pseudohypertrophy occurs with dehydration, and the sizes of the left heart chambers vary with hydration, which may lead to an erroneous diagnosis of cardiac disease or mask its presence.

2 SPONTANEOUS FELINE CARDIOMYOPATHY AS A MODEL FOR DIASTOLIC HEART FAILURE (DHF): IS COLOR M-MODE TRANS-MITRAL FLOW PROPAGATION VELOCITY SENSITIVE ENOUGH?

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Traditional Doppler measures of trans-mitral or pulmonary venous flow patterns often prove technically challenging and/or confounded by loading conditions. Transmitral propagation velocity (Vp) is considered both a sensitive and a load-independent gauge of diastolic function.

The feasibility of measuring Vp of feline early transmitral flow was investigated in comparison to 15 other diastolic function parameters (including mitral annular tissue Doppler velocities). Data were retrospectively collected from a total of 28 non-sedated, non-treated normal cats (N), cats with spontaneous hypertrophic cardiomyopathy (H) and cats with spontaneous restrictive cardiomyopathy (R) of comparable age, gender distribution, body weight, and heart rate. Descriptive statistical values were compared between N and each of the other 2 groups, as well as between N and H&R combined, using a two-tailed t-test assuming unequal variances. A P-value ≤0.05 was defined as suggestive of statistical significance.

When compared between N (0.07 ± 0.01) versus H (0.18 ± 0.06) and versus R (0.12 ± 0.04), E/E′ differed statistically significantly ($P = 0.0375$ and $P = 0.0363$, respectively). When compared between N and both H and R combined, statistical significance was higher at $P = 0.0027$. Vp was readily obtainable in 82%, 86%, and 70% of animals from N, H, and R, respectively. When compared between N (55 ± 24 cm/sec) versus H (30 ± 14 cm/sec) and versus R (24.3 ± 15.5 cm/sec), Vp differed statistically significantly more than did E/E′ ($P = 0.008$ and $P = 0.008$, respectively). When compared between N and both H and R combined, statistical significance was similar at $P = 0.009$. Vp, E′, and E/E′ demonstrated highly statistically significant differences, despite the relatively small sample size studied. Vp differed statistically significantly between N, H, and R cats more than did E/E′ and more consistently than did E′. Relative to N, other diastolic parameters demonstrated trends of impaired relaxation in H and restrictive filling in R, reaching no or peri-borderline statistical significance.

Vp holds promise of becoming a sensitive, rate and load independent and readily attainable parameter of diastolic dysfunction in spontaneously occurring feline cardiomyopathy. Larger scale, controlled, and blinded validation studies are warranted to enable taking full advantage of this spontaneously and physiologically occurring animal model of human DHF.

3 DOPPLER ECHOCARDIOGRAPHIC ASSESSMENT OF E:Ea AND E:Vp AS INDICATORS OF LEFT VENTRICULAR FILLING PRESSURE IN NORMAL CATS AND CATS HYPERTROPHIC CARDIOMYOPATHY.

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Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease of cats. While most affected cats are asymptomatic, HCM can progress to congestive heart failure (CHF). CHF leads to pulmonary venous congestion, pleural effusion, and pulmonary edema, which is recognized clinically by analysis of thoracic radiographs. The development of pulmonary edema is predicted largely by the magnitude of left ventricular (left atrial) filling pressure (LVFP). Measurement of LVFP requires cardiac catheterization, which is not suitable in clinical patients. Novel, recently introduced echocardiographic indices (E:EA and E:VP) combining spectral Doppler peak early transmitral flow velocity (E) with mitral annulus peak early diastolic tissue Doppler velocity (EA) or Doppler peak early diastolic flow propagation velocity (VP) have been shown useful in the Doppler-echocardiographic prediction of LVFP in people. In this study we hypothesized that the Doppler derived E:EA and E:VP ratios predict the radiographic diagnosis of CHF.

A total of 131 cats, 52 normal cats, 56 cats with HCM and without CHF, and 23 cats with HCM and CHF were studied. Diagnostic procedures included Doppler echocardiography (DE), thoracic radiography, and blood pressure measurement. Normal cats were used to establish reference values for E:EA and E:VP. Cats with HCM were compared to normal cats matched for age, body weight, heart rate, blood pressure, and left ventricular shortening fraction (n = 26).

In normal cats, E:EA (mean, 95% confidence interval) measured at the septal or lateral mitral valve annulus was 6.82 (6.20 to 7.45) and 7.37 (6.68 to 8.06), respectively ($P = 0.268$). The E:VP ratio was 1.16 (1.01 to 1.30). Age, heart rate, blood pressure, and left ventricular shortening fraction did not affect E:EA. Body weight had an effect on E:EA ($r = -0.32$, $P = 0.028$); age influenced E:VP ($r = 0.44$, $P = 0.003$). The E:EA ratio was increased ($P < 0.05$) in cats with non-congestive HCM (8.6, 7.9 to 9.4) and cat with HCM and CHF (17.1, 15.4 to 18.9), as was the E:VP ratio (1.45, 1.21 to 1.70 versus 2.38, 1.81 to 2.94). Using a cut-off of **12.0** for E:EA and **2.0** for E:VP to separate cats with HCM and CHF from cats with HCM and without CHF, sensitivity was 87% and 63%, specificity was 90% and 81%, positive predictive value was 76% and 59%, and negative predictive value was 95% and 83%, respectively. Kappa statistics revealed a Kappa of 0.74 (''substantial agreement'') and 0.43 (''moderate agreement'') for E:EA and E:VP in comparison with radiography in the prediction of CHF.

The E:EA ratio appears to be a useful Doppler-echocardiographic index of CHF in cats with HCM.

4 DOPPLER ECHOCARDIOGRAPHIC ASSESSMENT OF THE E:Ea RATIO AS AN INDICATOR OF LEFT VENTRICULAR FILLING PRESSURE IN NORMAL DOGS AND DOGS WITH HEART DISEASE.

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Left sided congestive heart failure (CHF) is a common clinical syndrome characterized by cardiac dysfunction, neurohormonal activation, renal sodium and water retention, and elevation of left ventricular filling pressure (LVFP). CHF leads to pulmonary venous congestion and edema, which is recognized clinically by analysis of thoracic radiographs. The development of pulmonary edema is predicted largely by the magnitude of left ventricular (left atrial) filling pressure. Measurement of LVFP requires cardiac catheterization. A novel, recently introduced echocardiographic index (E:Ea ratio) combines spectral Doppler peak early transmitral flow velocity (E) with mitral annulus peak early diastolic tissue Doppler velocity (Ea). This ratio has been shown to be a promising tool in the noninvasive prediction of LVFP in people and experimental dogs with mitral regurgitation. This study (1) aims at the determination of reference values for E:Ea and (2) addresses the general hypothesis that Doppler echocardiography (DE) can identify CHF by predicting radiographic abnormalities diagnostic of CHF in dogs.

A total of 132 dogs underwent DE studies: normal boxers (n = 24), normal Golden retrievers (n = 7), boxers with subaortic stenosis (SAS) but without CHF (n = 13), Golden retrievers with occult Duchennes Muscular Dystrophy (DMD; n = 18), dogs with degenerative mitral valve disease (MVD) and without (n = 37) and with (n = 10) CHF, and dogs with dilated cardiomyopathy (DCM) and without (n = 12) and with (n = 11) CHF. Dogs with SAS, MVD, and DCM underwent also thoracic radiography.

In normal dogs, E:Ea (mean, 95% confidence interval) measured at the septal or lateral mitral valve annulus, was 5.19 (4.78 to 5.60) or 5.41 (4.82 to 6.00), respectively ($P = 0.381$). Age, heart rate, body weight, and left ventricular shortening fraction did not affect E:Ea. Dogs with SAS (5.71, 4.44 to 6.98), occult

DMD (7.57, 7.04 to 8.10), and non-congestive MVD (7.41, 6.64 to 8.18) or DCM (6.84, 6.21 to 7.48) had similar E:Ea ($P > 0.05$). However, in dogs with CHF and MVD (14.34, 11.86 to 16.83) or DCM (12.26, 10.85 to 13.67), E:Ea was significantly elevated ($P < 0.0001$). Using a cut-off of 11.0 for E:Ea to separate dogs with CHF from dogs without CHF, sensitivity was 82%, specificity 93%, positive predictive value 82%, and negative predictive value 93% in the echocardiographic prediction of CHF determined by radiography. Kappa statistics revealed a Kappa of 0.86, indicating an ''almost perfect'' agreement between Doppler-echocardiographic and radiographic diagnosis of CHF.

The E:Ea ratio appears to be a useful Doppler index of CHF. Invasive studies comparing LVFP with Doppler indices of LVFP are needed to validate our findings.

5 ARTERIOSCLEROTIC CHANGES IN MYOCARDIUM, LUNG AND KIDNEY IN DOGS WITH CHRONIC CONGESTIVE HEART FAILURE AND MYXOMATOUS MITRAL VALVE DISEASE.

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Intramural arteriosclerosis and fibrosis in the myocardium of dogs with myxomatous mitral valve disease (MMVD) is previously described but the association between the two problems has not been established and the clinical significance of the arterial changes has not been evaluated. 21 dogs with naturally occurring congestive heart failure (CHF) and MMVD and 21 age and weight-matched control dogs with no signs of CHF and no or minimal findings compatible with MMVD underwent an extensive pathological and histopathological examination. Morphometry was used to measure arterial narrowing and fibrosis in the myocardium, arterial narrowing in the lung and kidney and intimal thickness and plaque formation in the aorta and pulmonary artery of the animals. CHF-dogs had significantly more arterial narrowing in the left ventricle ($P < 0.003$), lung ($P < 0.0001$) and kidney ($P = 0.02$) than control dogs. CHF-dogs also had significantly more fibrosis in the left ventricle ($P < 0.0001$) and more intimal-medial thickening in the pulmonary artery ($P = 0.04$). They did not, however, have significantly more plaque-formation or intimal-medial thickening in the aorta than control dogs. There was significantly more arterial narrowing in the papillary muscle than in all other locations in the CHF-dogs ($P < 0.002$), while the control dogs had similar changes in the papillary muscle and septum. This is the first study demonstrating an association between MMVD and intramural myocardial arteriosclerosis in the dog, and the association of arteriosclerosis in other organs to MMVD, possibly implying that this could be a more generalised disease process. The study also questions if the myocardial changes seen in these dogs are not involved in the pathogenesis of CHF in dogs with MMVD.

6 DECREASED SYSTOLIC FUNCTION AND INAPPROPRIATE HYPERTROPHY IN SMALL BREED DOGS WITH CHRONIC MITRAL VALVE DISEASE.

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Chronic mitral valve disease (CMVD) is the most common acquired heart disease of dogs. Systolic dysfunction has been reported as an uncommon finding in affected small breed (SB) dogs, and if present, it has been considered an end-stage CMVD or the effect of complicating factors such as multiple small intramural myocardial infarcts. Recently in experimentally induced chronic mitral regurgitation studies it has been suggested that inadequate hypertrophy developing with this condition would be responsible for the left ventricular dysfunction observed both in people and in experimental dogs. The aim of this study was to investigate systolic function and left ventricle hypertrophy in a group of SB dogs (<15 kg) with naturally occurred CMVD and moderate to severe heart failure.

33 SB dogs with CMVD and class 2 ISACHC of heart failure and 17 normal SB, with regard to heart status, dogs were included in the study. All dogs underwent an echocardiographic examination, which included transthoracic 2-D and M-mode. Right parasternal M-mode recordings were obtained from short-axis views with the dogs positioned in right lateral recumbency. A mean of three consecutive measurements was considered for each variable. M-mode measurements included left ventricular end-diastolic diameter (EDD), end-systolic diameter (ESD), fractional shortening (FS), and left ventricular diastolic wall thickness (h). From EDD and ESD using Teicholz formula end-diastolic (EDV-I) and end-systolic volume indexes (ESV-I) were calculated. Left ventricular radius (R) was calculated by dividing EDD by two. Systolic function was investigated using FS and ESV-I, whereas the h/R ration was used as a parameter of left ventricular hypertrophy. Data are presented as mean ± SD.

Affected dogs were significantly older (8.7 ± 3.5 vs 11 ± 3.2 yrs). There were no differences concerning sex and weight between the 2 groups. Affected dogs presented a significant increased FS (45.6% ± 8.04 vs 40.06% ± 8.9) and ESV-I (30.0 ml/m^2 ± 2.3 vs 21.18 ml/m^2 ± 13.9). The h/R was obtained in 18 affected and in all normal dogs. The h measures was not significantly different between the 2 groups (0.71 cm ± 0.04 vs 0.74 cm ± 0.18), whereas the h/R ratio was significantly decreased in affected dogs (0.41 ± 0.12 vs 0.53 ± 0.11).

Data from this study show that although FS in affected dogs was increased, as it should be expected in volume overload caused by CMVD, the increased ESV-I in these dogs suggests that a mild systolic dysfunction was present also with moderate heart failure. In fact, ESV-I is believed to represent a more reliable indicator of systolic function. Inadequate hypertrophy of left ventricle could be responsible for this finding, as it has been demonstrated in experimental models.

7 SURVIVAL IN DOGS WITH DILATED CARDIOMYOPATHY AND CONGESTIVE HEART FAILURE TREATED WITH DIGOXIN, FUROSEMIDE AND PROPRANOLOL: A RETROSPECTIVE STUDY OF 62 CASES.

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The objective of the study was to retrospectively evaluate the effect of ß-blocker therapy in dogs with DCM. Inclusion criteria were as follows: 1) echocardiographic evidence of left ventricular eccentric hypertrophy, left atrial dilatation and fractional shortening <25%; 2) radiographic evidence of left-sided or biventricular cardiac enlargement and pulmonary edema or pleural effusion; 3) medical treatment consisting of digoxin, furosemide and propranolol. Survival analysis was based on the Kaplan-Meier method.

Sixty-two dogs of 25 different large and medium-sized breeds were included in the study. Forty-five dogs (73%) were male and 17 (27%) dogs were female. Age at initial presentation ranged from 10 months to 12.5 years (mean, 7 ± 2.5 years). Body weight ranged from 12 to 69 kg (mean, 36.2 ± 12.7 kg). Echocardiographic measurements, indexed according to Kittleson and Kienle, were as follows: LVEDD ranged from 15.5 to 31.2 (mean, 21.4 ± 3.3), LVESD ranged from 9.7 to 20.5 (mean, 13.7 ± 2.5), LA ranged from 5.6 to 28.5 (mean, 15.8 ± 3.7) and Ao ranged from 2.5 to 9.11 (mean, 6.5 ± 1.3). LA/Ao ranged from 1.5 to 3.4 (mean, 2.04 ± 0.35) and FS ranged from 4 to 22% (mean, 12 ± 4). Pulmonary edema was present in 60 dogs, and peural effusion in 2 dogs. Heart rate ranged from 140 to 270 beats/min (mean, 186 ± 38). Thirty-one dogs (50%) presented with atrial fibrillation, and ventricular premature complexes were found in 9 dogs. All dogs were initially treated with digoxin (mean dose 0.009 mg/kg per day) and furosemide (mean dose 3.6 mg/kg per day). Propranolol (mean dose 2.4 mg/kg per day) was added after signs of CHF had been resolved, approximately one week after initial presentation. Additional treatment consisted of levothyroxine (15 dogs), spironolactone (4 dogs) and enalapril (2 dogs).

Survival time ranged from 8 to 1335 days (median, 126 days, mean 336 days). Nine dogs were censored in the analysis, 8 of them because euthanasia was performed for reasons unrelated to cardiac disease, and 1 dog was lost on follow-up. Fifty-two dogs were euthanized, nine dogs died suddenly. Post mortem examination was performed in 33 dogs where the attenuated wavy fiber form of DCM was found in 32 dogs and the fatty-infiltration-degenerative form in 1 dog.

The safety and efficacy of the use of ß-blocking agents in the treatment of congestive heart failure (CHF) in dogs have been debated. In comparison with previous studies, the present study shows a prolonged survival time when propranolol is added to conventional treatment with digoxin and furosemide, and that survival time in dogs with DCM and CHF treated with digoxin, furosemide and propranolol is comparable to conventional treatment including ACE-inhibitors.

8 ATHLETE HEART OR DCM IN A SPRINGER SPANIEL FAMILY?

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An athletic heart (AH) indicates the presence of morphological (increased LV diameter and wall thickness) and functional cardiac changes (reduced FS, increased SV, bradycardia) as a result of strenuous repetitive exercise. In humans and horses training also influences the development of atrioventricular valvular (AV) regurgitation, but this finding is less clear in dogs. The dilated form of cardiomyopathy (DCM) is characterised by impaired systolic function (reduced FS, EF) of the ventricular myocardium leading to progressive dilation of first the ventricle(s) and later the atria. AV valve insufficiency develops secondary to annular stretch and dilation. English Springer Spaniels (ESS) are known to develop DCM and they tend to show a rapid course. The resting echocardiogram of endurance athletes may be confused with early dilated cardiomyopathy (DCM), and the aim of the study was to show that this might also be the case in some dogs.

This study describes the long-term follow-up (4 y) of a family of non-working English Springer Spaniels (n = 5; 3 females/2 males, age 2–4 y) with different levels of fitness. The very fit dogs (n = 3) showed low heart rates (mean 64 BPM, sinus arrhythmia) but none of them were overtly bradycardic. Two out of 3 had audible systolic murmurs at the level of the left heart apex (max grade 2/6). Electrocardiography excluded the presence of atrial standstill, which has

been associated with the development of DCM in Springer Spaniels. The fit dogs showed initial echocardiographical characteristics of early DCM, where the non-fit dogs had normal echocardiographical parameters. All fit dogs had LVDd in the higher range of normal, LVDs out of reference range, normal ventricular wall thicknesses, but FS < 25% (18–23%). Obvious mitral and tricuspid valve regurgitation was present in all fit dogs, despite the AV valves having a normal appearance. No atrial enlargement was visible. Pulmonic insufficiency was visible in 2/3 dogs. Plasma taurine levels were within reference range (>50 nmol/ml). At long-term follow-up none of the dogs showed clinical signs. One of the non-fit dogs had now reached the level of fitness of the fit dogs. At this time all fit dogs had audible murmurs over the left apex (1–3/6), and one dog had an audible murmur over the tricuspid valve. Exercise did not make the murmurs disappear. Echocardiographically, LVDd had now further increased exceeding reference range, as did LVDs. FS had continued to decline in 2 dogs (min 13%), but remained stable (<25%) in the others. Mitral and tricuspid regurgitation was more marked in all dogs without associated atrial enlargement.

In conclusion, traditional echocardiography might be very misleading in the differentiation between AH and DCM. The dog's physical condition should be taken into consideration and long-term follow-up is advised before condemning these animals.

9 NEUROHORMONAL AND CIRCULATORY EFFECTS OF SHORT-TERM TREATMENT WITH ENALAPRIL AND QUINAPRIL IN DOGS WITH ASYMPTOMATIC MITRAL REGURGITATION.

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Mitral regurgitation (MR) is a common cause of heart failure in dogs. Cavalier King Charles Spaniels (CKCS) are predisposed to the disease and often develop asymptomatic MR at a young age, which seems to be associated with a decreased production of nitric oxide (NO) (measured as the stable metabolites nitrate and nitrite in plasma (NOx)). Activation of angiotensin-converting enzyme (ACE) in heart failure might lead to reduced endothelial NO release. Accordingly, ACE inhibition has been shown to improve endothelial function in humans, most likely by increasing the availability of NO. In that context, quinapril has been shown to have a superior effect on endothelial function compared to enalapril. In CKCS with MR enalapril does not prolong the asymptomatic period where endothelial dysfunction seems to be present. Quinapril, however, might be able to prolong the asymptomatic period of MR—provided that the results seen in humans regarding improved endothelial dysfunction can be reproduced in dogs.

The aim of the study was to compare the effect enalapril and quinapril on neurohormonal and circulatory parameters in CKCS with asymptomatic MR.

Ten CKCS with mild to severe untreated MR completed a protocol were they were treated with quinapril and enalapril (0.5 mg/kg PO SID for 7 days), in a double-blind cross-over study with a wash out period of 7 days between treatments. Blood samples were drawn and echocardiography was performed on days 0, 7, 14 and 21. Both treatments reduced ACE activity ($P < .001$) and increased renin activity ($P < .001$) and atrial natriuretic peptide concentration ($P < .005$). The ACE inhibitors had no effect on the plasma concentration of NOx or asymmetric dimethylarginine (ADMA). On day 0, a lower NOx concentration ($P = .02$) was found in samples taken in the clinic as compared to samples taken in the home of the dogs. Quinapril caused a significant reduction in a greater number of variables reflecting the severity of MR (e.g. jet size and left ventricular end diastolic diameter) than was found with enalapril. However, in terms of specific parameters, there was no significant difference between the effects of the two treatments on MR.

These results suggest that ACE inhibitors do not affect NOx and ADMA concentrations in asymptomatic dogs with MR, however, stress in connection with clinical examination may influence NOx concentrations in these dogs.

10 ANATOMICAL DISTRIBUTION AND ELECTROPHYSIOLOGICAL PROPERTIES OF ACCESSORY PATHWAYS IN SEVEN DOGS WITH ORTHODROMIC ATRIOVENTRICULAR RECIPROCATING TACHYCARDIA.

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Anatomic position and conduction pattern of six accessory pathways (AP) has been reported in five dogs suffering from orthodromic atrioventricular reciprocating tachycardia (OAVRT). In all but one, AP conduction was successfully abolished with radiofrequency (RF) catheter ablation. To better define electrophysiological properties, and anatomical distribution of APs in dog, it was the aim of this study to describe location, and type of conduction of 12 further APs, in a group of dogs undergone RF catheter ablation for symptomatic OAVRT. A second aim of the study was to assess long term efficacy of this treatment on the abolition of APs conduction.

Ten dogs were referred to our electrophysiology laboratory for symptomatic supraventricular tachycardia (SVT). Each dog underwent a complete cardiovascular examination before performing invasive electrophysiological study (EPS), as previously described. Conduction properties of AP, and normal AV-His-Purkinje system were assessed during sinus rhythm, and programmed electrical pacing (PES). Atrial entrainment, and resetting of tachycardia were used to characterize SVT type. RF catheter ablation was performed with a thermocouple-tipped stereable catheter, and ablation targets isolated using bipolar, and unipolar electrograms guidance. Abolition of AP conduction was evaluated 45 minutes post ablation with PES, and checking for OAVRT recurrence with serial Holter in the following six months.

Seven dogs had OAVRT: 4 Labrador Retriever, 3 Boxer, and 1 Beagle. They were all males, with a mean age of 21.71 ± 18.33 months, and a mean weight of 29.28 ± 10.09 kg. During EPS in all dogs, a narrow QRS complex and tachycardia was inducible with PES, mean cycle length was 226.42 ± 49.81 ms. Mapping tricuspid valve annulus a total of 12 APs was found. Four dogs presented single AP, two dogs multiple. APs resulted distributed along the AV groove as following: right posterior (n = 5), postero-septal (n = 3), right medioseptal (n = 2), right anteroseptal (n = 1), left lateral (n = 1). Mean AP retrograde effective refractory period was 149.16 ± 9.96 ms, and 7 APs presented unidirectional retrograde conduction. All APs were ablated, and conduction was lost after RF delivery with a mean duration of 5.28 ± 1.51 seconds, at each target zone. No AP conduction recovery within 45 minutes post-ablation was observed. Two dogs needed DC-shock for sustained atrial fibrillation. No reoccurrence of OAVRT was seen on serial Holter monitoring.

According to our findings, dogs seem to have a predominance of right-sided APs. All AV bypass tracts present very rapid retrograde conduction, while antegrade is often absent. Furthermore our data suggest that APs conduction and OAVRT can be safely and long-term eliminated with RF catheter ablation.

11 CORONARY FLOW RESERVE MEASURED BY POSITRON EMISSION TOMOGRAPHY IN HEALTHY CATS: ADENOSINE DOSE FINDING STUDY.

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In humans, positron emission tomography (PET) is the gold standard to measure myocardial blood flow (MBF). Coronary flow reserve (CFR) is defined as the ratio of maximal MBF, e.g. stimulated by administration of vasodilators, divided by resting MBF. An adenosine constant rate infusion for 7 minutes is used to determine the maximal MBF, and normally coronary flow increases 2.5–4 fold. The purpose of this study is to establish a protocol to determine MBF and CFR by PET in healthy cats for future reference in cats with hypertrophic cardiomyopathy. Emphasis was placed on the adenosine dose to produce maximal CFR and its adverse effects.

In the first part MBF was measured at rest and during adenosine at the human standard dose and rate of 0.14 ug/kg/min using PET with ^{13}N-ammonia ($^{13}NH_3$) and ^{15}O-water ($^{15}H_2O$). There was no increase of MBF and $^{15}H_2O$ was found to be unsuitable. For the main study, in 4 healthy cats anesthesia was induced with propofol, maintained with isoflurane/oxygen by inhalation. In two cats (cat 1 and 2) also direct arterial blood pressure (BP, indicated as mean, MAP) was monitored invasively through a catheter placed into the femoral artery. After MBF baseline measurement, each cat was challenged with adenosine infusions at different doses 50 min apart, randomly assigned. The doses were 0.28 (2 cats), 0.56 (3 cats), 0.84 (2 cats) and 1.12 ug/kg/min (1 cat) for 7 minutes.

In the individual cats baseline MBF were 1.72, 1.59, 1.54, 1.38 ml/g/min, maximal MBF were 2.68, 2.45, 2.32, 2.51 ml/g/min, resulting in a CFR of 1.55, 1.54, 1.50, 1.81. MBF did not correlate with adenosine dose, instead MBF decreased in 2 cats at 0.84 and 1.12 ug/kg/min, respectively. In cat 1 with invasive BP measurement there was MAP decrease from a baseline of 89 mmHg to 56 mmHg and 61 mmHg during 0.28 ug/kg/min and 0.84 ug/kg/min adenosine, respectively, returning to baseline within one min at the end of infusion. In cat 2 there was no relevant MAP decrease at 0.56 ug/kg/min and 1.12 ug/kg/min. At 1.12 ug/kg/min adenosine, the cat developed palpebral reflex and a rise in heart rate from 159 to 172/min, which ceased/returned to baseline at the end of infusion. ECG abnormalities occurred only in one cat at 0.56 ug/kg/min adenosine, consisting of a short AV-block of 3 p-waves not followed by a QRS-complex; in the same cat, 1.12 ug/kg/min did not result in any abnormalities.

In conclusion, CFR assessed by PET under adenosine infusion is markedly lower in anesthetized cats compared to awake healthy humans. Increasing adenosine doses do not relevantly increase MBF, but even high doses are tolerated without relevant adverse effects. A dose of 0.56 ug/kg/min seems adequate to study CFR in cats.

12 ANALYTICAL VALIDATION OF VARIOUS IMMUNOASSAYS FOR THE QUANTIFICATION OF CARDIOVASCULAR PEPTIDES IN DOGS.

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Measurement of cardiovascular peptides may play a role in the diagnosis and prognosis of spontaneous cardiovascular disease in dogs. In veterinary medicine usually human assays are used, however, there is scarce literature on assay validations. The aim of this study was to validate commercially available immunoassays. With one exception all of these are sold for use in dogs.

Assays evaluated were an ELISA for proAtrial Natriuretic Peptide (Vetsign CardioScreen, Guildhay Ltd. Guildford, England: proANP), two radioimmunoassays for Brain Natriuretic Peptide (Peninsula Lab. Inc, Belmont, CA: BNP_{Pen}; Phoenix Pharmaceuticals Inc, Belmont, CA: BNP_{Phoe}), three assays for Endothelin-1 (ELISA, Biomedica, Vienna, Austria: ET-1_{Bio}; EIA, Phoenix Parmaceuticals, Belmont, CA: ET-1_{Phoe}; ELISA, IBL-Hamburg, Germany: ET-1_{IBL}), and two assays for Big Endothelin-1 (ELISA, Biomedica, Vienna, Austria: Big-ET_{Bio}; EIA for rat Big ET-1, IBL-Hamburg, Germany: Big-ET_{IBL}). Validation included determination of intra-assay variability and dilutional parallelism. Intra-assay variability was determined by calculating the coefficients of variation (CV) for 10 replicates of 3 samples. Dilutional parallelism was assessed by serial dilution of 3 high-concentration or spiked samples. Results were evaluated for dilutional parallelism comparing observed with expected values. The correlation coefficient between observed and expected peptide concentrations was calculated.

For proANP the CVs of 3 plasma samples were 4.9%, 10.3% and 4.2%, for BNP_{Pen} 47.2%, 21.2% and 2.4%, for BNP_{Phoe} 8.2%, 12.7% and 21.8%, for ET-1_{Bio} 2.1%, 2.4% and 5.3%, for ET-1_{Phoe} 7.3%, 8.0% and 7.9%, for ET-1_{IBL} 17.5% and 18.8%, for Big-ET_{Bio} 5.0%, 2.4% and 8.6%, and for Big-ET_{IBL} 3.8%, 5.6% and 9.5%. Observed to expected (O/E) ratios for four serial dilutions of three plasma samples ranged from 100.3–206.2% (median: 122.2%) with a correlation coefficient (R) of 0.992 for proANP, 59.2–146.5% (99.4%) and R = 0.953 for BNP_{Pen}, 71.7–123.6% (108.6%) and R = 0.996 for BNP_{Phoe}, 161–608.3% (254.2%) and R = 0.844 for ET-1_{Bio}, 9.4–460.2% (85.7%) and R = 0.667 for ET-1_{Phoe}, 56.3–205.6% (95.2%) and R = 0.996 for ET-1_{IBL}, 241.9–1407.7% (579.1%) and R = 0.608 for Big-ET_{Bio}, and 96.5–132.9% (111.8%) with a correlation coefficient of 0.992 for Big-ET_{IBL}.

In conclusion, our study showed large differences between the performance of the assays. Considering high CVs and poor correlations and recovery in dilution studies several assays do not seem to be appropriate for use in dogs. Nevertheless, the assays for proANP, BNP_{Phoe}, ET-1_{IBL} and Big-ET_{rat} are expected to give reliable results in dogs, and, depending on additional validating studies, may be useful to study cardiovascular compensatory mechanisms in this species.

13 NEUROENDOCRINE EFFECTS OF DIGOXIN ON EARLY MITRAL REGURGITATION IN DOGS.

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Mitral regurgitation (MR) secondary to myxomatous valvular disease (MVD) is the most common acquired cardiac disease of dogs. There are well established therapeutic protocols for dogs once CHF develops. However, an ideal goal would be to find a drug that slows the progression of disease and delay the onset of CHF. There is experimental evidence that the sympathetic nervous system (SNS) is activated relatively early in the course of cardiac disease and the digitalis glycosides have been shown to reduce this SNS activity. We explored the effects of digoxin (D) on neuroendocrine parameters in dogs with early MR secondary to MVD. This was a placebo-controlled test-pair pilot study. The goals of the study were to 1) determine if there are altered neuroendocrine parameters early in the course of MR secondary to MVD and 2) does digoxin therapy in early MR secondary to MVD alter these neuroendocrine parameters.

Ten dogs were randomly allocated to either (D) or placebo (P) in test pairs where the first dog was given (D). Serum (D) levels were measured and the dose adjusted until a therapeutic level was achieved. Neuroendocrine parameters [ANP, BNP, aldosterone (ALD), plasma renin activity (PRA), epinephrine(EPI) and norepinephrine NEPI)] were measured at baseline and then repeated 4–6 weeks after a therapeutic (D) level was achieved (P dogs were repeated within the same week as the digoxin-treated dog within the pair). 5 normal dogs served as the control group. There were no significant differences (NSD) in neuroendocrine parameters between 1) all MR dogs at baseline and the control group 2) (D) dogs and (P) dogs at baseline or 3) (D) dogs and (P) dogs at end of study. There were also NSD in the subjective severity of MR or class of heart failure (modified NYHA, ISACHC). Within the (P) group, there were small but significant increases in ANP and NEPI and small but significant decreases in ALD, PRA and EPI. Within the (D) group, there were small but significant increases in ANP, ALD, PRA, EPI and NEPI. However, none of the measurements in any of the MR dogs were generally considered outside of normal limits.

From this study we concluded 1) there does not appear to be significant increases in neuroendocrine systems early in the course of MR secondary to MVD 2) this gives supportive evidence to previous studies where there is no evidence for RAAS activation early in the course of disease suggesting against the use of ACE- at this stage of disease 3) (D) did not alter the neuroendocrine parameters and does not appear to play a role in early MR secondary to MVD and 4) when (D) is administered at normal doses, adverse effects are rare.

14 MOLECULAR DIAGNOSIS OF CANINE FILARIASIS—TAKING THE GUESSWORK OUT OF MICROFILARIAL IDENTIFICATION.

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Canine dirofilariasis (HW) is usually diagnosed by specific antigen testing and/or identification of microfilariae (MF) by recognizing subtle morphological features, often necessitating examination by trained parasitologists. In 1–2% of (HW) cases, dogs can be antigen-negative (Ag−), but microfilaremic (MF+). However, at least 6 other filariae can infest dogs, producing microfilaremias that are HW Ag−. Discriminating these can be of clinical importance. Disparate diagnoses by 2 diagnostic laboratories in an Ag−, MF+ dog recently imported into the US from Europe prompted us to develop a simple molecular method of identifying different MF that would resolve this diagnostic dilemma and improve the accuracy of diagnosing canine filariasis.

Genomic DNA was extracted from *Dirofilaria immitis* and *Acanthocheilonema (Dipetalonema) reconditum* MF and from MF of the imported dog. Primers were designed from published sequences for the ITS2 regions and cytochrome oxidase I genes of *D. immitis, A. reconditum* and *D. repens* that would specifically identify each species using standard PCR techniques. An additional primer set was designed to differentiate *D. immitis* and *A. reconditum*. PCR with species-specific and multi-species primers identified the MF in the imported dog as *D. repens.*

Subsequently, we examined MF from 5 additional Ag−, MF+ dogs. In 3 dogs the MF had been morphologically identified as *A. reconditum,* as *D. immitis* in 1 dog and were unidentified in 1 dog. Our results were concordant with the morphological diagnosis in 2 dogs (1 *A. reconditum,* 1 *D. immitis*), discordant in 2 dogs (morphologic diagnosis *A. reconditum*; PCR diagnosis *D. immitis*), and the unidentified MF were diagnosed as *D. immitis* by PCR.

We further demonstrated that PCR using our multi-species ITS2 primers amplified DNA from *Brugia pahangi* and *B. malayi* filariae, two filariae related to *Dirofilaria* that can also infest dogs.

Our results highlight the complexity of traditional identification of MF in dogs. Our method allowed identification of a filaria previously unrecognized in the US (*D. repens*). Finally, we have developed a pan-filarial primer set that allows simple differentiation of most filarial species that infest dogs. This should improve the ability to identify and appropriately treat canine filariasis, as well as alert veterinarians to newly emerging filarial diseases.

15 USE OF ELECTRONIC STETHOSCOPE FOR DIAGNOSING MILD MITRAL REGURGITATION IN DOGS.

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Auscultatory findings in dogs with mild mitral regurgitation depend on observer experience and how difficult the dog is to auscultate. The aim of the study reported here was to evaluate the use of an electronic stethoscope system (The Stethoscope®, version 1.0, Meditron) for diagnosing mild mitral regurgitation in dogs with myxomatous mitral valve disease.

Two groups of dogs from ongoing screening programs were included: 75 Cavalier King Charles Spaniels (CKCS) and 75 Dachshunds. Clinical examination and echocardiography were performed in all dogs. Apart from mitral regurgitation murmur, no dogs had signs of cardiac or systemic disease. Auscultation was performed by two observers with different auscultation experience (7 and 3 years): first with an ordinary stethoscope and thereafter with the electronic stethoscope. Electronically recorded phonocardiograms (PCGs) were later evaluated for presence of murmur, murmur frequency and quality of the recording, by a third experienced observer.

In 46 dogs, mild mitral regurgitation (evaluated by colour flow mapping as jet size in percentage of left atrial area >10% and <55%) was found. Among these dogs, a murmur was found by the experienced observer in 52% of the dogs, but only in 11% of the dogs by the less experienced observer. On the phonocardiograms (PCGs) a systolic murmur was found in 20% of the recordings made by the experienced observer and in 30% of the recordings made by the less experienced observer. The specificity (assessed as percentage of dogs without jet diagnosed as having no murmur) were not different between the two observers (93%), however, lower for the PCG method (72%). The quality of the PCG recordings were especially influenced by shivering among Dachshunds. The murmur frequency was typically between 50 and 400 Hz.

In conclusion, a low percentage of murmurs was found on the PCGs from dogs with mild mitral regurgitation. Still, electronic stethoscope might improve the auscultatory diagnostic performance of less experienced observers. Further studies are needed to evaluate the use of the newer versions of the electronic stethoscope system for diagnosing mild mitral regurgitation in dogs.

16 EXPERIENCES WITH DUAL-CHAMBER PACEMAKER IMPLANTATION IN DOGS.

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Between December 1997 and November 2004 in 33 dogs (median body weight 27.0 kg, range 7.0–40.0 kg; median age 95.2 months, range 21.1–160.6 months) with a second (n = 3) or third (n = 30) degree AV block, unresponsive to a medical treatment and clinical signs like syncope or exercise intolerance, a dual-chamber pacemaker system was implanted. The most common breeds were German Wirehair Pointer (n = 7) Cocker Spaniels (n = 2), Labrador Retrievers (n = 2) and mixed breed dogs (n = 9).

Under general anaesthesia and fluoroscopic control one lead was placed into the right ventricle and the second lead into the right atrium by a transvenous access. Lead fixation to the myocardium was accomplished by an active screw in tip or passive by a lead tip with silastic tines. With a pacing system analyzer the threshold voltage, the system impedance and the amplitude of R- and P-wave were measured. The pulse generator was placed beneath the skin of the neck prescapular. Postoperative care included a neck bandaging and antibiotic treatment with amoxicillin/clavulanic acid (20 mg/kg body weight) over a 10-day period. In the time of hospitalization the programming was checked and if necessary reprogrammed by telemetry.

In all 33 patients (100%) a dual-chamber pacemaker implantation and a programming in a DDD pacing mode was possible. One patient died postoperatively because of a larynx edema. Malfunctions of the ventricular lead did not occur. Atrial lead disturbances were present in four patients and included lead dislodgement (n = 2), lead malfunction (n = 1) and improper atrial sensing (n = 1). In all four dogs these problems were eliminated by a second intervention. Consequently in 32/33 patients an atrioventricular synchrony was restored.

In conclusion the transvenous implantation of a dual-chamber pacemaker in dogs with AV block and clinical signs is possible. A reconnection of atrium and ventricle could be achieved by programming the pacemaker in a DDD- or DDDR-mode. The heart frequency adaptation is then achieved accordingly to the physiological centre, the sinus node. This results in a normal exercise efficiency.

17 EFFECT OF MILD OVERHYDRATION ON PLASMA EXOGENOUS CREATININE CLEARANCE TEST IN HEALTHY CATS.

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Determination of glomerular filtration rate (GFR) in companion animal practice allows early diagnosis of impaired renal function. Assessment of GFR by plasma exogenous creatinine clearance (PECC) test has been described in dogs and cats. Hydration status may affect GFR value. The aim of this study was to evaluate the effect of IV mild overhydration on GFR estimated by pClCr. Besides, 2 analytical conditions were compared for determination of creatinine.

PECC was measured for GFR assessment in 10 healthy cats (6M and 4F; 7 months to 17 years, 3.6 to 6.5 kg). Health was based on clinical examination and biochemistry panel results were obtained in all cats. Creatinine (40 mg/kg) was injected by IV bolus in normohydrated 10-hours fasted conscious animals and jugular blood samples were obtained before, 30, 120 and 600 minutes after injection. Immediately at the end of the first test, cats were placed under perfusion with balanced fluid(Ringer lactate) using an infusion pump, at a rate of 4 ml/kg/hour during 48h; the test was repeated after 38h of infusion. Water was given ad libitum throughout the whole procedure. Plasma was stored at −20°C at the end of each test and all plasma creatinine values (Pl-creat) were assayed simultaneously by an enzymatic method (Hitachi 917). Additionally, Pl-crea were also analyzed at the end of each experiment by enzymatic method in clinical conditions (Ménarini Spotchem SP4410)

All values are given as mean ±SE [range]. During the first test and using the Hitachi assay, basal Pl-creat was 1.53 ± 0.10 mg/dL [0.8–1.9] and GFR was 2.25 ± 0.19 mL/min/kg [1.3–3.3]. Double maintenance IV perfusion failed to induce a significant change in Pl-creat (1.46 ± 0.09 mg/dL, [0.8–1.8] and did not significantly influence GFR (2.45 ± 0.41 mL/min/kg [1.5–3.0]) (anova test, $P \leq 0.05$). Similar observations were made using the Spotchem assay although absolute Pl-creat values were significantly lower (13–14%) than using the Hitachi assay.

As a conclusion, balanced IV perfusion. at a rate of 4 mg/kg failed to induce a significant effect on the GFR measurements in healthy cats, independently from the creatinine assay selected.

18 POLYURIA-POLYDIPSIA IN DOGS: USEFULNESS OF GFR ASSESSMENT THROUGH CREATININE CLEARANCE TESTING FOR DIFFERENTIAL DIAGNOSIS.

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The plasma exogenous creatinine clearance test (PECCT) in blood was established by Watson et al (2002) and has been found to be a reliable estimate of the glomerular filtration rate (GFR).

The objectives of this study were to determine GFR-values in a group of healthy dogs from different sizes and to evaluate the usefulness of the PECCT in clinical cases with polyuria/polydipsia (pu/pd) without an obvious etiology.

Thirty healthy dogs of 15 different breeds entered the study (mean age 3 years ± 1.9; mean weight 16.1 kg ± 9.3). Eight dogs, all of different breeds (mean age 3.8 years ± 3.5) with pu/pd without azotemia entered the study.

Basal serum creatinine was determined before a dose of exogenous creatinine (20–70 mg/kg) was administered intravenously. Blood sampling was scheduled at 10, 60, 120, 360 and 600 minutes after creatinine administration and the exact time of prelevation was noted. The GFR was calculated based upon the clearance of creatinine (i.e. the actual dose administered divided by the area under the serum concentration versus time curve using a Winnonlin noncompartimental analysis program).

The healthy dogs and the clinical cases had a mean GFR of respectively 3.1 mL/kg/min ± 0.7 and 3.1 mL/kg/min ± 1.5 and ranged from respectively 2.1–4.4 mL/kg/min and 1.3–5.8 mL/kg/min. Two dogs of the second group had a decreased GFR (both 1.3 mL/kg/min) when compared with the healthy dogs and were diagnosed with renal insufficiency without azotemia. As the GFRs of the 6 other dogs were normal, further diagnostic work-up was undertaken as needed. Psychogenic diabetes insipidus was diagnosed in 2 dogs, partial nephrogenic diabetes insipidus in 1 dog and normal renal function in 2 dogs. The owners of the 6th dog declined further testing.

Interestingly, a two-fold difference in GFR (1.3 and 2.8 mL/kg/min) was noted in 2 dogs with similar basal serum creatinine concentrations (96 and 94 μmol/L).

The PECCT proved to be a valuable diagnostic tool in the work-up of pu/pd-patients, allowing to detect early renal insufficiency in 2 patients without azotemia. In the dogs with normal GFRs additional testing assessed etiologies of pu/pd different from renal insufficiency in 4 out of 5 cases. The PECCT required a 10-hour hospitalisation and was easy to perform, requiring nothing more than accurate sample prelevation and handling. The results of this study suggest that the PECCT is a useful aid in the diagnostic work-up of pu/pd in the absence of an obvious etiology.

19 BACTERIURIA IN FELINE LOWER URINARY TRACT DISORDERS (FLUTD).

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A recent study from the US reported that lower urinary tract signs afflicted approximately 1.5% of cats presented to private companion animal clinics. University-based studies (US and UK) during the last forty years operate with the following numbers: less than 2% of the cats presented with non-obstructive FLUTD have bacterial infection. 2% of the cats with obstructive FLUTD have a combination of uroliths and bacterial infection.

In 2003 and 2004, 134 cats presented with signs of lower urinary tract disorders were included in a study at NSVS. The majority of the cases were first opinion cases (>95%). All the cats went through a physical examination, and blood samples for haematology and clinical chemistry were collected. The urinary analysis included urine stix, specific gravity, and microscopic examination of sediment as well as culture and sensitivity tests. An ultrasound examination was done in most cases, but a few had x-rays done instead. Information about previous/concurrent disease, feeding regime, the cats' environment (out-door access etc), the cats' temperament and the weather when the signs first were observed, was registered through a standardized questionnaire. The cats received individual treatment, depending on clinical findings and diagnosis.

Our results diverge from those of other studies: the most striking finding was the large proportion of cats with bacteriuria. Only samples cultured on the day of collection, with growth $>10^4$ CFU/ml urine, were considered indicative of bacterial infection. Of the cats with non-obstructive FLUTD, 22% had bacterial infection, 1% a combination of uroliths and bacterial infection, and 5% a combination of crystals and bacterial infection. Of the cats with obstructive FLUTD, 5% had bacterial infection, 4% a combination of uroliths and bacterial infection, and 5% a combination of crystals and bacterial infection.

In the US and UK the majority of cats taken to the veterinarian are indoor cats, while many of the Norwegian cats are free to roam outdoors. This may influence e.g. diet, degree of exercise and water intake. The owners' control of the cats' drinking, eating and urinating habits may also be limited, thus influ-

encing the duration of signs before detection by the owners. The possible consequences of sleeping outside, in an often cold/moist environment, may also be significant.

The importance of these differences is yet to be determined, and further studies are needed, preferably also in other European countries. It would be interesting to see whether there is a difference in occurrence of the various causes of FLUTD between the US and UK and the rest of Europe.

20 ASSOCIATION BETWEEN GLOMERULAR FILTRATION RATE, PROTEINURIA AND HYPERTENSION IN DOGS WITH NATURALLY OCCURING RENAL AND NON-RENAL DISEASES.

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Naturally occurring chronic renal failure is a progressive disease, often resulting in uremia and death. Two major findings, proteinuria and systemic hypertension, are a consequence of renal disease and lead to further loss of functional kidney tissue.

The magnitude of proteinuria has been associated with the rate of progression of renal disease. Persistent proteinuria is associated with greater frequency of renal morbidity and overall mortality.

Dogs with higher systolic blood pressures (SBP) have a significantly greater risk for development of uremic crisis and mortality. A direct relationship between higher SBP and greater magnitude of proteinuria has also been reported.

Sixty dogs were enrolled in the present study. Complete blood counts, chemistry profiles, urinalyses, UP/C ratios, systolic blood pressure (SBP) and exogenous creatinine plasma clearance rates (GFR) were performed in all dogs. Dogs in our study were mixed breeds (n = 26), Bernese Mountain dogs (n = 6), Boxers (n = 4), German Shepherds (n = 3), Beagles (n = 3) and other breeds (n = 18). Final diagnoses included neoplasia (n = 21), infectious diseases (n = 18), chronic renal failure (n = 15), endocrine diseases (n = 3) and others (n = 2). Age ranged between 5 months and 15 years (median 5 years).

The following data were considered normal: GFR $\geq$ 3 ml/min/kg, SBP $\leq$ 140 mmHg, UP/C $\leq$ 0.3. There was a good overall correlation between GFR and SBP (r = 0.59, $P < .0001$) in all patients. Correlation between GFR and UP/C was weaker (r = 0.43, $P < .0007$). Dogs were divided into 4 groups according to their renal function. In group 1, despite normal renal function (n = 20), 15% of dogs had an elevated SBP and 35% had an increased UP/C value. In group 2, dogs with slightly impaired renal function (n = 24), 17% were hypertensive and 67% were proteinuric. Group 3 included dogs with moderate renal failure (n = 10), 90% had an elevated SBP and 80% showed an increased UP/C ratio. In group 4, dogs with severe renal failure (n = 6), all were hypertensive and 67% were proteinuric.

The present data reveal that there is a strong correlation between GFR, hypertension, and proteinuria once renal disease is present; however, this study also suggests that non-renal diseases can lead to significant hypertension and proteinuria in the absence of renal failure. In these patients every effort should be undertaken to search for other causes of hypertension and proteinuria. Measurement of GFR is recommendable to elucidate the origin of hypertension and proteinuria.

21 OVARIAN REMNANT SYNDROME IN QUEENS: 147 CASES (1997–2002).

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The ovarian remnant syndrome (ORS) is a condition that occurs when a neutered queen returns to oestrus behaviour and is a complication of castration. Although ORS is defined by the presence of functional ovarian tissue, the observed oestrus signs may have different underlying causes and a specific diagnosis is crucial to appropriate clinical management. Little information is currently available about ORS and the appropriate diagnostic method. The aims of the present study were to identify those historical, clinical and diagnostic findings which might assist the clinician in making a diagnosis. Case records of cats referred to our laboratory between 1997 and 2002 were reviewed. 147 cats were suitable for inclusion in the study. Estradiol (E2), progesterone (PG) and prolactin (PRL) were assayed. ORS was diagnosed in 70 cats (group A) by hCG stimulation test (England, 1997) whereas no stimulation test had been performed on 77 cats (group B). We usually only recommend the hCG stimulation test when E2 is increased in the first sample.

The queens ranged from 1 to 14 y of age (mean 4 $\pm$ 2.7 y). 125 were European short-hair. Duration of clinical signs prior to presentation ranged from 1 week to 5 years (mean 3.5 mo). Clinical signs included typical signs of oestrus (114), vocalisations (123), aggressiveness (9), polyuria/polydipsia (7), weight loss (3), polyphagia (8). Age at neutering was documented in 79 cases and the mean age was 2.6 y. Only 21 cats had been neutered before 1 y. In group A, diagnosis was confirmed for 51/70 cats. Ovulation was not induced in 13 cats with hyperE2 (120 $\pm$ 18 pmol/L) anamnesis suggesting an iatrogenic cause. E2 and PG values in 6 cats remained within the normal range suggesting an adrenal origin of oestrus signs. In group A, the frequency of ORS diagnosis was significantly higher in queens neutered after 1y (p = 0.01). Treatment was documented in 29 cats. 24 cats underwent surgery with 88% success. Medical management had been used in 2 cats and unexpectedly, signs resolved spontaneously in 3 cats. Diagnosis in group B, was only conclusive for 12/77 cats: 5 queens had hyper PG (12 $\pm$ 4 nmol/L) indicating that ovulation has occurred hence confirming ORS and 7 showed hyperPRL (25 $\pm$ 5 ng/mL) with low E2 and PG values. This study confirms the suitability of the hCG stimulation test as method of diagnosis and indicates that clinical signs can be seen months to years after castration. It was apparent in our population that late castration increased the risk of ORS. It should be stressed that the clinical signs of ORS cannot always be treated by surgery.

22 THYROID ENLARGEMENT AND ITS RELATIONSHIP TO SERUM T4 STATUS IN CLINICALLY SUSPECTED HYPERTHYROID CATS.

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Thyroid enlargement has been described as typical clinical finding in hyperthyroid cats. However, there is increasing evidence that the presence of a goiter alone is a poor indicator of symptomatic hyperthyroidism. Aim of the present study was, to relate thyroid size to serum T4 status in a group of cats with suspected hyperthyroidism.

The ventral neck of 161 cats presented to the Clinic for Small Animal Internal Medicine, University of Zurich with clinical sings consistent with hyperthyroidism was examined by 2 independent observers experienced with thyroid palpation using a semi-quantitative palpation system. Individual thyroid gland size of each side was scored between 0 (non-palpable) and a maximum of 6 (nodule >25 mm), with score 1 = 1–3 mm, score 2 = 3–5, score 3 = 5–8, score 4 = 8–12, score 5 = 12–25 mm. Allocation into scores was performed according to the largest detected nodule. In addition, total score sum of all palpable lobes of each cat was calculated. In cases, in which discrepancy between the 2 observers existed, mean score values of the largest lobe and mean score sum values were calculated. Serum T4 concentrations were determined and compared to thyroid palpation results.

In 117 of the 161 cats, one or more thyroid nodules were palpable by both observers with a score of 1 to 4. Based on the results of the T4-measurements, only 17 of the cats were classified as hyperthyroid and 144 as euthyroid. Thus, all of the hyperthyroid and 69.4% of the cats with a normal T4 had an enlarged thyroid gland.

Median (range) palpation scores and score sum of the hyperthyroid cats were 3.5 (1–4) and 4 (1–7) and in the euthyroid 1 (0–4) and 1.5 (0–6), which was statistically significantly different. Evaluation of the cats with a palpable nodule revealed a mild but significant positive correlation between the score of the largest lobe and the T4 concentration and between the score sum of all palpable lobes and the T4 concentration. However, three cats with a normal T4 had a score of 3.5, 3.5 and 4, respectively.

Results of our study show that a considerable number of cats with palpable thyroid glands has a normal T4 concentration. Therefore, no reliable conclusion on functional status of the thyroid can be drawn using its size. However, the likelihood of goiter increases with increasing size of the gland.

23 SOMATIC MUTATIONS OF THE THYROID STIMULATING HORMONE RECEPTOR GENE IN FELINE HYPERTHYROIDISM.

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The aetiopathogenesis of feline hyperthyroidism is complex and multifactorial and has not been fully elucidated. Dietary, environmental and genetic factors have all been implicated, but there has been little work on the genetic lesions associated with hyperthyroidism in cats. In hyperthyroid humans with toxic nodular goitre (TNG) or functional thyroid adenomas, activating mutations of the thyroid stimulating hormone receptor (TSHR) gene are common, but to date similar mutations have not been found in cats. In humans, most of these mutations occur in exon 10 of the transmembrane domain of the TSHR gene. At the amino acid level, the feline TSHR is 92% homologous to the human TSHR, and feline hyperthyroidism and human TNG are clinically and histopathologically similar. Our hypothesis was that similar mutations exist in hyperplastic/adenomatous nodules in hyperthyroid cats.

Genomic DNA was extracted from 134 hyperplastic/adenomatous nodules (from 50 hyperthyroid cats), and analysed for the presence of mutations in exon 10 of the TSHR gene (codons 399 to 684) by polymerase chain reaction (PCR) amplification and sequencing of PCR products. Eleven different mutations were detected, one silent and 10 mis-sense (three somatic mutations, one germline mutation/polymorphism, and six unknown). Twenty eight of the 50 cats (67/134 nodules) had at least one mis-sense mutation. The mis-sense mutations were codon met452thr in 17 cats (35 nodules), ser504ala (two different mutational forms) in two cats (two nodules), val508arg in one cat (three nodules), arg530glu in one cat (two nodules), val557leu in 13 cats (36 nodules), thr631ala or

thr631phe (each in one nodule of one cat), asp632tyr in six cats (10 nodules), and asp632his in one cat (one nodule). Five of these mutations have previously been reported in association with human hyperthyroidism. Additionally, of the 41 cats for which more than one nodule was available, not every nodule harbored a mutation, and 14 had nodules with different mutations within the same thyroid gland.

The identification of a potential genetic basis for feline hyperthyroidism is novel, increases our understanding of the pathogenesis of this significant feline disease, and confirms its similarity to human TNG.

24 AUTONOMIC NEUROPATHY IN DOGS WITH NATURALLY OCCURRING DIABETES MELLITUS.

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Diabetic autonomic neuropathy (DAN) is frequently recognised in human patients with diabetes mellitus. Parasympathetic dysfunction seems to be more profound than sympathetic dysfunction in those affected resulting in decreased heart rate variability and a higher resting heart rate than controls. Those diagnosed with dan have a calculated five year mortality rate of 56%. Analysis of heart rate variability has been useful in detecting diabetic autonomic neuropathy in humans. Previous studies have examined basic time domain analyses of heart rate variability in dogs with experimentally induced diabetes mellitus and have failed to demonstrate evidence of DAN.

This study used the neuroscope as an indicator of cardiac vagal tone. The neuroscope provides a real time measure of instantaneous cardiac vagal tone from a high resolution time domain analysis of R-R interval data. The output of the neuroscope is termed the Cardiac Index Of Parasympathetic Activity (CIPA), which is measured in units of the linear vagal scale.

The purpose of this study was to look for the existence of differences in autonomic tone between a population of dogs with naturally occurring diabetes mellitus and a population of control dogs. As part of the study, we wished to determine whether there was any correlation between cardiac vagal tone and duration of diabetes and adequacy of glycaemic control.

23 healthy dogs and 25 dogs with diabetes mellitus were used in the study, and CIPA values were determined for each dog over a period of 2600 beats. Mean, median and modal CIPA values in the diabetic population were found to be significantly lower than corresponding values in the control population (unpaired *t*-test $P = 0.0008$, $P = 0.0006$, $P = 0.0004$ respectively) supporting the existence of diabetic autonomic neuropathy in dogs. No relationship was demonstrated between CIPA values and duration of disease ($P = 0.1493$) or recent glycaemic control ($P = 0.9426$) in the diabetic population, however, there was a significant inverse relationship between CIPA values and body weight ($P = 0.0085$) in the diabetic population, that was not shown by the normal dogs, perhaps suggesting a greater proclivity for diabetic autonomic neuropathy in larger breed dogs.

The demonstration that DAN exists in dogs is a new finding. Future studies will be required to follow canine diabetics from diagnosis through their disease progression, to establish whether DAN has similar effect on survival as in their human counterparts.

25 EFFECT OF AMYLIN ON PLASMA CONCENTRATION OF GLUCOSE, INSULIN AND GLUCAGON IN AN ARGININE STIMULATION AND MEAL RESPONSE TEST IN CATS.

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Diabetes mellitus (DM) is one of the most common endocrinopathies in cats. Most diabetic cats are routinely treated with insulin. This, however, does not always lead to normalization of the metabolic situation. Because pancreatic beta-cells produce not only insulin but also amylin, it was recognized, that DM is a disease of insulin *and* amylin deficiency. Amylin reduces pancreatic glucagon secretion in various species. Hence, lack of amylin may contribute to postprandial hypersecretion of glucagon in diabetic individuals. It was therefore the aim of the present study to establish a dose-response relationship for amylin's effect to reduce pancreatic glucagon secretion in cats.

Our preclinical study was performed in 12 healthy male castrated cats (2.4 ± 0.23 years; 4.9 ± 0.4 kg). The effect of amylin on blood levels of glucose, insulin and glucagon was tested in an arginine stimulation (AST) and a meal response test (MRT). In the AST, amylin (5–10 μg/kg SC) was injected in 12h food deprived cats 5 min before arginine (0.2 g/kg IV). In the MRT, amylin was injected in 24h food deprived cats 5min before a meal which corresponded to 50% of average daily food intake. All cats consumed the meal within 10 min after presentation. Serial blood samples were obtained in both the AST and the MRT.

In the AST, amylin did not influence the arginine-induced increase in glucose and insulin concentration. Amylin significantly decreased plasma glucagon levels at some time points after injection. Over the whole 30min experimental period, amylin did not affect glucose excursion above baseline as analyzed by area under the curve (AUC). However, insulin and glucagon AUC were slightly but not significantly reduced by amylin. In the MRT, glucose AUC was reduced by 50% by amylin. Interestingly, the lower amylin dose appeared to produce a stronger effect than the higher dose. Lower glucose AUC was paralleled by (non-significantly) lower insulin and glucagon excursions.

We conclude that amylin may contribute to a better metabolic control although these effects were only minor in healthy cats. We expect, however, to see stronger effects of these amylin doses in diabetic cats which have deficient endogenous amylin levels. This will be tested in future studies in diabetic cats presented to the small animal clinic.

26 ACROMEGALY DUE TO A SOMATROPH ADENOMA IN A DOG.

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A 10-year-old intact male Dalmatian dog was referred because of gait abnormalities consisting of chronic progressive stiffness and rigidity. Other complains were polyphagia associated with weight gain, polyuria and polydipsia, and excessive panting. The owner had noticed progressive thickening of the skin and enlargement of the tongue during the last three years. In line with these observations, physical examination revealed thickening of the skin, especially at the level of the head and the neck, redundant skin folds and clear enlargement of the tongue compared to the standard of the breed. In addition, an inspiratory stridor was present. Neurological examination revealed an abnormal gait characterized by minimal tetraparesis, pacing, stiffness and slight proprioceptive defects. The only abnormalities found on routine laboratory examination were mild anaemia and increased circulating levels of CK and cholesterol. Circulating concentrations of total thyroxine, free thyroxine, and cTSH, and the results of an ACTH stimulation test were all within reference ranges. The basal plasma growth hormone (GH) concentration was markedly elevated (23 ng/ml; reference range 2–5 ng/ml) and did not decrease after glucose infusion (1 g/kg b.w.) or after somatostatin administration. The plasma insulin-like growth factor-I concentration was also markedly elevated (1254 μg/L; reference range 137–425 μg /L). Abdominal ultrasonography showed no abnormalities. Survey radiographs of the vertebral column showed diffuse spondylosis extending from the cervical to the lumbar spine. CT scan of the skull showed an enlarged pituitary gland with normal enhancement pattern.

The dog was dismissed without therapy. After 3 months the owner elected euthanasia because of deterioration of the gait abnormalities. On postmortem examination, the skin was about three times thicker than normal skin of this breed. The whole vertebral column appeared as a single and inflexible structure due to the presence of uniform and diffuse vertebral exostosis. Microscopic examination of the skin revealed a moderate regular epidermal hyperplasia and an increased amount in dermal collagen. An increased amount of fibrous tissue and fat cells was present in the interstitium of the myocardium. In the pituitary gland an acidophilic adenoma was found and the tumour immunostained positively for GH (and negative for ACTH and alpha-MSH). A final diagnosis of acromegaly due to a GH producing pituitary adenoma was made. To the authors' knowledge, this is the first description of acromegaly due to a somatotroph adenoma in the dog.

27 EFFECT OF TRILOSTANE ON CORTISOL AND CORTISONE CONCENTRATIONS IN DOGS WITH PITUITARY-DEPENDENT HYPERADRENOCORTICISM.

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Trilostane is used increasingly as a first line treatment for dogs with pituitary-dependant hyperadrenocorticism. From studies in human medicine assumptions about its mechanism of action in dogs have been drawn, but have not been confirmed. Recently we have proven that trilostane has an inhibitory effect on the 3β-hydroxysteroid dehydrogenase enzyme system (3β-HSD) in dogs. In this preliminary study we hypothesized that trilostane inhibits the 3β-HSD incompletely and has an additional effect more distal in the enzyme cascade, either on the 11β-hydroxylase or the 11β-hydroxysteroid dehydrogenase (11β-HSD).

The objective of our study was to investigate the effect of trilostane on cortisol and cortisone concentrations and on the cortisone-cortisol-ratio in dogs with PDH.

Cortisol and cortisone concentrations were evaluated in 8 dogs before and 1 hour after injection of synthetic ACTH on day 0 (t0), weeks 1–2 (t1), and weeks 3–7 (t2) of trilostane treatment.

Serum cortisol and cortisone concentrations before ACTH stimulation did not change significantly during trilostane treatment. Concentrations of cortisol and cortisone after ACTH stimulation were significantly decreased at t1 and t2. Cortisol values decreased about 70.5–98.5% (median: 92.9) at t1 and about 85–

98.5% (median: 93.6) at t2. Cortisone values decreased significantly less, about 0–84.6% (median: 46.4) at t1 and about 44.6–84.6% (median: 61.7) at t2. The cortisone-cortisol-ratio before and after ACTH-stimulation showed a significant increase at t1 and t2.

These results show that trilostane depresses the cortisol concentrations to a greater extent than the cortisone concentrations, resulting in a significant increase of the cortisone-cortisol-ratio. An influence of trilostane on the interconversion of cortisol and cortisone by the 11-HSD, which exists in two isoforms in humans, could explain these findings.

Further work is required to define, if these two isoforms also exist in dogs and if so, which one is influenced by trilostane.

28 TRH-INDUCED GH SECRETION IN DOGS WITH PRIMARY HYPOTHYROIDISM (PH).

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Primary hypothyroidism (PH) in dogs is associated with elevated basal GH secretion (Lee et al. 2001). This elevated GH secretion has endocrine significance as illustrated by elevated plasma IGF-1 levels and some physical changes mimicking acromegaly (Lee et al. 2001). In PH the hypothalamic release of TRH is increased. In addition, expression of TRH receptor mRNA has been demonstrated in somatotrophs of hypothyroid rats (Konako et al. 1997). We therefore tested the hypothesis that the increased GH release in PH dogs is due to a paradoxical response of the somatotrophs to TRH. In 10 dogs with ages ranging from 3 to 11 years, the diagnosis PH was based upon clinical signs, a plasma T4 concentration below 2 nmol/l, a plasma TSH concentration above 1.0 μg/l (range 1.6 to 9.5 μg/l), and insufficient uptake of the radioactive isotope of pertechnetate on thyroid scintigraphy. Blood samples for the determination of the plasma GH concentration were collected −15, 0, 5, 10, 20, 30, and 45 minutes after the i.v. administration of 10 μg TRH per kg body weight.

The mean basal GH concentration in the hypothyroid dogs was relatively high (2.5 μg/l) compared with that in healthy dogs. In 8 of the 10 hypothyroid dogs TRH administration resulted in a significant rise in plasma GH concentration (Figure 1). In healthy dogs, TRH administration does not result in changes of the plasma GH concentration (Rutteman et al. 1987). The results of this study indicate that the elevated plasma GH levels in dogs with PH may at least in part be due to the increased release of TRH.

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3. GR Rutteman et al. Acta Endocrinol 1987; 114: 275–282.

29 FELINE HYPERTENSION: THE ASSOCIATIONS BETWEEN LONG TERM BLOOD PRESSURE CONTROL AND SURVIVAL.

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Feline hypertension has frequently been documented in association with chronic renal failure and hyperthyroidism. To date, a large-scale study examining the association between hypertension, the adequacy of systolic blood pressure (SBP) control and survival is lacking. The aim of this study was to examine whether there is a relationship between adequacy of blood pressure control and long-term survival in a large population of naturally occurring hypertensive cats.

Hypertensive cats, from geriatric feline clinics held at two central London first opinion practices since 1998, were selected retrospectively. SBP was measured using the Doppler technique and hypertension diagnosed as SBP>170mm Hg on two occasions or once but in association with clinical manifestations of hypertension. Initial investigations where possible included full physical examination, indirect fundic examination, plasma biochemistry, urinalysis and total T4 if findings were indicative of hyperthyroidism. All hypertensive cats were treated with amlodipine besylate (initially 0.625 mg SID) and the majority of cats were re-examined within 3 weeks of diagnosis to ensure efficacy of medication. Cases were subsequently examined and SBP measured at approximately 6–12 week intervals until death or the study end point (1st March 2005). A time-averaged mean SBP was calculated to evaluate SBP over the survival period. Based on the time-averaged mean SBP, cats were divided into quartiles. The Kruskal-Wallis test was used to compare the survival times between the four groups representing differing levels of control of BP. In addition, clinical laboratory measures of renal function, age and SBP at diagnosis were compared between groups.

One hundred and forty one hypertensive cats were entered into the study. The median SBP at diagnosis for each quartile was 190.6 mmHg (Q1), 191 mmHg (Q2), 191.2 mmHg (Q3) and 208 mmHg (Q4) and the time averaged median SBP for each quartile was 139.8 mmHg (Q1), 151.5 mmHg (Q2), 155.9 mmHg (Q3), 174.4 mmHg (Q4). The median survival time for Q1 to Q4 respectively was 290, 286.5, 244, and 132.5 days. These data showed an apparent relationship between time averaged SBP and SBP at diagnosis ($P = 0.013$) and between time averaged SBP and survival ($P = 0.016$). Neither renal function nor age at diagnosis were significantly different between the four groups.

The univariate analysis of these data suggest that blood pressure control with amlodipine may influence survival in hypertensive cats. Furthermore, the lower the blood pressure at diagnosis the better the control on treatment is likely to be. Further investigation of these data using multivariate techniques is warranted.

30 EFFECT OF SEDATION ON RESISTIVE AND PULSATILITY INDEX IN DOGS.

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Resistive index (RI) and pulsatility index (PI) are indirect measurements of the blood flow resistance that may be measured on small arteries by pulsed Doppler ultrasonography. They had been used to evaluate vascular changes in renal and ophthalmologic disease. Chemical restraint is mandatory to perform ultrasonography in some animals. Thereafter, sedation may potentially alter RI and PI. Then, the purpose of this study is to detect modifications of RI and PI due to sedation in healthy animals.

Fifteen healthy Beagle dogs were studied. Duplex Doppler interrogation was performed in interlobar or arcuate arteries of the kidney, and in long posterior ciliar artery (LPCA). Heart rate was determined in the femoral artery from pulsed Doppler records. At least, three waveforms were registered in three separate sites (cranial, medial and caudal) within each kidney (9 waveforms) and three in the LPCA within each eye. The RI and PI were calculated for each waveform. In addition, indirect systemic blood pressure was measured by means of the conventional Doppler system on the tibial cranial artery. Finally, midazolam (0.2 mg/kg) IM and butorphanol (0.2 mg/kg) IM were administered and all ultrasonographic measurements were carried out again. An additional group of 12 healthy non-Beagle dogs were also studied without sedation.

The values of RI and PI obtained in the Beagles before sedation were compared with the healthy mixed dogs following a Student's *t* test (normal distributed values) or a Mann-Whitney *U*-test (non normal distributed values). The results obtained in the Beagles before and after sedation were also compared following a Student's *t* test or a Wilcoxon test. A correlation study (Pearson for normal distribution or Spearman for non normal distribution) was applied between RI, PI and blood pressure and heart rate.

No significant differences were found between the renal indexes of the non Beagle dogs and the beagles before sedation. The normal renal RI was 0.62 ± 0.05 for the right kidney and 0.62 ± 0.04 for the left kidney. The normal renal PI was 1.14 ± 0.19 for the right kidney and 1.14 ± 0.15 for the left kidney. The RI and PI of arcuate arteries were significantly higher in sedated Beagles than in nonsedated Beagles. The values for RI and PI in the sedated Beagles were 0.72 ± 0.03 and 1.53 ± 0.20 for the right kidney and 0.73 ± 0.04 and 1.58 ± 0.26 respectively for the left kidney.

There is no correlation between renal indexes and blood pressure nor heart rate. In conclusion, we may sedate dogs to get the renal indexes, but we shall consider that the results will vary; sedation with midazolam and butorphanol increases these indexes.

31 EFFECT OF ADAPTATION ON INDIRECT BLOOD PRESSURE MEASUREMENT IN CONSCIOUS UNTRAINED BEAGLE DOGS.

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Blood pressure is regulated through a complex interaction of neural, chemical and hormonal feedback systems affecting both cardiac output and peripheral resistance. In dogs, unknown people or foreign environment may cause a change in sympathetic tone which may cause a transient increase in blood pressure, called the ''white coat'' effect. To overcome this phenomenon, several measurements are usually obtained on one occasion and the first measurements disregarded. However, no study has been published about the effect of training on blood pressure measurement in dogs. The aim of this study was thus to evaluate the effect of an adaptation period on blood pressure measurement in conscious untrained Beagle dogs.

Systolic blood pressure was measured in twelve young adult Beagle dogs by use of a Doppler Flow Detector (Parks Medical Electronics, Inc., Aloha, Oregon). Measurements were performed on the right forelimb with the dogs in lateral recumbency. A cuff width of around 40% of the leg circumference was selected. Approximately 5 minutes of restraint preceded blood pressure measurements. The first 3–5 measurements were disregarded, and the arithmetic mean of the next five measurements was used for data analysis. Blood pressure was measured on day 0 (d_0), 9 (d_9), 10 (d_{10}), 35 (d_{35}) and 94 (d_{94}). On day 14 (d_{14}), 16 (d_{16}) and 17 (d_{17}) additional blood pressures were determined by the oscillometric technique (Memoprint, S+B medVET GmbH, Babenhausen, Germany). Data were analysed by nonparametric tests. Values are reported as median and range. Significance was defined at a $P < 0.05$.

Blood pressure values obtained by Doppler showed a gradual and significant decrease over time. Values on the individual days were 164 (153.6–199.6) mmHg on d_0, 145 (119.2–176.0) on d_9, 138 (118.0–165.2) on d_{10}, 127 (111.2–139.4) on d_{35}, and 124 (115.2–143.2) mmHg on d_{94}. Compared to d_0 all subsequent measurements were significantly lower. Also, the decrease at d_{35} compared to d_9 and d_{10} was significant. The oscillometric values (d_{14}: 131, 114.2–157.6; d_{16}: 121,

110.4–151.6; d_{17}: 122, 115.6–146.0) were not different from those obtained by the Doppler method on d_{35} and d_{94}, respectively, indicating that a plateau was reached after around 2 weeks, respectively 4–5 days of training. Male dogs had higher values than females on each occasion, which was significant on d_{94} (133, 123.2–143.2 versus 120, 115.2–127.2).

In conclusion, systolic blood pressure in dogs during an adaptation period decreases markedly and significantly. Short term adaptation followed by serial measurements on one day is inadequate to obtain accurate blood pressure in untrained dogs. However, after an adaptation period, i.e. serial measurements on several subsequent days, a plateau is reached and reproducible blood pressure measurements can be obtained.

32 IS THERE A DIFFERENCE BETWEEN ENERGY BALANCE OF CATS HOSPITALISED FOR SURGICAL OR FOR MEDICAL REASONS?

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During hospitalisation, most cats are thought to be in negative energy balance (NEB). NEB can induce hepatic lipidosis, increase morbidity, mortality and has the potential of undermining proper medical or surgical management of hospitalised cases. The objectives of this study were to estimate percentage of cats in NEB and, to determine the reasons of NEB, to observe if there was a difference between medicine and surgery units. We included 75 cats (29 from medicine and 46 from surgery) hospitalised at the veterinary school of Liège for at least 2 days from November 2003 to March 2004 and from November 2004 to March 2005 into equivalent conditions (same room and same medical staff). For each cat, breed, gender, age, disease, length of hospitalisation, body condition score (BCS)[1] on a 6-point scale and physical status score (PSS; from 1 = normal patient to 5 = moribund)[2] were recorded. Their energy requirement (ER) during hospitalization ER was calculated using the equation BW x 70 kcal[3], multiplied by a factor (of 1.2 to 1.6) to derive the illness ER (IER). When 80% of IER was covered by spontaneous feeding or by nasoesophageal tube at day 2, patient was considered in positive energy balance. Correlation analyses were performed using the SAS system.

Domestic Shorthair cats represented 81% of patients and 57% were female in the 2 units. The average age was 6.2 years in medicine and 4.3 years in surgery. BCS averaged 3 in the 2 populations, and it was associated with gender (males were heavier) and correlated positively with age ($P < 0.05$). The cats had an equal average hospital stay of 5 days for the 2 units.

A significant relationship between PSS (mean PSS = 2.6 in medicine vs 1.6 in surgery) and unit ($P < 0.05$) was observed and was reflected by the mortality rate (10.3% in medicine and 4.3% in surgery).

Energy balance was negative in 52% of cases without difference between medicine and surgery: 30% were due to lack of compliance with written feeding orders and 22% resulted from the cat refusing to eat any or all of the food offered.

BCS was not a significant predictor of PSS. PSS was neither correlated with length of hospitalisation nor with NEB.

[1]Scarlett et al, Internal Journal Obesity, 1994, 18, 22–28.
[2]Remillard et al, Veterinary Therapeutics, 2001, 2, 301–310.
[3]NRC, 1986.

33 THE EFFECT OF DIETARY FISH OIL ON PUPPY TRAINABILITY.

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The long chain polyunsaturated fatty acid (LCPUFA), docosahexaenoic acid (DHA) is known to support correct neural development in mammalian species. A study was undertaken to examine the effect of DHA in the canine species. The objective was to determine the effect of maternal and post-weaning dietary fish oil on trainability and DHA status in Beagle puppies.

Twenty-eight (28) Beagle bitches (parity 2–3) were randomly assigned to 3 treatment (TRT) diets at breeding and maintained on those diets through lactation and weaning. Puppies selected from these litters (58 total puppies) were maintained on the respective dam diets from weaning through to 16 weeks of age for trainability assessment by Discrimination Task Testing using a Two-Arm T-maze. In preparation for trainability testing, each puppy received daily socialisation and exposure to the testing environment which concluded with 5 days of pre-test T-maze training at 9 weeks of age. Trainability testing was conducted from 10–16 weeks of age with puppies participating in 2 sessions/day of 7–10 trials/session, 5 days/week for 30 days. A success criterion was achieved when a puppy achieved a correct score in at least 80% of trials for 2 consecutive sessions. In addition, all puppies were assessed for fatty acid (FA) status at 7, 11, and 15 weeks of age based on RBC membrane FA profiles. TRT diets were poultry and grain based and formulated using the same base-matrix to contain 31.5% protein and 20.75% fat with 0% (TRT A), 0.58% (TRT B) or 1.10% (TRT C) of the diet from fish oil.

Fatty acid profiles (maternal and puppy) were significantly altered by diet, particularly regarding omega-3 fatty acids. RBC membranes from TRT C-reared puppies (1.10% fish oil) contained approximately 4× and 2× the DHA content compared with puppies from TRT A (0% fish oil) and TRT B (0.58% Fish Oil), respectively. Trainability scores in the puppies were also found to be sensitive to TRT, with a greater percentage ($P < 0.05$) of puppies from TRT C (n = 19) achieving at least 1 success criteria compared with puppies from TRT A (n = 20). Puppies (n = 19) from TRT B did not differ in success criterion from puppies from either TRT A or C.

These data demonstrate the importance of dietary lipid sources (fatty acids) on neurological function (trainability) and nutrient status in the canine during critical developmental periods.

34 MALIGNANT LYMPHOMA IN THE DOG: RESULTS OF TREATMENT WITH A 12-WEEK MAINTENANCE-FREE CHEMOTHERAPY PROTOCOL.

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Malignant lymphoma is one of the most common neoplastic diseases in the dog. Traditionally, canine malignant lymphoma has been treated with long-term chemotherapy with remission rates up to 94% and remission times of approximately one year. It was the aim of the present study to investigate the efficacy and toxicity of a short, maintenance-free combination chemotherapy protocol of 12-week duration in the treatment of malignant lymphoma in the dog and to compare these treatment results to a historical control group treated with a similar protocol comprising a prolonged maintenance phase. Patients with histologically or cytologically confirmed diagnosis of malignant lymphoma were treated with a 12-week combination chemotherapy protocol consisting of L-asparaginase, vincristine, cyclophosphamide, doxorubicin and prednisolone. Kaplan-Meier product limit analysis was used for intent-to treat remission and survival analysis. Cox forward multivariate regression analysis was used to evaluate patient variables for their influence on remission and survival times. The influence of patient factors on whether each dog reached complete remission was analyzed by multivariate logistic regression analysis.

77 Patients were entered into the study. Overall response rate (CR + PR) was 89.5%. Multivariate logistic regression analysis showed that substage and immunophenotype had a significant influence on the likelihood of a dog achieving CR. Median duration of first remission was 210 days (range 0–1191 d). The 6-month-, 1-year- and 2-year-remission rates were 53%, 22%, and 10%, respectively. In the multivariate analysis of patient variables only clinical stage and presence of anemia and thrombocytopenia remained significant concerning the influence on remission time. Median remission duration following the completion of chemotherapy was 189 d (range 6–1123 d). The factor immunophenotype showed a significant influence on chemotherapy-free remission duration. Median overall survival amounted to 265 days (range: 6–1207 d). The 1-, 2-, and 3-year survival rates were 41.5%, 18.4%, and 12.2%, respectively. In the multivariate analysis of the patient variables only substage remained significant concerning the influence on overall survival. Toxicity, when present, was generally mild with the exception of one treatment-associated death. No significant difference in efficacy was found in the comparison with the historical long-term chemotherapy protocol.

In conclusion, in this group of dogs the 12-week maintenance-free chemotherapy protocol was a well-tolerated treatment regimen leading to satisfactory results comparable to its long-term counterpart demonstratingthat maintenance therapy does not seem necessary for the initial treatment of canine lymphoma.

35 CHANGES OF FREE RADICAL AND ANTIOXIDANT PARAMETERS IN BLOOD OF DOGS WITH LYMPHOMA DURING THE COURSE OF TREATMENT.

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We examined 21 female and 20 male 2–11 years old dogs with lymphoma, belonging to different breeds. Sixteen dogs had diffuse follicular centre cell-, 19 diffuse large B cell-, 1 MALT-, 4 precursor T-lymphoblastic- and 2 B-cell lymphoblastic leukaemia, lymphoma. Four dogs were in stage III/a, 3 in III/b, 14 in IV/a, 14 in IV/b, 2 in V/a and 2 in V/b of the disease. Free radical and antioxidant parameters were measured from blood samples taken before and during COPA-therapy (cyclophosphamide, vincristine, prednisolone, doxorubicin. Following parameters were measured: reduced glutathione concentration (GSH) in blood plasma and red blood cell hemolysate (RBC-hem), oxidized glutathione concentration (GSSG) in plasma and RBC-hem, GSH/GSSG in plasma and RBC-hem,

glutathione-peroxidase activity (GSH-Px) in RBC-hem, malondialdehyde concentration (MDA, thiobarbituric-acid-reactive substance indicating lipid peroxidation) in RBC-hem, superoxide-dismutase activity (SOD) in RBC-hem, total antioxidant status, based on the reduction of iron (FRAP) and vitamin-C concentration in plasma.

Values of the members of the antioxidant defence system in plasma showed a decrease from the basal values in various periods of the treatment then they started to raise after. Levels of vitamin-C decreased by 93,9% at the 1st, FRAP by 26.5% at the 3rd and GSH/GSSG by 55% at the 5th week. In RBC-hem GSH-Px activity decreased by 20.7% at the 1st and GSH/GSSG by 53% at the 5th and MDA concentration elevated by 12% after the 2nd week. There was a positive correlation between SOD and MDA values both before and the 2nd week of treatment ($r = 0.69$, $P < 0.01$ and $r = 0.57$, $P < 0.001$), respectively.

These changes in antioxidant concentrations may be due to the acute and delayed effect of the first treatment (doxorubicin), and the fourth (cyclophosphamide) used in our protocol. This latter drug caused obvious depression in the glutathione redox system.

We think that decreased defence against free radicals makes the animals more prone to the worsening of the general state and systemic signs caused by secondary infections. We believe that the use of drugs supporting antioxidant defence system is advisable during COPA-therapy.

36 CLINICAL FEATURES OF LOW-GRADE LYMPHOMA IN 8 DOGS.

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Although the new WHO classification system of human non-Hodgkin's lymphoma is now considered to be applied to the histological classification of canine lymphoma, NCI Working Formulation (WF) has been widely used for the classification of canine lymphoma for its prognostic capabilities. Based on the WF classification, three malignancy grades (low/intermediate/high) were defined from the histological architecture and cell types (Appelbaum et al., 1984; Carter et al., 1986). The WF malignancy grade was shown to be a prognostic factor for the survival time (Teske et al., 1994). Since the clinical findings of canine low-grade lymphoma have not been detailed, the present study demonstrates the clinical features of 8 dogs histologically diagnosed as low-grade lymphoma.

From 2002 to 2005, 8 of 166 lymphoma dogs referred to the Veterinary Medical Center of the University of Tokyo were categorized into low-grade lymphoma based on the WF classification of their histological findings. The 8 dogs were middle-aged ranging from 6 to 9 years old and included 4 Golden Retrievers and 4 Shi-Tzus. Two dogs were male (1 neutered) and 6 dogs were female (2 spayed). All of the 8 dogs showed generalized peripheral lymphadenopathy. Hepatomegaly and splenomegaly were shown in 3 and 2 dogs, respectively. Complete blood cell count revealed slight to remarkable lymphocytosis (7 dogs), thrombocytopenia (2 dogs), and anemia (1 dog). Fine needle aspiration of the lymph nodes revealed the monomorphic population of small lymphocytoid cells with slightly atypical morphology such as relatively abundant cytoplasm and/or cytoplasmic projections. Histopathological diagnosis of the resected lymph nodes were diffuse small lymphocytic lymphoma (5 dogs) and follicular small cleaved cell lymphoma (3 dogs) both classified into low-grade lymphoma. Flowcytometric analysis of the tumor cells revealed the clonally expanded cell population with T-cell (2 dogs), B-cell (1 dog), and T- and B-cell (1 dog) surface phenotypes in 4 dogs examined. Two dogs were treated with melphalan and prednisolone because of the excessive lymphadenopathy and anemia, respectively, but 6 dogs were periodically rechecked without chemotherapy. All of the 8 dogs with low-grade lymphoma are still alive 286 to 1006 days after the first admission.

Low-grade lymphoma constitutes an infrequent peculiar group of lymphoma in dogs. For its treatment, standard CHOP-based protocols may not be applied to most of the cases. Moreover, the survival time of the cases is very long even without any chemotherapy.

37 COARSE FRACTIONATED RADIATION THERAPY FOR PITUITARY TUMOURS IN CATS: A RETROSPECTIVE STUDY OF 8 CASES.

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Pituitary tumors are uncommon in cats. Signs may be due to either an expansile mass or paraneoplastic effects (acromegaly and/or unstable diabetes mellitus). There are a few small case series providing evidence of efficacy for radiotherapy of pituitary tumors in cats. This retrospective study describes the outcome of eight cats with pituitary tumors treated with course-fractionated radiation.

The medical records of cats with MRI-confirmed pituitary tumors that underwent radiotherapy were reviewed. A standard coarse-fractioned radiation protocol was used; 37Gy in 5 once-weekly fractions using two parallel-opposed 4MeV X-ray beams. Survival times were calculated from date of first radiation dose.

Eight cats with pituitary tumors underwent radiotherapy: 4 had CNS signs and 4 had evidence of growth hormone excess. The median diameter of the pituitary mass was 10 mm. One cat with pre-existing severe CNS signs died of unknown causes before completing the radiation course. Of the remaining 3 with CNS signs, 1 had complete resolution of signs and two showed partial improvement. Of the 4 cats with unstable diabetes, the dose of insulin could be reduced and in 3, the diabetes mellitus resolved fully.

The median survival time was 72.6 weeks. 4 cats died: 1 without completing the radiation course, 1 from unrelated VAFS, 1 from chronic renal failure and 1 from progression of CNS signs. 4 cats remain alive (range 60–217 weeks).

Radiation therapy is confirmed as an effective treatment for pituitary tumors in cats giving extended survival and control of both direct mass effect and paraneoplastic signs.

38 HOW TO IMPROVE THE TARGET/NON-TARGET RATIO—LOCOREGIONAL APPLICATION OF RADIOPHARMACEUTICALS.

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There are several systemic radiopharmaceutical treatment methods in human and veterinary oncology where the goal of further development is to improve the tumor target dose and paralelly to avoid the normal organs from radiation. In this present study we aimed to find the idealistic application route of ^{188}Re-colloid in multiple liver malignancies and ^{188}Re-HEDP, ^{177}Lu-EDTMP in osteosarcoma dog patients and in normal Beagle dogs.

Altogether 8 normal Beagle dogs (4 to liver pefusion studies, 2 for foreleg and 2 for hindleg perfusion studies) and 2 multiple liver tumor bearing (HSCs) and 5 osteosarcoma bearing (3 in forlegs, 2 in hindlegs) were used in locoregional treatments. Livers were injected by a. hepatica after laparatomie, forlegs were perfused via a. brachialis, and hindlegs were injected via a. femoralis while veins from the organs (v. hepatica, v. brachialis and v. femoralis) were clipsed for a 30 minutes hypoxia period. A dynamic study during hypoxia period (0–30 min), than 30 min, 1, 2, 4, 6 hs, 1, 2, 5 ds and 1, 2 ws after application whole-body scans were taken. Regions of interest were drawn around the tumor, contralateral sites, visualized parenchymal and excretional organs, then mean residency times and internal dosimetrical data were calculated using dedicated softvers (MIRDOSE and OLINDA).

The absorbed dose by the liver is 44–65% higher when ^{188}Re-colloid is applied via a. hepatica compared to the liver dose achieved by the conventional intravenous application. Due to the existing vein anastomoses in the fore- and hind legs, perfusion studies did not result very high overdose in the perfused region. Only 7–22% higher doses could be reached when radiophamaceutical is applied via arteries compared to the data of systemic intravenous injection.

Locoregional application of radiopharmaceuticals results a higher target/non-target ratio only if blood supply of the perfused organ is discrete. Thirty minutes hypoxic period in liver, fore- and hind leg was found to be well-tolerable for the dogs. Further studies are needed to elucidate the right place of intraarteric application and the vein-clips in the fore and hind legs in the dogs.

39 *HMGA2* EXPRESSION IN CANINE PROSTATIC TISSUES—A POTENTIAL DIAGNOSTIC TOOL?

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Beside humans the dog is the only known mammalian species that spontaneously develops prostate cancer. Both species show striking similarities in the development and progress of the disease. In both species adenocarcinomas show the same histopathology and represent a locally invasive disease affecting mainly older subjects. Also in both species, the tumors are likely to metastasize to the same distant regions by blood or lymphatic system and akin to their human counterparts canine prostatic cancers vary with respect to their clinical behaviour. Based on the histology of the lesions alone it is often not possible to recognize sufficiently the malignant potential of the tumour in terms of local invasiveness and metastatic spread. Thus, molecular indicators allowing for a valid prognosis of these cancers are of considerable interest.

In humans, *HMGA1* overexpression was recognized to be associated with a highly malignant phenotype of prostate cancer and is therefore considered a molecular marker in prostate cancer diagnosis. HMGA1 is a member of the High-Mobility Group Protein family comprised of HMGA1a, HMGA1b, and HMGA2. All three proteins show a high amino acid sequence homology and the human and canine proteins are highly conserved during evolution. HMGA proteins are abundantly expressed during embryogenesis and are almost undetectable in most normal adult tissues. Re-expression was detected in a variety of human malignancies with correlation of the expression level with the degree of neoplastic cell transformation and metastatic tumor progression.

In this study we report the *HMGA2* expression patterns as determined by real-time quantitative RT-PCR in prostatic tissues from 16 dogs with different histological findings comprising 4 samples with no abnormality detected, 3 hyperplasias, 3 cysts, and 6 carcinomas. The results show that expression of *HMGA2*

is low in tissues with no abnormality detected, rises in benign neoplasms with intermediate values for cysts and hyperplasias and increases at least 19-fold in carcinomas. In our study all malignant neoplasias showed expression levels beyond an assumed threshold of 50,000 transcripts per 250 ng total RNA, whereas none of the non-malignant tissues showed expression levels beyond this threshold. These results indicate that *HMGA2* expression analysis using real-time quantitative RT-PCR may provide a potential tool for better differentiation between varying degrees of malignancy in prostate carcinomas.

40 PRELIMINARY RESULTS OF THE USE OF CARPROFEN IN CANINE MAMMARY TUMORS.

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Mammary gland tumors are the most common type of tumor in female dogs, constituting up to 52% of all neoplasms. The reported frequency of malignancy is 50%. Over expression of COX-2 has been reported in various tumors both in humans and animals and COX-2 over expression has been found to be more frequent and more intense in malignant canine mammary tumors as compared to benign mammary tumors. The aim of this study was to investigate the possible role of COX-2 inhibitors in the treatment of canine mammary tumors. We evaluated the effect of treatment with carprofen in dogs with mammary tumors on the expression of COX-2, tumor size and histopathology results.

At this time 7 dogs have been entered into the study. At the time of diagnosis a complete blood count, biochemistry panel, 3 view thoracic radiographs, digital imaging of the tumor, measurement of the tumor and an incisional biopsy were performed. If the biopsy revealed a malignant tumor, treatment was initiated with carprofen (2 mg/kg q 12 hours) for 4 weeks. Two weeks following the biopsy, sutures were removed and the tumor was remeasured. Four weeks following the initial presention the tumor was measured, imaged, and surgically removed. All samples were sent for histology and immunohistochemistry for expression of COX-2.

Of the 7 dogs with malignant tumors, one tumor reduced slightly in size over the treatment period. Results of the incisional biopsies revealed anaplastic carcinoma (1), mixed adenocarcinoma (2), and adenocarcinoma (4). The histopathologic diagnosis after treatment with the COX-2 inhibitor did not reveal a change in six of the tumors. One tumor previously diagnosed as an adenocarcinoma was diagnosed as a mammary adenoma with hyperplasia and granulomatous inflammation on the excisional biopsy. Immunohistochemistry for COX-2 was performed on all of the tumors; four tumors had decreased COX-2 expression, one had stable COX-2 expression, and two tumors showed a slight increase in COX-2 expression.

These preliminary results are interesting, as a decrease in COX-2 expression was noted in the majority of dogs. These results warrant further evaluation of the role of COX-2 inhibitors in the treatment of canine malignant mammary tumors.

41 DEVELOPMENT OF QUANTITATIVE REAL-TIME RT-PCR ASSAYS FOR DETECTION OF METASTATIC DISEASE IN CANINE MELANOMA.

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Canine melanoma (CM) is the most common group of malignant tumors of the oral cavity and digits. Cutaneous CMs are usually benign, while oropharyngeal, uveal, and mucocutaneous neoplasms are often aggressive and have metastatic potential. Oral malignant CMs are characterized by rapid growth and local invasion. Metastatic rate is high, but metastases are not frequently observed until late during the course of the disease. As in humans, the prognosis is guarded if the diagnosis is late or if the cancer has disseminated. Correct staging and early discovery of residual disease is crucial for treatment plan and prognosis.

The aim of this study was to develop quantitative real-time reverse transcriptase-PCR (RT-PCR) methods for the determination of transcripts of canine melanoma associated antigens (MAAs) in primary tumors, regional metastases and cell cultures and to compare these techniques with similar analyses in man.

Biopsies from two canine oral melanomas with regional lymph node metastases were sampled at surgery and placed in RNA stabilization buffer. Specimens for primary culture were taken to cell medium and processed to cell lines. The protocol for human transcripts was used (Johansson et al. Melanoma Res 2000; 10:213–22) for tyrosinase (TYR), tyrosinase related proteins 1 and 2 (TRP-1, TRP-2) and Melan-A.

With human primers and probes we obtained positive results for TYR and TRP-1 but negative results for TRP-2 and Melan-A. Alignment studies of human and canine primer sequences showed marked differences in nucleotide sequences with the latter two transcripts and we therefore redesigned primers and probes for these to be specific for canine transcripts.

TRP-1 always gave positive results with canine melanomas. With redesigned primers and probes for Melan-A and TRP-2 one primary tumor was highly positive with these transcripts while only slightly positive for TYR. Similar pattern was obtained with a lymph node metastasis and with corresponding cell cultures. There was a strong connection between gene expression of pigment-related transcripts and melanocytic cells. In cultured cells, the expression is correlated with the level of pigmentation. TYR and Melan-A are considered to be sensitive melanoma markers.

Our findings show both similarities and dissimilarities between human and canine transcripts. Multimarker quantitative RT-PCR can detect small numbers of melanoma cells. Quantification of the studied markers in tumors and eventually in blood will be a useful complement to routine examination, in detection of metastasis, correlation to clinical stage and monitoring treatment response of melanoma patients.

42 IDENTIFICATION OF A SHORT INTERSPERSED NUCLEAR ELEMENT (SINE) INSERTION IN THE *GP100* GENE OF A POORLY PIGMENTED CANINE ORAL MELANOMA TUMOUR.

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Glycoprotein (gp100) plays a key role in melanin synthesis in melanocytes and melanoma tumors. Whilst investigating gp100 mRNA expression in canine malignant melanomas using RT-PCR, we identified an insertion mutation in the coding region of gp100 in a biopsy from a poorly pigmented oral malignant melanoma. Analysis of the tumour genomic DNA revealed this to be a short interspersed nuclear element (SINE).

RNA was extracted from the tumour biopsy and cDNA synthesised. PCR was performed using primers designed to amplify the full-length coding region of canine gp100. The amplicon produced was cloned and upon sequencing, was found to contain a 156 bp insertion when compared to the genomic sequence identified from the dog genome assembly (NCBI). Genomic DNA was also extracted from the tumour and used as the template for PCR, performed using primers designed in the gp100 sequence flanking the insertion. A single amplicon of the expected size (~792 bp) was generated from genomic DNA prepared from a control blood sample, whereas two amplicons (one of ~792 bp plus an additional larger amplicon) were detected from the melanoma sample. Cloning and sequencing of the larger amplicon yielded a product of 1034 bp. Sequence analysis indicated that the inserted element was the reverse complement of a SINE, with short direct nucleotide repeats at each end, a poly-A region, multiple CT repeats and potential internal RNA polymerase III transcriptional control regions.

SINEs are frequently repeated and dispersed throughout the dog genome, approximately every 5–8.3 Kb. There are ~360,000–600,000 copies in total, representing ~1.8–3% of the diploid dog genome. SINEs are thought to arise via retrotransposition, where RNA polymerase III transcripts (tRNA and 5SrRNA) are reverse-transcribed into DNA and subsequently integrated into the chromosome via an integrase enzyme. Staggered breakage and subsequent repair of the recipient chromosome results in flanking direct nucleotide repeats. In this tumour, the SINE was inserted within exon 11 of the gp100 gene. Such a mutation in the coding sequence would also disrupt the protein sequence in the C-terminal region, potentially resulting in a dysfunctional gp100 protein. Since gp100 is a key component of the melanin synthesis pathway, a SINE insertion at this location could account for the poor pigmentation of the tumour. If this phenomenon occurs in other canine melanomas, it could be one reason why such tumors can become amelanotic.

43 LONG TERM REMISSION OF A PRIMARY MALIGNANT LYMPHOMA OF THE URINARY BLADDER IN A DOG—A CASE REPORT.

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A 3 year old female spayed mixed breed dog was presented to Hofheim Animal Hospital with a 5 week history of hematuria and pollakisuria. A large infiltrative mass encompassing 2/3 of the bladder was identified by ultrasound. The bladder lumen was almost lost and the bladder wall thickness measured up to 1.8 cm with loss of its typical layered sonographic appearance. Mild distention of both ureters was present. Abdominal computed tomography, ultrasound, chest radiographs and blood work revealed no other abnormalities. A full thickness bladder biopsy was performed and was diagnosed as malignant lymphoma. On immunohistochemistry a CD79a positive and CD3 negative reaction was present which identified the mass as a B-cell lymphoma.

3 fractions of radiation therapy with 5 Gy each using a ^{60}Cobalt teletherapy unit were administered to the urinary bladder with 1 fraction per week and resulted in complete remission of the tumor. No side effects of the radiation treatment were noted. The radiation was followed by a polychemotherapy protocol using four cycles of Vincristin, Asparaginase, Cyclophosphamide, Cytosin Arabinoside, and Doxorubicin. There were mild and self limiting gastrointestinal side effects following each Doxorubicin application.

Follow up examinations by ultrasonography and blood work were performed on a regular basis and revealed no recurrence of the neoplasia or any other signs

of systemic spread. At present, the dog is in complete remission and free of symptoms for 24 months.

Primary malignant lymphoma of the urinary bladder is extremely rare in the dog and virtually no clinical data are available. In humans, less than 100 cases have been reported worldwide and most are B-cell lymphomas. Hematuria is reportedly the most common clinical symptom and the tumors usually respond well to therapy and have a favourable long term prognosis. It seems from this case that canine malignant lymphoma of the bladder resembles its human counterpart in both clinical symptoms and response to therapy.

44 PRIMARY PULMONARY HEMANGIOSARCOMA (HSA) IN A GERMAN SHEPHERD DOG WITH SPONTANEOUS PNEUMOTHORAX (SP).

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A 6-year-old male German Shepherd dog was referred for evaluation of dysnea. The referring veterinarian noticed fever, left shift neutrophilic leukocytosis and thrombocytopenia. Therapy prescribed included an antibiotic and an antipyretic. Abnormalities from physical exam were congestive oral mucous membranes, rapid capillary refill time, laboured breathing, tachycardia and abdominal distension. Radiology and ultrasound showed bilateral pneumothorax with collapse of caudal pulmonary lobes, hepatomegaly and splenomegaly. Liver and spleen cytological findings were consistent with systemic inflammatory response syndrome. Presumtive diagnosis was distributive shock and SP secondary to infectious, inflammatory or neoplastic pulmonary disease. Conservative medical treatment initially instaured was broad-spectrum antibiotherapy, low molecular weight heparin and thoracic drainage via thoracostomy tube. Clinical condition of the dog improved substantially and pleural fluid culture yielded no bacterial growth. Because SP persisted more than 48 hours, thoracotomy by means of median sternotomy was performed and multiple noduls were noticed in every lung lobe. Right cranial lobectomy was done to eliminate the source of air leakage. Histopathology revealed pleomorphic fusiform cells forming vascular type structures consistent with pulmonary HSA. Others primary sites were excluded. Owners refused chemotherapy, but 5 months later, the dog showed no evidence of progressive disease.

This report describes an unusual primary pulmonary HSA diagnosed because of acute SP as the main clinical sign. HSA is a highly malignant neoplasia of vascular endothelial origin with lung primary involvement not commonly reported.

SP related to pulmonary neoplasia is sparse in the veterinary literature and is a rare presentation as a respiratory oncologic emergency.

In this case SIRS was also encountered. Sepsis due to secondary pneumonia could not be ruled out as blood cultures were not performed.

To the authors' knowledge there are no prognostic studies about primary pulmonary HSA. However, and despite incomplete surgical excision and no chemotherapy, survival time of this case is surprisingly above the ones reported for dogs with HSA.

45 PATTERNS OF CANINE CANCER INCIDENCE IN SOUTH AFRICA (1998–2004).

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No information exists on the relative proportions or incidences of diagnosis of canine cancer in South Africa, barring one survey of histopathology reports[1]. Standard veterinary oncology texts quote data from various US and European studies, which may be subject to wide geographic variations.

In this retrospective analysis, the medical database of the Onderstepoort Veterinary Academic Hospital (OVAH) was analysed for details of canine cancer patients admitted for the period 1998–2004 ($n = 1022$ including a small number of duplicate entries for multiple neoplasms of $N = 47{,}245$ admissions, or 2.163% of total canine admissions). Patients were categorised according to diagnosis (histopathology, cytology or presumed); primary tumour location and metastasis; survival (where known); and for evidence of *Spirocerca lupi* lesions (i.e. type of neoplasm associated with it, or whether granuloma was diagnosed).

Mesenchymal tumors formed the majority of neoplasms (47%), even when mast cell tumors (MCT) were considered as round cell tumors (thereby reducing incidence of mesenchymal tumors to 33%). Adenomas and carcinomas were the next most common tumors (32%), followed by round cell tumors (28% including MCT, 12% without MCT). 8% of tumors were undiagnosed or unclassifiable on the basis of incomplete records. The two most common cancers diagnosed were mammary (15.17%) and mast cell tumors (14.09%). Other notable tumors diagnosed were: lymphosarcoma (9.2%), non-*Spirocerca*-related osteosarcoma (8.51%), haemangiosarcoma (5.97%) and various sarcomas (5.28%).

The proportion of cutaneous squamous cell carcinoma was lower than expected (1.17%), given its frequency in a similar retrospective study in felines (55%), but this result may be artificially lowered by the high number of ''unclassified'' tumors (7.8%) and ''other carcinomas'' (6.46%). Mesenchymal tumors are the most common tumors in patients presenting to the OVAH. Mammary and mast cell neoplasia are the two most numerous types diagnosed. Early (pre-pubertal) ovariohysterectomy, which has a powerful protective effect against mammary cancer, should be more strongly advocated by all veterinarians in the Republic.

1. Bastianello SS (1983) *Onderstepoort J. of Vet Research* 50:105–110.

46 PATTERNS OF FELINE CANCER INCIDENCE IN SOUTH AFRICA: CONTRASTS WITH THE REST OF THE WORLD (1998–2004).

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No information exists on the relative proportions, incidences or outcomes of diagnosis and treatment of feline cancer in South Africa, barring one survey of histopathology reports.[1] Standard texts of veterinary oncology quote data from the Northern hemisphere, and geographic differences are apparent even within these figures.

In this retrospective analysis, the medical database of the Onderstepoort Veterinary Academic Hospital (OVAH) was analysed for details of feline cancer patients admitted for period 1998–2004 ($n = 73$ including 1 duplicate record for 2 different neoplasms on 1 patient out of $N = 4274$ feline admissions, or 1.71% of total feline admissions). Patient records were analysed for signalment and patients were categorised according to diagnosis (histopathology, cytology or presumed); tumour location and metastasis; survival (where known); whether or not any medical or surgical treatments were performed; and colouration (white/not white) in cats with squamous cell carcinoma.

In contrast to published reports of US, Australian and European data where lymphosarcoma is the most common cancer of cats, squamous cell carcinoma (SCC) forms the predominant neoplasm diagnosed at the OVAH (55% of feline cancer patients, 81% in white or part-white cats). Lymphoma was the second most common diagnosis (19%) followed by various carcinomas and adenocarcinomas (10% combined, but excluding 3% various mammary tumors). Only one putative case of vaccine-associated sarcoma was recorded, and this was based on a cytological diagnosis. A large proportion (55%) of patients received some form of treatment, but only 68% of neoplasms were confirmed by cytology or histopathology.

The average age of feline cancer patients was 9.5 years at presentation, and feline cancer represented 1.71% of the caseload at the OVAH. Squamous cell carcinoma, followed by lymphoma, form the majority of feline cancers diagnosed in South Africa (74% combined). SCC should reasonably form the major South African feline oncology research focus.

1. Bastianello, SS (1983). *Onderstepoort J. of Vet Research* 50:105–110.

47 PATTERN RECOGNITION RECEPTOR mRNA EXPRESSION AND RESPONSE TO STIMULATION IN A CANINE MACROPHAGE CELL LINE.

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In order to provide a first line of defence against invading organisms, the innate immune system has evolved to recognise conserved molecules (pathogen-associated molecular patterns—PAMPs) that are expressed by foreign organisms. This recognition process is facilitated by pattern recognition receptors (PRRs) which each recognise specific PAMPs. The predominant PRRs are Toll-like receptors (TLRs) or nucleotide-binding oligomerisation domain (NOD) proteins. Defects in PRR function can lead to disease (e.g. NOD2 in Crohn's disease). The aim of this project was to characterise PRR expression and function in a canine macrophage cell line (DH82) as a model system for studying canine innate immunity.

DH82 cell expression of the PRRs; NOD1, NOD2, TLR1, TLR2, TLR4, TLR5, TLR6 and TLR9 mRNA was determined by RT-PCR using canine-specific primers. Cultured DH82 cells were exposed to various PAMPs including muramyl dipeptide (NOD2), peptidoglycan (TLR-2), bacterial lipopeptide PAM3CSK (TLR1/TLR2), lipopolysaccharide (TLR-4) and CpG DNA (TLR-9). The response to stimulation was determined by PCR using canine cytokine-specific primers for IL-1 beta, IL-6 and TNF-alpha with GAPDH used as a housekeeping gene control. TNF-alpha cytokine mRNA expression was quantified by performing real-time PCR (DyNAmo SYBR Green PCR Kit; Opticon 2 DNA engine, MJ Research). The TNF-alpha concentration in the culture medium supernatants was measured using the L929 TNF-alpha bioassay.

DH82 cells expressed NOD1, NOD2, TLR1, TLR2, TLR4 and TLR6 mRNA but TLR5 and TLR9 mRNA could not be detected. Cytokine mRNA expression was rapidly upregulated in DH82 cells following culture with muramyl dipeptide, peptidoglycan, PAM3CSK and LPS. Cells did not respond to CpG DNA, consistent with a lack of expression of TLR9. Real-time PCR demonstrated peak levels of TNF-alpha mRNA expression after 2 hours of stimulation. TNF-alpha protein concentrations were found to be highest in the cell supernatant after 4 hours of stimulation.

Characterisation of DH82 cell expression of PRRs and their response to spe-

cific PAMPs has proved to be a useful model for studying innate immune reactivity of canine macrophages. Future work will focus on adapting these techniques for use with blood-derived monocyte/macrophages isolated from dogs with suspected innate immune deficiency.

48 SEROLOGIC RESPONSES OF DOGS AFTER VACCINATION WITH A COMMERCIAL LEPTOSPIROSIS VACCINE.

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The microscopic agglutination test (MAT) is the standard specific serologic test of choice for diagnosing leptospirosis. Vaccines against leptospirosis can either be whole-cell or subunit. The only vaccines available against current serovars causing most disease in dogs in the US (*Leptospira interrogans* serovars pomona and autumnalis, and *Leptospira kirschneri* serovar grippotyphosa) are subunit vaccines against pomona and grippotyphosa. Little is known about whether these vaccines cause elevations in MAT titers and if so, are these elevations serovar specific as has been reported for whole-cell vaccines against other serovars (canicola and icterohaemorrhagiae). Such vaccine-induced elevations in MAT titers often confuse veterinarians trying to diagnose active clinical disease based on MAT titers. The objective of this study was to measure MAT titers against serovars pomona, autumnalis, and grippotyphosa in puppies and mature dogs given a commercial vaccine against serovars pomona and grippotyphosa.

Forty 12-week-old beagle dogs were administered a commercial vaccine against serovars pomona and grippotyphosa at 12 weeks of age, then boosted 3 weeks later. Twenty mature beagle dogs were administered the vaccine once. Serum MAT titers against serovars pomona, autumnalis, and grippotyphosa were measured pre-vaccination and at 2, 4, 6, 10, and 16 weeks after the first or only vaccination.

Of the 40 puppies vaccinated, 40 (100%), 0 (0%), and 40 (100%) developed MAT titers >100 after vaccination, to serovars pomona, grippotyphosa, and autumnalis, respectively. MAT titers against serovar autumnalis reached higher levels and persisted in some dogs for 16 weeks (6 weeks longer than for titers to serovar pomona). Of the 20 mature dogs, 13 (65%), 5 (25%), and 20 (100%) developed MAT titers >100 2 weeks after vaccination, to serovars pomona, grippotyphosa, and autumnalis, respectively. Titers against serovar pomona reached higher levels and persisted in some dogs beyond 16 weeks post-vaccination while those for serovar pomona and grippotyphosa persisted for 10 and 6 weeks, respectively.

These results show that subunit vaccines against serovars pomona and grippotyphosa induce MAT titers not only to homologous antigens, but also to serovar autumnalis which could lead to a miss-diagnosis of leptospirosis due to serovar autumnalis. Further, a failure of puppies and most mature dogs to generate MAT titers to grippotyphosa in this study varies considerably from results published by the vaccine company.

49 PREVALENCE OF *ANAPLASMA PHAGOCYTOPHILUM* IN DOGS IN GERMANY.

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Anaplasma phagocytophilum is a gram-negative intracellular organism transmitted by ixodid ticks. The purpose of this study was to evaluate prevalence and significance of *Anaplasma phagocytophilum* infections in dogs in Germany.

A total number of 112 dogs were included in the study. 49 showed symptoms that could be attributed to *Anaplasma phagocytophilum* infection such as fever, lethargy, leukopenia, thrombocytopenia, reluctance to move, or shifting lameness (symptomatic dogs). 63 dogs were without clinical signs (asymptomatic dogs). Exclusion criteria for both groups were a stay abroad and previous treatment with tetracyclins, chloramphenicol, or imidocarb. For each dog an *Anaplasma phagocytophilum* 16S rRNA nested polymerase chain reaction (PCR) of ethylenediaminetetraacetic acid anticoagulated whole blood, a microscopic evaluation of a buffy coat and an indirect fluorescent antibody test (IFAT) of serum was performed.

Seven (6.3%) of the 112 examined canine blood samples were positive in the PCR. In all cases the PCR products were sequenced and confirmed infection with *Anaplasma phagocytophilum*. Six of the positive dogs were of the symptomatic group and one was of the asymptomatic group. Only two PCR positive dogs had morulae in neutrophilic granulocytes. Both dogs had symptoms attributable to *Anaplasma phagocytophilum* infection. Positive antibody titers were detected in 48 dogs (42.9%). There was no significant difference between the two groups (symptomatic group 22 [44.9%] versus asymptomatic group 26 [41.3%]). Dogs with very high antibody titers (≥1:1024, n = 17) had more frequently symptoms associated to *Anaplasma phagocytophilum* infection than those with low or no antibody titers (≤1:64, n = 70). In all dogs with positive PCR results very high antibody titers were detected. There was no significant correlation of overall positives or antibody titers to age, breed, sex, or usage of the dogs as family or working dogs. Dogs with no or very low tick infestation were significantly less often infected with *Anaplasma phagocytophilum* than those with high tick infestation.

In conclusion there seems to be a high risk of infection with *Anaplasma phagocytophilum* in Germany. Results of this study suggest that severe illness solely caused by *Anaplasma phagocytophilum* is possible. Subclinical infection was seen frequently in this group of dogs.

50 PREVALENCE AND CLINICAL IMPORTANCE OF THE TWO KNOWN AND A THIRD NOVEL FELINE HAEMOPLASMA SPECIES IN CATS IN SWITZERLAND.

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Haemobartonella felis, the causative agent of Feline Infectious Anaemia, has been reclassified within the group of haemotropic *Mycoplasma* (aka haemoplasmas). Two different species have been recognized: *Mycoplasma haemofelis* (*Mhf*) and '*Candidatus* M. haemominutum' (*CMhm*). Recently, we identified a third novel feline haemoplasma isolate (*Mnov*) in a cat with severe intravascular haemolysis. Experimental transmission of *Mnov* resulted in anaemia in two SPF cats. Phylogenetic analyses of the 16S rRNA gene revealed close relationship of *Mnov* to rodent haemoplasma species (Willi et al. 2005, JCM 43, in press).

The goal of the present study was to investigate the prevalence, clinical manifestation and risk factors for infections with the three feline haemoplasmas in representative Swiss cat populations. Blood samples from 586 ill and 86 healthy cats were collected over one year. DNA extracted from 200 µl of blood was analyzed using three newly designed specific TaqMan PCR assays. The 16S rRNA genes from several isolates were sequenced. Plasma samples were tested for FeLV and FIV infection by ELISA. The case histories and laboratory parameters of ill cats were evaluated.

CMhm infection was detected in 6.9% and 8.7%, and *Mhf* in 2.3% and 0.2% of healthy and ill cats, respectively. *Mnov* was detected only in ill cats (1.1%); 4 out of 7 of these cats were co-infected with *CMhm*. *CMhm* infection was associated with male gender, outdoor access and old age. *CMhm*-infected ill cats had higher blood levels of BUN, creatinine, protein, and lipase, and were more frequently diagnosed with renal insufficiency, than *CMhm*-uninfected ill cats. Haematological parameters were not different in haemoplasma-infected compared to uninfected ill cats. Haemoplasma infections were not associated with either retroviral infections or pretreatment with immunosuppressive drugs. They were more frequent in certain areas (South, West) of Switzerland. A tendency of increased *Mnov* prevalence in spring could be noted. Sequencing of the 16S rRNA gene of representative Swiss isolates (n = 14) revealed >98% similarity with published sequences.

In conclusion, we have demonstrated all three feline haemoplasmas in Switzerland. *CMhm* was the most prevalent. Co-infection with *Mnov* and *CMhm* could be explained by a similar way of transmission. The association observed between *CMhm* infection and signs of renal insufficiency could be causal. However, it could also be accounted for by, e.g., the increased age of infected cats. Our study demonstrates the usefulness of the newly developed TaqMan assays in detecting infections with all three feline haemoplasmas.

51 REVIEW OF CLINICO-PATHOLOGICAL FINDINGS AND COAGULATION DISORDERS IN 45 CASES OF CANINE BABESIOSIS.

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Canine babesiosis is a tick-borne disease caused by the hemoprotozoan parasite *Babesia*. The haematological and biochemical findings vary on geographic locations according to the virulence of parasite strains. The occurrence of hypoglycaemia and haemostatic disorders, particularly disseminated intravascular coagulation (DIC) are also reported, but the information are related to the highly infection by *B. canis rossi*, occurring in Africa. This retrospective study reports the clinico-pathological findings and the haemostatic disorders in dogs affected by babesiosis from *B. canis canis* in Spain.

45 dogs of both sex and of different breeds were examined during the period January 2003 to October 2004. The diagnosis of babesiosis was always confirmed by direct observation of the protozoa in a blood smear. All dogs were presented with a 1 to 3 day history of lethargy (40/45), fever (27/45) and mucosal membrane pallor (27/45). Hematuria (15/45) was also observed. The primary haematological abnormalities included a mild (PCV = 30.8 ± 11.1%) normocytic (MCV = 65.6 ± 3.6 fl), normochromic (35.3 ± 1.75 g/dl) anaemia and thrombocytopenia. All dogs (45/45) had the platelet counts below the lower reference range value (175×10^9 /L). Leukocyte abnormalities were inconsistently and might include leucopoenia (10/45 dogs), leukocytosis (6/45 dogs), neutropenia (18/45 dogs) and monocytosis (10/45 dogs). A disproportionate el-

evation in serum urea nitrogen (BUN) was observed in 21 of 45 dogs. Haemolysis, as it occurs in canine babesiosis, may cause non-renal elevations in serum urea, possibly due to the ammonia loading. Hyperglycaemia (>120 mg/dl) was common (21/45) but was never severe. The highest blood glucose concentration was 221.6 mg/dl. We observed an increase of fibrinogen in 33 of 45 dogs (74%). DIC syndrome was diagnosed (based on PT, aPTT, TT, D-dimers, Fibrinogen) in 9 of 45 dogs (20%).

The clinico-pathological finding observed in 45/45 cases was a moderate thrombocytopenia. In the absence of thrombocytopenia, babesiosis is an unlikely diagnosis. In a context of hypermetabolic illness such as babesiosis, hyperglycaemia was not a surprising finding. Hyperglycaemia in critical illness most often is caused by increase glucose mobilization and stress, and can be markedly increased by increased cortisol secretion. The results also indicated that *B. canis canis* infection may impair haemostasis suggesting induction of DIC, and that treat dogs in an early stage of infection might potentially avoid the possibility of developing and acute and uncompensated DIC.

52 HUMAN DANDER AS A POTENTIAL ALLERGEN SOURCE IN ATOPIC DOGS—ALLERGEN CHARACTERIZATION AND IgE PROFILING.

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Perennial indoor allergens are known to be important inducers of IgE-mediated hypersensitivity reactions in dogs. Human dander (HD), an important component of house dust, may also be a major cause of canine atopy with a reported prevalence of up to 68% (intradermal test data). As yet HD has been poorly characterized as an allergenic source. The aim of this study was to differentiate between HD extracts and select the most potent for further characterization and identification of IgE-binding proteins for dogs.

Several HD extracts were evaluated regarding their biological potency and protein patterns using a recently developed mediator release assay (RBL-test) and SDS-PAGE, respectively. Suitable extracts were included in a panel of common indoor (n = 8) and outdoor (n = 4) allergens to identify dogs with high HD-specific IgE levels. Four groups of dogs were tested including ''multi-source'' atopic dogs (n = 88), dogs with other skin diseases (n = 20), healthy ''household'' (n = 30) and healthy ''kennelled'' dogs (n = 40) using a solid-phase assay based on the ''grid-blot'' device. The reactions were graded semi-quantitively (0, 1, 2, 3) according to the concentration of allergen-specific serum IgE.

Whereas grade 1 reactions were common in all groups of dogs, grade 3 reactions were not seen in any of the dogs tested and grade 2 reactions were also an infrequent finding (''multi-source'' atopic dogs: 15%; dogs with other skin diseases: 0%; healthy ''household'' dogs: 3%; healthy ''kennelled'' dogs: 10%). Only sera with high HD-specific IgE levels (grade 2) were selected as probes to identify IgE-binding proteins by western blot. Six sera from the ''multi-source'' atopic dogs demonstrated IgE-binding to a protein with an apparent molecular weight of 11 kDa identified as human cystatin A by N-terminal microsequencing.

Human cystatin A is a cysteine protease inhibitor belonging to the superfamily of cystatins. These include the recently identified allergen Fel d 3, an IgE reactive protein for human beings which supports the assumption that human cystatin A may be of relevance for the development of canine atopy. Considering the so far reportedly high prevalence of HD-related atopy detected in intradermal tests, the low sensitization rate of dogs to HD in our study was remarkable. This may be due to the presence of cell-bound IgE, which is only detectable in an intradermal test but not in in-vitro assays.

53 CENTRAL AND PERIPHERAL *BABESIA CANIS ROSSI* PARASITAEMIAS AND THEIR ASSOCIATION WITH OUTCOME OF INFECTION.

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We present an interim analysis of 62 of 105 dogs enrolled in a prospective, cross sectional, descriptive study of clinical cases of canine babesiosis. The objectives were to compare the use of central and peripheral smears for the detection of parasites and to determine whether the level of the parasitaemia was associated with outcome. In addition, we quantified the repeatability of our sampling and scoring methods.

Previous investigators disagreed on whether central or peripheral smears are most sensitive at detecting *Babesia canis* parasites. In a similar human haemoprotozoal disease (malaria due to *Plasmodium falciparum*), outcome is related to parasite density and sequestration of parasitised red blood cells (pRBC) in microvascular beds. These aspects of canine babesiosis have not been investigated.

Dogs were enrolled if large babesias were found on capillary smears. Infection with *B. canis rossi* was confirmed by reverse line blot (RLB). Dogs in which RLB detected *Ehrlichia canis* (5) or *B. canis vogeli* (2) were excluded. Peripheral smears were made from an ear prick. Central smears were made from jugular or cephalic blood collected within 10 minutes of making the capillary smears and prior to treatment. Parasitaemias were manually counted and expressed as the percent pRBC. Repeated scoring of the same smear and of paired peripheral and central smears was performed. Scoring was blinded. Dogs were grouped according to the following outcomes: death (D), admission for treatment (A), treatment as outpatient (H).

Peripheral parasitaemia (median 1.34%, range 0.04–73.9%) was significantly greater than central parasitaemia (median 0.24%, range 0–30.6%) ($P < 0.001$), although three dogs had higher central parasitaemias. Both peripheral and central parasitaemias were higher in D vs H ($P < 0.001$ for both) and in D vs A ($P = 0.02$ for both). Pairs of peripheral smears showed a coefficient of variation (COV) of 103%. Pairs of central smears and repeated scoring of peripheral and central slides showed COVs of 31.5%, 38.7% and 45.9% respectively. There was insufficient evidence to show that the difference between central and peripheral parasitaemias increased with worsening outcome ($P = 0.35$).

We conclude that peripheral smears are usually more sensitive than central smears at detecting *B. canis rossi* parasites. Our results show that the group of dogs that died had significantly higher parasitaemias than dogs that survived. We document that peripheral parasitaemia can be highly variable between smears from the same animal and thus advise repeat sampling if parasites are not obvious on a single peripheral smear.

54 FIRST EUROPEAN REPORT OF *B. GIBSONI* (ASIAN GENOTYPE) INFECTION IN TWO AMERICAN PIT BULL TERRIERS WITHOUT STAYING ABROAD, DETECTION BY PCR AND SEQUENCING.

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Babesiosis, which is caused by intraerythrocytic parasites of the genus *Babesia*, is a well-known disease in dogs. Clinical signs ranged from severe haemolytic anaemia and thrombocytopenia to subclinical infections. Historically, two species of *Babesia* parasites have been attributed to infected dogs, *Babesia canis* and *Babesia gibsoni*. There are differences between these species concerning parasite size and geographic location in which the infection was acquired. The small *Babesia* sp. (1.0–2.5 μm) infecting dogs have been attributed to *B. gibsoni*. This small *Babesia* species has been reported to occur endemically in Asia, parts of Africa and sporadically in North America and Spain. Recent studies demonstrate that there are at least three genetically distinct forms. This has resulted in a separation of the small piroplasms from dogs originating from three different areas into three distinct taxonomic groups: the Asian genotype of *B. gibsoni* (Asian genotype), the North America genotype of *B. gibsoni* (USA/California genotype) and the small canine piroplasm (*Theileria annae*).

The first cases of Babesiosis caused by the Asian genotype in American Pit Bull Terriers (APBT) without travel history, were published in USA 1999 (Birkenheuer et al. JAHA 35:125–8) and in Australia 2002 (Mohlnickel et al Aust Vet J 80:606–10).

In 2002 and 2004 we diagnosed Babesiosis in two APBT, without travel history to endemic areas. Both dogs had a severe haemolytic anaemia, thrombocytopenia, leukocytosis, hyperproteinaemia and haemoglobulinuria. Small intraerythrocytic piroplasms were seen on blood smears. The final diagnosis was made by PCR, which amplifing a partial region of the 18S rDNA gene and sequencing. The sequenced 486bp PCR-products were 100% identical to *Babesia gibsoni* (Asian genotype) sequence in Gene Bank (accession numbers AF175300 and AF 175301. http://www.ncbi.nlm.nih.gov/BLAST/).

The other four APBTs of the same owners were PCR negative.

One dog was treated with Imidocarb, Doxycyclin and Phenamidine and was subsequently PCR negative on three occasions over a periode of two years. The other dog was treated with Imidocarb, Doxycyclin and Buparvaquone and while all clinical parameter improved markedly, PCR remaind positive.

The source of infection in these two dogs remains unknown.

55 HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CANINE NASOPHARYNGEAL LYMPHOID TISSUE.

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In rodents, the nasopharyngeal tonsil is involved in the development of local immune responses following intranasal vaccination. Intranasal vaccination induces effective immune protection in dogs, but the mucosa-associated lymphoid tissue (MALT) of the canine nasopharynx has not been investigated. Therefore, the aim of the present study was to characterize the leucocyte subsets and their organization in the nasopharyngeal mucosa of dogs without respiratory disease.

Mucosal biopsy samples were obtained within 15 minutes of euthanasia from four areas of the roof of the nasopharynx (i.e. A = caudal, B = mid-caudal, C = mid-rostral and D = rostral) in 6 puppies (1 to 8 months old) and 8 adult dogs (5.5 to 13 years old) without signs of respiratory disease and euthanized for unrelated reasons. Sections were stained with H&E and with toluidine blue for mast cells. Antibodies used for immunohistochemistry were directed against MHCII molecules, the myelomonocytic antigen L1, CD3, IgA, IgG and IgM.

Overall, lymphoid tissue was predominantly found in caudal areas (areas A and B) and it was more developed in puppies than in adult dogs. These areas contained lymphoid follicles (LF), diffuse aggregates of lymphoid tissue (DA) and scattered lymphoid cells (SC) in the lamina propria (LP) just beneath the epithelium. In contrast, only small DA and few SC were found in the LP of areas C and D. Mast cells were mostly found immediately beneath the epithelium but were also scattered deeper in the LP.

MHC class II^+ cells were mostly localized within LF and DA. These cells had the morphology of macrophages, B cells or dendritic cells. $MHCII^+$ cells with a dendritic morphology were also present within and immediately beneath the basement membrane of the respiratory epithelium. The number of $MHCII^+$ cells was higher in puppies than in adult dogs. Polymorphonuclear $L1^+$ cells were found in very low numbers throughout the LP. $CD3^+$ cells represented the vast majority of the lymphocytic cells. There were significantly more $CD3^+$ cells in areas A and B in puppies than in adult dogs. IgA^+ and IgM^+ plasma cells were mostly associated with the glandular tissue but were also scattered in LF and DA or immediately beneath the respiratory epithelium. IgG^+ cells were sparse. Overall, the number of plasma cells was higher in adult dogs than in puppies.

This study has demonstrated the presence of organized lymphoid tissue in the canine nasopharynx. The immunohistochemical findings of the present study will enable comparisons to be made with similar studies conducted in dogs suffering from nasopharyngeal diseases or in dogs intra-nasally vaccinated.

56 FREQUENCIES OF FELINE BLOOD TYPES IN CATS AT THE ROYAL VETERINARY COLLEGE, LONDON, UK.

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There have been three different feline blood types described: A, B, and AB. The importance of blood typing in cats before a transfusion cannot be overemphasised. The presence of naturally occurring pre-formed alloantibodies in cats requires typing of the donor and recipient blood to prevent a potentially fatal transfusion reaction. The prevalence of different blood types is known to have geographical and breed variation. Some breeds are known to show a higher prevalence of type B (i.e. British Short Hair cats); others type A (Siamese 100% type A); whereas the majority of non-pedigree cats are known to be predominantly type A. Previous reports have suggested the prevalence of blood type B in non-pedigree cats in the north of the UK and Scotland to be 7.9%. The highest percentage of type B non-pedigree cats in Europe was found in France with a prevalence of 14.9%. It was our aim to establish the prevalence of blood types in our hospital population and to compare the frequencies of blood types between pedigree and non-pedigree cats.

Medical records of all cats blood typed in the Queen Mother Hospital for Animals between January 2000 and November 2004 were reviewed. Blood typing had been performed using commercial feline blood typing cards (previously validated for feline blood typing) and saline agglutination test. Cats showing autoagglutination were eliminated from the study.

156 cats were included in the study. Four cats were not included due to in-saline autoagglutination of red blood cells, which prevented accurate determination of blood type. 51 (32.7%) cats were pedigree and 105 (67.3%) cats were non-pedigree. Of the 51 pedigree cats, the prevalence of blood types was as follows: Type A, n = 42 (82.4%); Type B, 7 (13.7%); Type AB, 2 (3.9%). Of the 105 non-pedigree cats, the prevalence of blood types was as follows: Type A, n = 71 (67.6%); Type B, 32 (30.5%); Type AB, 2 (1.9%).

The results of this study have demonstrated that the prevalence of blood type B in non-pedigree cats in our hospital population was 30.5%, much higher than in other previous studies in the UK. This apparent increased prevalence of blood type B in non-pedigree cats is likely a reflection of geographic variation of feline blood types in the United Kingdom. The clinical implication of this finding is that the increased percentage of type B cats in the non-pedigree cat population increases the risk of transfusion incompatibility if donor and recipient cats are not appropriately blood typed prior to administration of blood products.

57 EFFECTIVITY AND SAFETY OF DIFFERENT DOSAGES OF LOW MOLECULAR WEIGHT HEPARIN IN DOGS SUFFERING FROM GASTRIC VOLVOLUS/DILATATION COMPLEX.

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The objective of this study was to evaluate the effectiveness and safety of low molecular weight heparin (LMWH) in dogs suffering from gastric volvolus/dilatation syndrome (GVDS), a disease which is often associated with disseminated intravascular coagulation (DIC).

The study included 60 dogs suffering from GVDS. 19 dogs served as a control (group 1, without anticoagulatory treatment). In the remaining groups, treatment with subcutaneous injections of dalteparin-sodium every 8 hours for 7 days started after surgery. The individual dosage consisted of 75 anti-FXaU/kg BM (n = 21, group 2), 100 anti-FXaU/kg BM (n = 14, group 3), and 150 anti-FXaU/kg BM (n = 6, group 4).

At defined times, clinical examination and measurements of heparin plasma activity, platelet count, prothrombin time, activated partial thromboplastin time (APTT), factor V activity, antithrombin activity, soluble fibrin as well as a haematological and biochemical profile were performed.

All dogs of group 4 developed severe bleeding complications (mainly in the area of the surgical wound). Due to this fact, only 6 animals were treated with 150 anti-FXaU/kg BM 3 times daily (group 4) and a modified protocol with 100 anti-FXaU/kg BM 3 times daily was added to the study design (group 3). 6 of 14 dogs of group 3, 3 of 21 dogs of group 2 and none of the control dogs showed significant bleeding episodes. The mean plasma heparin activity in groups 2, 3, and 4 reached maximum values of 0.430.17 anti-FXaU/ml, 0.60 ± 0.21 anti-FXaU/ml and 1.01 ± 0.39 anti-FXaU/ml.

Other clinical parameters and measurements of soluble fibrin, factor V activity and prothrombin time did not show significant differences between groups. APTT values were significantly higher in dogs of group 4 when compared to all other groups. In addition, a dose-dependent decrease of antithrombin activity was observed.

The results of this study indicate that LMWH does not have a positive influence on the clinical outcome and consumption coagulopathy in dogs suffering from GVDS. Prolongation of APTT and decrease of antithrombin in dogs treated with high LMWH dosages reflect direct heparin effects. Regarding the safety aspect of heparin treatment, the results of our study indicate that dalteparin dosages >75 anti-FXaU/kg BM three times daily and heparin activity levels >0.5 anti-FXaU/ml dalteparin should be avoided in dogs after major surgery.

58 QUANTIFICATION OF mRNA ENCODING CYTOKINES AND CHEMOKINES IN NASAL BIOPSIES FROM DOGS WITH SINO-NASAL ASPERGILLOSIS.

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Canine sino-nasal aspergillosis is characterised by localised invasion of the nasal cavity and frontal sinus and is mostly caused by *Aspergillus fumigatus.* The pathogenesis of the condition is poorly understood but this disease occurs in otherwise healthy and apparently immunocompetent individuals. In the present study, we have used quantitative RT-PCR to investigate the nature of the local immune response mounted in the upper respiratory mucosa of affected dogs.

Quantitative RT-PCR was carried out on RNA isolated from nasal biopsies from diseased and control dogs, using specific assays designed to amplify mRNA encoding a panel of cytokines (IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-13, IL-18, IFN-γ, TNF-α and TGF-β) and chemokines (IL-8, MCP-1, -2, -3 and -4, and eotaxin-2 and -3). For each molecule, relative copy numbers obtained in nasal tissue from control dogs and dogs with sino-nasal aspergillosis were compared by means of the Wilcoxon-two-samples test using the NPAR1WAY procedure (PROC GLM, SAS Institute, Cary, NC).

Canine sino-nasal aspergillosis was associated with significantly increased expression of mRNA encoding MCP-1, -2, -3 and -4, IL-8, IL-10, IL-18 and TNF-α relative to controls ($P < 0.01$) but there was no difference between groups with respect to IL-4, IL-5, IL-6, IL-12, TGF-β, and eotaxin-2 and -3. Samples from individual diseased dogs showed elevation of IFN-gg transcript above the upper range of normal, but overall there was no significant difference between diseased and control tissues.

Our results suggest that proinflammatory cytokines (IL-18 and TNF-α and chemokines related to the influx of phagocytic cells (MCPs and IL-8) might account for the restricted localisation of the infection to the upper respiratory tract in dogs. IL-12 and IFN-γ are known to be essential for clearing fungal infections in mammals, so the lack of up-regulation of mRNA encoding Th1 cytokines (IL-12 and IFN-γ) in diseased tissues might account for the fact that infected dogs are generally unable to clear *Aspergillus* spontaneously. Failure to express mRNA encoding Th1 cytokines in nasal tissue from dogs with sino-nasal aspergillosis might be due to the up-regulation of mRNA encoding the immunomodulatory cytokine IL-10. IL-10 producing regulatory T cells might also be important in limiting the extent of local tissue destruction.

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59 PREVALENCE AND DEVELOPMENT OF MICROALBUMINURIA IN DOGS WITH LEISHMANIOSIS.

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Canine leishmaniosis, caused by the protozoan parasite *Leishmania infantum*, is a severe systemic disease highly prevalent in the Mediterranean basin. Due to the importance of glomerular injury in patent canine leishmaniosis, several tests have been used to detect early renal damage in canine leishmaniosis such as urinary protein/creatinine ratio and enzymuria to establish a correct prognosis and treatment. However, the detection of microalbuminuria (urine albumin concentrations between 1 and 30 mg/dL) in dogs with leishmaniosis to early discover nephron damage has not been applied. We study the prevalence of microalbuminuria in 61 dogs with patent leishmaniosis at diagnosis and in 25 dogs to follow-up over a year during anti-*Leishmania* treatment. We also compare microalbuminuria detection with urine protein/creatinine ratio in urine of dogs with

leishmaniosis at diagnosis and over a one year follow-up treatment. Diagnosis of canine leishmaniosis was made by serum titer of anti-*Leishmania* antibodies (ELISA) and identification of the parasite either by visualization in bone marrow cytology, skin biopsy or detection of DNA by polymerase chain reaction technique in dogs with compatible clinical signs and laboratory abnormalities. In all cases general biochemistry analyses and complete urinalysis (including urine protein/creatinine ratio and microalbuminuria detection test) were performed. Because the measurement of microalbuminuria was qualitative in nature, logistic regression was used to evaluate the results.

The prevalence of microalbuminuria in dogs with patent leishmaniosis at diagnosis was 73.8%. In these dogs, the prevalence of the different levels of microalbuminuria was 6.6% (one +), 13.1% (two +) and 54.1% (three +). The prevalence of microalbuminuria in dogs in follow-up during anti-*Leishmania* treatment was 66.7% (at the diagnosis), 55.6% (one month later), 76.5% (six months later) and 56% (one year later). Urine protein/creatinine ratio was not statistically significant different between dogs with and without microalbuminuria at six and twelve months of follow-up.

Based on these results, it is clear that the likelihood of microalbuminuria is high in dogs with patent leishmaniosis and, although the prevalence of microalbuminuria decreases during the anti-*Leishmania* treatment, it is still higher than 50% during all the follow-up. It also seems reasonable to conclude that microalbuminuria detection could be a good test to evaluate renal involvement in dogs with leishmaniosis at the diagnosis and during the follow-up. Further analysis is required to determine if increased prevalence will decrease with the addition of specific therapy for proteinuria to the anti-*Leishmania* treatment.

60 CLINICAL PRESENTATION AND LONG-TERM OUTCOME IN 21 CATS WITH THIRD DEGREE ATRIOVENTICULAR BLOCK (1997–2004).

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The impact of third degree atrioventricular block (3AVB) on clinical findings and long-term outcome in cats is unknown. Presentation, clinical findings and clinical outcome of 21 cats with 3AVB were studied retrospectively. Median age of cats studied was 14 years (range 7–19 years). Typical presenting signs included respiratory distress or collapse, but 29% were presented with no abnormal clinical signs. Most cats had concurrent systemic diseases common in this age group, including diabetes mellitus, hyperthyroidism and renal disease. Eight cats (38%) had congestive heart failure (CHF) at the time of diagnosis. Heart rates overall ranged from 80–140 bpm (median 120 bpm) and there was no difference in heart rate between cats with CHF and those without CHF. Eleven of 18 (61%) cats that had echocardiograms had structural cardiac disease (myocardial or valvular) noted; six cats had cardiac changes consistent with their systemic disease and 1 cat had no abnormalities noted. No atrioventricular nodal lesions were noted by echocardiography. One cat had histologic atrioventricular nodal lesions. Median survival of 14 cats that died or were euthanized was 386 days (range 1–2013 days). Survival did not differ between cats with or without CHF or between cats with or without structural cardiac disease ($P < 0.05$). One cat underwent successful pacemaker implantation. Three cats that had pacing recommended did not receive pacemakers; all three of these cats survived >1 year although clinical signs continued. Sixty-two percent of cats with 3AVB survived >1 year post-diagnosis regardless of presenting signs or underlying cardiac disease.

Third degree heart block in cats is often not immediately life-threatening. Survival in these 21 cats was not affected by the presence of underlying heart disease or congestive heart failure at the time of presentation. Even cats with overt clinical signs of collapse may survive >1 year without pacemaker implantation.

61 PREVALENCE OF ENTERAL VIRUSES IN 936 DOGS WITH ACUTE HAEMORRHAGIC DIARRHEA.

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Acute haemorrhagic diarrhea is one of the most common reasons for presentation of dogs in veterinary practice. Among the viruses suspected to be involved in naturally occurring diarrhea in dogs are parvoviruses, coronaviruses, paramyxoviruses, and rotaviruses. Not much is known about the prevalence of these viruses in dogs with diarrhea in the field as well as concerning differences in clinical and laboratory signs and outcome associated with different viral agents. Moreover, the pathogenicity of some enteral viruses is matter of discussion, since clinically healthy dogs may be asymptomatic carriers of these viruses. Aim of this retrospective study was to evaluate prevalence of intestinal viruses in dogs with acute bloody diarrhea and to compare signalment, clinical signs, and laboratory abnormalities among groups of dogs infected with different viruses to those that tested virus-negative.

In this retrospective study, 936 client-owned dogs were included that were presented for acute bloody diarrhea to the Veterinary Teaching Hospital of the Munich University over a period of 11 years. Virus detection was performed by electron microscopy from freshly collected fecal samples in each of these patients. Signalment, clinical signs, and laboratory abnormalities among different virus groups were evaluated statistically and compared to the parameters of the negative dogs.

Virus was detected in 55.2% of the dogs presented with acute bloody diarrhea. The highest prevalence was demonstrated for parvoviruses (16.6%), followed by coronaviruses (11.9%), and paramyxoviruses (9.3%). In none of the fecal samples, rotavirus was detected. Two or three virus species were demonstrated in 6.5% of all fecal samples. Dogs with parvovirus infection were statistically significantly younger when compared to dogs infected with other enteral viruses or dogs that tested negative. No significant differences were demonstrated concerning sex, breed, or clinical parameters among groups. Parvovirus-infected patients showed significantly lower leukocyte and erythrocyte counts as well as hematocrit and total protein and albumin levels compared to all other groups.

Parvoviruses still seem to be the most prevalent viral agents involved in acute diarrhea in dogs in Germany. Besides the young age, parvovirus infection is strongly associated with typical changes in laboratory parameters. Rotaviruses were not identified in dogs with haemorrhagic diarrhea. Coronaviruses and paramyxoviruses were present in significant percentages, their role as primary agents in these cases of haemorrhagic enteritis or their potential role as secondary invaders, however, still has to be clarified.

62 REPRODUCIBILITY OF ENDOSCOPIC COLLECTION OF DUODENAL JUICE AND EVALUATION OF SAMPLE STABILITY FOR 16S rDNA ANALYSIS OF THE DUODENAL MICROFLORA IN DOGS.

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It is now recognized that many bacteria escape identification using standard bacterial culture. 16S rDNA analysis has revealed previously uncharacterized bacteria present in fecal samples. The aims of this study were to evaluate the reproducibility of endoscopic collection of duodenal juice, to evaluate sample stability, and to describe the canine duodenal microflora in healthy dogs by direct 16S rDNA analysis.

Duodenal juice was collected endoscopically in duplicate using sterile disposable cytology brushes from 5 dogs. Duodenal juice was also collected by needle aspiration from 6 healthy dogs, euthanized for unrelated studies. Samples were divided into 4 aliquots: 3 aliquots were stored in liquid nitrogen, at −80°C, and −20°C, respectively. The fourth aliquot was stored at +4°C for 72 hours, aiming to simulate storage conditions for overnight shipment of cooled samples. Each sample was processed independently. Bacterial DNA was extracted and 16S rDNA amplified. The reproducibility between duplicates collected endoscopically (method reproducibility), and aliquots stored (sample stability) was determined by comparing the similarity of their banding patterns following denaturing gradient gel electrophoresis (Dice coefficient, 100% = complete identity). Bacteria present in duodenal juice from 6 healthy dogs were identified by sequencing of cloned 16S rDNA amplicons.

The mean (±SD) similarity between duplicate samples collected endoscopically was 93.7% (±3.0). Mean (±SD) similarity between the aliquots stored at different storage conditions from each dog were 92.3% (±5.5), 90.3% (±7.9), 91.1% (±3.1), 94.2% (±5.2), 91.7 (±4.5) and 90.0% (±8.1), with no significant difference between different storage conditions (ANOVA, $p = 0.893$). Sequencing data revealed 47 individual 16S rDNA sequences, 20 (42%) of which showed lower than 98% sequence similarity to 16S rDNA sequences of previously described microorganisms as identified in public databases (GenBank, Ribosomal Database Project), suggesting the presence of previously uncharacterized bacterial species in the canine duodenum. The majority of sequences identified belonged to the orders of *Clostridiales* (40%), *Lactobacillales* (21%), and *Enterobacteriales* (19%).

The canine duodenum harbors a complex microflora. Endoscopic collection of duodenal juice using a disposable cytology brush is a rapid and reproducible sampling technique and the specimens obtained show high storage stability. Direct 16S rDNA analysis revealed previously uncharacterized bacterial species in the duodenum of healthy dogs.

63 DETERMINATION OF INTESTINAL PERMEABILITY AND MUCOSAL ABSORPTIVE CAPACITY IN NORWEGIAN LUNDEHUNDS USING A FOUR-SUGAR BLOOD TEST.

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Many Norwegian Lundehunds are affected by gastrointestinal disease. Clinical signs may include diarrhea, vomiting, anorexia, weight loss, and in many cases gastrointestinal protein loss. The aim of this study was to evaluate intestinal permeability and absorptive capacity in Norwegian Lundehunds in the USA and Canada, in order to further characterize intestinal abnormalities in this breed.

A four-sugar blood test was performed in 11 Norwegian Lundehunds (mean age 3.4 years; range 0.5–7.5 years), none were exhibiting clinical signs of gas-

trointestinal disease at the time of the test, and in eight healthy control dogs of various breeds (mean age 4.6 years; range 1.5–8.0 years). Food was withheld from the dogs for a minimum of 15 hours before testing. A baseline blood sample was obtained from each dog immediately prior to oral administration of the sugar solution. The sugar solution contained 5.0 g/L methylglucose, 10.0 g/L rhamnose, 10.0 g/L xylose, and 10.0 g/L lactulose. Dogs of less than 10 kg body weight received 100 ml, dogs weighing 10–20 kg received 200 ml, dogs weighing more than 20 kg received 400 ml of the solution. Subsequent blood samples were collected at 60, 90, and 120 minutes after sugar administration. Serum concentrations of the four sugars were determined by HPLC with pulsed amperometric detection. Serum concentrations of all sugars at all time points were analyzed in control dogs and Norwegian Lundehunds using the Friedman test. Median ratios of serum lactulose to rhamnose concentrations (L/R ratio) and xylose to methylglucose concentrations (X/M ratio) were compared between control dogs and Norwegian Lundehunds at 90 minutes post-dosing (Mann Whitney test).

Oral administration of the sugar solution lead to significant changes in serum concentrations of all sugars at all time points compared to baseline ($P \leq 0.0015$ for all). Norwegian Lundehunds had significantly higher L/R and X/M ratios at 90 minutes post-dosing (medians: 0.049 vs. 0.021; $p = 0.0015$ and 1.272 vs. 0.595; $p = 0.0008$, respectively) than the control dogs.

These data suggest that intestinal permeability and mucosal absorptive capacity is significantly different in this group of healthy Norwegian Lundehunds than in a group of healthy control dogs. High L/R ratios suggest an alteration in small intestinal permeability, possibly reflecting disturbed tight junctions and decreased intestinal surface area. The increased X/M ratios seen in this group of Norwegian Lundehunds were predominantly due to lower serum methylglucose concentrations, suggesting decreased mucosal absorptive capacity for this analyte.

64 DYNAMICS OF JEJUNAL MICROBIOTA DURING FOOD DEPRIVATION.

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While the composition of the small intestinal microbiota is reported to have a significant impact on the health status of an animal, and food deprivation is a commonly recommended remedy in acute gastrointestinal disturbances in dogs, no data are available about the dynamics of the canine small intestinal microbiota during an unfed state. The aim of this study was to evaluate the dynamics of the jejunal microbiota before, during and after food deprivation.

Five healthy laboratory Beagle dogs with a permanent jejunal fistula located 60 cm distally from the pylorus were included into this study. Food was withheld for 5 days, but normal tap water was given *ad libitum.* Jejunal juice samples were collected daily for 5 days via the fistula using a sterile cytology brush before, during and after food deprivation. Thorough clinical examination was performed and serum biochemistry and hematological values were evaluated daily during food deprivation. Bacterial DNA was purified, the variable V6-V8 region of 16S rDNA was amplified with universal bacterial primers, and PCR amplicons were subsequently separated by denaturing gradient gel electrophoresis (DGGE). Variation in the jejunal microbiota before, during and after food deprivation was evaluated by comparing similarity indices (Dice coefficient; 100% represents complete identity) of DGGE profiles using gel analysis software. Friedman two-way analysis of variance followed by multiple comparison test was used to test for the differences in the similarity percentages between the three phases.

No clinical, biochemical or hematological abnormalities were observed over the duration of the study. Mean (±SD) similarity indices of DGGE profiles before, during and after food deprivation were 76.9 (±3.1), 57.0 (±7.6), and 64.1 (±4.6), respectively. Food deprivation led to significant changes ($P < 0.05$) in DGGE profiles when compared to profiles before the food deprivation period. However, the jejunal microbiota remained relatively stable during the food deprivation period, and significant daily changes were not observed. After food deprivation, jejunal microbiota returned close to the baseline determined before food deprivation.

This is the first study to show that there is a complex bacterial population in the canine jejunum during food deprivation. Also, the jejunal microbiota found after food deprivation differs significantly from the microbiota determined during periods of feeding. The fasted phase microbiota is hypothesized to be closely adhered to intestinal mucosa. More studies are warranted to determine the importance of these mucosa-associated bacteria (MAB) to the host.

65 PROBIOTIC LAB CAUSED PERSISTENT CHANGES IN THE CANINE JEJUNAL MICROBIOTA.

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The aim of the study was to investigate the stability of potential probiotic lactic acid bacteria (LAB) in the canine small intestine. Most of the earlier studies have examined the competitive exclusion of pathogenic bacteria, whereas reports of the potential effects on the endogenous LAB population are scarce.

Five lactic acid bacteria strains isolated from dog faeces (*Lactobacillus fermentum, L. salivaruius, L. rhamnosus, L. mucosae* and *Weissella confusa)* were evaluated in this study. The strains were fed to five permanently fistulated dogs for 7 days and the stability of the LAB strains were monitored for 17 days by plating jejunal content on mLBS. The chromosomal DNA of the bacterial colonies was cloned by PCR and run in DGGE to detect the living bacteria passing through the intestine. The effect of the supplemented LAB on the normal intestinal LAB microbiota was determined by DGGE.

All the fed LAB strains survived the passage through the upper GI tract but disappeared within 7 days after the supplementation had ceased. However, a significant and persistent change in the indigenous LAB microbiota caused by the fed LAB lasted more than 17 days after the cessation of LAB supplementation.

We conclude that this long-term modification of the indigenous intestinal LAB microbiota may be an important mechanism for probiotic LAB to exert their beneficial health effects.

66 REAL TIME-PCR QUANTIFICATION AND GENETIC IDENTIFICATION OF HELICOBACTER STRAINS IN A GROUP OF DOGS WITH DIGESTIVE DISORDERS.

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Helicobacter spp. are frequently found in the dog stomach but the association between gastritis and *Helicobacter* spp. infection is not clear. In a former study on a group of dogs with chronic digestive disorders, we did not find any clear relationship between clinical signs, gastritis and *Helicobacter* spp infection as assessed by silver staining, urease test and PCR (abstract in JVIM 2003, 17: 447). The aims of this further study were 1. quantification of *Helicobacter* infection with Real Time-PCR and assessment of its relationship with microscopic quantification and gastritis, 2. characterization of *Helicobacter* species infecting this group of dogs.

Thirty-six dogs with either chronic vomiting (n = 14), diarrhea (n = 21) or no clinical signs (n = 6) were included in the study. Gastric endoscopic biopsies were performed in all dogs. The gastritis was histologically scored from absence to mild, moderate or severe. *Helicobacter* spp. infection was assessed by urease test, Warthin Starry staining and *Helicobacter* specific Real Time-PCR of 16S RNA gene on specimens from 3 gastric parts (fundus, body and antrum). Cloning and sequencing of amplicon of the urease gene were performed to further characterize *Helicobacter* species as compared with reference sequences from GenBank Database.

All dogs were infected with *Helicobacter* spp. The Real Time-PCR quantification was significantly lower in antrum when compared with fundus or body. There were no correlation between silver stain and Real Time-PCR quantification and no relationship between PCR quantification and histological gastritis scoring. As revealed by sequencing analysis performed in 31/36 dogs, 23/31 dogs were infected with more than one *Helicobacter* spp. strain. The prevalence of the identified strains was: *H. heilmannii* in 25/31 dogs, *H. bizzozeronii* in 18/31 dogs and *H. salomonis* in 1/31 dogs. *Helicobacter pylori* was identified in one single dog. In 19/31 dogs, the sequences were not matching with the *Helicobacter* spp sequences available on GenBank (maximal homology of 83% with *H. felis, bizzozeronii or heilmannii*) but were clearly *Helicobacter* spp. as shown by 16S RNA. These unknown sequences are relatively homogenous with phylogenetic analysis. No specific distribution was observed for the different *Helicobacter* strains but *H. pylori* was found only in the body and *H. salomonis* only in the fundus and body. No definitive relationship was observed between *Helicobacter* species infection and gastritis.

This study did not show any relationship between *Helicobacter* spp. infection and gastritis in dogs. Moreover, none of the *Helicobacter* species characterized has been specifically related to gastric inflammation in any part of the stomach in our group of dogs.

67 NO CHANGES IN HISTOLOGICAL SCORING, TOTAL NUMBER OF INFILTRATING CELLS AND NUMBER OF T CELLS AFTER TREATMENT IN DOGS WITH CHRONIC ENTEROPATHIES.

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Histology remains the mainstay of diagnosis in dogs with chronic enteropathies (CE). In human beings with inflammatory bowel disease (IBD), histology has been shown to be useful for assessing clinical remission and correlates well with clinical activity of disease. In dogs with CE, the main infiltrate in the lamina propria consists of plasma cells and lymphocytes. T cells and production of proinflammatory cytokines have been implicated in the pathogenesis of the canine disease. However, no studies have been undertaken so far to show if histological

scoring and enumeration of infiltrating lymphocytes and T cells can be used to assess clinical remission and severity of disease in dogs.

The purpose of this study was to assess changes in histological severity, and total numbers of infiltrating lymphocytes and T cells in mucosal biopsy samples of dogs with CE before and after standardized treatment protocols. Twenty dogs diagnosed with CE were retrospectively grouped into either diet responsive CE (FRD, n = 10) or CE requiring treatment with prednisolone (IBD, n = 10). Severity of clinical signs was assessed before and after 10 weeks of treatment using the Canine IBD Activity Index (CIBDAI; Jergens et al 2003). In addition, all biopsies were histologically graded for severity of intestinal infiltration by one board-certified pathologist according to previously published criteria. Total numbers of infiltrating lymphocytes and T cells expressing CD3 were determined in five biopsies for each dog before and after treatment, and expressed as numbers per 10,000 μm^2 lamina propria.

Median CIBDAI decreased significantly after treatment in both groups (FRD: 5.9 before to 1.4 after treatment [$P = 0.005$]; IBD: 8.0 before; 4.9 after treatment [$P = 0.04$]). Total number of lymphocytes and T cells did not differ between the two groups. The median histological severity score of endoscopically collected biopsies did not change after treatment in either group. Moreover, there was no statistically significant difference in total numbers of infiltrating lymphocytes or in total numbers of T cells after treatment in either group. Finally, histological scoring, total lymphocyte counts and T cell numbers did not correlate with CIBDAI.

The fact that inflammatory infiltrates did not change after treatment in either group is a new and unexpected finding. Based on these results, criteria for diagnosis, grading of disease severity, and assessment of therapeutic response in dogs with CE need to be reassessed.

68 COIL-EMBOLIZATION OF EXTRAHEPATIC PORTOSYSTEMIC SHUNT IN DOGS.

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The coil embolization of extrahepatic shunts showed a relatively low survival rate (4/7 patients) and one of the four surviving dogs developed acquired extrahepatic shunts (Leveille et al., 2003). Up to now no study proved the combination of coil embolization with antithrombotic treatment in dogs with a congenital extrahepatic portosystemic shunt.

Between July 2000 and October 2003 we diagnosed a congenital extrahepatic portosystemic shunt in 22 dogs. 3 dogs were managed only with dietary treatment because of owner decision. One dog was excluded because of an ultrasonographic measured to large shunt diameter. 18 dogs were included in the prospective study. From jugular vein a catheter was placed through the shunt into the portal vein for angiography. Coils (Gianturco or Tornado coils 0.035–0.038 inches, COOK) were modified to be detachable from an introducer wire. Dependent on the hemodynamic result one or more coils were implanted. Just before coil implantation heparin treatment was started by injection into portal vein (100 IU/kg) followed by continuous intravenous infusion (25 IU/kg/h) and subcutaneous injections (200 IU/kg TID), additionally intravenous injections were done to obtain an activated coagulation time 1.5 to 2.0 times higher than the basic value. Heparin treatment lasted as long as abdominal effusion was found in ultrasound examination but for a minimum of two days. Additionally interventions were done if residual shunt persisted longer than three months.

The median age of the patients was 10.0 months (range 5.0 to 80.5), the median body weight 5.4 kg (range 1.5–8.0). The following shunt morphologies were found: port-caval shunt (n = 14), porto-azygos shunt (n = 3) and portophrenico shunt (n = 1). Coil migration into the heart or pulmonary artery occurred in three cases and the coils were removed. A median number of 2 (range 1 to 5) coils were implanted in the first intervention. In four dogs we found subcutaneous hematoma and the heparin treatment was reduced or stopped. One of these dogs developed severe portal hypertension but was treated successfully. Three dogs died after the first intervention and one dog was not presented for reexamination. A control-angiography showed a complete shunt occlusion in 10/14 dogs and acquired shunts in one of them. One of four dogs died after the second intervention. In the other 3 dogs the shunt could be occluded in the second (n = 2) or third intervention (n = 1).

19 dogs with an extrahepatic shunt were studied. One dog could not be treated because of the shunt size and four dogs died. The other 14 dogs were treated successfully and only one of these showed extrahepatic acquired shunts. The heparin treatment seems to reduce the risk of portal hypertension but the treatment protocol has to be improved in the future.

69 PREVALENCE OF CHRONIC PANCREATITIS AT POST MORTEM EXAMINATION IN AN UNSELECTED POPULATION OF FIRST OPINION DOGS.

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The prevalence of chronic pancreatitis (CP) dogs is unknown. Previous studies have focussed on acute pancreatitis and/or have used a highly biased and selected population of second opinion and critical care cases. This study aimed to assess the prevalence of chronic pancreatitis in an unselected population of first opinion dogs.

Sections were obtained from 100 consecutive canine post mortems presented to Glasgow Veterinary School from surrounding first opinion practices. Most dogs were assessed as middle-aged to old. In each case, 3 sections of pancreas were taken: one from each limb and one from the body. Sections were preserved in formalin and stained with H&E and Sirius red. They were examined histologically, blind to signalment. Clinical details were not available. The dogs were grouped according to histological findings: (a) sections too autolysed to interpret; (b) no abnormalities visible; (c) non specific/non significant changes; (d) chronic or acute-on-chronic pancreatitis; (e) acute pancreatitis with no chronic changes and (f) other disease. Prevalence of each group was calculated and relative risk of CP and autolysis were calculated for different breeds.

Prevalence of autolysis was 27%, a shortcoming of this type of study. Autolysis was common in large breed dogs due to slow cooling of core temperature (e.g German shepherd dogs relative risk of autolysis 3.8) so relative risk of CP could not be calculated in large breeds. The pancreas had no histological abnormalities in only 13% of dogs. Non specific changes were observed in 24% of dogs. Acute pancreatitis had a low prevalence of 2%. The prevalence of CP was 29% and breeds with a high relative risk included Cavalier King Charles spaniels (CKCS): relative risk 4.1 and Jack Russell terriers: relative risk 2.4. 6/6 CKCS had CP, with 1/6 having end stage disease and 1/6 having concurrent pancreatic neoplasia. The lesions observed are likely to correlate with clinical disease: one out of four published cases of end stage CP with exocrine pancreatic insufficiency (EPI) was a CKCS and a further 2/7 biopsy-confirmed cases of chronic pancreatitis seen at the Queen's Veterinary School Hospital since 2002 were CKCS, both of which had EPI and one of which had diabetes mellitus. We conclude that CP is common in the first opinion dog population and that, like in the liver, end stage disease can be considered as a distinct clinically significant entity. There are strong breed-associations in CKCS and JRT, suggesting a possible genetic basis to the disease in these breeds.

70 COPPER-ASSOCIATED CHRONIC HEPATITIS IN LABRADOR RETRIEVERS: 15 CLINICAL PATIENTS AND THEIR FAMILY.

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Chronic hepatitis is a histological diagnosis, characterized by the presence of fibrosis, inflammation and hepatocellular apoptosis and necrosis. Cirrhosis can result as the end-stage of the disease (''Liver Diseases and Pathology Standardisation Research Group'', presented at the ACVIM forum 2003). The term chronic hepatitis is used irrespective of the cause of the disease, which is usually unknown in spontaneous canine chronic hepatitis, although some cases have been associated with infections, as well as treatment with anticonvulsant drugs, and copper accumulation. Inherited copper toxicosis is a well described disease in the Bedlington Terrier, where a genetic mutation causes accumulation of copper in hepatocytes, resulting in chronic hepatitis. The Labrador retriever is known as a dog breed with increased risk to develop chronic hepatitis. Evaluation of data retrieved from this study revealed copper as associated factor in chronic hepatitis of Labrador retrievers.

This study presents clinical, laboratory, and pathologic examination results of 15 Labrador retriever patients with copper-associated chronic hepatitis. The patient group consisted of 11 female and four male Labrador retrievers, all of which were registered at the breed club. The average age was 7 years at clinical presentation (range 2.5–10.5 years). All dogs were presented for gastrointestinal clinical signs, including anorexia in all, and vomiting in 8 of 15 dogs. Chronic hepatitis or cirrhosis and copper accumulation were diagnosed based on histologic examination of liver biopsies in all patients. The inflammatory infiltrate was mononuclear in variable amounts and co-localized with copper accumulation in zone 3, in all biopsies. A relative increase in ALT activity far above a relative increase in AP activity, as well as the hepatic localization of copper in zone 3 suggested a primary copper storage disease rather than primary cholestatic liver disease.

Eight family members of two former patients were examined for subclinical liver disease. The mean hepatic copper concentration measured in this group of 8 clinically healthy siblings and offspring was 1317 $\mu g/g$ dry weight liver (range 402–2576 $\mu g/g$). This was different from examination results of a healthy control group of six unrelated Labradors, which revealed a mean hepatic copper concentration of 233 $\mu g/g$ dry weight liver (range 120–304 $\mu g/g$).

Our objective was to describe the clinicopathologic findings in 15 Labrador retrievers with copper-associated chronic hepatitis and to reveal if a genetic basis of the disease is likely by examination of family members from these patients. Our findings support a genetic defect to underlie copper-associated chronic hepatitis in the Labrador retriever.

71 DETECTION OF FELINE AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE (AD-PKD) BY REAL-TIME PCR GENOTYPING.

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AD-PKD is the most prevalent inherited genetic disease of cats, particularly affecting Persians. Until now AD-PKD testing in the Europe has centred on ultrasound screening, but this is time-consuming and registered scanning can only be carried out by an approved ultrasonographer and in cats over 10 months of age. Recently, a genetic mutation has been identified in the PKD 1 gene of Persian cats in the USA which is linked to AD-PKD. A single nucleotide polymorphism (SNP) in *PKD 1* results in the production of an abnormal truncated protein. A conventional polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assay detecting this SNP reliably detects AD-PKD affected cats in the USA.

The aim of this study was to develop a rapid, accurate and sensitive multiplex real-time PCR genotyping assay to detect the *PKD1* SNP in genomic DNA isolated from feline blood samples, and to evaluate this assay's accuracy in diagnosing AD-PKD. A PCR assay that amplifies a 130 base pair fragment from *PKD 1*, with the SNP located near the centre, was designed. Two locked nucleic acid Taqman probes were used; one (labelled with a Fam fluorophore) to detect the wild type allele, and one (labelled with a Hex fluorophore) to detect the mutant allele. Extensive assay testing over a range of annealing temperatures showed these probes to be specific for the wild type and mutant alleles respectively. Seventy-two EDTA blood samples from Persian and Exotic Shorthair cats undergoing AD-PKD ultrasound screening at the University of Bristol were obtained. DNA was extracted from 100l of blood using a Nucleospin Blood kit (Macherey-Nagel) and subjected to the multiplex real-time PCR and the conventional PCR-RFLP assay previously described (the latter was run both at the Universities of Bristol and California, Davis). Of the 72 UK cats tested, 29 were positive for the *PKD 1* SNP (all heterozygous) and 43 were negative (all homozygous wild type); no homozygous mutant PKD cats were identified. All 72 samples showed 100% agreement with the conventional PCR-RFLP assay previously described. All 29 cats which generated positive AD-PKD PCR tests were positive for PKD on ultrasonography. Of the 43 cats which generated negative AD-PKD PCR results, 41 were negative and 2 were equivocal by ultrasonography.

AD-PKD in Persians and Exotic Shorthair cats in the UK appears to be caused by the same SNP in *PKD 1* identified in the USA. PCR detection of AD-PKD negates the need for ultrasonography for definitive diagnosis and relies on easily performed blood sampling that can be done in cats less than 10 months old. Additionally, compared to the conventional PCR-RFLP assay, real-time PCR genotyping is quicker (90 minutes vs. 5 hours), less labour intensive and significantly reduces the risk of false positives due to amplicon contamination.

72 REAL-TIME QUANTITATIVE PCR ASSAYS FOR THE DIAGNOSIS OF THREE HAEMOPLASMA SPECIES IN FELINE BLOOD SAMPLES.

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Two distinct species of feline haemoplasmas are recognised in the UK; *Mycoplasma haemofelis* (Mhf) and '*Candidatus* Mycoplasma haemominutum' (CMhm). These species differ in pathogenicity as Mhf infection often results in a severe haemolytic anaemia whilst CMhm infection usually results in few clinical signs. A novel feline species of haemoplasma (Mnov), most closely related to rodent haemoplasmas, has recently been reported in Switzerland and has been associated with anaemia. Real-time quantitative PCR (QPCR) assays are excellent diagnostic tools for haemoplasma infection since they allow development of highly sensitive and specific quantitative assays. The purpose of this study was to develop Taqman QPCR assays for detection of all three feline haemoplasma species, together with an internal control. To confirm the specificity of the new assays, and determine whether Mnov is present in UK cats, these assays were applied to 60 stored blood samples previously tested at the School of Clinical Veterinary Science for the presence of Mhf and CMhm infection by a previously described QPCR assay.

Primers and Taqman probes were designed against published 16S rDNA sequences. Each of the three haemoplasma assays were combined with a feline 28S rDNA-specific assay to produce three duplex assays, thus allowing confirmation of the presence of cat DNA in the PCR (as an internal control). The three haemoplasma assays were sensitive enough to detect 1–2 copies of a sequence-specific plasmid per PCR in the duplex reaction. None of the assays showed cross-reactivity with the other haemoplasma species when tested with 1×10^6 copies of the sequence-specific plasmids. DNA was isolated from 60 samples of EDTA blood (100 μl) using the Macherey-Nagel Nucleospin Blood kit. QPCR was performed on each sample with Qiagen HotStarTaq Master Mix using a Bio-Rad i-Cycler-IQ.

All samples were positive for feline 28S rDNA in all assays performed. Of the samples which had previously tested haemoplasma positive [Mhf (n = 2) and CMhm (n = 27)], all were positive with the new assays, except one CMhm sample. Additionally, one sample was positive for CMhm with the new assay but had previously been negative. Both discordant samples were at the limit of detectability of the assays. One sample was dual positive for both CMhm and Mnov. The remaining samples were negative for all three haemoplasma species on both assays (n = 30). The results of this study demonstrate the utility of new duplex PCR assays for the detection of haemoplasma infection and that the recently described Mnov species is present in the UK cat population. Further studies are required to determine the prevalence of, and risk factors for, this species in the UK.

73 QUANTITATIVE REAL-TIME RT-PCR FOR FELINE CORONAVIRUS RNA IN THE BLOOD AND TISSUES OF CATS.

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The aim of the study was to compare the levels of feline coronavirus (FCoV) RNA in the blood and organs of cats with and without feline infectious peritonitis (FIP). Serum and whole blood (collected ante mortem), ascitic fluid (when present: 5 cats) and tissue samples (collected at post mortem into ''RNA later'') were taken from six cats that had clinical signs and laboratory data suggestive of FIP. RNA was isolated from the fluids (100 μl) and tissues (30 mg) using a Macherey-Nagel Nuclecleospin RNA II kit, which includes a DNAse step. From five of the cats, RNA was also isolated from whole blood using the Qiagen ''PAXgene'' Blood RNA kit which includes an RNA stabilisation reagent. Histopathology ruled out a diagnosis of FIP in two of the cats whilst the remaining four cats were confirmed as having FIP.

A two-step quantitative real-time RT-PCR was performed using random hexamer primed cDNA and a FCoV specific Taqman based real-time RT-PCR assay. The assay was designed around the highly conserved MN gene junction from five different FCoV isolates. A feline G3PDH real-time PCR assay was performed before and after reverse transcription on each RNA sample to confirm successful cDNA synthesis. Threshold cycle (C_T) values were calculated and used to compare relative amounts of FCoV RNA in the samples.

The four cats with confirmed FIP all had high levels ($C_T < 30$) of FCoV RNA in the stomach, intestines, liver, spleen, kidney, body wall and intra-abdominal lymph nodes. Lower levels ($C_T > 30$) were found in the eye and blood. FCoV RNA levels in the tonsil, heart, lung, brain, bone marrow, peripheral lymph nodes and ascitic fluid varied considerably between cats. The non-FIP cats were both negative for FCoV RNA in the majority of organs tested but extremely low ($C_T > 35$) FCoV RNA levels were found in occasional samples.

In conclusion, high levels of FCoV RNA were found throughout the abdominal organs of cats with FIP compared with negative or extremely low levels in the non-FIP cats. Given the problems of interpreting FCoV antibody titres in suspected FIP patients, these findings may have diagnostic relevance in helping to distinguish cats with FIP from those with non-FIP disease. Unfortunately blood, the most accessible body sample to obtain for diagnostic purposes, was not found to be the most useful in this respect. These data represent a small preliminary study and more samples, particularly in the non-FIP group, are being collected to confirm and extend our findings. It will also be interesting to compare the results of 'effusive FIP' cats with those of cats with ''dry (non-effusive) FIP'' disease.

74 TRYPTOPHAN METABOLISM IS ALTERED IN CATS INFECTED WITH FELINE IMMUNODEFICIENCY VIRUS.

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Feline immunodeficiency virus (FIV) is a lentivirus that shares many properties with human immunodeficiency virus (HIV). FIV infection occurs in approximately 6% of healthy cats and 19% of sick cats in the UK, and is a significant cause of feline morbidity. Humans infected with HIV have been shown to have reduced serum tryptophan concentrations, mediated by up-regulation of the γ-interferon-induced enzyme indoleamine dioxygenase. Tryptophan concentrations progressively fall as HIV-associated disease develops, and treatments aimed at reversing the tryptophan depletion have decreased HIV-associated disease progression and improved survival in man.

The aim of this study was to measure the serum tryptophan and kynurenine (a major metabolite of tryptophan) concentrations in FIV-infected and uninfected cats, to see if the tryptophan changes reported in HIV-infected humans are mirrored by similar changes in FIV-infected cats.

Surplus feline serum and plasma submitted to commercial diagnostic laboratories was used for this study. All samples were tested for FIV antibody using an enzyme-linked immunosorbent assay (PetCheck; Idexx, UK). Samples from 69 FIV-negative and 76 FIV-positive cats were analysed by high performance liquid chromatography to determine tryptophan and kynurenine concentrations. No attempt was made to match samples with respect to age, sex or breed. Sam-

ples were initially treated by the addition of an equal volume of 2M trichloroacetic acid to precipitate proteins and to allow subsequent clarification of the sample. FIV-negative and FIV-positive cats were statistically compared using *t*-tests, following demonstration of a normal distribution of logged data.

FIV-infected cats had significantly lower serum tryptophan concentrations compared to uninfected cats (39.6 μM vs. 50.5 μM, $P = 0.012$). FIV-infected cats had significantly higher kynurenine concentrations compared to uninfected cats (21.9 μM vs. 10.2 μM, $P < 0.0001$). The [kynurenine]:[tryptophan] ratios of FIV-infected cats were significantly higher than those of uninfected cats (0.55 vs 0.20, $P < 0.0001$).

These findings show that tryptophan metabolism is altered in FIV-infected cats, paralleling the situation with HIV infection. Pharmacological manipulation of tryptophan levels may be a feasible means of ameliorating clinical signs and disease progression in cats infected with FIV.

75 SEROPREVALENCE OF FIV AND FELV INFECTION AND DETERMINATION OF FIV SUBTYPES IN SICK DOMESTIC CATS IN SOUTH AFRICA.

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The prevalences of Feline leukaemia virus (FeLV) and Feline immunodeficiency virus (FIV) infections have been shown across the world to be higher in sick than in healthy cats and to vary widely from study to study and from country to country. The prevalence of FIV and FeLV infections, as well as the subtypes of FIV infecting domestic cats in South Africa is currently unknown.

The aim of this study was to determine the prevalence of these viral infections in sick domestic cats and to determine the subtype(s) of FIV virus that exist in South Africa.

Serum was collected from 454 sick cats presenting to the Onderstepoort Veterinary Academic Hospital over a 7-year period from 1998 to 2004. The serum was submitted for detection of specific antibodies directed to FIV *gag* (group antigen) or *env* (envelope) gp40 proteins and the group-specific p27 core antigen of FeLV by ELISA. In addition, heparinised whole blood was collected from FIV positive cats, consisting of 11 of the above cats and a further 20 cats from 3 different centres in the country, viz Cape Town (12), Durban (4) and Johannesburg (4). The whole blood samples were subjected to polymerase chain reaction (PCR) amplification and sequences were determined for the V3-V5 region of the *env* gene of FIV.

Fifty-six out of 454 (12.3%) samples were positive for FeLV antigen and 101 out of 454 (22.2%) samples were positive for FIV antibody. Sixteen out of 454 cats (3.5%) were co-infected with both viruses. Twenty-two out of 31 (71%) samples revealed FIV subtype A and 9 out of 31 (29%) samples revealed FIV subtype C.

The prevalence rates of these two viruses in sick cats in South Africa is in line with prevalences encountered in the rest of the world, with the FIV prevalence rating amongst the highest in the world, closely resembling that found in two separate studies of cats in Australia and Italy and only slightly higher than rates from the UK and France. The prevalence of FIV is approximately twice that of FeLV in sick cats in South Africa. Although early literature suggested that each FIV subtype was limited in its geographical distribution, the data presented here add to growing evidence that many FIV subtypes are widely distributed around the world. This study thus provides supportive data that the introduction of the FIV vaccine containing subtype A, should protect the majority of cats in South Africa.