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Oral Platform Presentations

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HEMOSTATIC ABNORMALITIES AND DISSEMINATED INTRAVASCULAR COAGULATION ARE COMMON IN CANINE HEAT STROKE AND ARE RISK FACTORS FOR MORTALITY. **I. Aroch, E. Klement, J. Saragusty, E. Finkelstein, Y. Bruchim.** School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel.

Canine heat stroke (CHS) is a severe illness characterized by core temperatures $>41^{\circ}\text{C}$, central nervous system dysfunction and a systemic inflammatory response syndrome leading to multiorgan dysfunction. It results from exposure to a hot and humid environment or to strenuous physical exercise. The aims of this study were to record the hemostatic abnormalities in CHS, and to assess their association with mortality. The medical records of 54 dogs presented to the Hebrew University Veterinary Teaching Hospital (1999–2004) and diagnosed with CHS were retrospectively reviewed. The data included the history, clinical and clinicopathological signs upon admission, treatment and outcome. Statistical analysis included Fisher's Exact, chi square and Mantel tests for dichotomous variables, statistical significance assessment of independence between nominal variables consisting of more than 2 categories and of linear trend association, respectively and ROC analysis. Disseminated intravascular coagulation (DIC) was diagnosed if dogs had thrombocytopenia as well as 2 of the following: prolongation ($>25\%$) of the prothrombin time (PT) or activated partial thromboplastin time (aPTT) and clinical signs compatible with DIC (i.e., bleeding). Thrombocytopenia was recorded in 31/50 dogs and 45/54 dogs upon admission and during hospitalization, respectively, but was not a risk factor for mortality, while prolongation of the prothrombin (PT) and activated thromboplastin (aPTT) times was recorded in 27/46 dogs. DIC was diagnosed in 28/54 of the cases and was a significant ($P=0.013$) risk factor for mortality. The mortality in dogs with concurrent DIC and acute

renal failure (ARF) was significantly ($P=0.001$) higher compared with dogs without either DIC or ARF (91.7% vs. 29%, respectively). The overall mortality rate was 50%. Using ROC analysis of the PT and aPTT ranges, cutoff points were set at 18 and 30 sec, respectively. Dogs with prolonged PT (>18 sec) and aPTT (>30 sec) had a significantly ($P=0.05$, and $P < 0.001$, respectively) higher mortality. Mean PT and aPTT (logarithmically transformed) in the non-survivors were significantly ($P=0.004$ and $P < 0.001$, respectively) longer compared to the survivors (mean 14.8 vs. 10.2 sec and 28.1 vs. 16.5 sec, respectively). Necropsy (6 dogs) revealed diffuse hemorrhage in serosal surfaces and skeletal muscles, multiple petechiae and diffuse gastrointestinal bleeding (5/6) and cerebral hemorrhage (1/6). Canine HS is a life-threatening condition, resulting in serious hemostatic complications and high mortality despite appropriate and aggressive treatment.

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INITIAL EVALUATION OF TOTAL BLOOD CELL COUNTS IN A COMPARATIVE CLINICAL STUDY OF SEVEN POINT-OF-CARE AUTOMATED HAEMATOLOGY SYSTEMS. **M. Becker^{1,2}, A. Moritz¹, U. Giger^{2,3}.** ¹Small Animal Clinic, Justus-Liebig-University, Giessen, Germany; ²Clinical Diagnostic Solutions, Inc, Fort Lauderdale, Florida, USA; ³Section of Medical Genetics, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

A complete blood cell count (CBC) has become an integral part of the routine clinical assessment of companion animals. While in the past most complete blood cell counts have been performed by clinical pathology laboratories, various automated haematology instruments have recently become available for use in clinical practice. The technologies and breadth of measured parameters vary greatly, and the usefulness and accuracy of the different point-of-care and laboratory instruments have not been compared among the different instruments and against reference methods. The purpose of this clinical field study was to evaluate 7 point-of-care and 2 laboratory haematology instruments, and initial comparative results of the total cell counts are presented here. Over a 3-month period EDTA blood samples from healthy and diseased dogs

(n=265) and cats (n=111) from 3 veterinary clinics in South Florida were analysed within 4-6hrs after collection (Advia <24hrs). Two laboratory systems (Advia 120 and CellDyn 3500), 1 laser-based (LaserCyte) and 1 laser- and impedance-based (ForCyte) system providing CBCs with a 5-part white blood cell (WBC) differential, platelet and reticulocyte count and 4 impedance-based systems providing CBCs, platelets and a 5-part (MS4-5) or 3-part WBC differential (CBC-Diff, VetScan HMT, ABC) and one buffy coat analyser with a 2-part WBC differential (QBC VetAutoread) were tested; PCVs were also measured. In comparison with the Advia 120, excellent correlations for RBC counts (correlation coefficient $r \geq 0.95$) and haemoglobin concentrations ($r \geq 0.97$) were observed with all instruments, and the r for MCV ranged from 0.77 to 0.94 depending on the system. Comparing the PCV to the hematocrit the r ranged from 0.92 (ForCyte) to 0.99 (QBC VetAutoread). Excellent correlations between laboratory and in-house systems were also found for the total WBC count ($r=0.93$ to 0.99). The correlation for the platelet count between the 2 laboratory methods was good ($r=0.84$) in dogs and fair in cats ($r=0.76$), while the correlations for the platelet count between the Advia 120 and the point-of-care systems were fair to good for both cats ($r=0.68$ [VetScan HMT] to 0.86 [LaserCyte]) and dogs ($r=0.78$ [VetScan HMT] to 0.92 [ABC]). The correlation of the absolute reticulocyte counts between the Advia and LaserCyte was also good for dogs ($r=0.79$) as well as cats ($r=0.86$). The total blood cell counts obtained by the various point-of-care instruments compared satisfactorily with the 2 laboratory methods and thus appear useful for clinical practice. In addition, the laser-based instruments (LaserCyte) can offer more valuable information (an absolute reticulocyte count) to classify anaemic patients.

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COMPARISON OF MICROALBUMINURIA, URINE PROTEIN: CREATININE RATIO AND URINE ELECTROPHORESIS IN DOGS. **N.H. Bexfield, A.G. Jakins, M.E. Herrtage, J. Archer.** Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge, Cambridge, UK.

Microalbuminuria (MALB) is defined as a urinary concentration of albumin greater than normal, but below the limit of detection of conventional urine dipstick methodology. In humans, MALB is associated with a variety of diseases, and can be an early predictor of proteinuria and renal disease. MALB has recently gained attention as a possible early predictor of nephropathy in veterinary medicine. No published data exist comparing MALB with more conventional methods of urinary protein measurement such as urine protein: creatinine ratio (UPC) or urine electrophoresis. A point-of-care semi-quantitative immunoassay, using an antibody specific against canine albumin, has recently become available for detection of MALB in dogs (E.R.D. Healthscreen; Heska Corporation). The aim of this study was to compare urinary protein concentrations recorded by two conventional methods to the results obtained using the MALB immunoassay. Voided urine samples were collected from healthy dogs (n=26) or clinically ill dogs (n=95) presented to the Queen's Veterinary School Hospital over a 7-month period. All samples were tested for MALB according to the manufacturer's instructions. In addition, measurement of UPC and electrophoresis (to determine urinary albumin concentration) was performed on each sample and compared with the MALB result by group (MALB negative, low positive, medium positive, high positive, very high positive). There was a trend for UPC and albumin to increase as MALB increased although a wide range of values was obtained in each group. UPC by group (median [range]): MALB negative 0.13 [0.07-0.28]; MALB low 0.20 [0.11-0.44]; MALB medium 0.39 [0.18-0.97]; MALB high 1.28 [0.30-3.85]; MALB very high 3.68 [1.06-23.9]. Albumin g/l by group (median [range]): MALB negative 0.02 [0.0-0.26]; MALB low 0.02 [0.01-0.12]; MALB medium 0.16 [0.05-0.47];

MALB high 0.48 [0.32-2.26]; MALB very high 1.25 [0.47-2.88]. The majority of healthy dogs had MALB results in the negative category (n=21 of 26), compared with unhealthy dogs that mainly fell into the medium category (n=49 of 95). It was not possible to predict which MALB category results would fall into solely by examining results of UPC or urine albumin. Some of the discrepancies in results obtained with different methods might be due to the semi-quantitative subjective nature of the MALB test, which relies on operator assessment of colour change, in comparison with the quantitative UPC and urine albumin tests. Further studies are required to determine the significance of MALB in canine patients suffering from different conditions.

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HEMATOLOGICAL PARAMETERS IN YOUNG, PRE-TRAINING HEALTHY GREYHOUNDS. **R.E. Shiel¹, S.F. Brennan¹, M. McCullough², C.T. Mooney¹.** Departments of ¹Small Animal Clinical Studies and ²Veterinary Pathology, University College Dublin, Ireland.

Canine hematological reference intervals are rarely breed- or age-specific although these can markedly influence results. For instance, it is widely recognized that adult greyhounds exhibit higher hematocrit and hemoglobin concentrations and lower neutrophil, monocyte, lymphocyte and platelet counts compared with the general canine population. However, as a breed, greyhounds represent a heterogeneous group, and the influence of life-stage and lifestyle on hematological parameters has not been fully evaluated. The aim of this study was to assess hematological findings in young, healthy pre-training greyhounds, and to assess the effect of age and sex on these findings. Jugular blood samples were collected from 45 healthy pre-training greyhounds, aged between 5 and 13 months. Twenty seven of the dogs were male, and 18 were female. Samples were analysed using an Abbott CELL-DYN 3500R system. The mean and median were calculated for each hematological parameter, and reference limits produced based on the 5th and 95th percentiles. A Mann Whitney U test was used to assess differences between males and females. Spearman's rank correlation was performed to assess the relationship between age and each individual hematological parameter. All results are expressed as median and reference limits. The greyhounds had higher hematocrit (0.58, 0.45-0.66 L/L) and hemoglobin concentration (190, 145-223 g/L) limits compared with the standard laboratory limits (0.37-0.55 L/L and 120-180 g/L, respectively). Total white blood cell (8.4, 5.4-12.2 $\times 10^9$ /L), neutrophil (5.61, 3.9-8.8 $\times 10^9$ /L), lymphocyte (1.39, 0.89-2.39 $\times 10^9$ /L) and platelet (104, 129-267 $\times 10^9$ /L) count limits were lower than our standard laboratory reference limits (6-17, 3-11.5, 1-4.8 and 200-500 $\times 10^9$ /L, respectively). No sex-related differences were found. Hematocrit had a positive correlation with age ($r=0.7556$, $p < 0.0001$), and total white blood cell ($r=-0.33$, $P < 0.05$), lymphocyte ($r=-0.36$, $P < 0.05$) and platelet ($r=-0.34$, $P < 0.05$) counts were all negatively correlated with age. These results confirm that significant differences exist in breed-specific reference limits. However, these differences were less marked in young healthy greyhounds compared with those reported for older greyhounds. Intra- as well as interbreed variation must be considered when interpreting hematological profiles of greyhounds.

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RETROSPECTIVE REVIEW OF ABNORMAL FELINE SERUM PROTEIN ELECTROPHORESIS RESULTS. **S.W. Tappin, S.J. Dodkin, K. Papasouliotis, S. Tasker.** School of Clinical Veterinary Science, University of Bristol, Bristol, UK.

Serum protein electrophoresis (SPE) is performed to investigate protein abnormalities in feline patients. Although dysproteinemias

are reported to occur in reviews of specific diseases and individual case reports, to the authors' knowledge, there are no currently published general reviews of abnormal SPE results in the cat. The aim of this study was to describe the abnormal changes found on SPE in a population of referral cats in the UK and to relate these changes to the underlying diagnosis. Between January 2000 and September 2004 SPE was performed in 119 cats as part of their diagnostic investigations at the University of Bristol Feline Centre. SPE was performed on cellulose acetate gel and read by a single operator (S.J.D.) using a densitometer. These were compared with previously reported values for normal healthy cats (Paltrinieri *et al*, 2001, mean \pm 2 SD). Of these 119 cats, 36 had abnormal SPE values. Information was collated from the case records of these 36 cats. Common presenting signs were weight loss (11/36), anorexia/inappetance (8/36) and lethargy (7/36). The most common clinical examination findings were pyrexia (7/36), lymphadenopathy (4/36) and dyspnoea (3/36). Twenty-four cats had elevated gamma globulins (48.6 ± 9.5 g/l [mean \pm SD]; reference limits 7.4–34.6 g/l). Twelve of these cats were diagnosed with feline infectious peritonitis (FIP) (7 confirmed by histopathology, 5 with a strong clinical suspicion based on clinical findings and laboratory data such as ascites [5/5], lymphopenia [5/5], hyperglobulinaemia [5/5] and non-regenerative anemia [4/5]). Three cats had lymphoma (1 alimentary, 1 hepatic and 1 multicentric) and one cat had each of the following diagnoses: splenic plasmacytoma, feline asthma, diffuse bronchial neoplasia, cerebellar infarction, and lymphoblastic leukaemia. There were 4 cats in which diagnoses were not reached. Of the cats with elevated gamma globulins, three cats had a monoclonal pattern. These were the cat with alimentary lymphoma, the splenic plasmacytoma case and one cat in which a final diagnosis was not reached. Eleven cats had decreased gamma globulins (5.8 ± 1.5 g/l; range 7.4–34.6 g/l). One cat had each of the following diagnoses: hepatic lipidosis, idiopathic chylothorax, portosystemic shunt, acute renal failure, dilated cardiomyopathy, FIP, calicivirus infection, and nephrogenic diabetes insipidus. No final diagnosis was made in 3 cats. One cat had increased α -2 globulins (14.26 g/l; reference limits 0.8–12 g/l) and was diagnosed with pyogranulomatous pneumonia. Dysproteinemias occur in a wide variety of disease processes. The most frequent abnormality seen in this study was raised gamma globulins, which was most commonly associated with FIP and lymphoma.

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ANALYTICAL GOALS FOR THROMBOELASTOGRAPHY PARAMETERS BASED ON ANALYSIS OF BIOLOGICAL VARIATION. B. Wiinberg¹, M. Kjelgaard-Hansen¹, A.L. Jensen¹, R. Røjkjær², P. Johansson³, L.P. Gade², A.T. Kristensen¹. ¹Small Animal Clinical Sciences, Royal Veterinary and Agricultural University, Copenhagen, Denmark; ²Novo Nordisk A/S; and ³Copenhagen University Hospital.

Thromboelastography (TEG) enables global assessment of hemostatic function in whole blood with evaluation of both plasma and cellular components and it may be a valuable supplement to the traditional coagulation parameters currently used in most clinical pathology laboratories. Objective standards for imprecision, inaccuracy and applicability of population-based reference limits for the hemostasis parameters can be assessed on the basis of observations on biological variation. The objective of the study was to investigate the biological variation of four parameters from TEG on citrated canine plasma from clinically healthy dogs to 1) assess the utility of conventional population-based reference limits and 2) set objective analytical performance standards for each of the four TEG parameters: reaction time (R), angle (α), split point (SP), and maximum amplitude (MA). Samples were collected after a set protocol once weekly for 5 consecutive weeks from 8 healthy dogs (4 males and 4 females). Plasma was collected and stored at -80°C

until analysis. Randomized duplicate TEG analyses with Tissue-Factor as activator at a concentration of 1:50,000 were performed on all plasma samples within one day. The data were analyzed for outliers ($p < 0.01$) and subsequently subjected to nested analysis of variance to obtain the coefficient of analytical, intra-individual and inter-individual variation (CV_a , CV_i and CV_g , respectively). From these, the objective analytical performance standard for imprecision ($CV_{max} = \frac{1}{2}CV_a$) and the index of individuality ($R_i = [CV_a^2 + CV_i^2]^{1/2} / CV_g$) were calculated to assess the utility of conventional population-based reference limits (applicable when $R_i > 1.4$). No outliers were detected. The observed CV_a ; CV_{max} were: R=6.7%; 10.4%, SP=6.1%;9.1%, α =8.4%;6.6% and MA = 7.9%;9.3%. CV_g were: R=7.2%, SP=6.9%, α =10.4% and MA=7.8%. CV_i were: R=20.9%, SP=18.3%, α =13.2% and MA=18.6%. The calculated R_i were: R: 3.03, SP: 2.77, α : 1.50 and MA: 2.6. In conclusion, all 4 measured parameters met the objective analytical performance standard for imprecision except α , for which analysis in duplicate could be necessary. The observed degrees of individuality would make the use of conventional population-based reference limits a sensitive interpretation criterion for the clinical use of TEG.

Poster Presentations

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BICLONAL GAMMOPATHY IN A DOG AND A CAT WITH PLASMA CELL NEOPLASIA. W. Bertazzolo¹, D. Zuliani², U. Bonfanti³, G. Avallone⁴, P. Roccabianca⁴. ¹Pronto Soccorso Veterinario, Lodi, Italy; ²Clinica Veterinaria Tibaldi, Milano, Italy; ³Clinica Veterinaria Gran Sasso, Milano, Italy; and ⁴Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Sezione di Anatomia Patologica e Patologia Aviare, Università di Milano, Italy.

Biclonal gammopathy is a rare plasma protein dyscrasia usually caused by multiple myeloma and extramedullary plasmacytoma. To date, only 4 cases (3 dogs and 1 cat) have been described in the veterinary literature. Case 1: a 15-year-old, spayed female, domestic shorthaired cat was presented because of anorexia, depression and weight loss. Splenomegaly and abnormal splenic and hepatic echogenicity were found at clinical examination and by diagnostic imaging. CBC, serum biochemical profile and urinalysis showed moderate anemia and thrombocytopenia, mild azotemia, moderate hyperproteinemia with mild hypoalbuminemia and marked hyperglobulinemia. Urine SDS-PAGE for qualitative evaluation of proteinuria showed a 26–28,000D band consistent with tubular proteinuria or Bence-Jones proteinuria. Capillary electrophoresis showed a biclonal gammopathy with both spikes in the γ -globulin region. Agar gel diffusion precipitation assay using antisera specific for feline μ , α and γ heavy chains showed precipitin lines only against γ heavy chains, suggesting that both paraproteins were of the IgG class. Splenic and hepatic ultrasound-guided fine-needle aspirates showed a pleomorphic population of atypical plasmacytoid cells. Atypical plasma cells were less than 3% of all nucleated cells on bone marrow examination. Immunocytochemistry showed marked positivity for CD79a and IgG and mild positivity for λ light chains. To our knowledge, this is the first described case of a cat with biclonal gammopathy of the IgG class associated with extramedullary hepato-splenic plasma cell neoplasia. Case 2: a 9-year-old, spayed female, Rottweiler dog was referred because of depression and polyuria/polydipsia. Pale mucous membranes and hepatosplenomegaly were found on clinical examination. Blood work showed hypercalcemia, moderate hyperproteinemia with mild hypoalbuminemia and marked hyperglobulinemia. Marked mixed

glomerular and tubular proteinuria was found on urinalysis. Capillary electrophoresis showed a biclonal gammopathy with two spikes, one in the β -globulin and one in γ -globulin region. Agar gel diffusion precipitation assay showed precipitin lines against α and γ heavy chains, suggesting a mixed IgG and IgA paraproteinemia. Splenic, hepatic, lymph nodal and bone marrow cytologic examination revealed infiltration with atypical plasma cells, suggesting a final diagnosis of multiple myeloma.

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CYTOLOGIC FINDINGS IN 11 CASES OF CANINE ABDOMINAL EFFUSION ASSOCIATED WITH OVARIAN TUMOURS. **U. Bonfanti¹, W. Bertazzolo², C. Masserdotti³, D. De Lorenzi⁴, S. Ferro⁵.** ¹Clinica Veterinaria Gran Sasso, Milan, Italy; ²Pronto Soccorso Veterinario, Lodi, Italy; ³Laboratorio Biodiversity, Brescia, Italy; ⁴Clinica Veterinaria San Marco, Padua, Italy; and ⁵Department of Public Health, Comparative Pathology and Veterinary Hygiene, University of Padua, Italy.

Ovarian tumours are uncommon in dogs, probably due to spaying often performed at an early age. Primary tumours of the ovary are divided into four main types: sex cord-stromal (gonadostromal) tumors (granulosa cell tumor, thecoma, luteoma), germ cell tumors (dysgerminoma, teratoma, embryonal carcinoma), epithelial tumors (papillary adenoma, papillary adenocarcinoma, rete adenoma) and mesenchymal tumors. A total of 11 dogs with abdominal effusion associated with a histologically confirmed ovarian neoplasm were included in the study: 8 were associated with a papillary adenocarcinoma, 2 with a granulosa cell tumour, and 1 with a mixed gonadostromal tumour (granulosa cell tumour and luteoma). Neoplastic cells were observed in 7 out of 8 effusions from dogs with papillary adenocarcinomas. Cytology of the effusions revealed high cellularity and large tridimensional clusters of cells either arranged in a branching pattern or in tightly rounded clusters. Small aggregates of cells with microacinar arrangement were also evident. Tightly cohesive, roundish, oval or cuboidal cells with moderate anisocytosis and anisokaryosis, variable nuclear atypia, and scant to moderate amounts of sometimes vacuolated grey cytoplasm were present. The 2 granulosa cell tumours were associated with serohemorrhagic effusion containing erythrocytes, non-degenerate neutrophils, macrophages and mesothelial cells; neoplastic cells were not detected. The mixed gonadostromal tumour caused a serosanguineous effusion containing neoplastic cells. Smears of the effusion were characterized by high cellularity, aggregates of moderately cohesive cells with intercellular eosinophilic substance, dysmetric nuclei, prominent and multiple nucleoli and large clear, often micro- and macrovacuolated cytoplasm. Moreover, many vacuolated macrophages were present in the background. In conclusion, ovarian papillary adenocarcinomas were frequently associated with a carcinomatous effusion. In fact papillary adenocarcinoma often causes widespread peritoneal implantation and a consequent malignant cell effusion. Sex cord stromal tumours are uncommonly associated with abdominal effusions despite a prevalence similar to epithelial neoplasms. In this study we describe an unusual case of a malignant cell effusion caused by a gonadostromal ovarian tumour.

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VALIDATION OF A HUMAN IMMUNOTURBIDIMETRIC ASSAY FOR MEASURING CANINE C-REACTIVE PROTEIN. **F. Gentilini¹, D. Mancini¹, F. Dondi¹, L. Ingra¹, M.E. Turba², M. Forni², P. Famigli Bergamini¹.** ¹Veterinary Clinical Department and ² Department of Veterinary Morphophysiology and Animal Production, University of Bologna, Italy.

C-reactive protein (CRP) is an extremely powerful prognostic marker for a wide variety of diseases. In human medicine immunoturbidimetric assays (ITAs) are replacing the ELISA test due to their high degree of accuracy, high-throughput and low turnaround time. Until now, a canine-specific ELISA test has represented the gold standard in veterinary medicine for measuring serum CRP. Even though there are contrasting data concerning the cross-reactivity of human and canine CRP, a previous study has nevertheless demonstrated that a commercially available human ITA is reliable for measuring canine CRP without method modification. Since many automated analyzers are furnished with proprietary standardized reagents, the aim of this study was to verify whether a commercially available human ITA (Olympus system reagent, CRP ITA, Olympus, Italy) different from the previous validated assay is also reliable for accurately measuring canine CRP. The CRP concentrations of 35 canine sera collected from either healthy dogs or dogs with infectious and neoplastic diseases were assayed by ELISA and ITA on an automated chemistry analyzer (Olympus AU 400). The ELISA test was carried out following the manufacturer's instructions while the ITA measurement was initially performed using the settings and calibration of the human assay. The results, which were compared using Passing and Bablok regression analysis, showed that the ITA underestimated canine CRP both with an absolute and a proportional inaccuracy. To overcome this pitfall, the ITA was repeated after calibration of the automated analyzer with a standard specimen obtained by pooling 3 canine sera with high CRP concentrations. The standard was assayed 6 times in the same run using the ELISA test. The CV was 5.8% and the mean concentration of the 6 replicates was assumed to be the actual concentration of the standard. The modified assay (ITA-c) was used to evaluate the same 35 serum samples in duplicate. The results were again compared with the ELISA test by Passing and Bablok regression analysis which showed a good correlation ($y=1.18x - 0.69$). Moreover, two samples with 23.7 mg/dl and 21.7 mg/dl of CRP were serially diluted and assayed in duplicate. The ITA-c was linear in the verified range ($y=0.9988x + 0.2$ and $y=0.9982x + 0.07$, respectively). In conclusion, this study confirms that any commercially available human CRP ITA should be validated before its routine application in dogs. Moreover, the partial cross-reactivity between human and canine CRP allowed us to reliably measure canine CRP after calibration of the ITA with a standard pooled sample having a known concentration of canine CRP.

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RELATION BETWEEN *p53* GENE MUTATION OF TUMOR CELLS AND CLINICAL RESPONSE TO ANTINEOPLASTIC AGENTS IN DOGS WITH LYMPHOMA. **A. Koshino, Y. Kishida, I. Yamana, A. Setoguchi, Y. Fujino, K. Ohno, H. Tsujimoto.** Veterinary Medical Center, The University of Tokyo, Tokyo, Japan.

Development of drug resistance is a major obstacle limiting the survival time in dogs with lymphoma treated with chemotherapy. In human patients with non-Hodgkin's lymphoma, mutation of the *p53* gene was shown to be a negative prognostic factor because of the inhibition of antineoplastic drug-induced apoptosis. We and others previously reported mutations of *p53* gene in canine lymphomas (Veldhoen et al., 1998; Setoguchi et al., 2001). The aim of the present study was to examine the relationship between *p53* mutation of the tumor cells and clinical response to the commonly used antineoplastic agents in dogs with lymphoma. Thirty dogs with lymphoma were included in this study. Mutation of *p53* gene (exon 4-8) in the chromosomal DNA of tumor tissues/cells was examined by PCR-SSCP (polymerase chain reaction - single strand conformational polymorphisms) analysis, followed by nucleotide sequencing. Response to each antineoplastic agent was evaluated by the size of the lymph nodes 1-2 weeks after administration of the

drugs, at the first remission induction chemotherapy, or at rescue chemotherapy. Of the lymphoma tissues/cells, 12 had mutation of the *p53* gene, however, 18 were devoid of the *p53* gene mutation. The overall response rates (complete response + partial response) to vincristine, doxorubicin, and L-asparaginase were 25%, 57%, and 45%, respectively, in dogs with lymphoma having the *p53* mutation, whereas they were 68%, 80%, and 65%, respectively, in dogs with lymphoma devoid of the *p53* gene mutation. By χ^2 analysis, the overall response rate to vincristine was significantly lower in dogs with lymphoma having the *p53* mutation than in dogs without the *p53* gene mutation ($P=0.014$). In contrast, there was no significant difference in the response rates to L-asparaginase and doxorubicin between the dogs with lymphoma having the *p53* mutation and those with lymphoma without the *p53* gene mutation. In conclusion, *p53* gene mutation analysis of tumor cells can be useful for selecting antineoplastic agents for the chemotherapy of canine lymphoma.

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CHLAMYDOPHILA FELIS INFECTION - CLINICAL FEATURES IN FIVE CATS. D. Pavlin¹, A. Dovč², S. Suhadolc¹, N. Tozon¹.
¹Small Animal Clinic and ²Institute for Poultry Health, Veterinary Faculty, University of Ljubljana, Slovenia.

Infection with *Chlamydomphila felis*, formerly known as *Chlamydia psittaci*, in cats is primarily associated with conjunctivitis in infected animals. Signs of upper respiratory tract disease, especially in adult animals, are believed to be less common. In this report, clinical signs of severe upper and lower respiratory tract disease in 5 clinical cases are presented. In all 5 animals routine physical examination, CBC and white blood cell differential count were performed. In 2 cats with severe systemic disease, chest radiographs were made. Based on results of rapid ELISA test (IDEXX, Westbrook, Maine, USA – Feline Leukemia Virus Antigen/Feline Immunodeficiency Virus Antibody Test Kit[®]), all patients were free of Feline Leukemia Virus (FeLV) and specific antibodies against Feline Immunodeficiency Virus (FIV). *Chlamydomphila felis* positivity was evaluated in oropharyngeal swabs using a rapid EIA (Clearview Chlamydia MF, Unipath Limited[®]) and a direct immunofluorescence method (Chlamydia Direct IF, Biomerieux[®]). All 5 cats were positive by both methods. For detection of specific antibodies against *Chlamydomphila felis* in feline sera an indirect immunofluorescence test (Feline Chlamydia IgG IFA KIT, Fuller Laboratories[®]) was used. Four of 5 cats had specific IgG antibodies (titre 1:40 to 1:320). Three cats, 1 young (5 months old) and 2 adult cats (6 and 13 years old) from multi-cat households were presented with signs of upper respiratory disease, without signs of systemic illness. Both adult cats had intermittent recurrent nasal discharge for the past 2–3 years. Clinical signs in the youngest cat (coughing and nasal discharge) had a more acute onset. Only the oldest cat had previously been treated for these problems and had responded well to inhalation oxytetracycline. All 3 patients had an unremarkable CBC. Another 2 young cats were presented with severe systemic disease and clinical signs attributable to lower respiratory tract involvement. The first patient was a 6-month-old fully vaccinated mainly indoor cat with dyspnea. The CBC was within reference limits. Chest radiographs revealed extensive bilateral alveolar infiltrations. The second patient was a 7-month-old outdoor non-vaccinated cat presented in a febrile state with a cough of 1 month duration and conjunctivitis. This patient had an elevated WBC count ($27 \times 10^9/L$) and radiographic changes showing generalized interstitial infiltration of the lung. All patients were successfully treated with doxycycline (5 mg/kg b.w./day for 3 weeks). They showed rapid clinical improvement shortly after therapy and remained free of clinical signs of respiratory infection for the next five months. Based on these results, we conclude that *C. felis* can be considered a primary or secondary pathogen, which can potentially cause severe respiratory tract infections in young and adult cats.

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ASSESSMENT OF A NEW PORTABLE BLOOD GLUCOSE METER FOR MEASURING BLOOD GLUCOSE CONCENTRATION IN DOGS. M.I. Rodríguez¹, L. Espino¹, J. Marey¹, G. Santamarina¹, C. Carrera², L.F. de la Cruz³.
¹Depto. de Ciencias Clínicas Veterinarias, ²Hospital Clínico Veterinario Rof Codina, and ³Depto. de Fisiología, Facultad de Veterinaria de Lugo, Spain.

Portable blood glucose meters (PBGM) are readily available and inexpensive and can rapidly provide results from small quantities of blood and many veterinarians have used them to measure blood glucose concentration in dogs. **Objective:** The purpose of the study reported here was to compare glucose concentrations in blood samples from dogs using a reference method and a new commercially available portable blood glucose meter, Ascensia[®] Confirm[™] (Bayer). In addition, we wanted to compare concentrations using plasma obtained after centrifugation of blood anticoagulated with lithium heparin. **Experimental Protocol:** The experiment was carried out in two phases. First, we compared blood glucose concentrations obtained with the PBGM using fresh blood with concentrations obtained using the reference method (91 blood samples from different dogs). Then we compared blood glucose concentrations obtained with the PBGM using plasma (lithium heparin) with the results of the reference method (91 blood samples from different dogs). The blood was obtained from the cephalic vein, and in the case of anticoagulated blood was immediately placed in tubes containing lithium heparin, and these samples were assayed within 10 minutes after collection. In the case of fresh blood, the needle was placed in the cephalic vein and the blood was automatically collected onto the test strip. The PBGM was operated according to the manufacturer's directions. **Data Analysis:** Linear regression models of values obtained with the PBGM versus the reference method values were constructed. A general linearity test was used to determine whether the slope of the regression line was different from 1 and the intercept was different from 0. Values of $P < 0.05$ were considered significant. The correlation between glucose concentrations obtained by the PBGM and the reference method was determined using the Pearson correlation coefficient. **Results:** When linear regression models of results were examined, the null hypothesis that the slope of the regression line equaled 1 and the intercept equaled 0 was rejected. The Pearson correlation coefficient indicated that there was no correlation between the methods. **Discussion:** The PBGM used in this study is small and portable, requires use of only small quantities of blood, and provides results rapidly. However, the results obtained with this device do not provide accurate information with either fresh blood or plasma. We conclude that this device should not be used in veterinary medicine because the results obtained will often dictate the course of clinical care.

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CANINE BABESIOSIS IN SLOVENIA: CLINICAL FINDINGS IN DOGS INFECTED WITH BABESIA CANIS CANIS AND BABESIA CANIS VOGELI. N. Tozon¹, D. Duh², M. Pettrovec², K. Strasek², T. Avsic-Zupanc².
¹Small Animal Clinic, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia; and ²Institute of Microbiology and Immunology, Medical Faculty, Ljubljana, Slovenia.

A retrospective study of clinical cases of babesiosis in dogs examined at the Small Animal Clinic Veterinary Faculty in Ljubljana between 2000 and 2002 was conducted. Clinical manifestations, hematological alterations, and biochemical and urine parameters were analysed from clinical records of dogs with laboratory-confirmed *Babesia canis* infections. Fourteen of 238 (5.9%) evaluated dogs were infected with *Babesia* as detected by PCR and light microscopy. Two subspecies of *B. canis* were determined by

sequencing analysis, namely *B. canis canis* and *B. canis vogeli*. The prevalence rate of infection differed between subspecies: 4.6 % (11/238) for *B. canis canis* and 1.3 % (3/238) for *B. canis vogeli*. Clinical features were similar in all observed cases: 9/13 dogs had anorexia, 8/13 were depressed and 4/13 had fever. Vomiting, polydipsia, pale mucous membranes and chronic cough were noted in individual cases. In all cases specific antibodies for *Anaplasma phagocytophila* were found, however, *Ehrlichia* infection could not be determined by PCR. Observed hematological alterations were anemia (6/9), thrombocytopenia (9/9), leukocytosis (1/8), leukopenia (1/8) and lymphocytosis (3/7). One month later using the same treatment in all cases (Imidocarb dipropionate 3 mg/kg b.w. bid every 14 days),

improvements in hematological parameters were noted as follows: anemia (2/5), thrombocytopenia (4/5), lymphocytosis (3/4) and lymphopenia (1/4). The threat of canine babesiosis due to infection with *B. canis canis* and *B. canis vogeli* exists in Slovenia. Furthermore, coinfection with *Babesia* and *Ehrlichia* is suggested by the presence of specific antibodies for *A. phagocytophila* in dogs infected with *B. canis*. The clinical features and hematological, biochemical, and urinalysis alterations caused by these tick-transmitted agents are not specific. Therefore, distinguishing between them is very difficult and the use of molecular diagnostic methods is important. Moreover, treatment should be based on the diagnostic profile and the epidemiologic prevalence of subspecies of *Babesia*.