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## ABSTRACTS

# European Society for Veterinary Clinical Pathology (ESVCP) 2<sup>nd</sup> Annual Scientific Meeting

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**1**  
SERUM ADENOSINE DEAMINASE ACTIVITY IN DOGS: IT'S IMPORTANCE IN EXPERIMENTAL LIVER TOXICITY. N. Altug and Z.T. Agaoglu. Department of Internal Diseases, Faculty of Veterinary Medicine, University of Yuzuncu Yil, Van, Turkey.

Adenosine deaminase (ADA) activity was investigated together with clinical, biochemical, hematological, and histopathological findings in dogs experimentally intoxicated with CCl<sub>4</sub> to determine ADA's role in liver toxicity. In this study, 20 healthy crossbreed dogs were divided into 2 groups of 10 each (acute and chronic toxicity). CCl<sub>4</sub> was prepared in olive oil (1:1). Dogs in the acute toxicity group received 1.5 mL/kg CCl<sub>4</sub> only once. Dogs in the chronic toxicity group received 0.5 mL/kg CCl<sub>4</sub> twice a week for 12 weeks. The CCl<sub>4</sub> was administered by orogastric probe after 12 hours of fasting. Clinical, hematological and biochemical analyses were done in all dogs 2 days before the experiment; on days 1, 3, 5, 7, and 9 in the acute toxicity group; and 4 times at 21-day intervals in the chronic toxicity group. Liver biopsies were taken on days 4 and 9 (acute toxicity group), or at weeks 6 and 12 (chronic toxicity group). During the experiment, inappetance, lethargy, watery defecation, and mild dehydration were observed in acute toxicity dogs. Inappetance, weakness, light colored feces, dark orange urine, and depression were observed in dogs in the chronic toxicity group. Erythrocyte and hemoglobin levels increased significantly ( $P < .05$ ) on day 3 in acute toxicity dogs. Total leukocyte numbers increased significantly ( $P < .001$ ) in chronic toxicity dogs. Significant increases ( $P < .001$ ) were also observed in serum AST, ALT, ALP, and ADA activities in both groups of dogs. Histopathologically, centrilobular and midzonal regions showed hepatocellular hydropic degeneration, necrosis and dissociation of hepatic cords in acutely intoxicated dogs. In the chronic toxicity group, hepatic fibrosis was observed in portal regions and around central veins. As a result of these findings, we conclude that serum ADA activity may be useful in the assessment of liver degeneration caused by toxic hepatopathy, and in the differentiation of acute and chronic liver disease. ADA activity can be added to other routine biochemical tests used in the diagnosis of liver disease.

**2**  
CHANGES IN ANTIOXIDANT PARAMETERS IN FOALS DURING THE FIRST THREE WEEKS OF LIFE. N. Balogh, T. Gaál, P. Sz. Ribiczeyné, and M. Kovács. Szent István University, Faculty of Veterinary Science, Budapest, Hungary.

This study was undertaken to describe antioxidant system changes in horses during the first 3 weeks of life. Spectrophotometric measurement of malondialdehyde (MDA) concentration, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities of red blood cells (RBC), and ferric reducing ability of plasma (FRAP) and total antioxidant status (TAS) of plasma were completed. Blood samples were taken from mares ( $n = 17$ ) immediately after foaling and from foals ( $n = 17$ ) at the following times: after birth, and at 2, 3, 7, 14, and 21 days of age. Changes in MDA concentration, GSH-Px activity, and FRAP were significant over time ( $P < .05$ , 1-way ANOVA). The concentration of lipid peroxidation endproduct, MDA, was higher in foals after birth and showed a constant increase until the third day of life (mean  $\pm$  SD,  $505 \pm 47$  nmoL/g protein) followed by a return to the level found in mares ( $391 \pm 26$  nmoL/g protein). Foals had significantly lower GSH-Px activities (28-37 U/g protein) during the period of study than mares ( $44 \pm 4$  U/g protein), although a moderate but constant increase was observed. FRAP values of both the mares and the foals were lower than in other species. However the mean FRAP value in foals in the first 3 days of life ( $0.50 \pm 0.04$  mmol/L) was higher than that of mares ( $0.44 \pm 0.03$  mmol/L). Although overall changes in SOD activity were not significant, there was a tendency for changes in SOD activity to be opposite to changes in MDA concentration. There was no unequivocal pattern of change observed in plasma TAS.

## 3

THE DYNAMICS OF SOME OXIDOREDUCTASES IN SODIUM NITRATE SUBLETHAL DOSE INTAKE IN SHEEP. I. Chisu, L. Olariu, A. Trif, L. Stana, V. Curtui, and H. Sarandan. Faculty of Veterinary Medicine, Timisoara, Romania.

Nitrates and nitrites, chemical fertilizers, are present in high quantities and represent an increasingly toxic threat to the environment. The goal of this research was to complete the data concerning the activity of some blood protective enzymes (methemoglobin reductase, catalase, and glutathione reductase) in sheep with sublethal sodium nitrate intake, and to correlate enzyme activity with changes in blood levels of nitrate, hemoglobin, and methemoglobin over 24 hours. The study was carried out on 3 Turcana breed healthy rams with chronic rumen fistulas, on a diet of combined feed. The experiment started after 7 days of accommodation to the new feed, and consisted of 1 control (C) and 2 experimental ( $E_1$ ,  $E_2$ ) sheep. In  $E_1$ , sodium nitrate was administered intraruminally in a single dose representing  $1/3$  the lethal dose. In  $E_2$ , sodium nitrate was administered intraruminally in a single dose representing  $2/3$  the lethal dose. Samples of ruminal fluid were taken before feeding (0 hour), and at 1, 2, 3, 4, 5, 6, 9, 12, and 24 hours after feeding and administration of sodium nitrate. It was observed that blood nitrate levels correlated with the dosage of sodium nitrate. After a maximum at 2 hours, blood nitrate levels in  $E_1$  decreased continuously to 24 hours after administration, when values were similar to those at 0 hour. However, in  $E_2$ , nitrate values remained 6.2 times higher at 24 hours than at the start. Methemoglobin reductase activity correlated well with methemoglobin and blood nitrate values. Enzyme activity was stimulated with the administration of nitrate. Catalase and glutathione reductase activities were inversely correlated with nitrate and methemoglobin levels, and with methemoglobin reductase activity.

## 4

HEMATOLOGICAL AND BIOCHEMICAL ABNORMALITIES IN DAIRY COWS WITH CHRONIC ARSENIC POISONING: PRELIMINARY RESULTS. A. Fusari and A. Ubaldi. Istituto di Diagnostica e Tossicologia Sperimentale Veterinaria, Facoltà di Medicina Veterinaria, Parma, Italy.

To follow-up on suspected chronic poisoning of dairy cows by water arsenic content (35.8 ppm) in an artesian well, we studied the bovine metabolic status using laboratory diagnostic testing. Whole blood and serum samples were collected from 20 animals for analysis by routine laboratory methods. Atomic absorption (coupled with a graphite furnace for arsenic determination) and a colorimetric method (Mulei et al., *J Vet Med A*. 1988;35:522) were employed to determine serum and erythrocyte electrolyte concentrations. Subjects in the early lactation period showed an imbalance in erythrocyte maturation, which we attributed to the toxic action of arsenic. Moreover, during this delicate period, the concentration of methemoglobin increased to its highest value. On this basis, we can assert that the hematological abnormalities could be attributed to an increased ingestion of arsenic contaminated (but  $\text{NO}_3^-$  and  $\text{NO}_2^-$  free) water. In subsequent periods we noted a decrease in methemoglobin concentration. Total WBC counts were low in all periods of the production cycle, and were closely linked to the toxic action of arsenic compounds on lymphocytes in vitro. The mechanism of action of the arsenic compounds was likely also responsible for increased serum and decreased erythrocyte potassium concentrations, as a consequence of insult to the erythrocyte membrane. The hematological abnormalities correlated with the degree of toxicity and with recovery in the affected cows. The decrease in erythrocyte count and increased serum potassium levels reflect the insult of arsenic compounds on the integrity of erythrocyte membranes.

## 5

IMMUNO-ORGANS RESPONSE TO *ALLIUM SATIVUM* CONCENTRATED EXTRACT ADMINISTRATION IN RATS. L. Olariu, M. Sincai, I. Chisu, and L. Stana. Faculty of Veterinary Medicine, Timisoara, Romania.

The beneficial effect of *Allium sativum* is well known throughout the ages. In recent years, an important emphasis was placed on the study of the chemical composition of *Allium sativum* and its effects on animals. The purpose of our present work was to add new data about the effects of an *Allium sativum* pure extract on rat kidney, liver, and spleen. The research was carried out on 2 uniform groups, control and experimental, of 45 rats each. Control rats were administered 0.5 mL distilled water per os. The experimental group received 0.5 mL of pure *Allium sativum* extract (1.58 mg thiosulphinates/mL) IM daily for 16 days. Rats were sacrificed after 8, 14, and 16 days. Kidney, liver and spleen were removed for histologic examination. After 8 days of extract administration, lymphocytic proliferation was present in the lymphoid nodules and perivascularly in the red pulp. The volume of the lymphoid nodules was increased. Also, an increase in reactive lymphocytes was observed. After 14 days of extract administration, marked proliferation in the lymphoid nodules was observed. A high number of macrophages and lymphoplasmacytic cells was detected in the red pulp perivascular areas. Importantly, Heinz bodies also appeared (4-5/section). All of these modifications could be explained by an intensification of the immune response (the appearance of the proactive lymphocytes). In the liver, marked perinuclear and cytoplasmic hepatic vacuolation and hepatic cytolysis were seen. Dilatation of glomerular capsules and alteration of the capsular filtration barrier (which concerns either the podocytes of the visceral layer or the vascular glomerular capillary network) were observed in the kidney.

## 6

EFFECT OF HEAT STRESS DURING INCUBATION ON BLOOD PLASMA, BRAIN, AND LIVER ANTIOXIDANT PARAMETERS IN CHICK EMBRYOS. T. Gaál<sup>1</sup>, F. Husvéth<sup>2</sup>, L. Wagner<sup>2</sup>, N. Balogh<sup>1</sup>, and P. Sz. Ribiczeyné<sup>1</sup>. <sup>1</sup>Department of Internal Medicine, Szent István University, Faculty of Veterinary Science, Budapest, Hungary; and <sup>2</sup>Department of Animal Physiology, Veszprém University, Georgicon Faculty, Keszthely, Hungary.

Effect of artificial hyperthermia (39°C) during incubation was studied in ROSS broiler chick embryos and 1-day-old chicks (n = 40). Blood plasma, brain, and liver tissues were compared to those of embryos incubated at 37.5°C (controls, n = 40). Ferric reducing ability of plasma (FRAP), rate of lipid peroxidation (expressed as malondialdehyde, MDA) in brain and liver homogenates, as well as vitamin C concentration in brain and vitamin E concentration in the liver were measured. Pre- and post-hatch plasma FRAP was ~2 times higher in control chicks (range 2.0-5.0 mmol/L) than in hyperthermic ones (0.9-1.7 mmol/L). Heat stress caused a 2.5-fold increase in MDA concentration in both brain and liver tissue (control liver and brain MDA <60 µmol/g; hyperthermic group >130 µmol/g). In both groups, MDA concentration in the brain was higher than that in the liver. Heat-stress resulted in substantial loss of post-hatch brain vitamin C content (control group >300 µg/g; hyperthermic group <150 µg/g). Pre-hatch liver vitamin E levels increased in both groups, but higher post-hatch results (by some 30%) were found in the hyperthermic group. In conclusion, the FRAP method proved to be a useful tool in measurement of overall antioxidant capacity of blood plasma in chicks. Heat stress caused significant changes in chick plasma, brain, and liver antioxidant parameters. Brain proved to be more sensitive to heat stress than the liver.

## 7

PHARMACOKINETIC EVALUATION OF PRAZIQUANTEL FOLLOWING INTRAMUSCULAR AND ORAL ADMINISTRATION IN THREE-MONTH-OLD LAMBS. M. Giorgi, A.P. Salvatori, G. Soldani, and G. Mengozzi. Pharmacology and Toxicology Section, Department of Veterinary Clinics, Pisa, Italy.

Praziquantel (PZQ) is a broadly effective anthelmintic, widely employed in veterinary medicine. Several pharmacokinetic studies have been performed in both laboratory animals and humans, but few data are available in ruminants. Two groups of 3 lambs (3 months old) were treated with an intramuscular (Droncit®) or oral (Neomansonil®) dose of PZQ (15 or 30 mg/kg, respectively) according to a crossover experimental design. The last IM treatment was prolonged, at 60 mg/kg/day for 2 days. PZQ plasma concentrations were determined by HPLC according to Xiao, et al. (*J. Chromatogr.* 1983;275:127). PZQ metabolites were detected according to Hoogemann, et al. (*Arzeimittelforsch/Drug Res.* 1990;40:1159). Although the IM PZQ dosage was only one-half of that used for the oral route, the mean PZQ plasma concentration was higher after IM than after oral treatment. This low value can be explained by an intense first-pass effect. The oral treatment did not change the monooxygenase activities tested, suggesting that PZQ is well-tolerated by lamb liver; whereas, the administration of the drug at 60 mg/kg/day caused a significant decrease of 3A-dependent activity, suggesting a selective toxicity towards this subfamily. The main in vitro metabolite has been identified as PZQ 11b-OH. After selective inhibitions of 2B and 3A subfamilies, performed with chloramphenicol and troleandomycin, PZQ 11b-OH production declined by about 30% and 90%, respectively, suggesting 3A subfamily involvement in PZQ metabolism.

## 8

MEASUREMENT OF SELECTED SERUM BILE ACIDS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC) IN DOGS WITH PORTOSYSTEMIC SHUNTS: PRELIMINARY RESULTS. T. Humphrey and J. Archer. Department of Pathology and Infectious Diseases, The Royal Veterinary College, University of London, Hatfield Hertfordshire, United Kingdom.

The objective of this study was to develop a direct HPLC method for separating selected serum bile acids in dogs for monitoring the development and progression of liver disease. Current enzymatic methods for total bile acids quantitate both conjugated and unconjugated forms and may be affected by hemolysis and lipemia. Quantitation of individual bile acids is more sensitive and specific for hepatic disease in humans. In dogs, >80% of total bile acids consist of taurocholic (TC), taurodeoxycholic (TDC), and taurochenodeoxycholic (TCDC) acids. A direct HPLC method was developed and standardized for separation of TC, TDC, and TCDC in dog serum, utilizing reverse phase chromatography on a Phenomenex 5µ phenyl hexyl column (150×4.6 mm) and a Merck-Hitachi system with UV detection. Standards and sera were eluted at a flow rate of 1 mL/min using a 2-solvent mobile phase gradient. Limits of detection were 10-150 µg/mL (TC), 1-50 µg/mL (TDC), and 5-100 µg/mL (TCDC). Serum from 14 dogs with portocaval shunts contained 50-269 µmol total bile acids/L, with 16-69 µg TC/mL, 2-21 µg TDC/mL, and 3-40 µg TCDC/mL. Ratios of TC:TCDC, TC:TDC, and TCDC:TDC were 2:1, 5:1, and 2.5:1, respectively, in normal dogs; and 5.25 ± 2.73, 6.31 ± 5.85 and 2.58 ± 2.32 in dogs with shunts. The TC:TCDC ratio was significantly higher in portocaval shunt dogs. In conclusion, a simple reverse phase nonderivatized HPLC method sensitively and reliably detected taurine conjugates of bile acids in dog sera. Changes in the TC:TCDC ratio may be a more sensitive and earlier indicator of liver disease in dogs than total bile acids.

## 9

BLOOD PROGESTERONE, ESTRADIOL, AND TESTOSTERONE CONCENTRATIONS IN MARES WITH INFERTILITY. A. Özpınar<sup>1</sup> and H. Özpınar<sup>2</sup>. Departments of <sup>1</sup>Biochemistry and <sup>2</sup>Animal Nutrition, Faculty of Veterinary Medicine, University of Istanbul, Avclar, Istanbul, Turkey.

Increased reproductive performance of mares and stallions is very important for horse breeding. Nowadays, reproductive biochemistry should be well known due to biotechnological studies. Hormonal variations in pregnancy and estrus cycle of mares are different from those of other domestic animals. Animals with infertility could be improved by way of selection; however, the criteria for selection of mares are dependent on running, jumping, and training performance. The aim of this study was to investigate variations in serum progesterone, estradiol, and testosterone concentrations in mares with infertility. Fourteen mares bred in the Izmit study farm were used. They were 8 to 18 years old and had not become pregnant for 2 to 3 years. Blood samples were taken by jugular venipuncture 13 times at regular intervals over a 42 day period beginning in March. Samples were centrifuged after clotting, and serum was stored at -20°C until analyzed. Hormone assays were done by RIA on 182 samples. The mares were divided into 3 groups: healthy, metritis, and insufficient corpus luteum activity (ICLA; based on clinical findings and results of progesterone analysis) (Table). It was observed that serum progesterone, estradiol, and testosterone levels in mares were important in the diagnosis of infertility. Especially, serum testosterone levels <0.02 ng/mL indicated deficient ovarian function. Further studies regarding blood testosterone level and ovarian function in mares are needed.

**Table.** Serum progesterone, estradiol and testosterone concentrations (mean ± SD) in mares with infertility.

Group	Progesterone (ng/mL)	Estradiol (pg/mL)	Testosterone (ng/mL)
Healthy (n = 4)	4.32 ± 2.53 <sup>a</sup>	10.43 ± 5.10 <sup>a</sup>	0.032 ± 0.001 <sup>a</sup>
Metritis (n = 6)	2.00 ± 2.77 <sup>b</sup>	17.42 ± 12.93 <sup>b</sup>	0.022 ± 0.014 <sup>b</sup>
ICLA (n = 5)	1.31 ± 1.72 <sup>c</sup>	9.73 ± 5.93 <sup>ac</sup>	0.017 ± 0.007 <sup>c</sup>

<sup>a</sup>Means with different superscripts are significantly different from one another.

## 10

VALIDATION OF THE ADVIA 1650 CHEMISTRY SYSTEM FOR THE DOG AND RAT. A.J. Swain, J. Fuller, and P.J. O'Brien. Safety Assessment, SmithKline Beecham, Welwyn, United Kingdom.

The Bayer Advia 1650 is a novel, programmable, high-capacity chemistry system using small volumes and performing pre-assay dilutions. It was validated (using EP5-A, EP9-A; NCCLS) vs. the Roche Hitachi 717 for ALT, AST, ALP, total protein, albumin (Alb), Ca, Na, K, urea, creatinine (Cr), cholesterol (Chol), and glucose at 37°C in rat and dog plasma. Analyzer vendor materials were used, except ALT/AST reagents for the Advia 1650, and QC and non-electrolyte calibrators for the Hitachi 717 (Randox). Rat and dog plasma pools, and low, normal, and elevated QC (Randox) were analyzed 2X, 2 runs/day, for 21 days. Ninety rat and 73 dog plasma samples were analyzed in duplicate; 48 dog and 64 rat samples were spiked. Enzyme factor values needed correction by the vendor. Bias was calculated from least-squares lines. Evaporation was lower for the Advia 1650 (2% vs. 4%/hour) due to lower sample cup size (0.5 mL vs. 2.0 mL). Data rejection was higher for the Advia 1650 due to undersampling (2.0% vs. 0.2%) following inadvertent operator misspecification of sample-cup size. Methods correlated highly ( $r^2 > 0.95$ ) except for rat Alb ( $r^2 = 0.91$ ). At the upper and lower limits of rat reference ranges, net bias was <2% for electrolytes and <10% for other analytes, except at the lower limit for ALP (-16%) and ALT (14%), and at lower/upper limits, respectively, for Chol (-13%/20%) and Alb (18%/20%). Average 1650-717 imprecision (within-run/run-run/day-day/total), was 0.5-0.5/0.8-1.4/0.9-1.8/1.3-2.3 for electrolytes, 1.0-1.6/0.6-1.2/3.3-0.9/3.5-2.3 for enzymes, and 0.7-1.5/0.6-1.1/1.2-0.5/1.6-2.1 for other analytes. Average Advia 1650 imprecision was ~50% less for electrolytes (run-run/day-day/total) and other analytes (within-run/run-run), but, largely due to reagent instability (ALP, Cr) or inadvertent use of expired reagents (ALT, AST), day-day was ~3X more and total imprecision was ~50% more for enzymes. The Advia 1650 is considered validated for dog and rat plasma, highly precise, and highly correlated with the Hitachi 717.

## 11

TEACHING VETERINARY CLINICAL PATHOLOGY THROUGH THE WEB USING CASE SIMULATION: A COMPLEMENTARY TEACHING AID. A. Lanevski-Pietersma<sup>1</sup> and D. Harvey<sup>2</sup>. Départements de <sup>1</sup>Pathologie et Microbiologie, et des <sup>2</sup>Sciences Cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada.

Our objective was to construct a website for students that required the application of concepts in clinical pathology for the interpretation of laboratory data. The website was constructed using Microsoft Frontpage® and included 2 databases: (1) simulated clinical cases representing a variety of diseases in different species; and (2) an image library corresponding to common clinical pathology terms. The clinical case format consisted of a signalment, brief history, outstanding physical examination observations, laboratory results, and photomicrographs. The user was asked to describe laboratory changes (through the selection of options), and to enter a cytological description and a differential or final diagnosis. The answers were then posted. The student could print a summary of the case. The website was offered to students to complement their learning while taking the core hematology course in the second year, and in an elective laboratory medicine course in the final year of the veterinary program. Twenty percent of final-year students responded to the request to fill out an evaluation form. From these evaluations, and from informal student-teacher exchange regarding the website's usefulness, common observations included the perception that the website aided in preparation for the national certifying examination, self-testing of skills and review of concepts in clinical pathology, as well as knowledge of diseases. Most student-teacher interaction occurred in person, despite inclusion of the teacher's e-mail address on the website. We concluded that case-based learning is a useful complementary tool in clinical pathology, and increases case exposure. The website was greeted enthusiastically by students as a self-testing and study tool.

## 12

EXTERNAL QUALITY ASSURANCE IN VETERINARY PATHOLOGY. B. Kelly. Department of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh, Scotland.

Veterinary pathologists are aware of the importance of good quality, consistently well-prepared stained sections as an aid to accurate diagnosis. Although histologic techniques are well established, it is essential to have procedures in place to control, maintain, and improve their quality. Quality control measures are universally recognized in all aspects of clinical laboratory medicine with External Quality Assessment (EQA), the most accurate and authoritative means of monitoring performance. An EQA scheme for the technical aspects of veterinary pathology in the UK and Ireland has been in operation since 1993, following a pilot study (*Vet Record*. 1993;133, 94-96). The scheme initially operated independently, but since 1999 has been a constituent part of the national scheme in the UK. The scheme assesses the output of participating laboratories in 2 ways. Firstly, archival H&E sections are assessed using criteria covering all aspects of slide production, from fixation to final mounting. Secondly, participants are asked to stain an unstained section using a "special stain" selected from a list of commonly performed techniques. These sections are assessed using criteria specific for the particular technique. Experienced technologists and veterinary pathologists carry out all assessments. A results package containing the slides, assessment sheets, a histogram of all the participants' marks, and videoprints of the sections obtaining the highest marks is returned to each participant. There are 6 assessments per annum. Confidentiality is maintained by use of a laboratory code, and confidential support is offered to laboratories that perform poorly. The scheme is well received and has participants in Europe and Australia as well as the UK.

## 13

SIRS, MODS AND DIC IN SMALL ANIMAL VETERINARY MEDICINE (PLENARY LECTURE). G. Lubas<sup>1</sup>, M. Caldin<sup>2</sup>, and T. Furlanello<sup>2</sup>. <sup>1</sup>Department Veterinary Clinic, University of Pisa, Pisa, Italy; and <sup>2</sup>San Marco Veterinary Clinic, Padova, Italy.

Systemic inflammatory response syndrome (SIRS) describes a clinical condition resulting from the action of complex intrinsic mediators of the acute phase reaction. Sepsis is a clinical syndrome complicating severe infection. In SIRS and/or sepsis there is inflammatory "dysregulation", with massive and uncontrolled release of mediators (cytokines and lipid substances). The host response in sepsis and/or SIRS is responsible for multiple organ dysfunction syndrome (MODS). MODS can be primary (organ dysfunction occurs early), or secondary (organ failure is induced by the host response). MODS represents the more severe end of an illness characterized by SIRS and/or sepsis. Coagulation is activated in acute inflammatory responses because the trigger stimuli are the same mediators involved in sepsis/SIRS. Inflammatory cytokines convert endothelium to a prothrombotic surface, and then activate both the extrinsic and intrinsic coagulation systems. The fibrinolytic system and the protein-C anticoagulant pathways are initially activated but subsequently inhibited, leading to a marked imbalance of coagulation and fibrinolysis. When thrombin generation and platelet activation exceed the body's capacity to inactivate or remove them, disseminated intravascular coagulation (DIC) results. DIC is a thrombohemorrhagic disorder with paradoxical simultaneous widespread microvascular thrombosis and hemorrhage; DIC contributes to MODS. Several molecules have been identified to assess SIRS and/or DIC in veterinary medicine. For example, C-reactive protein, valuable in identifying inflammatory disease that may potentially lead to SIRS, and D-dimer, which shows good sensitivity and specificity for DIC, have been recently validated in dogs. In cats, alpha-1-acid glycoprotein, serum amyloid-A, and haptoglobin have proven useful as acute phase proteins.

## 14

A POSSIBLE RESEARCH MODEL FOR SPECIFIC PROPHYLAXIS AND TREATMENT OF BACTERIAL PNEUMONIA AND DIARRHEA IN YOUNG ANIMALS. Ü. Pavel<sup>1</sup>, J. Kumar<sup>1</sup>, and A. Karus<sup>2</sup>. <sup>1</sup>Estonian Agribiological Center, Tartu, Estonia; and <sup>2</sup>Estonian Agricultural University, Institute of Animal Science, Tartu, Estonia.

The aim of this study was to evaluate the use of neuropeptides for potentiating vaccine response, as a model for the prophylaxis and treatment of bacterial diarrhea and pneumonia in young animals. The dose of the chosen bacteria, *Salmonella typhimurium* 34-96 was equal to ~100% the lethal dose, ie,  $1.4 \times 10^4$  cells, intraperitoneally or subcutaneously. The neuropeptide, 30 to 70 ng per mouse (1.6 to 3.6 ng/g, subcutaneously) was given 5 days before the experimental infection. Mortality was documented during postinfection days 1-5 and 6-10. Blood antibody titers and relative leukocyte profiles were determined. Results of the study indicated a marked immunoprotective effect of neuropeptides in mice. The best results were obtained in the group receiving the neuropeptide "CCb", in which mortality was 58.3% vs. 91.6% in control mice. Neuropeptides were also effective as potentiators of the vaccine. The neuropeptides markedly enhanced the antibody titer of mice during the 5 days investigated, starting on the 5th day after the vaccination, ie, in the period when the effect of the vaccine was not yet fully manifested. Concerning the relative blood leukocyte profile, there was a tendency for lymphocyte counts to increase, and for granulocyte counts to decrease. We conclude that it is reasonable to potentiate vaccines with neuropeptides for the purpose of compensating the low rate of antibody synthesis in its lag phase.

## 15

ASSESSMENT OF BIOCHEMICAL PARAMETERS FOR THE EVALUATION OF INTESTINAL BACTERIAL OVERGROWTH IN GROWING DOGS. M. Weber<sup>1</sup>, L. Martin<sup>1,2</sup>, H. Dumon<sup>1</sup>, P. Nguyen<sup>1</sup>, F. Stambouli<sup>1</sup>, and B. Siliart<sup>2</sup>. <sup>1</sup>Laboratoire de Nutrition et Alimentation and <sup>2</sup>Laboratoire des Dosages Hormonaux LDH/GNVN, Department of Biology and Pharmacology, Ecole Vétérinaire, Nantes, France.

The aim of this study was to correlate the parameters commonly used to assess small intestinal bacterial overgrowth in growing dogs. We used 17 growing dogs: 6 Great Danes, 6 Giant Schnauzers, and 5 Medium Schnauzers. Diet was formulated to meet the NRC (1974) recommendations for all macronutrients. Experimental protocol was as follows: feces scoring was recorded daily as grade 1 (hard and dry) to grade 5 (watery diarrhea); breath hydrogen test (Quintron) was performed monthly for a 7-hour period after a meal. Blood assays were performed for folate and vitamin B<sub>12</sub> concentrations at 6-week intervals starting at 10 weeks of age (Simul Trac-SNB RIA Kit, Diasorin). In adult dogs, usual values for B<sub>12</sub> and folate are <350 µg/L and >12 µg/L, respectively. In the growing dogs, there was no relationship between serum levels of B<sub>12</sub> and folate ( $r^2 = 0.09$ ;  $n = 84$ ). The poorest feces quality was recorded in dogs with serum folate values >18 µg/L and serum B<sub>12</sub> values <350 µg/L. The serum B<sub>12</sub> level correlated with the amount of expired H<sub>2</sub> produced during the 7-hour period ( $r^2 = 0.598$ ;  $P < .0001$ ;  $n = 26$ ) indicating bacterial overgrowth when the B<sub>12</sub> value was <350 µg/L. However, serum levels of folate did not correlate with expired H<sub>2</sub> values. Based on results of the breath hydrogen test, serum B<sub>12</sub> concentration was a better indicator of bacterial overgrowth than serum folate concentration. It was apparent from the feces scoring study that the threshold value for folate may be higher in growing dogs (18 µg/L vs. 12 µg/L); whereas, the same threshold value for vitamin B<sub>12</sub> could be used for both adult and growing dogs.

## 16

CILIOCYTOPHTORIA IN THE BRONCHOALVEOLAR LAVAGE OF AN FIV-POSITIVE CAT. C. Masserdotti. Clinica Veterinaria S. Antonio, Salò, Italy.

Cilioctophthoria is a peculiar degeneration of the ciliated respiratory epithelium. The purpose of this study was to describe the cytologic features of this rare cellular change. A 5-year-old, female, DSH cat was referred to our clinic for evaluation of chronic coughing and mild dyspnea. Physical examination revealed an alert, responsive cat. Thoracic auscultation, with the exception of mild alveolar murmur, was unremarkable. An ELISA test for anti-FIV antibodies was positive. Thoracic radiography revealed diffuse, miliary radioopacity of the pulmonary areas. To assess infectious or parasitic diseases secondary to the viral immunodeficiency, a bronchoalveolar lavage was performed. Scanty, yellowish, mucoid material was obtained from repeated lavages. This material was gently spread directly onto slides, and stained using H&E and Hemacolor. All 7 slides showed a diffuse, mild increase in neutrophils, without evidence of nuclear degeneration or phagocytosis. Epithelial cells exhibited changes consistent with benign reactive atypia of the ciliated respiratory cells and hyperplasia of the reserve cells. In one slide we observed diffuse cilioctophthoria, a pinching-off of the distal ciliated portion of cells that results in formation of anucleated ciliated tufts and nucleated cytoplasmic remnants. Cilioctophthoria refers to the small ciliated tufts that appear to be broken-off tops of ciliated bronchial cells. Cilioctophthoria is a significant feature of some inflammatory conditions of the lung, especially viral pneumonia. The cytologic features of the bronchoalveolar lavage in this cat were consistent with nonspecific inflammation. There is no known correlation between FIV infection and damage to ciliated bronchial cells. It is our opinion that retroviral infection is a predisposing factor to secondary viral or bacterial infections, which are the true causes of the observed features.

## 17

EVALUATION OF THE NEW MULTISPECIES SOFTWARE OF THE HEMATOLOGY SYSTEM ADVIA 120. A. Moritz. Clinic of Veterinary Medicine and Forensic Affairs, Giessen, Germany.

Hematological examinations are an indispensable part of routine veterinary diagnostics, as physiological and pathological processes in the body can cause qualitative and quantitative changes of the blood cells. Due to the species-specific variations of blood cells, the examination of animal blood requires a high standard for modern automated hematology systems. Quality assurance in veterinary hematology requires not only a technical evaluation of the analyzer intended for use in routine diagnostics, but also a comparison with results obtained using established methods. Our evaluation of the ADVIA 120 automated hematology system included comprehensive precision studies, investigations on the linearity and carryover, and a comparison with reference methods and analyzers for blood cell counts (Sysmex F-800, microhematocrit centrifuge) and blood cell differentials (manual differentials on smears stained with panoptic Pappenheim stain or Wright's stain, peroxidase,  $\alpha$ -naphthyl acetate esterase, and supravital brilliant cresyl blue). Data obtained using the ADVIA 120 and reference methods were compared for 1665 animals, including dogs ( $n = 213$ ), cats ( $n = 275$ ), horses ( $n = 221$ ), pigs ( $n = 242$ ), cattle ( $n = 245$ ), sheep ( $n = 212$ ), and goats ( $n = 257$ ). The ADVIA 120 was well suited for routine blood counts in veterinary hematology due to its highly evolved technique of measurement and its comprehensive species-specific settings. Results obtained for CBC and white blood cell differentials demonstrated that the ADVIA 120 yielded concise differential counts for all species, so that it is especially well suited for the veterinary routine laboratory examination.

## 18

INDEX OF ANISOCYTOSIS IN ADULT DOGS. PRELIMINARY OBSERVATIONS. J. F. Guelfi, D. Perret, A. Diquérou, and C. Trumel. Ecole Nationale Vétérinaire de Toulouse, Toulouse, France.

The aim of this study was to evaluate the index of anisocytosis (the RDW) to classify the mechanism and cause of anemia and to better characterize canine diseases with disturbances of iron metabolism. Index of anisocytosis was evaluated using an automated cell counter (MS9, Melet Schloesing Laboratories) in adult dogs ( $\geq 1$  year of age) admitted to the Veterinary School of Toulouse from October 1998 to May 1999. Forty-five dogs were clinically healthy and 916 had various diseases, including 266 with anemia ( $Hb < 120$  g/L). Classification of anemia was based on reticulocytosis, MCV, MCH and RBC morphology. Nonregenerative anemia and babesiosis were characterized by a normal or low index of anisocytosis (Table). Conversely, a higher proportion of dogs with regenerative anemia and especially iron-deficiency anemia, had an increased index of anisocytosis, with values as high as 16. In diseased, non-anemic dogs, the index of anisocytosis was usually normal. Nevertheless, it was elevated in some cases, mainly with microcytosis (including 3 cases of portosystemic shunt) and in 1 case of Poodle macrocytosis. The index of anisocytosis was rarely decreased, mainly in inflammatory disease; the lowest value was 6. In conclusion, the index of anisocytosis can be a useful parameter to gain better knowledge about the etiology of anemia and to identify inflammatory or metabolic disease in dogs. These observations require additional confirmation, and evaluation of the sensitivity and specificity of this test.

Table. Index of anisocytosis in 961 dogs. Data are number and percentage of dogs in each category.

Index of Anisocytosis	Healthy	Diseased dogs with anemia				Diseased, nonanemic
		Nonregen. anemia*	Iron deficiency†	Regen. anemia‡	Babesia canis	
< 7.8	0	22 (11%)	0	1 (4%)	2 (9%)	11 (2%)
7.8 to 10.6	45 (100%)	178 (89%)	7 (39%)	14 (56%)	21 (91%)	610 (94%)
$\geq 10.9$	0	0	11 (61%)	10 (40%)	0	29 (4%)

\*Nonregenerative anemias were due to inflammation, tumors, or renal insufficiency.

† Iron deficiency anemias were due to chronic bleeding and usually had microcytosis and hypochromasia.

‡ Regenerative anemias were due to acute bleeding or hemolysis.

## 19

OCCURRENCE OF ABNORMAL RED BLOOD CELL AGGLUTINATION AND/OR HEMOLYSIS IN HORSE SAMPLES ROUTINELY SURVEYED FOR BLOOD GROUP TYPING: REPORT OF TEN YEARS EXPERIENCE. G. Lubas<sup>1</sup>, A. Gavazza<sup>2</sup>, B. Gugliucci<sup>1</sup>, A.J. Delgado<sup>1</sup>, and G. Savini<sup>2</sup>. <sup>1</sup>Department Veterinary Clinic, University of Pisa, Pisa, Italy; and <sup>2</sup>Istituto Zooprofilattico Sperimentale "G. Caporale" Teramo, Italy.

An unusual immunologic reaction (agglutination and/or hemolysis) was observed in 21 of 10,000 horse serum samples that were being hemotyped using standard techniques (according to the International Society of Animal Genetics). Ten samples were also tested using a species-specific direct antiglobulin test (DAT). All sera were screened for *Babesia equi*, *B. caballi*, and equine infectious anemia (EIA) antibodies. Four different reactions were observed (Table).

All sera were negative for *B. caballi* and EIA antibodies, and the owners and veterinarians reported no clinical signs. Most of the positive reactions on both the agglutination test and DAT were associated with the presence of *B. equi* antibodies. In contrast, in those samples where atypical hemolysis was the only phenomenon observed, antibodies to *B. equi* were not detected. In agreement with what is reported in the literature, these atypical reactions were not related to any clinical signs in horses. These phenomena suggest a low level of macrophage activity in the spleen and liver, which may induce a severe hemolytic process.

**Table.** Results for 21 equine serum samples showing atypical agglutination and/or hemolysis in relation to *Babesia equi* antibodies and direct antiglobulin test results.

No. Samples	Agglutination	Hemolysis	<i>Babesia equi</i>	DAT Test
8	Positive	Negative	Positive	Positive (3 of 3)
4	Positive	Positive	Positive	Positive (3 of 3)
4	Positive	Negative	Negative	Positive (2 of 2)
5	Negative	Positive	Negative	Negative (2 of 2)

## 20

ADAPTATION OF THE AGNOR METHOD TO CYTOLOGY SAMPLES DERIVED FROM CANINE LYMPH NODES AND BODY CAVITY FLUIDS. P. Vajdovich<sup>1</sup>, J. Schuller<sup>1</sup>, and J. Dunn<sup>2</sup>. <sup>1</sup>Department of Internal Medicine and Clinics, Szent István University, Faculty of Veterinary Science, Budapest, Hungary; and <sup>2</sup>The Queen's Veterinary School Hospital, University of Cambridge, Cambridge, United Kingdom.

The aim of our study was to adapt and evaluate the usefulness of the argyrophilic nucleolar organizer region (AgNOR) staining method in distinguishing neoplastic proliferation from reactive cells derived from lymph nodes and body cavity fluids using cytological techniques. Samples from 37 dogs were analyzed. Fine needle aspiration samples were taken from 21 lymph nodes and from 16 peritoneal or pleural effusions. Smears were stained as described by Lim et al. (*J Pathol.* 1992; 166:53-60). We contrast-stained the smears using Diff-Quik stain. The mean number of AgNOR per nuclei was counted from at least 100 cells in each sample microscopically at  $\times 400$  to  $\times 1000$  magnification. Configuration of AgNORs within the nuclei was also observed using the classification of Crocker et al. (*J Pathol.* 1988;158:185-188). Cells were classified to the 1st class if their nucleolus or nucleoli contained 1 AgNOR dot; to the 2nd class if their nucleolus or nucleoli contained more than 1 dot; and to the 3rd class if dots were found outside of the nucleoli or randomly within the nuclei. In neoplastic lymph nodes, the mean number of AgNOR per nuclei was 4.19, while in reactive nodes it was 1.62. Mean AgNOR number per nuclei in cells in neoplastic effusions was 4.68, while in "reactive" fluids it was 2.46. Association between the mean number of AgNOR per nuclei and the onset of neoplastic disease was significant ( $P < .001$ ) in both types of specimens. Samples classified into the 1st and 2nd classes were found to be primarily normal or reactive ( $P < .01$  for lymph nodes,  $P < .05$  for effusions); whereas, samples in the 3rd class were mostly neoplastic.

## 21

ACUTE MYELOID LEUKEMIA (M4) IN A DOG: HEMATOLOGY, FLOW CYTOMETRY, CYTOCHEMISTRY, AND BLOOD CELL METABOLISM. S. Comazzi, S. Paltrinieri, R. Guglielmino, B. Miniscalco, M. Caniatti, C. Di Palma, and C. Cortelezzi. Istituto di Patologia Generale Veterinaria, Milano, Italy.

A 4-year-old Saint Bernard dog was presented with fever, anemia, and hepatosplenomegaly. CBCs were done on days 0, 3, 9, and 10. On day 9, a bone marrow aspirate was performed and flow cytometry was done with the following antibodies: CD21, IgG, IgM, CD5, CD3, CD4, CD8, CD45RA, CD49d, and CD45. Cytochemical staining was done for peroxidase, alkaline phosphatase,  $\alpha$ -naphthyl acetate esterase, and chloracetate esterase. On days 0 and 3, RBC pyruvate kinase and glucose-6-phosphate dehydrogenase activities, 2,3 DPG concentration, and osmotic fragility were determined. On day 10, neutrophils were isolated using a discontinuous Percoll density gradient. Adherence in microtiter plates and chemotaxis in a modified Boyden chamber were evaluated. Severe, nonregenerative, normocytic, normochromic anemia was present. Total leukocyte number increased from  $18.1 \times 10^6/\text{mL}$  (day 0) to  $35.4 \times 10^6/\text{mL}$  (day 3) and  $78.9 \times 10^6/\text{mL}$  (day 10), with immature cells seen on days 9 and 10. The bone marrow M:E ratio was 33:1, with 34% blasts. Flow cytometry was negative for lymphocyte markers and positive for CD45 and CD49d. A total of 38.5% of cells was positive for CD4, a lymphocyte antigen also expressed in canine neutrophils. Blasts were positive for all cytochemical reactions. A diagnosis of myelomonocytic leukemia (M4) was made. Erythrocytes showed strong increases in enzyme activities and 2,3 DPG concentration compared to reference values, probably due to hypoxia caused by anemia. After isolation, neutrophils were detected in the lower density band, and monocytes and myeloid precursors in the other band, suggesting altered neutrophil density. Granulocyte movements were increased in the neutrophil-rich population. These results, together with increased chemotactic activity, suggest changes in mature granulocyte function.

## 22

FLOW CYTOMETRIC ANALYSIS OF CIRCULATING LYMPHOCYTE SUBSETS IN CATS WITH SPONTANEOUS FELINE INFECTIOUS PERITONITIS. S. Paltrinieri<sup>1</sup>, W. Ponti<sup>2</sup>, S. Comazzi<sup>1</sup>, F. Di Mauro<sup>2</sup>, and P.F. Moore<sup>3</sup>. <sup>1</sup>Istituto di Patologia Generale Veterinaria, Milano, Italy; <sup>2</sup>Istituto di Microbiologia e Immunologia Veterinaria, Milano, Italy; and <sup>3</sup>Department of Pathology, Microbiology and Immunology, University of California-Davis, USA.

To investigate changes in circulating lymphocyte subsets during feline infectious peritonitis (FIP) infection, 17 cats with FIP and 18 healthy control cats were sampled. One cat with FIP was tested twice: at the diagnosis of disease and 10 days later. After a CBC and differential leukocyte count, lymphocyte subsets were investigated by flow cytometry using the following antibodies against feline leukocyte markers: FE1.B11 (CD5, pan-T cells), FE1.7B12 (CD4, T cells), FE1.10E9 (CD8, T cells), CA2.1D6 (CD21, B cells). As secondary antibody, the F(ab')<sub>2</sub> antibody-binding fragment of goat anti-mouse phycoerythrin-conjugated immunoglobulin was used. Results from the 2 groups were compared by U-Mann Whitney tests. Compared to controls, cats with FIP showed relative and absolute lymphopenia ( $P < .001$ ), a significant decrease in the percentages of pan-T, CD4, and CD8 lymphocytes, and a significant decrease in lymphocyte number in all subsets (Table). In contrast, the percentage of CD5<sup>+</sup>/CD21<sup>-</sup> lymphocytes increased. Strong individual variability might have been due to the stage of disease at which the tests were performed. In the cat with repeated analysis, in fact, the decrease in percentage and number of lymphocytes was mainly due to decreased CD4 and B lymphocytes.

**Table.** Percentage and number (mean  $\pm$  SD) of cells positive for leukocyte markers in control cats and cats with feline infectious peritonitis (FIP).

	CD5	CD4	CD8	CD21	T+B
Control (%)	64.0 $\pm$ 11.7	37.5 $\pm$ 8.0	25.5 $\pm$ 8.0	28.1 $\pm$ 9.6	92.1 $\pm$ 7.3
FIP (%)	44.4 $\pm$ 23.2*	30.1 $\pm$ 20.4*	13.8 $\pm$ 9.3 <sup>†</sup>	21.1 $\pm$ 16.0	65.6 $\pm$ 29.0 <sup>†</sup>
Control ( $\mu\text{L}$ )	2.72 $\pm$ 1.56	1.55 $\pm$ 0.74	1.13 $\pm$ 0.84	1.07 $\pm$ 0.30	—
FIP ( $\mu\text{L}$ )	0.49 $\pm$ 0.42 <sup>†</sup>	0.37 $\pm$ 0.41 <sup>†</sup>	0.14 $\pm$ 0.11 <sup>†</sup>	0.23 $\pm$ 0.24 <sup>†</sup>	—

Significantly different from control cats: \* $P < .05$ ; <sup>†</sup> $P < .001$ .