Veterinary Clinical Pathology ISSN 0275-6382

ABSTRACTS

European Society of Veterinary Clinical Pathology (ESVCP) and European College of Veterinary Clinical Pathology (ECVCP) 11th Annual Congress

Thessaloniki, Greece – October 7–9, 2009

DOI:10.1111/j.1939-165X.2009.00195.x

Oral Platform Presentations: Abstracts 1-14
Poster Presentations: Abstracts 15-66

1 CLINICAL APPLICABILITY OF A POINT-OF-CARE PATIENT-SIDE CANINE-SPECIFIC COMMERCIALLY AVAILABLE QUANTITATIVE ASSAY FOR DETERMINATION OF C-REACTIVE PROTEIN. **M. Kjelgaard-Hansen.** Department of Small Animal Clinical Sciences, University of Copenhagen, Copenhagen, Denmark.

2 HOMOCYSTEINE IN CANINE SERUM INCREASES IN SEVERE KIDNEY DISEASE BUT NOT IN INFLAMMATORY BOWEL DISEASE. **G. Rossi[1], S. Breda[1], A. Giordano[1], A. Zatelli[2], S. Paltrinieri[1].** [1]Dept. of Veterinary Pathology Hygiene and Public Health, University of Milan, Italy; and [2]Veterinary Clinic Pirani, Reggio Emilia, Italy.

3 PERFORMANCE EVALUATION OF THE SYSMEX POCH-100IV DIFF HEMATOLOGY ANALYZER FOR CANINE, FELINE, EQUINE AND BOVINE BLOOD. **B. Riond, S. Weissenbacher, R. Hofmann-Lehmann, H. Lutz.** Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland.

4 REFERENCE VALUES OF BLOOD COMPONENTS IN HIGH-PRODUCING GOATS. **B. Siliart[1,2], L. Jaillardon[1,2], L. Martin[1], H. Dumon[1]**. [1]Endocrinology and Nutrition Unit and [2]Hospital Clinical Laboratory, Department of Biology and Pathology, National Veterinary School, Nantes, France.

5 PREVALENCE OF GREY EOSINOPHILS IN RACING HOUNDS AND COMPARISON OF MANUAL AND INSTRUMENT COUNTS. **L. Giori[1], S. Gironi[2], P. Scarpa[2], M. Gualtieri[2], S. Paltrinieri[1].** Departments of [1]Veterinary Pathology Hygiene and Public Health and [2]Veterinary Clinical Science, University of Milan, Milan, Italy.

6 AUTOMATIC DIGITAL IMAGE ANALYSIS SYSTEM (CELLAVISION DM96) USED FOR CANINE LEUKOCYTE DIFFERENTIAL COUNTING. **I. Lilliehook[1], H. Tvedten[2].** [1]University Animal Hospital and [2]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.

7 ACQUIRED MYELOPEROXIDASE DEFICIENCY IN DOGS. **S. Klenner, J. Richartz, N. Bauer, A. Moritz.** Department of Veterinary Clinical Sciences, Clinical Pathology, and Clinical Pathophysiology, Justus-Liebig-University Giessen, Giessen, Germany.

8 BIOCHEMICAL AND ENDOCRINE PROFILE OF 78 OBESE CATS. **L. Jaillardon[1,2], B. Siliart[1,2], L. Martin[1].** [1]Endocrinology and Nutrition Unit and [2]Hospital Clinical Laboratory, Department of Biology and Pathology, National Veterinary School, Nantes, France.

9 NEW INDIRECT ELISAS FOR DIAGNOSIS OF ENZOOTIC BOVINE LEUKOSIS. **S. Safi[1], B. Mohammadi[2], F. Hemmatzadeh[3].** [1]Department of Clinical Pathology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Tehran, Iran; [2]Islamic Azad University, Tehran, Iran; and [3]Department of Microbiology, Faculty of Veterinary Medicine, Tehran University, Iran.

10 *BABESIA GIBSONI* INFECTION IN ISRAEL. **G. Baneth[1], T. Molad[2], Y. Yisaschar-Mekuzas[1], E. Yas[1], G. Segev[1], L. Fleiderovich[2], V. Shkap[2].** [1]School of Veterinary Medicine, Hebrew University of Jerusalem, Israel; and [2]Division of Parasitology, Kimron Veterinary Institute, Israel.

11 ASSESSMENT OF LACTATE DEHYDROGENASE (LDH), ALKALINE PHOSPHATASE (ALP) AND ASPARTATE AMINOTRASFERASE (AST) ACTIVITIES IN MILK FOR THE DIAGNOSIS OF SUBCLINICAL MASTITIS IN SHEEP AND GOATS. **P.D. Katsoulos[1], G. Christodoulopoulos[1], A. Minas[2], K. Pourliotis[3], M.A. Karatzia[3], S.K. Kritas[4].** [1]Clinic of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece; [2]Laboratory of Microbiology, Faculty of Health Professions, Technological Educational Institution of Larissa, Larissa, Greece; [3]Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; and [4]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

12 STEP-BY-STEP INTRODUCTION OF QC VALIDATION AND APPLICATION OF SIGMA METRICS USING THE HAEMATOLOGY ANALYSER CELL-DYN® 3500 AS AN EXAMPLE. **E. Leidinger[1], E. Hooijberg[1], K.P. Freeman[2].** [1]Invitro Veterinary Laboratory, Vienna, Austria; and [2]IDEXX Laboratories, Grange House, Wetherby, West Yorkshire, UK.

13 EVALUATION OF 9 NOVEL RENAL URINARY BIOMARKERS. **M. Pinches, C. Betts, S. Bickerton, L. Burdett, N. Derbyshire, H. Jones, H. Caddick, M. Moores, S. Sourial.** Safety Assessment AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK.

14 APPLICATION OF SIGMA METRICS: A "REVERSE APPROACH". **I. Schwendenwein, C. Johler-Weißenbacher, A. Guija de Arespacochaga.** Division of Clinical Pathology, University of Veterinary Medicine, Vienna.

15 STUDY OF THE EFFECTS OF INFECTIOUS BURSAL DISEASE (GUMBORO) IN NATURAL ENVIRONMENTAL CONDITIONS ON SERUM ACUTE PHASE PROTEINS HAPTOGLOBIN AND α-1-ACID GLYCOPROTEIN IN BROILERS. **S. Safi[1], F. Mohammadi[2], M. Rahimi[3], A. Rahimi[4].** [1]Department of Clinical Pathology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Tehran, Iran; [2]Veterinary Group, Islamic Azad University, Kermanshah, Iran; [3]Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran; and [4]Department of Epidemiology & Biostatistics, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

16 PTX3: A MARKER FOR LOCAL INFLAMMATION? **E. Ramery, F. Bureau, T. Art, P. Lekeux.** Functional Sciences, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

17 A RARE CASE OF HAEMANGIOPERICYTOMA IN AN AMAZON PARROT. **P. Loukopoulos, A. Komnenou, V. Tsiouris, M. Zavlaris, A.-L. Thomas, I. Georgopoulou.** Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece.

18 THE EFFECTS OF STORAGE TIME AND TEMPERATURE ON SELECTED HEMATOLOGICAL VARIABLES IN CANINE BLOOD SAMPLES. **T.S. Maysam[1], J.P. Mohsen[2], M.A. Seyed[3], V.H. Amin[3], A.J. Shahriar[3], M.A. Amir[3], G.A. Seyed[3].** [1]Faculty of Veterinary Medicine, Islamic Azad University, Garmsar, Iran; [2]Faculty of Veterinary Medicine, Ferdowsi Uuniversity, Mashad, Iran; and [3]Student, Islamic Azad University, Garmsar, Iran.

19 CYTOLOGICAL ASPECTS AND HISTOLOGICAL CORRELATION OF EPITHELIAL ODONTOGENIC TUMORS WITHOUT ODONTOGENIC MESENCHYME IN CANINE SPECIES. **U. Bonfanti[1], W. Bertazzolo[2], M. Gracis[3], S. Ferro[4].** [1]Accelera-NMS, Nerviano, Italy; [2]Clinica Veterinaria Tibaldi, Milan, Italy; [3]Clinica Veterinaria San Siro, Milan, Italy; and [4]Faculty of Veterinary Medicine, Padua, Italy.

20 EVALUATION OF STABILITY OF RAT EDTA BLOOD SAMPLES STORED AT DIFFERENT CONDITIONS. **U. Bonfanti, M. Rocchetti, A.M. Giusti, P. Colombo.** Accelera – Nerviano Medical Sciences, Nerviano, Italy.

21 THE SENSITIVITY AND SPECIFICITY OF FIBRINOGEN AND TOTAL PROTEIN:FIBRINOGEN RATIO IN SCREENING HEALTHY AND DISEASED DAIRY CATTLE AT ABATTOIRS. **Gh. Ghalamkari[1], Sh. Safi[2], S.R. Jafarzadeh[3], R. Sedaghat[4].** [1]Department of Animal Science, Agriculture Faculty, Islamic Azad University, Isfahan, Iran; [2]Faculty of Specialized Veterinary Medicine, Islamic Azad University, Tehran, Iran; [3]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, USA; and [4]Faculty of Medicine, Shahed University, Tehran, Iran.

22 MACROTHROMBOCYTOPENIA IN GROUP OF RELATED NORFOLK TERRIERS: A CONDITION SIMILAR TO MACROTHROMBOCYTOPENIA OF CAVALIER KING CHARLES SPANIELS? **G.F. Tutino[1], E. Pogliani[1], W. Bertazzolo[1], M.E. Gelain[2].** [1]Clinica Veterinaria Tibaldi, Milan, Italy; and [2]Department of Veterinary Pathology, Hygiene and Health, University of Milan, Italy.

23 UTERINE LEIOMYOSARCOMA AND PYOMETRA IN A 7-YEAR-OLD DOG. **V.G. Tsioli[1], P.G. Gouletsou[1], P. Loukopoulos[2], M. Zavlaris[2], A.D. Galatos[1].** [1]Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece; and [2]Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

24 CLINICAL EVALUATION OF THE BC-2008 VET HEMATOLOGY ANALYZER FOR USE IN VETERINARY PRACTICE. **C. Masserdotti.** Laboratorio di Analisi Veterinarie San Marco, Padova, Italy.

25 CLINICAL PATHOLOGY FINDINGS IN FIVE CATS AFFECTED BY ABSOLUTE ERYTHROCYTOSIS. **A. Gavazza, G. Lubas, B. Gugliucci.** Dept. of Veterinary Clinic, University of Pisa, Italy.

26 ASSESSMENT OF MOLYBDENUM STATUS IN DAIRY COWS. **L. Martin, L. Jaillardon, B. Siliart, E. Paniez, H. Dumon.** Laboratoire des Dosages Hormonaux, Ecole Nationale Vétérinaire, Nantes, France.

27 IMPACT OF IN VITRO HEMOLYSIS ON KAOLIN-ACTIVATED THROMBELASTOGRAPHY PARAMETERS AND ADVIA 2120 PLATELET ACTIVATION INDICES IN CANINE SPECIMENS. **N. Bauer, O. Eralp, A. Moritz.** Department of Veterinary Clinical Sciences, Clinical Pathology, and Clinical Pathophysiology, Justus-Liebig-University Giessen, Giessen, Germany.

28 DEVELOPMENT AND APPLICATION OF A PCR METHOD FOR THE DETECTION OF *BRUCELLA* SPP. FROM SHEEP, GOAT AND BOVINE NON-PASTEURISED MILK. **A. Katsiolis[1], A. Minas[2], C. Hadjichristodoulou[3], A. Vantarakis[1].** [1]Department of Public Health, Medical School, University of Patras, Greece; [2]Department of Medical Laboratories, Technological Educational Institution of Larissa, Greece; and [3]Department of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, Karditsa, Greece.

29 PRELIMINARY RESULTS: BIG ENDOTHELIN-1 IN SERUM FROM DOGS WITH KIDNEY DISEASE. **A. Giordano[1], G. Rossi[1], A. Virtuoso[1], A. Zatelli[2], S. Paltrinieri[1].** [1]Dept. of Veterinary Pathology Hygiene and Public Health, University of Milan, Italy; and [2]Veterinary Clinic Pirani, Reggio Emilia, Italy.

30 SERUM AND URINARY IMMUNOFIXATION ELECTROPHORESIS IN A DOG WITH MULTIPLE MYELOMA. **A. Miglio[1], F. Birettoni[1], M.T. Antognoni[1], P. Lalli[2], V. Mangili[1].** [1]Dipartimento di Patologia, Diagnostica e Clinica Veterinaria, University of Perugia; and [2]Azienda Ospedaliera, Perugia, Italy.

31 *HEPATOZOON* SPP. INFECTION IN STRAY CATS FROM A FELINE COLONY IN NORTHEASTERN ITALY DETECTED BY BLOOD SMEAR EXAMINATION AND MOLECULAR TESTING. **M. Trotta[1], E. Carli[1], M. Nicetto[1], R. Chinelli[2], T. Furlanello[1], L. Solano-Gallego[3].** [1]Laboratorio d'Analisi Veterinarie San Marco, Padova, Italy; [2]DVM, Trieste, Italy; and [3]Royal Veterinary College, UK.

32 THE UTILITY OF FINE-NEEDLE ASPIRATION CYTOLOGY IN THE DIFFERENTIAL DIAGNOSIS OF CUTANEOUS MASSES CONTAINING KERATIN. **G. Ghisleni[1], N. Pinto da Cunha[1], M. Santos[2], M. Florenti[3], M. Caniatti[1], P. Roccabianca[1].** [1]Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, University of Milan, Italy; [2]Instituto de Ciências Biomédicas de Abel Salazar, Porto, Portugal; and [3]Clinica Veterinaria Gran Sasso, Milan, Italy.

33 *CYTAUXZOON* SP. INFECTION IN CATS FROM NORTHEASTERN ITALY. **E. Carli[1], M. Trotta[1], R. Chinelli[2], S. Tasca[1], T. Furlanello[1], L. Solano-Gallego[3].** [1]Private Laboratory San Marco, Padua, Italy; [2]DVM, Trieste, Italy; and [3] Royal Veterinary College, London, UK.

34 COMPARISON BETWEEN ADVIA 120 AND MANUAL LEUKOCYTE DIFFERENTIAL COUNTS. **F. Riondato, E. Maggi, B. Miniscalco.** Dipartimento di Patologia Animale, University of Torino, Italy.

35 CYTOLOGICAL ACCURACY IN THE DIAGNOSIS OF THYMOMA IN RABBITS. **A. Guija de Arespachochaga[1], I. Schwendenwein[1], A. Fuchs-Baumgartinger[2], A. Bilek[3], K. Hittmair[4], J. Hassan[4], F. Künzel[3].** [1]Institute of Immunology: Laboratory Medicine, [2]Institute of Pathology and Forensic Medicine, [3]Clinic of Internal Medicine and Infectious Diseases, and [4]Clinic of Diagnostic Imaging, Veterinary University of Vienna, Austria.

36 LARGE GRANULAR LYMPHOCYTOSIS IN DOGS WITH BABESIOSIS. **A. Guija de Arespacochaga[1], W. Gerner[2], I. Schwendenwein[1], M. Leschnik[3].** Institute of Immunology: [1]Laboratory Medicine and [2]Clinical Immunology and [3]Clinic of Internal Medicine and Infectious Diseases, Veterinary University Vienna, Austria.

37 THE MOLECULAR INVESTIGATION OF WIDESPREAD *SALMONELLA* SEROVARS, *S. TYPHIMURIUM* AND *S.ENTERITIDIS*, INVOLVED IN *SALMONELLA* INFECTION OF CATTLE AND SHEEP ON A FARM AROUND TEHRAN, IRAN. **F. Ghazisaeedi[1], N. Atyabi[1], T. Zahraei Salehi[2], I. Ashrafi[2].** [1]Clinical Pathology Department and [2]Microbiology Department of Veterinary Medicine, Faculty of Tehran University, Tehran, Iran.

38 EVALUATION OF SERUM HAPTOGLOBIN OF NEWLY CALVED HEALTHY HEIFERS AND THOSE SUFFERING FROM SUBACUTE AND ACUTE METRITIS. **N. Atyabi, F. Gharagozlu, Z. Bolandnaza, M. Hasemian.** College of Veterinary Medicine, University of Tehran, Tehran, Iran.

39 ANALYTICAL BLOOD CHANGES IN *LEISHMANIA*-POSITIVE DOGS: A RETROSPECTIVE STUDY. **R. Barrera, D.M. Beristain, J. Duque, P. Ruiz.** Departamento de Medicina Animal, Universidad de Extremadura, Cáceres, Spain.

40 PLASMA FIBRINOGEN AND IRON CONCENTRATIONS VERSUS LEUKOCYTE RESPONSE AS INDICATORS OF SYSTEMIC INFLAMMATORY DISEASES IN HORSES. **R. Barrera, M. Martín, P. Ruiz, V. Vieitez, D.M. Beristain, J. Duque, L.J. Ezquerra.** Departamento de Medicina Animal, Universidad de Extremadura, Cáceres, Spain.

41 DETERMINATION OF FRACTIONAL EXCRETION AND URINARY OSMOLALITY IN DOGS WITH CHRONIC KIDNEY DISEASE. **D.M. Beristain, C. Zaragoza, C. Rodríguez, J. Duque, P. Ruiz, R. Barrera.** Departamento de Medicina Animal, UEx, Cáceres, Spain.

42 LIMITS OF STANDARDISATION OF PROTHROMBIN TIME MEASUREMENTS IN CANINE SAMPLES. **R. Mischke.** Small Animal Clinic, Hannover School of Veterinary Medicine, Hannover, Germany.

43 A SURVEY OF THE RELATIONSHIP BETWEEN MORPHOLOGICAL CHANGES OF WHITE BLOOD CELLS AND SOME BIOCHEMICAL PARAMETERS IN CALVES INFECTED BY *SALMONELLA* AND OTHER PATHOGENS IN IRAN. **F. Aslani[1], Z. Khaki[2], T. Zahrahi Salehi[3].** [1]Islamic Azad University, Tehran, Iran; and Departments of [2]Clinical Science and [3]Pathobiology, Faculty of Veterinary Medicine University of Tehran, Iran.

44 PATHOLOGICAL FINDINGS DUE TO *N. CANINUM* IN THE BRAIN OF BOVINE ABORTED FETUSES AND PLACENTA FROM SEROPOSITIVE DAIRY CATTLE. **H. Haddadzadeh[1], N. Salehi[1], P. Kazraee nia[2].** Departments of [1]Parasitology and [2]Clinical Science/Section of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

45 A CLINICAL PATHOLOGY STUDY OF PIGEON TUBERCULOSIS. **P. Khazraiinia, J. Ashrafi Halan, M. Ahmadi, H.A. Mahpeikar, P. Yousefi.** Veterinary Faculty, University of Tehran, Tehran, Iran.

46 EFFECT OF DIETARY SUPPLEMENTATION OF XANTHURENIC ACID, KYNURENIC ACID, ANTHRANILIC ACID, NICOTINIC ACID AND TRYPTOPHAN ON LIPID RESPONSES IN HYPERLIPEMIC GUINEA PIGS.

M. Abbasalipour Kabir rah[1], P. Asadian[2], F. Asadi[1], N. Atyabi[3], A. Shahriari[4]. [1]Department of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Department of Clinical Pathology, School of Veterinary Medicine, Lorestan University, Khoramabad, Iran; [3]Department of Clinical Pathology, School of Veterinary Medicine, University of Tehran, Tehran, Iran; and [4]Department of Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran.

47 SERUM LIPID AND LIPOPROTEIN PARAMETERS OF PERSIAN BUFFALO. **F. Asadi[1], A. Rezaee[1], P. Asadian[2], M. Pourkabir[1], A. Shahriari[3].** [1]Department of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Department of Clinical Pathology, School of Veterinary Medicine, Lorestan University, Khoramabad, Iran; and [3]Departmaent of Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran.

48 ALTERATIONS IN LIPID PARAMETERS IN RESPONSE TO KYNURENIC ACID, XANTHURENIC ACID, ANTHRANILIC ACID AND NICOTINIC ACID IN HEPG2 CELLS. **P. Asadian[1], F. Asadi[2], N. Atyabi[3], A. Shahriari[4], M. Pourkabir[2].** [1]Department of Clinical Pathology, School of Veterinary Medicine, Lorestan University, Khoramabad, Iran; [2]Departmaent of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran, Iran; [3]Department of Clinical Pathology, School of Veterinary Medicine, University of Tehran, Tehran, Iran; and [4]Department of Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran.

49 ELECTROPHORETIC PATTERN OF HEMOGLOBIN IN IRANIAN HORSES: TURKMAN, IRANIAN ARAB, CASPIAN PONY, DARESHORI, AND KURD. **A. Shahriari[1], F. Asadi[2], M. Shahabzadeh[2], P. Asadian[3], M. Pourkabir[2].** [1]Department of Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran; [2]Department of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran, Iran; and [3]Department of Clinical Pathology, School of Veterinary Medicine, Lorestan University, Khoramabad, Iran.

50 LYMPHOCYTE SUBSET REFERENCE RANGES IN SPANISH PUREBRED HORSES. **K. Satué[1], A. Hernández[1], C. Lorente[2].** [1]CEU-Cardenal Herrera University, Moncada-Valencia, Spain; and [2] Dermatology Clinic (ADERVET), Madrid, Spain.

51 HEMATOLOGY IN THE ANDALUSIAN BROODMARE OF CARTHUSIAN STRAIN: EFFECTS OF PREGNANCY, AGE OF THE MARE, SEX OF FETUS AND MATING SEASON. **K. Satué, A. Muñoz.** CEU-Cardenal Herrera University, Moncada-Valencia, Spain.

52 IS THERE A NEED FOR IRON SUPPLEMENTATION IN SPANISH PUREBRED MARES DURING PREGNANCY? **K. Satué, P. Montesinos.** CEU-Cardenal Herrera University, Moncada-Valencia, Spain.

53 SERUM RENIN, ANGIOTENSIN II AND ALDOSTERONE CONCENTRATIONS IN SPANISH MARES DURING PREGNANCY. **K. Satué, R. Domingo.** Animal Medicine and Surgery Department, CEU-Cardenal Herrera University, Valencia, Spain.

54 HEMATOLOGICAL PROFILE IN SPANISH PUREBRED HORSES: EFFECTS OF AGE AND GENDER. **K. Satué[1], A. Hernández[1], C. Lorente[2] .** [1]CEU-Cardenal Herrera University, Moncada-Valencia, Spain; and [2]Dermatology Clinic (ADERVET), Madrid, Spain.

55 COMPARISON OF THREE DIFFERENT METHODS TO DETECT IN VITRO ACTIVATED PLATELETS IN WHOLE BLOOD OF RATS. **A. Pankraz[1], D. Ledieu[2], D. Pralet[2], A. Moritz[3], A. Provencher-Bolliger[4].** [1]Biocontrol Veterinary Laboratories, Ingelheim, Germany; [2]Clinical Pathology, Preclinical Safety, Novartis Institutes for BioMedical Research, Basel, Switzerland; [3]Department of Veterinary Clinical Sciences, Clinical Pathophysiology, and Clinical Pathology, Justus-Liebig University; and [4]Charles River Laboratories, Preclinical Services, Quebec H9X 3R3, Canada.

56 EVALUATION OF A PORTABLE GLUCOSE METER FOR USE IN SHEEP. **P.D. Katsoulos[1], M.A. Karatzia[2], K. Pourliotis[2], A. Minas[3], G. Christodoulopoulos[1].** [1]Clinic of Medicine, School of Veterinary Medicine,

University of Thessaly, Karditsa, Greece; [2]Clinic of Farm Animals, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; and [3]Laboratory of Microbiology, Department of Medical Laboratories, Technological Educational Institution of Larissa, Larissa, Greece.

57 SERUM IRON CONCENTRATIONS IN HORSES COVERING DIFFERENT DISTANCES IN ENDURANCE EVENTS. **A. Muñoz[1,2], C. Riber[1,2], P. Trigo[2], K. Satué[3], F.C. Castejón[2].** [1]Animal Medicine and Surgery Dept., [2]Equine Sport Medicine Centre, CEMEDE, University of Córdoba; and [3]Animal Medicine and Surgery Dept., Cardenal Herrera-CEU University, Valencia, Spain.

58 AGE AND GENDER SHOULD BE CONSIDERED WHEN INTERPRETING SERUM CONCENTRATIONS OF IGF-1 IN SPANISH FOALS. **C. Riber[1,3], A. Muñoz[1,3], K. Satué[2], P. Trigo[3], F.C. Castejón[3].** [1]Animal Medicine and Surgery, Córdoba University, Spain; [2]Animal Medicine and Surgery, Cardenal Herrera-CEU University, Valencia, Spain; and [3]Equine Sport Medicine Centre, Córdoba University, Spain.

59 COULD SERUM IRON BE USED AS A MARKER OF MUSCLE INFLAMMATION IN ENDURANCE HORSES WITH EXERTIONAL RHABDOMYOLYSIS? **A. Muñoz[1,2], C. Riber[1,2], P. Trigo[2], K. Satué[3], F.C. Castejón[2].** [1]Medicine and Surgery Dept and [2]Equine Sport Medicine Centre, Córdoba University, Spain; and [3]Medicine and Surgery Dept., Cardenal Herrera-CEU University, Valencia, Spain.

60 REFERENCE VALUE ADVISOR: FREEWARE TO HELP COMPUTE REFERENCE LIMITS. **A. Geffré[1], D. Concordet[2], J.P. Braun[1,2], C. Trumel[1].** [1]Département des Sciences Cliniques (Groupe de Recherche en Animaux de Compagnie) and [2]UMR 181 Physiopathologie et Toxicologie Expérimentales, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France.

61 ANTIAGGREGANTS FOR PLATELET COUNTING IN FELINE BLOOD. **F. Granat[1], A. Geffré[1], J.P. Braun[1, 2], C. Trumel[1].** [1]Département des Sciences Cliniques Groupe de Recherches Animaux de Compagnie and [2]UMR 181 Physiopathologie and Toxicologie Experimentales INRA, ENVT, Ecole Nationale Vétérinaire, Toulouse, France.

62 EVALUATION OF THE EFFECT OF THE COLLECTION REGIME ON RENAL URINARY BIOMARKERS. **M. Pinches, C. Betts, S. Bickerton, L. Burdett, N. Derbyshire, H. Jones, H. Caddick, M. Moores, S Sourial.** Safety Assessment AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK.

63 COMPARISON OF THREE DIFFERENT METHODS OF URINE CANINE SEDIMENT PREPARATION FOR MICROSCOPIC ANALYSIS. **C. Layssol[1], A. Geffré[1], J.P. Braun[1,2], C. Trumel[1].** [1]Department of Clinical Sciences, Groupe de Recherche des Animaux de Compagnie and [2]UMR 181 Physiopathologie and Toxicologie Expérimentales INRA, Ecole Nationale Vétérinaire, Toulouse, France.

64 HEMATOLOGY AND PLASMA BIOCHEMISTRY REFERENCE VALUES IN THE FREE-LIVING BLACK VULTURE *(AEGYPIUS MONACHUS)* IN THE DADIA FOREST RESERVE, THRACE, GREECE. **M. Kritsepi-Konstantinou[1], A. Komnenou[2], I. Georgopoulou[3], A.-L. Thomas[2], D. Vasilakis[4], Th. Skartsi[4].** [1]Diagnostic Laboratory, [2]Clinic of Companion Animal Medicine, and [3]Clinic of Avian Medicine, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece; and [4]Dadia Project, WWF Greece, Athens, Greece.

65 STABILITY OF HEMATOLOGY ANALYTES IN FELINE BLOOD STORED FOR 2 DAYS AT ROOM TEMPERATURE. **N. Bourgès-Abella[1], F. Granat[1], A. Geffré[1], J.P. Braun[1,2], C. Trumel[1].** [1]Département des Sciences Cliniques Groupe de Recherches Animaux de Compagnie and [2]UMR 181 Physiopathologie and Toxicologie Experimentales INRA, ENVT, Ecole Nationale Vétérinaire, Toulouse, France.

66 STABILITY OF HEMATOLOGY ANALYTES IN CANINE BLOOD STORED FOR 2 DAYS AT ROOM TEMPERATURE. **N. Bourgès-Abella[1], P. Deshuilliers[1], A. Geffré[1], J.P. Braun[1,2], C. Trumel[1].** [1]Département des Sciences Cliniques Groupe de Recherches Animaux de Compagnie and [2]UMR 181 Physiopathologie and Toxicologie Experimentales INRA, ENVT, Ecole Nationale Vétérinaire, Toulouse, France.

Oral Platform Presentations

1

CLINICAL APPLICABILITY OF A POINT-OF-CARE PATIENT-SIDE CANINE-SPECIFIC COMMERCIALLY AVAILABLE QUANTITATIVE ASSAY FOR DETERMINATION OF C-REACTIVE PROTEIN. **M. Kjelgaard-Hansen.** Department of Small Animal Clinical Sciences, University of Copenhagen, Copenhagen, Denmark.

Background: Canine C-reactive protein (CRP) is a major acute phase protein in dogs, and is reported as a sensitive, specific and quantitative marker of systemic inflammation. Canine CRP is especially useful for detection of inflammation during establishment of diagnosis and for monitoring inflammatory activity during treatment. Laboratory-based methods are available; however, reliable quantitative methods for patient-side operation are warranted. Objective**:** Evaluation of a novel point of care (POC) canine-specific commercially available quantitative assay for determination of CRP. Methods: CRP was determined by a commercially available magnetic permeability-based two-site immunoassay for canine C-reactive protein (LifeAssays, Sweden). Intra- and inter-assay imprecision was assessed by running pooled canine serum with low (37 mg/L) and high (89 mg/L) concentrations of CRP repetitively (n = 7) within-day and across days, respectively. Linearity was investigated by an equal-step dilution (n = 8) of a high content sample (180 mg/L). Inaccuracy was investigated by method comparison (range 10–182.4 mg/L, n = 17) and by 'spike and recovery'. A previously validated automated immunoturbidimetric assay (Kjelgaard-Hansen et al., 2003) was used for method comparison. Purified canine CRP (LifeDiagnostics, USA) was used to spike the low pool to final concentrations of A) 103 mg/L and B) 152 mg/L. Results: Acceptable intra-assay coefficient of variation (CV %) (9.6% and 8.5%) and inter-assay imprecision (12% and 11%) were observed for the low and high pool, respectively. Linearity was acceptable. Method comparison revealed a proportional overestimation above 100 mg/L, confirmed by recoveries of 115% and 127% for A and B, respectively. Conclusions: The quantitative patient-side POC canine specific CRP assay performed acceptably for clinical purposes; however, direct comparison with results obtained by other methods should be made with care. Patient-side POC operation and short run-time (15 min) should facilitate routine use.

2

HOMOCYSTEINE IN CANINE SERUM INCREASES IN SEVERE KIDNEY DISEASE BUT NOT IN INFLAMMATORY BOWEL DISEASE. **G. Rossi[1], S. Breda[1], A. Giordano[1], A. Zatelli[2], S. Paltrinieri[1].** [1]Dept. of Veterinary Pathology Hygiene and Public Health, University of Milan, Italy; and [2]Veterinary Clinic Pirani, Reggio Emilia, Italy.

Background: In humans, homocysteine (Hcy) increases in inflammatory bowel disease (IBD) and chronic kidney disease (CKD). In a preliminary study hyperhomocysteinemia (HHcy) was detected in dogs with CKD (which, however, was not staged) but not with gastrointestinal disorders different from IBD. Objective: To assess whether dogs with IBD have HHcy and whether HHcy is associated with the severity of IBD or of CKD. Methods: This study was done on 111 serum samples from 98 dogs (6 of which were repeatedly sampled during the follow-up). Based on clinical chemistry, haematology, urinalysis, and histopathology of intestinal biopsies, dogs were classified as controls (n = 16), IBD (n = 18) or CKD (n = 64). The latter were subgrouped based on serum creatinine concentration, urine protein/creatinine ratio and blood pressure, as suggested by the International Renal Interest Society (IRIS). Results: Hcy values were not significantly different between controls (mean ± SD = 6.2 ± 2.7; median = 6.3 μmol/L) and dogs with IBD (6.5 ± 3.6; 6.1, regardless of the type of lesions) or CKD (9.7 ± 10.4; 6.7). Among dogs with CKD, Hcy was significantly higher ($P < 0.05$) in dogs with stage IV CKD (12.9 ± 12.8; 9.5), proteinuria (11.3 ± 12.4; 7.0) or hypertension (16.6 ± 15.2; 13.1). During follow-up, Hcy decreased in dogs with improvement of creatinine or blood pressure. Conclusions: In spite of histological changes usually associated with malabsorption, canine IBD is not associated with HHcy. By contrast, HHcy is present in dogs with severe CKD or with CKD associated with proteinuria or hypertension. The potential role of Hcy as a prognostic factor in patients with CKD merits further investigation.

3

PERFORMANCE EVALUATION OF THE SYSMEX POCH-100IV DIFF HEMATOLOGY ANALYZER FOR CANINE, FELINE, EQUINE AND BOVINE BLOOD. **B. Riond, S. Weissenbacher, R. Hofmann-Lehmann, H. Lutz.** Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland.

The Sysmex PocH-100iV Diff is one of the recent automated hematology analyzers developed for use in veterinary practice using impedance technology only. Apart from a CBC, a three-part WBC differential (lymphocytes, eosinophils and "others") is available for dogs and cats. A two-part differential (lymphocytes and "others") is provided for equine and bovine samples. The purpose of this study was to validate the PocH-100iV Diff for its use in blood from diseased dogs, cats, horses and cattle. Fresh EDTA-blood samples (115 dogs, 94 cats, 91 horses and 78 cattle) were analyzed on the PochH-100iV (software version 17) and the Cell-Dyn 3500. Results from the WBC differential were compared with a manual differential. Accuracy, precision, linearity and carryover of the PocH-100iV Diff were assessed. Coefficients of correlation, the intercept and slope with 95% confidence intervals calculated by Passing-Bablok regression, and biases with 95% limits of agreement using Bland-Altmann plots were reported for each analyte and species. In all species tested, most of the CBC results obtained by the PocH-100iV correlated very well with the Cell-Dyn 3500. Slightly lower correlation was observed for canine MCV and hemoglobin. Feline platelets showed a very strong negative bias. The mixed cell population "others" correlated very well in all tested species. Lymphocytes correlated well in horses and cattle, whereas feline and canine lymphocytes correlated less well. Moderate correlation was found in feline eosinophils. Canine eosinophils were overestimated by the instrument. The overall performance of the Sysmex PocH-100iV Diff is excellent with limitations for feline platelet counts. The provided WBC differential is an accurate screening tool for veterinarians, in conjunction with the evaluation of a blood smear.

4

REFERENCE VALUES OF BLOOD COMPONENTS IN HIGH-PRODUCING GOATS. **B. Siliart[1,2], L. Jaillardon[1,2], L. Martin[1], H. Dumon[1].** [1]Endocrinology and Nutrition Unit and [2]Hospital Clinical Laboratory, Department of Biology and Pathology, National Veterinary School, Nantes, France.

Background: The metabolic profile of high-producing cows is well known and can be used to evaluate the risk of acidosis, ketosis and periparturient disease. Reference values of biological parameters, which could be used to determine the metabolic profile of high-producing goats, have never been published. We determined the reference values of some major biochemical parameters and their variations during lactation and gestation in goats. Protocol: Five herds were selected. In each herd, 25 healthy (< 300,000 cells/mL milk), high-producing (between 700 and 850 kg milk) goats, between the second and fourth lactation were selected for blood sampling. Blood was collected monthly for 12 months. Metabolic profiles were established for 20 blood components. Results (percentiles 2.5/97.5, data non-normally distributed): haemoglobin (Hb) 4.4/7.2 mmol/L, packed cell volume (PCV) 21/33%, glucose (Glc) 1.1/3.3 mmol/L, cholesterol (C) 1.8/4.9 mmol/L, triglycerides (TAG) 0.1/0.9 mmol/L, NEFA 0/0.7 mmol/L, urea (U) 3.3/11.6 mmol/L, creatinine(C) 53/106 µmol/L, total protein (TP) 61/90 g/L, BHB 0.2/1.7 mmol/L, Na^+ 151/162 mmol/L, K^+ 3.8/6.0 mmol/L, Cl^- 106/109 mmol/L, Mg^{++} 0.8/1.3 mmol/L, Ca^{++} 1.8/2.6 mmol/L, $PO4^-$ 1.2/3.2 mmol/L, ASAT 63/165 U/L, ALP 81/1050 U/L, GGT 24/82 U/L, and CK 52/579 U/L. Hb, Ht, C, TAG, TP, BHB, NEFA, Na^+, Cl^-, Mg^{++}, $PO4^-$ were lower ($p < 0.05$) when the ration was richer in hay than in corn silage; Hb and Ht were lower ($p < 0.05$) during the dry period; Glc, TAG, TP, BHB, Na^+, Cl^-, and Ca^{++} were lower during gestation than during the first two thirds of lactation; C and NEFA increased during the dry period. Conclusion: These reference values are useful in evaluating metabolic diseases in goats, but must be interpreted with regard to herd data and physiologic states.

5

PREVALENCE OF GREY EOSINOPHILS IN RACING HOUNDS AND COMPARISON OF MANUAL AND INSTRUMENT COUNTS. **L. Giori[1], S. Gironi[2], P. Scarpa[2], M. Gualtieri[2], S. Paltrinieri[1].** Departments of [1]Veterinary Pathology Hygiene and Public Health and [2]Veterinary Clinical Science, University of Milan, Milan, Italy.

Background: Grey eosinophils (GE), which have been reported in Greyhounds and only occasionally in other breeds, have small non-stained granules and optically empty cytoplasmic vacuoles, likely due to abnormal staining properties rather than structural abnormalities. Objectives: 1) to investigate the prevalence of GE in racing hounds other than Greyhounds; 2) to assess the ability of haematology analyzers to identify GE. Methods: Blood from 20 Greyhounds (GH), 29 Italian Greyhounds (IG) and 24 Whippets (W) was analyzed with the Advia 120 (Siemens) and Sysmex XT2000iV (Sysmex) analysers. Two hundred cells were counted on May Grunwald Giemsa-stained smears by 2 independent observers. Differences in the prevalence of GE in the breeds were assessed using a Pearson's chi-square analysis. Instrumental and manual results were compared to each other using a Wilcoxon test, and the agreement between methods was assessed using Passing & Bablok and Bland-Altman tests. Results: GE were found in all breeds (GH: 9/20, 45%; IG: 10/29, 34.5%; W: 15/24, 62.5%) without significant differences. The percentage of eosinophils in samples containing GE (4.1 ± 2.1, manual differential) was significantly higher ($P < 0.01$) than that recorded by the Advia (3.1 ± 2.4) or Sysmex (3.1 ± 2.2). No significant differences were found in samples without GE ($4.6 \pm 2.6\%$, $4.7 \pm 2.5\%$ and $4.8 \pm 2.8\%$, respectively). A slight constant error between instrumental and manual counts was found on samples containing GE. Results from the two instruments were not always significantly different from each other and had good agreement. Conclusions: GE can be found in blood smears from racing hounds other than Greyhounds. When GE are present, haematology analyzers can underestimate eosinophil counts.

6

AUTOMATIC DIGITAL IMAGE ANALYSIS SYSTEM (CELLAVISION DM96) USED FOR CANINE LEUKOCYTE DIFFERENTIAL COUNTING. **I. Lilliehook[1], H. Tvedten[2].** [1]University Animal Hospital and [2]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.

CellaVision DM96 (CellaVision AB, Lund, Sweden) is a computerized microscope and software system that automatically takes digital photos of leukocytes and structures that might be leukocytes in stained blood smears and pre-classifies them into 16 or more groups. It then displays the photographs on a computer screen and facilitates final classification by a person. We evaluated the CellaVision DM96 as a potentially useful tool for canine leukocyte differential counts in a clinical setting. Blood smears from107 fresh samples from canine patients were stained with May-Grunwald-Giemsa stain and analysed with the DM96. The DM96 was set to count 125 leukocytes. After analysis excellent quality photographs of pre-classified cells were well displayed in different formats. It was possible to enlarge the pictures, compare different cell groups to each other and move cells to proper classifications. On average 174 cells/structures per blood smear were photographed and displayed. The overall agreement of DM96 pre-classification and after reclassification was 78%. On average 38 cells/structures per smear had to be reclassified. Pre-classification was in agreement with final classification for 91% of the lymphocytes, 88% of the neutrophils, 69% of the monocytes and 97% of the NRBCs. The DM96 initially identified only 37% of the eosinophils and 2% of the basophils. CellaVision requires excellent staining quality and a good and consistently placed monolayer area. Weak staining sometimes caused many neutrophils to be erroneously pre-classified as artefacts. DM96 was easy to work with and showed excellent pictures of all cells in spite of the fact that the instrument only has human software. Pre-classification facilitated differential leukocyte counting especially in samples with abnormal cells or leukopenia.

7

ACQUIRED MYELOPEROXIDASE DEFICIENCY IN DOGS. **S. Klenner, J. Richartz, N. Bauer, A. Moritz.** Department of Veterinary Clinical Sciences, Clinical Pathology, and Clinical Pathophysiology, Justus-Liebig-University Giessen, Giessen, Germany.

Background: ADVIA 120/2120 haematology analyzers differentiate leukocytes according to their cellular volume and

myeloperoxidase activity. Shifting of the neutrophil population in the monocyte cluster and/or a low mean peroxidase activity index (MPXI) are indicative of neutrophil myeloperoxidase deficiency (MPOD), which has been described in humans but rarely in dogs. The aim of our study was to characterize disease conditions associated with acquired MPOD in dogs and to investigate the diagnostic use of the MPXI compared with the visual evaluation of ADVIA scattergrams. Methods: Peroxidase scatter plots indicative of MPOD were reviewed and the severity of MPOD was classified semiquantitatively in three groups: MPOD grade 1 (MPOD-1): all neutrophils correctly classified but abnormal shift of the population; MPOD-2: approximately 50% of the neutrophils extending into the monocyte cluster and therefore misclassified; and MPOD-3: all neutrophils misclassified due to their location in the monocyte cluster. Sex, age, breed, diagnosis, and MPXI were recorded. Results: 34 dogs with 43 analyses consistent with MPOD were found. Dogs included 11 females and 23 males of 23 different breeds. Age varied from 2 months to 14 years. Diseases were characterized by leukocyte consumption and included parvovirus infection (10/34), DIC/sepsis (5/34), pyometra, pyothorax, aspiration pneumonia, pancreatic abscess, and septic cystitis. Seventeen dogs were classified as MPOD-1 (MPXI = 16.95 ± 9.2 [mean ± standard deviation]), 16 as MPOD-2 (MPXI = 10.08 ± 9.3), and 10 as MPOD-3 (MPXI = 6.8 ± 11.8). Between the means of the MPXI in MPOD-1 and MPOD-3 a significant difference ($P < .05$) could be found. Conclusion: Acquired MPOD occurs in dogs, especially in patients with diseases associated with neutrophil consumption or impaired production. The diagnostic use of the MPXI for detection of MPOD is limited by its high standard deviation.

8

BIOCHEMICAL AND ENDOCRINE PROFILE OF 78 OBESE CATS. **L. Jaillardon[1,2], B. Siliart[1,2], L. Martin[1].** [1]Endocrinology and Nutrition Unit and [2]Hospital Clinical Laboratory, Department of Biology and Pathology, National Veterinary School, Nantes, France.

Obesity in pets is increasing and seems to be a key health problem in cats. Many biological disorders have been proven to be associated with obesity. We therefore aimed to compare biochemical and hormonal profiles in obese and lean cats. Seventy-eight obese (group OB) and 88 healthy (group H) cats were included in the study. Most obese cats (41 females, 33 spayed; 34 males, 33 castrated) were common domestic cats (94.4%) ranging from 1 to 14 years old (7.1 ± 3.04 years). Mean body weight was 8.3 ± 1.8 kg (from 4 to 13 kg). The biochemical profile was unremarkable. Prolactin (PRL = 30.3 ± 33.2 ng/mL), leptin (LEP = 14 ± 11.5 ng/mL) and insulin-like growth factor (IGF1 = 555 ± 250 ng/mL) were significantly higher ($p < 0.001$) in OB than in H (PRL = 3 ± 3.7 ng/mL, LEP = 3.6 ± 1.4 ng/mL, IGF1 = 248 ± 82.5 ng/mL) as well as free thyroxine (FT4 = 22.9 ± 4.8 pmol/L for OB versus FT4 = 21.1 ± 6.2 for H $p = 0.02$).There was no significant difference for cortisol both before and after an ACTH stimulation test between OB (106.3 ± 66.4 and 203.2 ± 88.1 nmol/L) and H (102.2 ± 68.8 and 208.9 ± 116.8 nmol/L). Mean insulin concentration in OB was 25.7 ± 53.7 µUI/mL and was not correlated with glucose concentration (1.54 ± 0.78 g/L, $p = 0.6$). Feline obesity is associated with significant increase of prolactin, leptin, IGF1 and FT4. As these hormones are strongly implicated in adipose tissue development and/or glucose homeostasis, it would be interesting to assess the prognostic value of these parameters and their relationship to the onset of diabetes mellitus.

9

NEW INDIRECT ELISAS FOR DIAGNOSIS OF ENZOOTIC BOVINE LEUKOSIS. **S. Safi[1], B. Mohammadi[2], F. Hemmatzadeh[3].** [1]Department of Clinical Pathology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Tehran, Iran; [2]Islamic Azad University, Tehran, Iran; and [3]Department of Microbiology, Faculty of Veterinary Medicine, Tehran University, Iran.

Enzootic bovine leukosis (EBL) is a common retroviral infection in cattle, which is widely distributed in the cattle farms of many countries. Once a cow is infected it remains a virus-carrier for life, and such a state is correlated with a specific antibody, which is detectable by several serological tests. In the present study, two new indirect ELISAs were developed, based on various antigens including: 1) FLK cells lysed chemically (LYS) and 2) supernatant from infected cultures (SUP). To compare these ELISAs with a commercial reference kit (Svanova, Sweden), 360 serum samples were collected from adult cows and tested using the ELISAs and the reference kit. In comparison with the reference kit, the LYS ELISA had a sensitivity (Se), specificity (Sp), negative predictive value (NPV) and positive predictive value (PPV) of 84%, 70%, 83% and 70%, respectively. The correlation between the two tests was 68%. For SUP ELISA Se of 83%, Sp of 87%, NPV of 83% and PPV of 88% were obtained. The correlation between the two tests was 81%. The SUP ELISA had acceptable Se, Sp, NPV and PPV with the reference kit and can be used as an alternative test in monitoring and control programs considering its reasonable cost.

10

BABESIA GIBSONI INFECTION IN ISRAEL. **G. Baneth[1], T. Molad[2], Y. Yisaschar-Mekuzas[1], E. Yas[1], G. Segev[1], L. Fleiderovich[2], V. Shkap[2].** [1]School of Veterinary Medicine, Hebrew University of Jerusalem, Israel; and [2]Division of Parasitology, Kimron Veterinary Institute, Israel.

Reports of canine babesiosis from Israel have included documentation of only large *Babesia* forms and genetic characterization has identified *B. canis vogeli*. A fourteen-year-old mongrel dog, brought from Hong Kong to Israel 12 years earlier, was referred to the Hebrew University and splenectomized due to neoplasia. It was readmitted with anemia, spherocytosis and thrombocytopenia a month later. Following the tentative diagnosis of immune-mediated anemia and thrombocytopenia, treatment was initiated with immunosuppressive doses of prednisone. It was presented again with a hemolytic crisis and forms of a small *Babesia* species were detected in blood smears a week later. PCR assays targeting a fragment of the *Babesia*18S rRNA gene and the ribosomal internal transcribed spacer and sequencing confirmed that the dog was infected with *B. gibsoni*. *B. gibsoni* infection is endemic in Southeast Asia and has been reported sporadically in Europe. Pitt Bull terriers are frequently infected and may be subclinical carriers. In order to investigate if *B. gibsoni* is prevalent in dogs in Israel and whether disease in the infected dog was likely to have been imported or autochthonous, a PCR survey that included Pitt Bull terriers (n = 24) and dogs from rural villages (n = 159) was done. Twelve (7.5%) of the dogs from the rural villages and none of the Pitt Bull terriers were positive for *Babesia*. Sequencing confirmed that all dogs were infected with *B. canis vogeli*. The lack of previous reports of *B. gibsoni* in Israel and the preliminary results of this study suggest that *B. gibsoni* is probably not endemic in Israel

and that the first described case of this infection was likely to have been imported.

11

ASSESSMENT OF LACTATE DEHYDROGENASE (LDH), ALKALINE PHOSPHATASE (ALP) AND ASPARTATE AMINOTRASFERASE (AST) ACTIVITIES IN MILK FOR THE DIAGNOSIS OF SUBCLINICAL MASTITIS IN SHEEP AND GOATS. **P.D. Katsoulos[1], G. Christodoulopoulos[1], A. Minas[2], K. Pourliotis[3], M.A. Karatzia[3], S.K. Kritas[4].** [1]Clinic of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece; [2]Laboratory of Microbiology, Faculty of Health Professions, Technological Educational Institution of Larissa, Larissa, Greece; [3]Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; and [4]Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Objective: To evaluate whether the determination of LDH, ALP and AST in milk is valuable for the diagnosis of subclinical mastitis in sheep and goats. Materials and Methods: 217 milk samples (109 from sheep and 108 from goats) from normal and 151 samples (97 from sheep and 54 from goats) from subclinically infected udder halves were used. LDH, ALP and AST were determined using enzymatic kinetic methods in the milk. The optimum cut-off values, offering the highest diagnostic sensitivity (DSn) and diagnostic specificity (DSp), for each enzyme were determined by ROC analysis. Results: LDH activity in milk was identified as the best indicator of subclinical mastitis in both species. The optimum cut-off values for LDH activity were determined at 197 U/l, 185 U/l and 197 U/l for sheep, goats and both species, respectively. The DSns for sheep, goats and both species for these cut-off values were 92.8%, 98.1% and 94.0%, whereas DSps were 95.4%, 96.3% and 96.3%, respectively. Conclusion: LDH activity in milk can be used for the laboratory assessment of subclinical mastitis in sheep and goats.

12

STEP-BY-STEP INTRODUCTION OF QC VALIDATION AND APPLICATION OF SIGMA METRICS USING THE HAEMATOLOGY ANALYSER CELL-DYN 3500 AS AN EXAMPLE. **E. Leidinger[1], E. Hooijberg[1], K.P. Freeman[2].** [1]InVitro Veterinary Laboratory, Vienna, Austria; and [2]IDEXX Laboratories, Grange House, Wetherby, West Yorkshire, UK.

QC validation is the approach to evaluation of the whole process of statistical quality control (QC). The purpose of QC validation is to determine a) whether statistical QC procedures are appropriate for detecting medically important errors and b) the quality of performance required by different tests. QC validation is documented in a few papers in the veterinary literature, but there is little information on how to apply QC validation in practice. The purpose of this study is to present an easy-to-follow, stepwise introduction to EZ-Rules 3 (Westgard)-software-based QC validation and performance of sigma metrics as flow diagrams. The QC validation is carried out according to the following five steps: 1. Define the quality required for a test (e.g., WBC) as total allowable error (TE_a) or clinical decision level, derived from the literature. 2. Determine method imprecision (CV) and inaccuracy (bias) for the methods of choice and calculate the total error. 3. Identify candidate internal quality control (IQC) procedures by OPSpecs charts. 4. Predict IQC performance by the criteria: probability of error detections (P_{ed}), probability of false rejection (P_{fr}) and critical systematic or random error. 5. Calculate the sigma metrics; set performance goals and select appropriate IQC procedures. The whole validation process is explained using the example of the Cell-Dyn 3500 (Abbott) haematology analyser. We explain how the process of QC validation was incorporated into the existing ISO 9001:2000 QMS, how improvements can be achieved by applying the process of QC validation, and where the limitations, mainly based on the analyser's performance for certain parameters and the quality control material used, are seen.

13

EVALUATION OF 9 NOVEL RENAL URINARY BIOMARKERS. **M. Pinches, C. Betts, S. Bickerton, L. Burdett, N. Derbyshire, H. Jones, H. Caddick, M. Moores, S. Sourial.** Safety Assessment AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK.

Background: Numerous novel urinary biomarkers have been proposed for the detection and monitoring of renal injury in the rat. Recently new multiplex kits containing assays for these biomarkers have become widely available for the first time. Objective: The aim of this study was to evaluate over time and against histopathologic changes, 5 traditional (plasma urea, plasma creatinine, urinary total protein, urinary glucose and urinary NAG) and 9 proposed (albumin, aGST, calbindin d28, cystatin C, GSTYB1, lipocalin, KIM-1, osteopontin, and RPA-1) markers of renal injury in the rat following the administration of a nephrotoxicant. Methods: Multiple groups of 10 male han-wistar rats were given a single dose of 0.1 mg, 1 mg or 2.5 mg Cisplatin. Multiple urine samples were taken at days 2, 3, 5, 8, 15 and 22, and blood sampling and necropsy was performed on days 5, 8 and 22. Results: ROC curves and associated AUC values were generated against the principal pathology of note (S3 segment proximal tubular necrosis) at days 5 and 8. At day 5, biomarker AUCs were KIM-1 (0.97), albumin (0.94), glucose (0.94), osteopontin (0.91), GSTYb1 (0.89), RPA-1 (0.89), lipocalin (0.89), NAG (0.87), aGST (0.83), urine TP(0.82), creatinine (0.82), calbindin d28 (0.81), urea (0.79), and cystatin C (0.67). At day 8, biomarker AUCs were KIM-1 (0.99), RPA-1 (0.90), NAG (0.89), osteopontin (0.86), lipocalin (0.83), albumin (0.83), glucose (0.83), cystatin C (0.71), urea (0.67), GSTYb1 (0.62), creatinine (0.62), urine TP (0.61), aGST (0.56), and calbindin d28 (0.53). By day 22, all biomarker values had returned to levels similar to concurrent control values with the exception of osteopontin and KIM-1, which remained 2.8 and 1.9 fold higher, respectively. **Conclusions:** Many of the proposed novel biomarkers have higher sensitivity and specificity than the traditional biomarkers in this model.

14

APPLICATION OF SIGMA METRICS: A "REVERSE APPROACH". **I. Schwendenwein, C. Johler-Weißenbacher, A. Guija de Arespacochaga.** Division of Clinical Pathology, University of Veterinary Medicine, Vienna.

Individual tailoring of quality control (QC) procedures for each analyte ensures analytical quality and cost efficacy. Overall analytical goals are set by determining the required total allowable

error (TEa) by merging clinical and analytical aspects. In veterinary medicine guidelines such as CLIA recommendations are under development. Thus, a reverse approach applying sigma metrics was chosen. QC data from the Advia 120 (10 analytes, 3 control levels) and the biochemistry analyzer Hitachi 911 (32 analytes, 2 control levels) were accumulated; coefficient of variation and bias were calculated. Then different levels of TEa starting from 5% were evaluated for each analyte and increased stepwise until a good method performance (sigma > 4) was achieved by sigma metrics. Results were compared with published data of critical differences obtained in another study in our laboratory. Control rules were defined by use of operating specifications (OPSpecs) charts. We aimed for a 90% level of error detection. Hematology: 1_{3s} rule can be applied for all analytes, but with a probability for error detection (Ped) of 75% in the low control for platelets and hematocrit, and 80% for haemoglobin in the normal and high level. When a Ped of 90% is required, a $1_{3s}/2$ or $3_{2s}/R_{4s}$ rule can be used. Biochemistry: the 1_{3s} rule can be applied to 26 out of 32 analytes. For 10 analytes, TEa had to be increased. Still, for total protein, albumin, bile acids, sodium, potassium, and chloride, the 1_{2s} rule is necessary. Most of the parameters can be controlled with a 1_{3s} rule. For the remaining, 1_{2s} is necessary to obtain a high Ped. We consider this a time- and cost-saving improvement compared to the previously used multirule procedures.

Poster Presentations

15

STUDY OF THE EFFECTS OF INFECTIOUS BURSAL DISEASE (GUMBORO) IN NATURAL ENVIRONMENTAL CONDITIONS ON SERUM ACUTE PHASE PROTEINS HAPTOGLOBIN AND α-1-ACID GLYCOPROTEIN IN BROILERS. **S. Safi[1], F. Mohammadi[2], M. Rahimi[3], A. Rahimi[4].** [1]Department of Clinical Pathology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Tehran, Iran; [2]Veterinary Group, Islamic Azad University, Kermanshah, Iran; [3]Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran; and [4]Department of Epidemiology & Biostatistics, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

One hundred thirty-eight blood samples, including 118 from poultry infected with infectious bursal disease (Gumboro) and 20 from healthy poultry, were taken from poultry from one of the broiler farms in Kermanshah Province, Iran. Serum samples were submitted for measurement of α-1-acid glycoprotein and haptoglobin and were also used to confirm the disease by ELISA test. The present study showed that the mean ($\pm$SD) concentration of α-1-acid glycoprotein in the healthy group was 437 ± 253 mg/L and in the diseased group was 1558 ± 680 mg/L. The mean ($\pm$SD) concentrations of haptoglobin were 0.10 ± 0.044 g/L and 0.22 ± 0.092 g/L in the healthy and diseased groups, respectively. The mean concentration of α-1-acid glycoprotein in the diseased group was significantly higher than the control group ($P < 0.05$). This increase can be attributed to the direct relationship between serum level of α-1-acid glycoprotein and the severity of pathologic lesions in Gumboro disease and bursa involvement. The haptoglobin level was significantly higher ($P < 0.05$) in the diseased group in comparison to the control group. To the best of the authors' knowledge there is no report on reference intervals for haptoglobin levels in poultry and the findings of the present study can provide novel information in this regard. The increase in haptoglobin levels can be attributed to the pathological lesions in this study.

16

PTX3: A MARKER FOR LOCAL INFLAMMATION? **E. Ramery, F. Bureau, T. Art, P. Lekeux.** Functional Sciences, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

Background: The long pentraxin 3 (PTX3) is a recently discovered soluble protein of the acute phase of inflammation. In contrast to short pentraxins (e.g., CRP, SAP), it is produced at the site of inflammation. Objective: The purpose of this study was to evaluate PTX3 production in the respiratory tract in response to an inflammatory challenge in horses. Methods: Two groups of six horses, healthy or affected by heaves, were submitted to a 10-day hay dust challenge. At days zero and ten, clinical and functional parameters were evaluated, broncho-alveolar lavages (BAL) were collected and BAL cell counts were performed. PTX3 released was evaluated by western blotting in the BAL supernatant. In addition, bronchial sections were obtained from two heaves-affected horses in crisis and two healthy horses. PTX3 production by the different cell types was evaluated by immunohistochemistry. Results: In the healthy group, slight inflammation occurred in the respiratory tract (assessed by function tests and BAL cell counts) with no clinical signs. In the heaves-affected group, an important inflammatory response developed with the typical clinical signs of heaves. Following dust exposure, PTX3 was increased in both groups but the level of PTX3 detected in the supernatant of heaves-affected horses was markedly higher. The immunohistochemistry revealed that macrophages and bronchial epithelial cells were the major cellular sources of PTX3 in the airways. Moreover, epithelial cells of heaves-affected horses contained higher levels of PTX3 than those of healthy horses. Conclusion: PTX3 levels in BAL supernatant may be a good sensor of airway inflammation, even without the presence of clinical signs. Its level of excretion may help in evaluating the severity of inflammation.

17

A RARE CASE OF HAEMANGIOPERICYTOMA IN AN AMAZON PARROT. **P. Loukopoulos, A. Komnenou, V. Tsiouris, M. Zavlaris, A.-L. Thomas, I. Georgopoulou.** Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece.

A 20-year-old male Amazon parrot (*Amazona ochrocephala*) was presented with a history of a painless mass developing over six months. On clinical examination a firm, well defined, 2.5×2 cm mass was palpated at the right pelvic area. Haematology and biochemistry were normal while radiography revealed that there was no bone involvement. The tumour was removed under general anaesthesia. The bird recovered fully and remains tumour-free two years post-surgery. The tumour was ovoid, whitish and moderately hard in cut section. Histologically, the mass was well demarcated and composed of spindle or fusiform cells sparsely arranged in perivascular whorls or in loose interlacing bundles, giving the lesion a translucent appearance. Tumour cells were

mildly pleomorphic with few mitoses. Based on the above, a diagnosis of benign haemangiopericytoma was offered, the first reported in this species. However, haemangiopericytoma, which is usually reported in dogs, is mainly composed of polygonal or spindle cells with uniform, ovoid, plump nuclei in that species. Furthermore, the presence of small wavy spindle cells partly arranged in interlacing bundles, albeit not a dominant feature, was reminiscent of a benign peripheral nerve sheath tumour (BPNST/schwannoma) and allowed this diagnosis to be entertained. For this reason, immunohistochemistry was performed employing a streptavidin-biotin-peroxidase protocol. Tumour cells were positive for S-100 and aSMA but not GFAP. Most cells arranged perivascularly or in bundles were positive for PCNA and vimentin, suggesting that they belong to the same tumour cell population. The immunohistochemical profile was consistent with and further established the diagnosis of haemangiopericytoma. This report demonstrates the value of immunohistochemistry, through the application of a specific panel of antibodies, in differentiating between haemangiopericytoma and BPNST.

18

THE EFFECTS OF STORAGE TIME AND TEMPERATURE ON SELECTED HEMATOLOGICAL VARIABLES IN CANINE BLOOD SAMPLES. **T.S. Maysam[1], J.P. Mohsen[2], M.A. Seyed[3], V.H. Amin[3], A.J. Shahriar[3], M.A. Amir[3], G.A. Seyed[3].** [1]Faculty of Veterinary Medicine, Islamic Azad University, Garmsar, Iran; [2]Faculty of Veterinary Medicine, Ferdowsi University, Mashad, Iran; and [3]Student, Islamic Azad University, Garmsar, Iran.

Background: Most hematological analyses are performed immediately after blood sampling, but occasionally samples are stored in a refrigerator. Objective: The aim of this study was to compare the results of PCV, Hb, RBC, WBC, MCV, and MCH before and after storage at room temperature and in a refrigerator. Methods: Thirty-four K3-EDTA canine blood specimens from animal husbandry centres were analyzed within 2, 12, 24, and 48 hours by a manual method. Blood samples were split into 2 aliquots and stored at 30°C or 5°C. Results: PCV did not change 12 hours after blood sampling in samples stored at room temperature or refrigerated, but increased gradually thereafter under both storage conditions after sampling (1% and 1% increase at 24 hours and 3% and 2% increase at 48 hours after sampling in samples stored at room temperature or refrigerated). The RBC count did not change at 12 and 24 hours after blood sampling, but decreased slightly after 48 hours. The increased RBC count was more prominent in room temperature-stored samples. Hb and MCH remained stable. MCV increased slightly at 24 hours after blood sampling in both room temperature-stored and refrigerated samples. The increase in MCV was more pronounced at 48 hours after blood sampling in comparison with 24 hours. Differences in WBC and platelet counts varied with the specimen, independently of the initial value. Conclusion: Blood samples stored for up to 24 hours in a refrigerator are suitable for cell count and Hb measurement. Although CBCs should be performed immediately after blood sampling, under certain conditions samples can be stored for 24 hours in a refrigerator. However, potential variations have to be known to avoid misinterpretations, especially near the decision limits.

19

CYTOLOGICAL ASPECTS AND HISTOLOGICAL CORRELATION OF EPITHELIAL ODONTOGENIC TUMORS WITHOUT ODONTOGENIC MESENCHYME IN CANINE SPECIES. **U. Bonfanti[1], W. Bertazzolo[2], M. Gracis[3], S. Ferro[4].** [1]Accelera-NMS, Nerviano, Italy; [2]Clinica Veterinaria Tibaldi, Milan, Italy; [3]Clinica Veterinaria San Siro, Milan, Italy; and [4]Faculty of Veterinary Medicine, Padua, Italy.

Background and Objective: Ameloblastoma and acanthomatous ameloblastoma represent tumor originating from odontogenic epithelium without odontogenic mesenchyme. The aim of the study was to describe the cytological features of ameloblastoma and acanthomatous ameloblastoma, and to compare them with histological findings. Materials and Methods: Evaluation of cytological characteristics of oral masses histologically classified as tumors of odontogenic origin was performed. Oral neoplasia involved maxilla (7) and mandible (2). Nine dogs were included in the study. There were 3 mixed-breed dogs, and one each of the following breeds: Fox Terrier, Labrador Retriever, Bobtail, Schnauzer, Schipperkee, Shih-Tzu. Age ranged from 7 months to 17 years. Results: Histological diagnoses included acanthomatous ameloblastoma (6) and ameloblastoma (3). Cytological diagnoses included ameloblastoma (4), acanthomatous ameloblastoma (3), and one low-grade epithelial neoplasia. One sample was hemodiluted. Cytological examination of ameloblastomas revealed tightly packed clusters of small basaloid-type cells, as well as larger interdigitating neoplastic cells showing mild pleomorphism and minimal anisocytosis and anisokaryosis. In cases of acanthomatous ameloblastoma, cytological examination often revealed variably sized clusters of oval to polygonal cells, and sometimes variable nuclear:cytoplasmic maturation asynchrony with characteristics of squamous differentiation. Histologically ameloblastoma was characterized by islands of proliferating odontogenic epithelium with low nuclear atypia. In acanthomatous ameloblastoma cords of odontogenic epithelium showed a more plexiform pattern. Keratinization was sometimes present. Discussion and conclusion: The most striking characteristics of the odontogenic neoplasms consisted of tightly packed clusters of epithelial cells, oval to polygonal in shape. Some cellular atypia and asynchronous maturation of the nucleus and cytoplasm were more commonly detected in acanthomatous ameloblastoma.

20

EVALUATION OF STABILITY OF RAT EDTA BLOOD SAMPLES STORED AT DIFFERENT CONDITIONS. **U. Bonfanti, M. Rocchetti, A.M. Giusti, P. Colombo.** Accelera – Nerviano Medical Sciences, Nerviano, Italy.

Background and Objective: The CBC represents an important diagnostic procedure used in every toxicological study in research animals. The objective of the study was to evaluate possible alterations that may occur in rat blood samples stored at different conditions, in case analysis is delayed for two to three days after sampling. Materials and methods: EDTA blood samples were drawn from 20 healthy Sprague Dawley rats (10 males and 10 females). Samples were analyzed with an ADVIA 120 hematology analyzer within 1 hour after collection (T0), after 24 hours of storage at room temperature, and after 24, 32, 48 and 72 hours of storage at 4°C. The following parameters were considered: RBC, Hgb, HCT, MCV, MCH, MCHC, CHCM, RDW, HDW, reticulocytes, MCVr, CHr, platelets, MPV, PCT, PDW, total WBC and differential

count. After appropriate ANOVA on log-transformed data, 90% simultaneous confidence interval (CI) of the mean ratio of the values observed at the different storage conditions versus the corresponding value at T0 were obtained. Stability of each parameter was evaluated plotting the limits of these CI versus pre-selected reference intervals: 0.8–1.25 (±20%), 0.9–1.1 (±10%), 0.95–1.05 (±5%). Results: For both genders, after 24 hours at room temperature, 6/23, 11/23 and 19/23 parameters showed mean ratio 90% CI within ±5%, 10% and 20%, respectively; after 24 hours at 4°C, 11/23, 14/23 and 19/23; and after 72 hours at 4°C, 5/23, 13/23 and 17/23. Conclusion: Sample storage for 24 hours at 4°C improves the stability for the majority of parameters examined, with respect to storage at room temperature. Storage for up to 72 hours at 4°C allows adequate interpretation of most of the parameters.

21

THE SENSITIVITY AND SPECIFICITY OF FIBRINOGEN AND TOTAL PROTEIN:FIBRINOGEN RATIO IN SCREENING HEALTHY AND DISEASED DAIRY CATTLE AT ABATTOIRS. **Gh. Ghalamkari[1], Sh. Safi[2], S.R. Jafarzadeh[3], R. Sedaghat[4].** [1]Department of Animal Science, Agriculture Faculty, Islamic Azad University, Isfahan, Iran; [2]Faculty of Specialized Veterinary Medicine, Islamic Azad University, Tehran, Iran; [3]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, USA; and [4]Faculty of Medicine, Shahed University, Tehran, Iran.

Fibrinogen is one of the acute phase proteins that changes in response to infection and inflammation. As it is important to discriminate between healthy and diseased cattle during meat inspection, the objective of this study was to measure the sensitivity and specificity of fibrinogen and total protein:fibrinogen ratio of healthy and diseased dairy cattle from abattoirs, using the post-mortem findings as the gold standard. The plasma concentrations of fibrinogen and total protein and the fibrinogen total protein:fibrinogen ratio were determined in 30 healthy dairy cows with no pathological findings and 50 cows with pathologic conditions. The means of fibrinogen and total protein:fibrinogen ratio were significantly different between groups. Using ROC curves, the cut-off points for fibrinogen and total protein:fibrinogen ratio were 0.5 g/dL and 12.6 g/dL, respectively, and the sensitivity and specificity of fibrinogen were 56% and 76.67% and for total protein:fibrinogen ratio were 68% and 76.67%, respectivly. Also, the positive predictive values for fibrinogen and total protein:fibrinogen ratio were 65.15% and 80.40%, respectively. The results of the present study indicated that the total protein: fibrinogen ratio had better sensitivity and specificity than fibrinogen alone and could be used for animal screening at abattoir.

22

MACROTHROMBOCYTOPENIA IN GROUP OF RELATED NORFOLK TERRIERS: A CONDITION SIMILAR TO MACROTHROMBOCYTOPENIA OF CAVALIER KING CHARLES SPANIELS? **G.F. Tutino[1], E. Pogliani[1], W. Bertazzolo[1], M.E. Gelain[2].** [1]Clinica Veterinaria Tibaldi, Milan, Italy; and [2]Department of Veterinary Pathology, Hygiene and Health, University of Milan, Italy.

Asymptomatic inherited macrothrombocytopenia of Cavalier King Charles spaniel (CKCS) is characterized by a low platelet count (less than 100,000/μL), increased mean platelet volume and, as a consequence, a normal plateletcrit. The prevalence of macrothrombocytopenia in CKCS varies from 31–56% with an autosomal recessive type of inheritance. The aim of this work is to describe the presence of a similar macrothrombocytopenia in a small group of related Norfolk terriers. Eight Norfolk Terriers were included (4 males, 3 females). Blood samples were collected in EDTA tubes and a blood smear was immediately obtained for each sample. Samples were analyzed using a quantitative buffy coat analyzer (QBC, VetAutoreader, IDEXX) and an impedance cell counter (IC, HecoVet SEAC) to evaluate RBC, WBC, Hb, HCT and platelet number. On blood smears, evaluation of platelet aggregates and microscopic estimation (ME) of the platelet numbers were performed. All the Norfolk Terriers included in this study were clinically healthy without any history or signs of haemostatic dysfunction. Six dogs had platelet counts less than 100×10^9/L by IC and ME, whereas all dogs had a normal platelet count by QBC. Microscopic evaluation showed macrothrombocytosis only in dogs with low platelet counts. Pedigree analysis cannot reveal the inheritance pattern due to the low number of dogs. The group of Norfolk terriers described here showed a platelet dysplasia similar to CKCS macrothrombocytopenia. The platelet counts obtained through QBC, which is calculated from a measured platelecrit, showed a normal circulating platelet mass despite a low platelet count on IC and ME. This may be explained by the macrothrombocytosis found in NT with a low platelet count on ME and IC.

23

UTERINE LEIOMYOSARCOMA AND PYOMETRA IN A 7-YEAR-OLD DOG. **V.G. Tsioli[1], P.G. Gouletsou[1], P. Loukopoulos[2], M. Zavlaris[2], A.D. Galatos[1].** [1]Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece; and [2]Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Canine malignant uterine tumours are extremely rare. A 7-year-old intact female mongrel dog was presented with a two-day history of reduced appetite, abdominal distention and general dullness. Abdominal palpation revealed the presence of a large mass. Radiography showed a large mass occupying the left mid-abdominal area and loops od mild opacity occupying the right mid-abdominal area. Ultrasonography revealed a large heterogeneous mass with an anechoic area and some hyperechoic foci, indicative of calcification, in the mid-abdominal area. Anechoic areas were also found in the mid- and caudal abdominal area, and were presumed to be the fluid-filled uterus. On laparotomy, a 10.5 × 14.5-cm, firm mass was found in the uterine body while the uterine horns were filled with a thick red-brownish exudate; ovariohysterectomy was subsequently performed. Histologic examination of the mass showed a malignant mesenchymal tumour with cells arranged in large interlacing bundles or streams with little stroma, as well as severe endometritis. Immunohistochemistry was performed employing a streptavidin-biotin-peroxidase protocol. The tumour cells were immunohistochemically positive to desmin and alpha-smooth muscle actin, indicating their smooth muscle origin and establishing the diagnosis of intermediate grade uterine leiomyosarcoma associated with pyometra. The use of immunohistochemistry allowed the differentiation between tumours of smooth muscle origin and other tumour types, particularly fibrosarcoma and poorly differentiated tumours. To the best of our knowledge, this is one of the few cases of uterine leiomyosarcoma described in the dog.

24

CLINICAL EVALUATION OF THE BC-2008 VET HEMATOLOGY ANALYZER FOR USE IN VETERINARY PRACTICE. **C. Masserdotti.** Laboratorio di Analisi Veterinarie San Marco, Padova, Italy.

Background: The BC-2008 VET is a completely automated impedance hematology analyzer, which yields an 18-parameter blood count including a 3-part leukocyte differential. The software provides electronic printable results on a screen, with 3 histograms on the basis of the cell distributions. Objective: The aim of this study was to examine the operational potential of the analyzer and its value for use in veterinary practice. Methods: The analyzer was tested for precision and accuracy. Comparison methods included the H8-SEAC analyzer and a 100-cell manual WBC differential. EDTA-anticoagulated blood samples for comparison of the methods were obtained from 58 dogs and 20 cats. Linear regression and difference plot were used to analyze results. Results: Correlations of RBC and WBC count, HGB and HCT measurement were very good between the BC2008 VET and the H8-SEAC. Platelet counts showed a constant bias both in dogs and cats. Lymphocyte, neutrophil and monocyte differentials compared poorly between the BC2008-VET and manual counts. Conclusions: The BC2800VET is a useful hematology analyzer for clinical purposes. RBC, WBC, HGB and HCT values obtained with canine and feline samples on the BC-2008 VET were highly comparable with another impedance counter (H8-SEAC). The BC-2008VET had moderate precision and accuracy for platelet counts, probably due to software limitations; in fact, it is not possible to manually correct the WBC histogram, where, particularly in the cat, an overlap between platelet aggregates and leukocytes can affect the count. Implementation of the software would improve these results. The main disadvantages of the BC2800VET were poor precision in counting leukocyte subpopulations and poor concordance with manual differentials, as previously observed with all in-office analyzers.

25

CLINICAL PATHOLOGY FINDINGS IN FIVE CATS AFFECTED BY ABSOLUTE ERYTHROCYTOSIS. **A. Gavazza, G. Lubas, B. Gugliucci.** Dept. of Veterinary Clinic, University of Pisa, Italy.

Absolute erythrocytosis is rarely described in cats and can be a primary myeloproliferative disorder of the stem cell that is erythropoietin (EPO)-independent or secondary to disorders involving increased EPO production. From 2004 to 2009 five cases of absolute erythrocytosis were referred to our facility. The diagnostic plan (DP) included a CBC, serum biochemical profile and protein electrophoresis, urinalysis, FIV-FeLV serology, bone marrow (BM) aspirate evaluation, full body radiology, abdominal ultrasound and serum EPO measurement. The most striking findings were: #1 Persian, F, 15 mos - Hct .527 l/l, RBC 11.5×10^{12}/L, Hgb 16 g/dL, RDW 17.2, TP 8.4 g/dL- no additional diagnostic investigation. #2 DSH, M, 42 mos – Hct .513 l/l, RBC 13.2×10^{12}/L, Hgb 17.2 g/dL, RDW 18.9, TP 8.1 g/dL- no additional diagnostic investigation. #3 DSH, M, 30 mos – Hct .527 l/l, RBC 12.8×10^{12}/L, Hgb 16.5 g/dL, RDW 18.3, TP 8.7 g/dL, enlarged spleen removed and histopathologically diagnosed as hemangiosarcoma – mild erythrocytosis (Hct .52 l/l) persisted after the spleen removal for 1 month. #4 DSH, F, 36 mos – Hct .653 l/l, RBC 16.9×10^{12}/L, Hgb 22 g/dL, RDW 21.2, CRP 3.48, PLT 1198 - follow-up: Hct adjusted around .65 l/l for 2.5 years. #5 DSH, F, 54 mos - Hct .747l/l, RBC 16.02×10^{12}/L, Hgb 23.2 g/dL, RDW 19.8, CRP 3, PLT 600, TP 8.1 g/dL - follow-up: Hct adjusted around .60l/l for 2.5 years. In all cases BM exam showed erythroid hyperplasia and EPO levels were 0.1–1 mU/mL (ref int 0.1–5). Case #3 could be related to tumour-related EPO synthesis even if the serum EPO was low (0.19 mU/mL). In cases #4 & #5 reticulocyte counts revealed regeneration both at initial DP and during hydroxyurea (HU) or pipobroman administration. Additionally, during HU therapy Heinz bodies were observed.

26

ASSESSMENT OF MOLYBDENUM STATUS IN DAIRY COWS. **L. Martin, L. Jaillardon, B. Siliart, E. Paniez, H. Dumon.** Laboratoire des Dosages Hormonaux, Ecole Nationale Vétérinaire, Nantes, France.

Molybdenum (Mo) is involved in vital biochemical processes, but excess intake can lead to disease and toxicity. In particular, Mo forms a complex with copper (Cu) and sulphates, which is absorbed but cannot be metabolized and is excreted through the kidney. It is therefore important to determine the total dietary concentration of Mo and Cu to better interpret bioavailability. Objectives: To establish reference values for plasma Mo and Cu for routine control in dairy cows. Superoxide dismutase (SOD) concentrations were also assayed as Cu is involved in defence against oxidative stress. Materials: 111 healthy lactating dairy cows from 8 different herds (2 to 11 yrs old). The rations were balanced with the same mineral mix. Mo and Cu were assayed in plasma by ICP-MS, SOD in whole blood by spectrophotometry (BP 144, Kit Ransod, Randox©), and haemoglobin (Hb) by reflectometry (Reflotron, Scil©). Daily intake of minerals was calculated.Results: (i) Centiles [5%; 95%] were [3.0; 21.0] µg/L for Mo, [825; 1200] µg/L for Cu, and [1235; 2900] U/g Hb for SOD. (ii) Plasma Mo was not correlated with ration composition and stage of lactation but was significantly correlated with milk cell count ($p < 0.0002$). There was a strong herd effect ($p < 0.0001$). (iii) Plasma Mo and Cu were unrelated and were not correlated with blood SOD. (iv) Plasma Mo was not correlated with dietary Cu.Conclusion: reference ranges for plasma Mo in dairy cows are [3–21 µg/L]. There was no correlation between plasma Mo and plasma Cu in apparently healthy animals, and plasma Mo was unrelated to dietary Cu. Nevertheless, Mo concentration is closely related to herd condition and decreases when the cell count in milk increases.

27

IMPACT OF IN VITRO HEMOLYSIS ON KAOLIN-ACTIVATED THROMBELASTOGRAPHY PARAMETERS AND ADVIA 2120 PLATELET ACTIVATION INDICES IN CANINE SPECIMENS. **N. Bauer, O. Eralp, A. Moritz.** Department of Veterinary Clinical Sciences, Clinical Pathology, and Clinical Pathophysiology, Justus-Liebig-University Giessen, Giessen, Germany.

Background: The impact of hemolysis on kaolin-activated thromboelastography (TEG) has not been evaluated before in dogs. Objective: To investigate the impact of two types of hemolysis, i.e., mechanical (HM)/freeze and thawing (HF) on canine kaolin-activated TEG®, platelet count (PLT) and mean platelet component concentration (MPC) reflecting platelet activation. Methods: TEG analyses were performed on blood from 17 healthy dogs. Citrated whole blood samples were divided into

aliquots serving as control, HM, and HF. TEG was performed after re-calcification, 1 hour after sample acquisition. Five TEG variables (R; K angle [α]; MA; G) were evaluated. MPC was measured flow cytometrically with the ADVIA 2120™ hematology analyser. Results: HM and HF resulted in a mean decrease in hematocrit PCV of 6.3% ± 5.4% and 39.3% ± 4.6% compared to controls. PLT decreased significantly after HM ($181 \pm 68 \times 10^9$/L) but did not change significantly after HF. TEG R-value was significantly shorter after HM than in controls (2.5 ± 0.9 minutes versus 5.2 ± 1.9 minutes; $p < 0.001$). HF induced a significant increase of K (15.2 ± 8.6 versus 5.3 ± 4.0; $p < 0.01$) and α (20 ± 11degrees versus 46 ± 17 degrees; $p < 0.001$) compared to the controls. MA was significantly lower after HF (26 ± 2 mm) than after HM (38 ± 8 mm) or in the control specimens (49 ± 9 mm; all: $p < 0.0001$). The same applied for G being 5.1 ± 1.8 Kdyn/cm^2 (controls), 3.2 ± 1.2 Kdyn/cm^2 (HM) and 2.0 ± 1.1 Kdyn/cm^2 (HF) (all: $p < 0.0001$). Hemolysis induced a significant (< 0.0001) decrease in MPC suggestive of platelet activation with means of 19.3 ± 2.0 g/dL (controls), 15.5 ± 3.4 g/dL (HM) and 14.3 ± 0.7 g/dL (HF). Despite a decrease in MPC and R-value indicating activated primary and secondary hemostasis, respectively, markedly reduced clot firmness most likely due to decrease in hematocrit value was observed. Conclusion: TEG and MPC analysis is significantly affected by hemolysis.

28

DEVELOPMENT AND APPLICATION OF A PCR METHOD FOR THE DETECTION OF *BRUCELLA* SPP. FROM SHEEP, GOAT AND BOVINE NON-PASTEURISED MILK. **A. Katsiolis[1], A. Minas[2], C. Hadjichristodoulou[3], A. Vantarakis[1].** [1]Department of Public Health, Medical School, University of Patras, Greece; [2]Department of Medical Laboratories, Technological Educational Institution of Larissa, Greece; and [3]Department of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, Karditsa, Greece.

Background: Brucellosis remains a significant problem for both humans and animals in Greece. *Brucella melitensis* causes the most important infectious disease from all the species of its family. *Brucella* spp. can be detected and identified with serologic, culture and molecular methods. Objective: The objective of this study is the development and application of a PCR method for the detection and identification of Brucella spp. in milk. Methods: Twenty samples from non-pasteurized milk from different parts of South-Western regions of Greece were analyzed. Because *Brucella* spp. mainly accumulates in the fat layer of the milk, it was cultured using Brucella Medium Base with Brucella selective supplement, horse serum, dextrose and methanon. Positive samples for *Brucella* spp. were confirmed by biochemical tests and PCR. The same PCR protocol was used for the direct detection of Brucella spp. in milk. PCR was performed using two set of primers, which amplify the gene encoding the 31-kDa *B. abortus* antigen (B4, B5) and a region of the 16S rRNA gene (F4, R2). Specificity and sensitivity of the PCR method for both set of primers were evaluated. Results: Two milk samples were found positive for *Brucella melitensis* (confirmed by PCR). Also, PCR direct detection of *Brucella* spp. in milk using B4, B5 primers was developed. Sensitivity (10^{-6} dilution) and specificity of the PCR method was determined. Conclusion: The PCR method is a promising method to perform a fast, reliable and specific detection of *Brucella* spp. in milk.

29

PRELIMINARY RESULTS: BIG ENDOTHELIN-1 IN SERUM FROM DOGS WITH KIDNEY DISEASE. **A. Giordano[1], G. Rossi[1], A. Virtuoso[1], A. Zatelli[2], S. Paltrinieri[1].** [1]Dept. of Veterinary Pathology Hygiene and Public Health, University of Milan, Italy; and [2]Veterinary Clinic Pirani, Reggio Emilia, Italy.

Background: Endothelin-1 (ET-1) is a potent vasoconstrictor potentially associated with microangiopathy and development of chronic kidney disease. It stimulates the rennin-angiotensin-aldosterone system and has positive inotropic and chronotropic effects. ET-1 arises from its precursor bigET-1 which has a longer half-life and thus is more easily detectable in serum than ET-1. Objective: To determine the serum concentration of bigET-1 in dogs with chronic kidney disease (CKD) and to assess whether bigET-1 varies, depending on the presence of inflammation or on the severity of CKD. Methods: BigET-1 was measured using a commercially available ELISA kit on serum samples from 56 dogs staged according to the classification of the International Renal Interest Society (IRIS). C-reactive protein was measured to identify inflammation. Serology for *Leishmania* was also performed on 53 dogs. Results: The level of bigET-1 was higher ($P < 0.001$) in dogs in stage IV (mean ± SD.: 28.0 ± 19.5; median: 21.8 pg/mL) compared with dogs in stage I (9.2 ± 6.5; 7.9), II (8.2 ± 3.4; 9.5) or III (10.4 ± 6.1; 9.1) kidney disease. No significant differences were found among proteinuric dogs (19.2 ± 18.1; 14.7), dogs with borderline proteinuria (13.4 ± 9.3; 12.4) and nonproteinuric dogs (7.3 ± 3.2; 6.9) or between dogs seropositive (15.8 ± 25.6; 9.1) and seronegative (17.7 ± 12.0; 15.6) for *Leishmania*. BigET-1 was correlated with creatinine ($P < 0.001$; $r^2 = 0.68$) and CRP ($P < 0.001$; $r^2 = 0.52$) but not with urinary protein/creatinine ratio ($P = 0.051$; $r^2 = 0.27$). Conclusions: Serum bigET-1 increases in dogs with severe CKD of inflammatory origin. *Leishmania* infection does not affect bigET-1 concentration. The pathogenic mechanisms of increased bigET-1 and its possible association with clinically evident leishmaniasis should be further investigated.

30

SERUM AND URINARY IMMUNOFIXATION ELECTROPHORESIS IN A DOG WITH MULTIPLE MYELOMA. **A. Miglio[1], F. Birettoni[1], M.T. Antognoni[1], P. Lalli[2], V. Mangili[1].** [1]Dipartimento di Patologia, Diagnostica e Clinica Veterinaria, University of Perugia; and [2]Azienda Ospedaliera, Perugia, Italy.

Multiple myeloma (MM), a neoplasm of plasma cells or their precursors, is a lymphoproliferative disorder usually associated with a monoclonal gammopathy. Immunofixation electrophoresis (IFE) is considered the gold standard to detect and identify the suspected monoclonal component (MC). A 10-year-old crossbreed dog was presented because of lethargy, weight loss, polyuria/polydipsia and exophthalmos. Blood tests revealed anemia, thrombocytopenia and hyperproteinemia with a narrow spike in the β_2-γ region of the electrophoretic pattern. Urinalysis showed a urinary proteine/creatinine ratio of 4.51 and a mixed incomplete proteinuria using sodium-dodecil-sulfate agarose gel electrophoresis. The bone marrow cytology showed the presence of 25% atypical plasma cells. The MC was identified as IgA-λ type both in serum and urine by IFE using mammalian immunoglobulins anti-human heavy (gamma, alpha and mu) and light

(kappa and lambda, both free and bound) chains. Paraproteins were quantified in the serum by densitometric scanning of high resolution agarose gel (72.4 g/L). Bence-Jones proteins were not found in the urine by IFE. Treatment with melphalan (2 mg/day PO), prednisone (12.5 mg/day PO) and benazepril (10 mg/day PO) was started. After 2 months of therapy complete clinical remission was obtained and serum paraprotein concentration decreased to 3 g/L. Atypical plasma cells were still detectable on bone marrow cytology, but were markedly reduced (15%). We highlight the cross-reaction between anti-human heavy and light chains (kappa and lambda, free and bound) and canine heavy and light chains. The use of IFE has proved crucial to the diagnosis of MM and to monitoring the course and treatment of disease.

31

HEPATOZOON SPP. INFECTION IN STRAY CATS FROM A FELINE COLONY IN NORTHEASTERN ITALY DETECTED BY BLOOD SMEAR EXAMINATION AND MOLECULAR TESTING. **M. Trotta[1], E. Carli[1], M. Nicetto[1], R. Chinelli[2], T. Furlanello[1], L. Solano-Gallego[3].** [1]Laboratorio d'Analisi Veterinarie San Marco, Padova, Italy; [2]DVM, Trieste, Italy; and [3]Royal Veterinary College, UK.

Hepatozoon spp. is an apicomplexan protozoan that infects a wide variety of hosts. *Hepatozoon* infection has been sporadically reported in cats. In Europe, *Hepatozoon* infection in cats has been described only in Spain. Therefore, there is limited information about clinicopathological and epidemiological aspects of *Hepatozoon* infection in cats. The aim of the study was to investigate the presence of *Hepatozoon* in stray cats from a colony in northeastern Italy. Fifty-seven free-ranging domestic cats from northeastern Italy that were captured and anesthetized for sterilization were studied. They were apparently healthy and belonged to town colonies. Signalment, physical examination, K_3EDTA blood samples and blood smears were available for all cats. Diagnosis of *Hepatozoon* infection was made by blood smear examination and molecular techniques. DNA extraction and 18S rRNA gene PCR were performed in all blood samples. Positive amplicons were further analyzed by direct sequencing. Sporadic *Hepatozoon* gamonts with an ellipsoidal shape and pleomorphic nucleus were detected in the cytoplasm of neutrophils by blood smear examination in two cats. PCR was only positive in the two cats (3.5%) in which parasites were observed on blood smear examination. The sequences obtained showed 95% identity with *Hepatozoon felis* sequences from Spain deposited in GenBank. We report the first detection and identification of *Hepatozoon* spp. in cats from Italy.

32

THE UTILITY OF FINE-NEEDLE ASPIRATION CYTOLOGY IN THE DIFFERENTIAL DIAGNOSIS OF CUTANEOUS MASSES CONTAINING KERATIN. **G. Ghisleni[1], N. Pinto da Cunha[1], M. Santos[2], M. Florenti[3], M. Caniatti[1], P. Roccabianca[1].** [1]Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, University of Milan, Italy; [2]Instituto de Ciências Biomédicas de Abel Salazar, Porto, Portugal; and [3]Clinica Veterinaria Gran Sasso, Milan, Italy.

Fine-needle aspiration (FNA) cytology is commonly used to evaluate canine cutaneous and subcutaneous masses in a clinical setting. Neoplastic and non-neoplastic canine cutaneous masses containing keratin (CMCK) are a common finding in dogs; however a specific presurgical diagnosis by cytology is useful for the correct surgical approach. Few studies have addressed the accuracy of FNA cytology in the evaluation of CMCK. The purpose of this study was to describe cytological features and compare the cytology and histopathology of CMCK in dogs. Specimens of 23 CMCK from 21 dogs were retrospectively evaluated. Cytological specimens were obtained by FNA prior to surgery. Post-surgical tissue specimens were routinely processed for histopathology. A cytological diagnosis of neoplasia was achieved in 6 cases confirmed by histology (all true positive). Negative cytology for neoplasia was obtained in 17 cases (10 true negative, 7 false negative). Cytology had an overall agreement of 69.6% with histopathology, with a specificity of 100%, a sensitivity of 46%, a positive predictive value of 100% and a negative predictive value of 58.8%. According to these results, cytology is a good diagnostic tool for neoplastic CMCK and should be utilized to assist in the definition of the best surgical approach for CMCK.

33

CYTAUXZOON SP. INFECTION IN CATS FROM NORTHEASTERN ITALY. **E. Carli[1], M. Trotta[1], R. Chinelli[2], S. Tasca[1], T. Furlanello[1], L. Solano-Gallego[3].** [1]Private Laboratory San Marco, Padua, Italy; [2]DVM, Trieste, Italy; and [3]Royal Veterinary College, London, UK.

Cytauxzoon sp. (*C.* sp.) infections have been sporadically reported in cats in Europe. Three sick cats were previously diagnosed with *C.* sp. infection in Trieste (northeastern Italy). The aim of this study was to investigate the presence of *C.* sp. infection in cats living in Trieste. Sixty-three apparently healthy stray cats living in town colonies and 49 healthy and sick client-owned cats subjected to a routine blood screening were studied. *C.* sp. infection was diagnosed by blood smear evaluation, PCR analysis for the18S rRNA gene fragment of 412 bp and sequencing. A CBC was also evaluated, if available. Sequencing was assessed in 4 positive cases. Small, single or occasionally in pairs, piroplasms of 0.5–0.9 μm diameter were observed inside erythrocytes in 15/63 stray cats. *C.* sp. infection was diagnosed in 4/49 (8%) client-owned cats and in 19/63 (30%) stray cats by PCR analysis. The sequencing revealed a 99% homology with *C.* sp. from GenBank isolated in wild and domestic felids in Mongolia and Spain. The owned infected cats showed mild to moderate non-regenerative anemia (2/4, 50%), normal leukocyte counts (3/4, 75%) and normal platelet counts (3/4, 75%). The stray infected cats had mild to moderate anemia (5/19, 26%), leukocytosis (15/19, 79%) and normal platelet counts (14/19, 74%). *C.* sp. infection in cats appears to be prevalent in Trieste (northeastern Italy) with a higher proportion of colony cats being infected than client-owned cats. It appears that *C.* sp. infection causes a subclinical infection in cats in northeastern Italy. Further studies are needed to determine if *C.* sp. infection might cause clinicopathological disorders in cats.

34

COMPARISON BETWEEN ADVIA 120 AND MANUAL LEUKOCYTE DIFFERENTIAL COUNTS. **F. Riondato, E. Maggi, B. Miniscalco.** Dipartimento di Patologia Animale, University of Torino, Italy.

Objective: To compare automated (ADVIA 120) with manual differential leukocyte counts and to evaluate the possibility of identifying cases with reliable vs unreliable automated counts.

Methods: EDTA-blood samples from 82 dogs, 52 cats, 30 cattle and 20 horses were used. ADVIA 120 results were allocated to two different groups, based on the evaluation of the perox cytograms: group A (reliable cytograms) and group B (unreliable cytograms). A 200-cell differential WBC count was performed by two observers (experienced and unskilled). Data comparison and methods agreement were assessed. Results: The t-test showed significant differences for many WBC classes. Differences in most cases only affected group B results and were always wider than in A. Differentials provided by the two observers showed larger differences between them than comparison between the skilled observer and the ADVIA 120. Agreement between methods was good for neutrophils and lymphocytes in group A, while it was poor or not reliable in group B. Monocyte counts did not agree or were unreliable in any species in both groups. In horses the confidence intervals were always too wide to be accepted. The ADVIA 120 tended to underestimate neutrophils and monocytes and overestimate lymphocytes in dogs, cats and cattle; the opposite happened in horses. Concerning monocytes, the bias was always linked to a proportional error. Conclusions: Statistical analysis revealed significant differences between automated and manual differentials; effects on clinical-hematologic interpretations appear unlikely. Evaluation of absolute counts in normal and pathologic leukograms is needed to confirm this supposition. Results suggest that automated counts are more reliable than manual counts provided by unskilled observers, particularly in cases with a normal leukogram. The different results reported in the two groups suggest that a skilled operator can reliably select the cases for which a manual differential is needed.

35

CYTOLOGICAL ACCURACY IN THE DIAGNOSIS OF THYMOMA IN RABBITS. **A. Guija de Arespachochaga[1], I. Schwendenwein[1], A. Fuchs-Baumgartinger[2], A. Bilek[3], K. Hittmair[4], J. Hassan[4], F. Künzel[3].** [1]Institute of Immunology: Laboratory Medicine, [2]Institute of Pathology and Forensic Medicine, [3]Clinic of Internal Medicine and Infectious Diseases, and [4]Clinic of Diagnostic Imaging, Veterinary University of Vienna, Austria.

Thymomas are uncommon tumours in the cranial mediastinum, arising from thymic epithelial cells with variable amounts of lymphocytic infiltration. Thus, they are sub-classified as lymphocyte-predominant, epithelial-predominant, or mixed. Thymic lymphoma is the most challenging differential diagnosis when a large number of lymphocytes are present. Accurate differentiation is required for prognosis and adequate treatment options. In a period of 3 years, 9 rabbits were presented with exophthalmos and dyspnoea: 8 were diagnosed with thymomas. In the remaining case, thymoma and thymic lymphoma could not been differentiated by cytology, and histology was not available. Samples were taken by ultrasound-guided fine needle aspiration. In 6 cases histology was available and confirmed the cytological diagnosis, resulting in a positive predictive value of 100%. Cytologically, thymomas were characterized by a mixed population of small and occasional large lymphocytes and various amounts of epithelial cells. In 3 out of 6 cases, subtypes suggested by cytology agreed with the histological results. This could be caused by an inherent sampling error in a tumour that often displays a patchy pattern. However, in one case that was diagnosed by cytology as lymphocyte-predominant thymoma, a differentiation between thymoma and thymic lymphoma could not be made by histological examination after surgical removal. Nevertheless, histology of the mass that had recurred after 6 months finally confirmed the cytological diagnosis. The results showed that cytology is an accurate diagnostic tool to identify thymoma in rabbits. Nevertheless, the subtype must be determined by histopathology.

36

LARGE GRANULAR LYMPHOCYTOSIS IN DOGS WITH BABESIOSIS. **A. Guija de Arespacochaga[1], W. Gerner[2], I. Schwendenwein[1], M. Leschnik[3].** Institute of Immunology: [1]Laboratory Medicine and [2]Clinical Immunology and [3]Clinic of Internal Medicine and Infectious Diseases, Veterinary University Vienna, Austria.

Reactive large granular lymphocytosis is an unusual haematological finding, commonly associated with chronic ehrlichiosis, and must be distinguished from lymphoproliferative disease of granular lymphocytes. In canine babesiosis, leucocyte abnormalities are inconsistent findings. The presence of activated lymphocytes without lymphocytosis has been reported. Blood immunophenotyping was performed in three dogs with babesiosis diagnosed microscopically. All patients showed a moderate anemia (mean hematocrit 26%), and severe thrombocytopenia ($12–33 \times 10^3/\mu l$) while the leucocyte count was inconsistent. Lymphocytosis was present only in one dog. The following antibodies for lymphocyte phenotyping by flow cytometry were used: anti-CD3,-CD4,-CD8,-CD21 and -CD11d. For two patients, blood immunophenotyping before and 10 days after infection was performed. Frequency of $CD11d^+$ cells prior to infection was $< 10\%$ and increased during the acute phase of infection to 40%. These frequencies correlated with absolute numbers of large granular lymphocytes (LGL) identified by microscopic differential counts where LGL number prior to infection was $< 550/\mu l$ followed by an increase to 3000 LGL/μl during infection. One of the dogs showed a slight increase of LGL compared to values before infection. However, the absolute LGL count did not show a difference at any time ($< 500/\mu l$). Ten days after infection, all haematological parameters improved and were similar to values prior to infection. Reactive large granular lymphocytosis is not only a unique condition in dogs with ehrlichiosis, but also with babesiosis. Our data indicate that this is an intermittent condition, with recovery to initial values after therapy. The presence of LGL in dogs can help in the diagnosis in cases of low parasitemia. Further studies are necessary to determine the correlation between parasitemia and LGL lymphocytosis.

37

THE MOLECULAR INVESTIGATION OF WIDESPREAD *SALMONELLA* SEROVARS, *S. TYPHIMURIUM* AND *S.ENTERITIDIS*, INVOLVED IN *SALMONELLA* INFECTION OF CATTLE AND SHEEP ON A FARM AROUND TEHRAN, IRAN. **F. Ghazisaeedi[1], N. Atyabi[1], T. Zahraei Salehi[2], I. Ashrafi[2].** [1]Clinical Pathology Department and [2]Microbiology Department of Veterinary Medicine, Faculty of Tehran University, Tehran, Iran.

Salmonella serovars cause worldwide public health problems. Salmonellosis is an important zoonotic disease causing foodborne poisoning in humans consuming animal products. Studies were conducted on different aspects of incidence, treatment and control of salmonellosis all over the world. In this study, using molecular (PCR and Multiplex PCR) and conventional tests,

Salmonella serovars *S. typhimurium* and *S. enteritidis*, isolated from an outbreak of salmonellosis in cattle herds and sheep flocks around Tehran, the capital city of Iran, in the summer of 2008, were evaluated. Samples were collected from 8 calves, 3 lambs and embryonic tissue from one cow abortus. The cases involved juvenile animals and the presence of *Salmonella* serovars was confirmed in all isolates. Infection with *S. enteritidis* was much more prevalent in comparison to *S. typhimurium*. In 2 calves both *S. typhimurium* and *S. enteritidis* were detected, and *S. typhimurium* was isolated from lthe iver in both cases. All isolates were sensitive to streptomycin, lincospectin, enrofloxacine and trimetoprim and resistant to doxycycline and erythromycin.

38

EVALUATION OF SERUM HAPTOGLOBIN OF NEWLY CALVED HEALTHY HEIFERS AND THOSE SUFFERING FROM SUBACUTE AND ACUTE METRITIS. **N. Atyabi, F. Gharagozlu, Z. Bolandnaza, M. Hasemian.** College of Veterinary Medicine, University of Tehran, Tehran, Iran.

Haptoglobin (Hp) is an acute phase protein, and its concentration increases in most infectious diseases and during pregnancy and parturition in humans and most animals. The aim of this study was to evaluate the levels of serum haptoglobin in newly calved heifers after parturition, especially in cows with metritis. Blood was taken from thirty Holstein heifers, at one week before parturition, and then in the first, second, third, fourth, sixth and eighth weeks after parturition. Cellulose acetate electrophoresis was performed for separation of haptoglobin, using hemolysate with 7.5 gm/dL hemoglobin, and the method of Compton et al (1976). O″diniazidine stain (Merck Co.) was used for specific staining of haptoglobin (Hp) and the complex of haptoglobin and hemoglobin (Hp+Hb). The concentration of Haptogobin was obtained by densitometric method using the Photo EP densitometer (Germany). The means of Hp were 15.37, 20.90, 12.33, 11.28, 9.53, 8.40, and 6.05 mg/dL before parturition and the first, second, third, fourth, sixth and eighth weeks after parturition, respectively. Results showed the concentration of Hp in the first week postpartum was higher than in the other weeks ($P \leq 0.05$). This was due to the physiological process of parturition, as there is an acute phase response. There were three heifers that developed postpartum metritis. The concentration of Hp from these animals remained high in serum during this investigation, with peak values of 26.45, 32.63 and 29.36 mg/dL at week six, and then decreased to normal values (5.63–21.32 mg/dL) after antibiotic therapy. We concluded that Hp can increase significantly in the first week postpartum in cattle and there is a significant correlation with uterine infection, especially postpartum metritis.

39

ANALYTICAL BLOOD CHANGES IN *LEISHMANIA*-POSITIVE DOGS: A RETROSPECTIVE STUDY. **R. Barrera, D.M. Beristain, J. Duque, P. Ruiz.** Departamento de Medicina Animal, Universidad de Extremadura, Cáceres, Spain.

Canine leishmaniasis is an enzootic disease in Extremadura, Spain. Adequate blood analysis allows veterinarians to suspect disease, assess patient condition, determine a prognosis and evaluate treatment response. The aim of this study is to evaluate commonly observed clinicopathological changes and their incidence in this geographic area. Blood samples from 75 dogs suffering from leishmaniasis diagnosed by ELISA procedures were analyzed (hematology and blood biochemistry). The most common hematological finding was slight to moderate normocytic, normochromic and non-regenerative anemia (62%), which is indicative of chronic disease. The leukocyte count was normal in most of patients (65%). Leukocytosis with neutrophilia was observed in 17% and leukopenia due to neutropenia and/or lymphopenia in 18% of cases. Monocytosis due to chronic inflammation was observed also (85%). Thrombocytopenia was found in 31% of cases. Biochemistry analysis showed hyperproteinemia due to hyperglobulinemia, associated with antigenic chronic stimulation (75%). Hypoalbuminemia as a result of chronic inflammation and kidney disease was common (72%). In advanced stages of leishmaniasis, dogs may develop chronic renal failure because of immune-mediated glomerulonephritis, interstitial nephritis and amyloidosis (rare). Azotemia was present in 45% of cases and was accompanied by anemia and proteinuria. Liver damage is not commonly observed (in 9% of cases we observed increases in alanine aminotransferase, accompanied by alkaline phosphatase and bile acids). Additionally, hypercholesterolemia was observed in 13% of cases, but its interpretation is difficult. These results suggest that blood analysis is very important in diagnoses, prognosis and disease control. Although anemia, hyperproteinemia and azotemia are commonly observed, there are other analytical changes that should be considered.

40

PLASMA FIBRINOGEN AND IRON CONCENTRATIONS VERSUS LEUKOCYTE RESPONSE AS INDICATORS OF SYSTEMIC INFLAMMATORY DISEASES IN HORSES. **R. Barrera, M. Martín, P. Ruiz, V. Vieitez, D.M. Beristain, J. Duque, L.J. Ezquerra.** Departamento de Medicina Animal, Universidad de Extremadura, Cáceres, Spain.

Conventionally, the leukocyte response has been used to assess inflammatory diseases in veterinary medicine. Recent studies suggest that fibrinogen and plasma iron concentrations are sensitive indicators of systemic inflammation in horses. The aim of this study is to evaluate the usefulness of both kinds of analyses. Hematologic and complete biochemistry testing was performed in 12 healthy horses and 25 hospitalized horses with diseases causing systemic inflammation (only the analysis at time of admission to hospital was included in this group). Low plasma iron concentrations (36.61 ± 31.51 microgm/dL) and high fibrinogen concentrations (647.12 ± 446.03 mgm/dL) were both sensitive indicators of systemic inflammation in horses. These alterations were observed in 96% and 92% of cases, respectively. However, results of the leukocyte responses were variable. Sixty percent of sick horses had normal leukocyte counts (7751 ± 2731 leukocytes/μL), 20% had leukopenia (3350 ± 1861 leukocytes/μL) as a result of neutropenia (918 ± 657 neutrophils/μL). Finally, 20% showed leukocytosis due to neutrophilia (15249 ± 9396 neutrophils/μL). Fibrinogen and plasma iron concentrations are reliable indicators of inflammation in horses. In contrast, changes in leukocyte numbers, which are usually considered to be hallmarks of infection and inflammation, do not appear to be useful in the diagnosis of these processes. However, more information is obtained when interpreting the results of iron and fibrinogen plasma concentrations in combination with the leukocyte response, because in many cases this combination helps to characterize the intensity and severity of the process.

41

DETERMINATION OF FRACTIONAL EXCRETION AND URINARY OSMOLALITY IN DOGS WITH CHRONIC KIDNEY DISEASE. **D.M. Beristain, C. Zaragoza, C. Rodríguez, J. Duque, P. Ruiz, R. Barrera.** Departamento de Medicina Animal, UEx, Cáceres, Spain.

Introduction and Objective: Evaluation of the ability to concentrate/dilute urine is the first step of urinalysis used to assess kidney function. Urinary osmolality (UOsm) and specific gravity (USG) are used routinely for assessing renal concentrating ability. Fractional excretion (FE) of a plasma solute is the fraction of this solute filtered by the glomerulus, which is excreted into urine. When renal dysfunction is suspected, FE sodium (FE_{Na}) and FE phosphorus (FE_P) are most often used and may increase up to 3.5 to 10-fold in dogs with chronic kidney disease (CKD) compared with healthy dogs. The goal of this study is to compare the USG, UOsm, and FE from healthy dogs and dogs suffering from CKD. Material and Methods: Group I included 11 healthy dogs. Group II included 16 dogs with symptoms and hematological and urinalysis findings and complementary tests compatible with renal disease. Haematology and biochemistry analysis were performed in this study. Urinalysis, USG (manual refractometry) and UOsm (by freezing point osmometer) were performed on all samples. Results: Group I: USG mean 1.038 ($\pm$0.014), UOsm mean 1528.64 mOsm/kg ($\pm$771.50). FE_{Na} mean 0.57 ($\pm$0.35), FE_K 15.34 ($\pm$7.02), and FE_P 24.49 ($\pm$9.8). Group II: USG mean 1.018 ($\pm$0.006), UOsm mean 739.75 mOsm/kg ($\pm$336.36). FE_{Na} mean 5.40 ($\pm$6.03), FE_K mean 154.80 ($\pm$110.43), FE_P 62.76 ($\pm$45.50). Conclusion: UOsm is the best method for determination of urinary concentrating ability; however, determining the FE of many constituents that are usually increased in CKD may help to better assess renal function.

42

LIMITS OF STANDARDISATION OF PROTHROMBIN TIME MEASUREMENTS IN CANINE SAMPLES. **R. Mischke.** Small Animal Clinic, Hannover School of Veterinary Medicine, Hannover, Germany.

Background: In contrast to humans, results of prothrombin time (PT) measurements in dogs and other animals are usually reported as coagulation time (in seconds). This does not allow direct comparison of results obtained with different reagents and may even require reagent batch (lot)-related reference values in case of significant batch-to-batch variability. Aims: The objectives of this study were to evaluate the batch-to-batch and inter-reagent variability of PT measurements in canine plasma and to investigate the effect of different methods of standardisation. Materials and Methods: PT standard test (PT_{ST}) and PT modified test (PT_{MT}; 1:20 sample dilution, fibrinogen supplementation) were measured using 5 different batches of each of two commercial reagents Thromborel S and Neoplastin Plus. In addition, inter-reagent variability was studied for PT_{MT} in artificially prepared canine samples with different factor VII activity levels (5 reagents) and in 86 canine samples (4 standardised reagents with ISI-values provided by the manufacturer) based on clotting times and after calculation of percent activity, prothrombin ratio (PTR) or international normalised ratio (INR). Results: PT_{ST} and PT_{MT} measurements showed only a small variation between different batches of the two investigated commercial reagents (CV $\leq$ 5%). Calculation of percent activity or PTR reduced differences between different reagents when compared with results reported as coagulation times. However, significant differences were still present. Calculation of INR using the ISI-values provided by manufacturers for use on humans receiving oral anticoagulants showed less favourable results. Conclusions: The results of this investigation indicate high reagent stability with respect to different batches and, therefore, standardisation does not seem to be necessary to further reduce batch-to-batch variability for the studied reagents. Possibilities for PT standardisation in order to achieve a reagent-independent result are limited.

43

A SURVEY OF THE RELATIONSHIP BETWEEN MORPHOLOGICAL CHANGES OF WHITE BLOOD CELLS AND SOME BIOCHEMICAL PARAMETERS IN CALVES INFECTED BY *SALMONELLA* AND OTHER PATHOGENS IN IRAN. **F. Aslani[1], Z. Khaki[2], T. Zahrahi Salehi[3].** [1]Islamic Azad University, Tehran, Iran; and Departments of [2]Clinical Science and [3]Pathobiology, Faculty of Veterinary Medicine University of Tehran, Iran.

Salmonella spp. have been implicated as a cause of diarrhea in dairy calves. With regard to the importance of diarrhea in dairy calves and the effects of pathogen endotoxins that cause diarrhea in dairy calves, we studied some hematological and biochemical changes. In this study, blood and fecal samples were collected from 35 calves with diarrhea and 18 healthy calves at 5–8 weeks of age. Each calf was examined clinically. In order to detect which bacterial agents were involved, appropriate diagnostic examination was done. *Salmonella* spp., *Klebsiella, E coli* and *Proteus* were detected. Diagnostic examinations were performed and *Cryptosporidium parvum* and *Eimeria* were detected as the causative protozoal agents. Based on the isolated pathogen, diarrheic calves were divided into 7 groups: *E coli* (E); *E coli, Klebsiella* (EK); *E coli, Salmonella* (SE); *E coli,Proteus* (EP); *E coli, Klebsiella, Cryptosporidium* (ECK); *E coli, Proteus, Cryptosporidium* (EPC); and *Klebsiella, Cryptosporidium* (CK), and one was considered as the control. Creatinine, uric acid and urea were measured by commercial kits using an Eppendorf autoanalyzer. For serum levels of TBARs and vitamin A a UV autoanalyzer was used. There were statistically significant differences in serum creatinine, urea and PP/F concentrations between the EPC and the control groups ($P < 0.05$). There was a significant difference in serum creatinine concentration between the EPC group and two other diarrheic groups (EK, EP). Temperature was significantly differenent between different diarrheic groups and the control group ($P < 0.05$). In contrast, there were no significant differences in hematological findings or biochemical parameters ($P > 0.05$) between groups.

44

PATHOLOGICAL FINDINGS DUE TO *N. CANINUM* IN THE BRAIN OF BOVINE ABORTED FETUSES AND PLACENTA FROM SEROPOSITIVE DAIRY CATTLE. **H. Haddadzadeh[1], N. Salehi[1], P. Kazraee nia[2].** Departments of [1]Parasitology and [2]Clinical Science/Section of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Neospora caninum infection has emerged as a serious disease of cattle and dogs worldwide and neosporosis is a major cause of abortion in cattle. Although aborted fetuses seem to be the most likely source of infective material for dogs, placentas from seropositive dams also appear to be a potential source of infective material. The objective of the study was to evaluate the presence

of *N. caninum* organisms in the brain of aborted fetuses and placentas of full-term calves born to seropositive cows. Over a one-year study period (2006–2007), a total of 12 brains of aborted calves from *Neospora*-seropositive cattle and 7 placentas from seropositive dams giving birth to full-term calves from two dairy farms located around the Tehran Province, Iran were examined histopathologically. Mild to severe placentitis was observed in 5 placentas. Severe hyperemia and perivascular and perineuronal edema was evident in all fetal brains. In 3 of 12 brains, scattered foci of hemorrhage, neuropilar necrosis and gliosis were present. Also, nonsuppurative encephalitis with severe lymphohistiocytic perivascular cuffing in one case and a small tissue cyst of *Neospora caninum* in the other calf, were observed. Our results confirmed the histopathologic findings of other studies on *Neospora caninum* infection and seem to support the hypothesis that *Neospora* infection is associated with bovine abortion in Iran.

45

A CLINICAL PATHOLOGY STUDY OF PIGEON TUBERCULOSIS. **P. Khazraiinia, J. Ashrafi Halan, M. Ahmadi, H.A. Mahpeikar, P. Yousefi.** Veterinary Faculty, University of Tehran, Tehran, Iran.

Avian tuberculosis is distributed all over the world, most commonly in small barnyard flocks with adult birds flying together, such as pigeons. The disease is caused by *Mycobacterium avium* or *Mycobacterium genavense*. The clinical signs are progressive weight loss, depression, and fluffing. Granulomatous nodules of varying size are found in many parts of the body, such as liver, spleen, bone marrow and subcutaneous tissue. Some flocks of pigeons in the area of Tabriz were suspected to suffer from tuberculosis. PCR, microbiology and pathology examination were done on the tissues of pigeons with suspicious clinical signs, and results proved the presence of tuberculosis. Before euthanasia, blood samples were taken from cervical vessels of 19 healthy pigeons as a control group, 28 infected pigeons without hepatic lesions and 18 pigeons with hepatic lesions. Sera were stored at -20°C until used for biochemical measurement. Cellulose acetate electrophoresis was done on serum samples and serum activity of ALT, AST, ALP, LDH and CPK were measured. The mean concentration of albumin and pre-albumin was decreased and the mean α, β, and γ globulin concentrations were increased in affected groups compared with the control group. The mean serum AST, ALT, LDH and CPK activities were significantly increased in the affected group, especially pigeons with liver granulomatosis, compared with the control group.

46

EFFECT OF DIETARY SUPPLEMENTATION OF XANTHURENIC ACID, KYNURENIC ACID, ANTHRANILIC ACID, NICOTINIC ACID AND TRYPTOPHAN ON LIPID RESPONSES IN HYPERLIPEMIC GUINEA PIGS. **M. Abbasalipour Kabir rah[1], P. Asadian[2], F. Asadi[1], N. Atyabi[3], A. Shahriari[4].** [1]Department of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Department of Clinical Pathology, School of Veterinary Medicine, Lorestan University, Khoramabad, Iran; [3]Department of Clinical Pathology, School of Veterinary Medicine, University of Tehran, Tehran, Iran; and [4]Department of Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran.

In the present study, the effects of dietary tryptophan-derived metabolic product supplementation on plasma and hepatic triglyceride (TG) and cholesterol (CHO) levels were evaluated in guinea pigs fed a lipid-rich diet. Six groups of male guinea pigs were fed one of the following diets for 10 days: lipid-rich diet (LRD), lipid-rich diet containing xanthurenic acid (LRX), lipid-rich diet containing kynurenic acid (LRK), lipid-rich diet containing anthranilic acid (LRA), lipid-rich diet containing tryptophan (LRT) and lipid-rich diet containing nicotinic acid (LRN). Results were analyzed among groups using a 1-way ANOVA. Plasma levels of CHO in guinea pigs fed LRX (65.45 ± 15.04 mg/dL), LRK (59.54 ± 11.88 mg/dL) and LRA (67.88 ± 5.35 mg/dL) were significantly lower than in guinea pigs fed LRD alone (86.78 ± 10.61 mg/dL). However, serum TG levels did not show any significant changes ($p > 0.05$). On the other hand, all treatment groups showed significant decreases in liver CHO levels compared with the LRD ($p < 0.05$). Furthermore, LRX and LRK decreased liver TG levels (499.48 ± 72.63 and 487.93 ± 46.34 mg/g liver, respectively) when compared with the LRD (395.46 ± 45.66 mg/g liver). These results suggest that supplementation of diet with xanthurenic acid and kynurenic acid have a lowering effect on the whole liver and serum CHO values in the LDL animal model.

47

SERUM LIPID AND LIPOPROTEIN PARAMETERS OF PERSIAN BUFFALO. **F. Asadi[1], A. Rezaee[1], P. Asadian[2], M. Pourkabir[1], A. Shahriari[3].** [1]Department of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Department of Clinical Pathology, School of Veterinary Medicine, Lorestan University, Khoramabad, Iran; and [3]Departmaent of Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran.

Lipid transport systems in animals have been evaluated both as experimental models for lipid and lipoprotein metabolism in humans and to gain insights into the lipid metabolism of specific animal breeds. Currently, there is little information about serum lipid and lipoprotein status in Persian Buffalo. The aim of the present study was to measure lipid and lipoprotein parameters and show lipoprotein electrophoresis patterns in this breed. We measured serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) in 52 clinically healthy Persian buffalo of both sexes ranging in age from 1 to 15 years. Agarose gel electrophoresis was used to evaluate serum lipoprotein patterns. Data were tested for normality, and reference limits were calculated as 95% range. The 95% range of values for serum TG, TC, FFA, HDL-C, VLDL-C and LDL-C values were as follows: 0.55–0.69 mmol/L, 2.05–2.26 mmol/L, 0.22–0.43 mmol/L, 1.63–1.94 mmol/L, 0.25–0.31 mmol/L and 0.41–0.87 mmol/L, respectively. The relative percentages of α- and β-lipoproteins in electrophoretic tracings were $66.99 \pm 3.43\%$ and $32.46 \pm 3.48\%$, respectively. Serum lipid and lipoprotein values in Persian buffalo are similar to those in cattle and may be useful for evaluating metabolic disorders in this species.

48

ALTERATIONS IN LIPID PARAMETERS IN RESPONSE TO KYNURENIC ACID, XANTHURENIC ACID, ANTHRANILIC ACID AND NICOTINIC ACID IN HEPG2 CELLS. **P. Asadian[1], F. Asadi[2], N. Atyabi[3], A. Shahriari[4], M. Pourkabir[2].** [1]Department of Clinical Pathology, School of Veterinary Medicine, Lorestan University, Khoramabad, Iran; [2]Departmaent of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran, Iran; [3]Department of Clinical Pathology, School of Veterinary Medicine, University of Tehran, Tehran, Iran; and [4]Department of Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran.

The aim of the present study was to determine if major metabolites of tryptophan (TRP) metabolism, kynurenic acid (KA), xanthurenic acid (XA), anthranilic acid (AA) and nicotininc acid (NA), can change triglyceride (TG) and cholesterol (CHO) secretion and cell content in a HepG2 cell culture system during both basal and glucose-stimulated TG synthesis conditions. The data indicated that XA, KA, AA, NA and TRP decreased CHO secretion after 24- and 48-hour incubations in both basal (30, 35, 7, 2, 21; 33, 41,72, 4.5, 39%, respectively) and glucose-stimulated TG synthesis conditions (12, 17, 22, 15, 4.5; 40, 21, 31, 41, 2%, respectively). Except for NA, this decrease was associated with an increase in the cellular CHO accumulation ($p < 0.05$). On the other hand, XA, AA, NA and TRP decreased TG secretion after 24- and 48-hour incubations in both basal (62, 62, 42, 50, 27; 51, 13, 21, 53, 56%, respectively) and glucose-stimulated TG synthesis conditions (28, 21, 12, 23, 22; 25, 14, 37, 16, 8.3%, respectively) when compared with the corresponding basal and stimulated controls. However, the decrease in TG secretion was associated with an increase in cellular TG degradation in 24- and 48-hour incubations for both basal (39, 13, 10, 9.5, 12; 11, 1, 39, 9, 4%, respectively) and glucose stimulated-TG synthesis conditions (40, 30, 33, 7, 34; 34, 17, 30, 14, 30%, respectively) when compared with the corresponding controls. The data suggest that XA and AA may be useful for manipulation of serum and cellular TG status.

49

ELECTROPHORETIC PATTERN OF HEMOGLOBIN IN IRANIAN HORSES: TURKMAN, IRANIAN ARAB, CASPIAN PONY, DARESHORI, AND KURD. **A. Shahriari[1], F. Asadi[2], M. Shahabzadeh[2], P. Asadian[3], M. Pourkabir[2].** [1]Department of Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran; [2]Department of Biochemistry, School of Veterinary Medicine, University of Tehran, Tehran, Iran; and [3]Department of Clinical Pathology, School of Veterinary Medicine, Lorestan University, Khoramabad, Iran.

The occurrence of multiple adult hemoglobins (Hb) in domestic animals has been investigated previously. The aim of the present study was to evaluate hemoglobin electrophoresis patterns in clinically healthy Turkman, Iranian Arab, Caspian pony, Dareshori and Kurd horses by cellulose acetate electrophoresis (CAE) and polyacrylamide gel electrophoresis (PAGE). Whole blood was collected and packed cell volume (PCV) and hemoglobin (Hb) concentrations were determined. Hemolysates were applied on cellulose acetate film and polyacrylamide gels, run, stained and scanned. While all horses showed a similar pattern with two hemoglobin bands on CAE, different patterns were shown on the PAGE. The flow rate ratios of fraction 1:fraction 2 on CAE were 1.062 ± 0.011, 1.05 ± 0.01, 1.047 ± 0.009, 1.05 ± 0.008, and 1.05 ± 0.004, respectively. These ratios on PAGE were 1.33 ± 0.01, 1.23 ± 0.018, 1.24 ± 0.014, 1.27 ± 0.029, and 1.24 ± 0.03, respectively. Fetal Hb was absent in Turkman horses. In the other horses, the flow rate ratios of fetal Hb:fraction 2 were 1.54 ± 0.016, 1.5 ± 0.06, 1.5 ± 0.067, and 1.47 ± 0.05, respectively. Kurd horses showed an extra fraction on PAGE so that its ratio of flow rates to fractions 1 and 2 were 1.15 ± 0.02 and 1.36 ± 0.03, respectively. In conclusion, hemoglobin electrophoresis showed different patterns among horses and may be useful for evaluating hose breeds.

50

LYMPHOCYTE SUBSET REFERENCE RANGES IN SPANISH PUREBRED HORSES. **K. Satué[1], A. Hernández[1], C. Lorente[2].** [1]CEU-Cardenal Herrera University, Moncada-Valencia, Spain; and [2]Dermatology Clinic (ADERVET), Madrid, Spain.

Introduction and Objectives: Simultaneous assessment of lymphocyte subsets is a most effective method for characterizing disorders related to the immune system (leukaemia, lymphomas, autoimmune and infectious diseases), offering a prognosis and evaluating the response to treatment. The aim of the present research was to establish normal reference values, estimating the age- and gender-related variations in Spanish purebred horses. Material and Methods: Analysis was done in samples from a total of 159 horses, 77 male and 82 females, classified in four age groups: Group A (1–2 years old), Group B (2–3 years old); Group C (3–4 years old) and Group D (4–7 years old). The animals were vaccinated and dewormed, did not have clinical signs of disease, and did not receive medications during the three months before the experiment. Blood samples were obtained from the jugular vein and were stored in tubes with EDTA as anticoagulant. The total number of lymphocytes, T and CD2+, Th and Tc lymphocytes and B lymphocytes were determined by flow cytometry. NK cells were calculated by a mathematical formula by subtracting CD2+lymphocytes from the sum of Th and Tc lymphocytes. Results: Whereas sex did not exert any influence over lymphocytes subsets, there was an age-related decrease in the number of T, CD2+, Th, Tc and B lymphocytes, and NK cells, without differences in the CD4/CD8 ratio. Conclusions: Populations of T, Th, Tc, B and NK cells are within the reported reference range for adult horses. Age had a significant influence on the immunophenotype of the Spanish Purebred horse. These results indicate a natural condition in healthy individuals and could be the origin of the immune dysfunction seen in older animals.

51

HEMATOLOGY IN THE ANDALUSIAN BROODMARE OF CARTHUSIAN STRAIN: EFFECTS OF PREGNANCY, AGE OF THE MARE, SEX OF FETUS AND MATING SEASON. **K. Satué, A. Muñoz.** CEU-Cardenal Herrera University, Moncada-Valencia, Spain.

This research assesses whether pregnancy, sex of the fetus, age of the mare and mating period influence the hematological profile in mares. Forty-four healthy Spanish broodmares of Carthusian strain were studied during pregnancy, which was divided into three periods of similar duration. The mares were classified according to: 1) their age in three groups: A (4–7 years of age), B (8–12) and C (13–17); 2) gender of the fetus; 3) and three periods

according to the month of the year the mares were mated: between November-January, February-April and May-July. Venous blood samples were collected every month during pregnancy. Blood samples was collected into tubes with EDTA and were analyzed by a semiautomatic analyzer. The number of red blood cells (RBC), hemoglobin concentration (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean hemoglobin concentration (MCH), mean corpuscular hemoglobin concentration (MCHC), and number of platelets (PLT) and WBC were recorded. On blood smears, the differential count was evaluated. In serum, total protein (TSP) was measured. Higher WBC counts were found in the 1st pregnancy period. PCV was higher and MCV, MCHC, neutrophils and N/L ratio were lower in the 2nd period. MCV was lower in the 3rd period. Older mares had lower PCV with higher MCV and MCH, lower WBC and lymphocytes and higher eosinophils and N/L ratio. Mares that had a male fetus had higher RBC counts, HB and N/L and lower MCV than mares that had a female fetus. Although the hematological parameters in broodmares of Carthusian strain are within the reference range for healthy adult horses, the effect of age of the mare, month of pregnancy and sex of the fetus should be taken into account.

52

IS THERE A NEED FOR IRON SUPPLEMENTATION IN SPANISH PUREBRED MARES DURING PREGNANCY? **K. Satué, P. Montesinos.** CEU-Cardenal Herrera University, Moncada-Valencia, Spain.

Introduction and Objectives: Quantifying the reserve maternal iron during pregnancy would determine the need for supplementation and thus reduce the adverse effects of deficiency of this metal during embryonic and fetal development in the neonate, minimizing the risk for cardiovascular disease in adulthood. The aim of the present research was to analyze the effect of pregnancy on FERR and Fe concentrations in the pregnant Spanish mare. Material and Methods: A total of 31 reproductive Spanish mares were analyzed during pregnancy. Pregnancy was divided into three periods of similar duration. Venous blood samples were collected monthly and always in the morning. The fractions were placed in glass tubes with activators of coagulation for FERR and Fe determinations. FERR concentrations were measured by a turbidimetric method using reagents from Spinreact® with an intra-assay coefficient of variation of 4.96%. Fe concentrations were measured using a spectrophotometer (Clima-M5-15®) and special reagents for this spectrophotometer (RAL®). Results: Mean ± ee FERR concentrations were 145.34 ± 2.3 µg/dl (42.90–357.10 µg/dl) and mean ± ee of Fe concentrations were 164.50 ± 2.83 µg/dl (6.20–399.60 µg/dl). Pregnancy in the Spanish mare was characterized by a significant increase in the concentrations of FERR and Fe. Conclusions: In conclusion, pregnancy significantly modifies the iron state in the Spanish mare. Concentrations of Fe during period I of gestation suggest a major utilization of Fe by the fetus and the placenta. The increase of the stores of Fe during periods II and III indicate a positive balance of Fe, which could be related to a decrease in mobilization, indicating there is no need for iron supplementation in these mares during pregnancy.

53

SERUM RENIN, ANGIOTENSIN II AND ALDOSTERONE CONCENTRATIONS IN SPANISH MARES DURING PREGNANCY. **K. Satué, R. Domingo.** Animal Medicine and Surgery Department, CEU-Cardenal Herrera University, Valencia, Spain.

Activation of the rennin-angiotensin-aldosterone system (RAAS) during pregnancy is important in adjusting cardiovascular and hemodynamic maternal demands and also has a direct influence on proper development of the fetus and on fetal viability. The main purpose of this research was to analyze pregnancy-related changes in serum renin (REN), angiotensin II (ANG-II) and aldosterone (ALDO). Jugular venous blood samples were taken every month during pregnancy in a total of 33 Spanish Purebred mares, 4–17 years in age. Serum REN, ANG-II and ALDO concentrations were measured by competitive immunoassay. Mean values of REN during the first five months of pregnancy ranged between 2.56 and 3.31 pg/dL. They increased progressively from the 6th month (3.26 pg/mL), achieving maximum mean values, three-fold higher than basal values, at the 10th (7.20 pg/mL) and 11th (7.23 pg/mL) months. Serum ANG-II fluctuated without significant changes between 0.05 and 7.66, with a mean value of 1.28 ng/mL. Mean ALDO, after a decrease in the second month, increased progressively from the third month reaching the average maximum level of (795.19 ± 71.22 pg/mL) in the fifth month. From the sixth to the ninth months a new significant decrease was seen ($p < 0.05$). In conclusion, the physiological state of gestation is characterized by a marked activity of the rennin-angiotensin-aldosterone system. Pregnancy in Spanish mares induces a progressive increase in REN concentrations, without modification of ANG-II and fluctuations of ALDO concentrations. These changes could possibly be associated with the interaction of certain metabolic and hormonal factors that occur during pregnancy in the mare.

54

HEMATOLOGICAL PROFILE IN SPANISH PUREBRED HORSES: EFFECTS OF AGE AND GENDER. **K. Satué[1], A. Hernández[1], C. Lorente[2].** [1]CEU-Cardenal Herrera University, Moncada-Valencia, Spain; and [2]Dermatology Clinic (ADERVET), Madrid, Spain.

The aim of this research was to establish reference values for the hemogram of Spanish Purebred horses and to assess the influence of age and gender on hematological parameters. Venous blood samples were obtained from a total of 159 horses, 77 male and 82 females, grouped in four age groups: A group (1–2 years old), B group (2–3 years old), C group (3–4 years old) and D group (4–7 years old). Immediately after sampling blood smears were made. Samples were placed into tubes with EDTA and lithium-heparin for hematological parameters and total plasma protein (TPP) determinations, respectively. Using a semiautomatic cell-counter (Sysmex F-820) the following parameters were assayed: red blood cell count (RBC), haemoglobin concentration (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean haemoglobin concentration (MCH), mean corpuscular haemoglobin concentration (MCHC), number of platelets (PLAQ) and white blood cell count (WBC). On the peripheral blood smear the differential white blood cell count was estimated by counting lymphocytes (LYMPH), band neutrophils (BAND NEUT), segmented neutrophils (SEGM NEUT), eosinophils (EOS), monocytes (MON) and basophils (BAS). TPP was analyzed by

spectrophotometry. Gender has a limited influence on these parameters. Increasing age caused a reduction in the number of RBC, WBC, PLAQ, LYMPH and SEGM NEUT with increases in MCV, MCH and N/L ratio, and no change in BAND NEUT, EOS, MON and BAS. In conclusion, we have established reference values for haematological parameters in Spanish Purebred horses. Age and, to a lesser extent, gender have a significant influence on the hemogram of Spanish Purebred horse, warranting the establishment of reference ranges specific for age and gender.

55

COMPARISON OF THREE DIFFERENT METHODS TO DETECT IN VITRO ACTIVATED PLATELETS IN WHOLE BLOOD OF RATS. **A. Pankraz[1], D. Ledieu[2], D. Pralet[2], A. Moritz[3], A. Provencher-Bolliger[4].** [1]Biocontrol Veterinary Laboratories, Ingelheim, Germany; [2]Clinical Pathology, Preclinical Safety, Novartis Institutes for BioMedical Research, Basel, Switzerland; [3]Department of Veterinary Clinical Sciences, Clinical Pathophysiology, and Clinical Pathology, Justus-Liebig University; and [4]Charles River Laboratories, Preclinical Services, Quebec H9X 3R3 Canada.

Activated platelets have been implicated in the pathogenesis of several diseases in people and animals. Rats are currently used as a model to investigate diseases like stroke, cardiac infarction, vascular damage, and thrombosis. The aim of the study was to compare the detection of CD62p expression on platelets, the detection of platelet-neutrophil aggregates, and the quantitative analysis of the shape change of the platelet population with flow cytometric methods as markers for platelet activation in whole blood of rats. CD62p expression on the outer cell membrane of platelets was detected using CD61 and CD62p-specific antibodies; for detection of platelet-neutrophil aggregates CD61 and CD45 antibodies were utilized. Shape change was calculated after applying a described gating strategy to a FSC/SSC dot plot. The determined reference ranges in non-activated samples were (n = 30; mean ± sd) 71.6 ± 40.42 for CD62p mean fluorescence intensity (CD62p MFI), 29.4 ± 7.9 for shape change, 1.4 ± 1.3 for platelet-neutrophil aggregates and 4.3/2.6–22.4% (median/95% percentile) for CD62p % positive cells (CD62p %). Using Spearman's rank correlation good correlation was determined for CD62p % and shape change ($r = -0.85$), CD62p MFI and shape change ($r = -0.82$), whereas the correlation of the number of aggregates with other platelet activation parameters (CD62p %, CD62 MFI, shape change) was only fair ($r = 0.69$, $r = 0.73$, and $r = -0.67$, respectively). Based on our data, the detection of CD62p expression as well as shape change are the best suited parameters to assess platelet activation in whole blood of rats.

56

EVALUATION OF A PORTABLE GLUCOSE METER FOR USE IN SHEEP. **P.D. Katsoulos[1], M.A. Karatzia[2], K. Pourliotis[2], A. Minas[3], G. Christodoulopoulos[1].** [1]Clinic of Medicine, School of Veterinary Medicine, University of Thessaly, Karditsa, Greece; [2]Clinic of Farm Animals, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; and [3]Laboratory of Microbiology, Department of Medical Laboratories, Technological Educational Institution of Larissa, Larissa, Greece.

Background and Objective: In farm animal veterinary practice determination of blood glucose values on the farm is often required. As there is no portable glucose meter developed for use in farm animals, the analytical accuracy of a human portable glucometer for use in sheep was evaluated. Materials and Methods: 101 sheep blood samples, obtained from the jugular vein, were used for the study. Glucose was determined in whole blood immediately after veinpuncture with a One Touch® Vita™ portable glucometer and in serum with a colorimetric method. The agreement between methods was evaluated using Passing and Bablok regression analysis. The precision and the accuracy of the measurements were tested using concordance correlation coefficient. Results: There is a strong linear relationship between the glucose values obtained with the portable glucometer and those with the laboratory method ($y = 0.1444 + 0.9474x$). The precision and the accuracy of the measurement were determined at 87.99% and 98.80%, respectively. The mean glucose values with the portable glucometer were significantly lower than those of the laboratory method. Conclusions: One Touch Ultra® portable glucometer is sufficiently accurate for use in clinical practice to determine blood glucose concentrations in sheep.

57

SERUM IRON CONCENTRATIONS IN HORSES COVERING DIFFERENT DISTANCES IN ENDURANCE EVENTS. **A. Muñoz[1,2], C. Riber[1,2], P. Trigo[2], K. Satué[3], F.C. Castejón[2].** [1]Animal Medicine and Surgery Dept., [2]Equine Sport Medicine Centre, CEMEDE, University of Córdoba; and [3]Animal Medicine and Surgery Dept., Cardenal Herrera-CEU University, Valencia, Spain.

Iron (Fe) is of essential importance for aerobic metabolism as a component of blood hemoglobin, cytochromes and several enzymes involved in energy-generating oxidative processes. Trainers frequently provide Fe, even though a positive effect on performance has not been confirmed. This research evaluates resting Fe concentrations in endurance horses, and assesses whether anemia exists and whether exercise influences serum Fe concentrations. Thirty-one performance endurance horses were studied, divided into two groups: A (that covered 80 km/day; n = 20) and B (that covered 160 km in two days, 80 km/day; n = 11). Blood samples were taken at rest and after each vet-gate, i.e., after 27, 60 and 80 km for one day (group A) and for two days (group B). Serum concentrations of Fe and plasma concentrations of total protein (TPP) and albumin (ALB) were measured. Packed cell volume (PCV) was also determined. The animals were not on Fe supplementation at the time of the study. No significant differences were found between both exercise types (A and B). Resting Fe was 129.7 ± 54.82 µg/dL and PCV was 36.90 ± 3.208%. Although serum Fe concentrations increased after 27, 60 and 80 km, they did not reach significant differences compared with resting values. Anemia was not found. Significant positive correlations were found between exercise velocity, ALB and Fe. It is concluded that serum Fe concentrations in our study were within the reference interval for adult horses, and Fe was not affected by the duration of the performed exercise. However, the correlations showed that mild but clinically insignificant changes could appear as a result of dehydration.

58

AGE AND GENDER SHOULD BE CONSIDERED WHEN INTERPRETING SERUM CONCENTRATIONS OF IGF-1 IN SPANISH FOALS. **C. Riber[1,3], A. Muñoz[1,3], K. Satué[2], P. Trigo[3], F.C. Castejón[3].** [1]Animal Medicine and Surgery, Córdoba University, Spain; [2]Animal Medicine and Surgery, Cardenal Herrera-CEU University, Valencia, Spain; and [3]Equine Sport Medicine Centre, Córdoba University, Spain.

Developmental orthopedic disorders (DOD) are common causes of pain and lameness in sport horses and include clinical entities such as osteochondritis dissecans, subchondral bone cysts, angular limb deformities, physitis and cuboidal bone abnormalities. Insulin-like growth factor 1 (IGF-1) is lower in healthy horses than in those with DOD. However, IGF-1 might be affected by breed, age and gender. This study establishes reference values for IGF-1 in DOD-free Spanish foals of both genders and different age. Eighty-seven Spanish foals were divided into 7 age groups: A (2 months old, 3 colts, 6 fillies), B (3 months old, 5 colts, 6 fillies), C (4 months old, 10 colts, 3 fillies), D (5 months old, 3 colts, 5 fillies), E (6 months, 3 colts, 4 fillies), F (1–2 years old, 5 colts, 5 fillies) and G (3–4 yeas old; 7 colts, 12 fillies). Mean values found in colts were 235.3 ± 32.68, 207.1 ± 26.39, 170.6 ± 28.52, 155.4 ± 20.05, 137.0 ± 25.91, 105.6 ± 39.78, 98.57 ± 28.72 ng/mL and in fillies were: 245.4 ± 32.68, 194.7 ± 39.29, 174.2 ± 23.68, 161.7 ± 19.85, 66.36 ± 11.67, 86.60 ± 23.03 and 73.59 ± 16.64 ng/mL for the different groups. Serum IGF-1 was significantly higher in colts than in fillies in group G. Colts between 2 and 6 months had higher IGF-1 and from this age on, significant differences linked to age were not detected. Fillies between 2 and 4 months had higher IGF-1 than fillies between 4 and 6 months and both had higher IGF-1 than fillies older than 1 year. In conclusion, IGF-1 decreased with age in Spanish horses, especially during the first 6 months of age. The influence of gender seems to be less marked.

59

COULD SERUM IRON BE USED AS A MARKER OF MUSCLE INFLAMMATION IN ENDURANCE HORSES WITH EXERTIONAL RHABDOMYOLYSIS? **A. Muñoz[1,2], C. Riber[1,2], P. Trigo[2], K. Satué[3], F.C. Castejón[2].** [1]Medicine and Surgery Dept and [2]Equine Sport Medicine Centre, Córdoba University, Spain; and [3]Medicine and Surgery Dept., Cardenal Herrera-CEU University, Valencia, Spain.

Significant decreases in serum iron concentrations occur within 24 hrs of experimentally induced inflammation in horses. Hypoferremia has been found in illnesses associated with infection, tissue injury and inflammatory processes. Equine rhabdomyolysis is common during or after prolonged exercise, with muscle cell disruption and inflammation. This research compared serum iron concentrations in endurance horses with and without rhabdomyolysis in order to verify whether iron could reflect acute muscle inflammation. Blood samples were collected at rest and after 80 km of competition from 6 horses with rhabdomyolysis (RHB; myoglobinuria, muscle pain, and stiffness and confirmed with serum CK higher than 1000 IU/L) and from 11 horses without clinical and/or laboratory evidence of muscle injury. No significant differences in Fe, serum muscle enzymes (CK, AST and LDH) and markers of hydration (PCV, total plasma proteins, TPP and albumin, ALB) were found between groups at rest. At the end of the competition, RHB showed higher CK (2036 vs. 311.8 IU/L), AST (337.3 vs. 221.4 IU/L), LDH (983.0 vs. 525 IU/L), PCV (55 vs. 48.2%), TPP (8.280 vs. 7.459 g/dl) and ALB (4.035 vs. 3.086 g/dL). Differences in serum Fe concentrations were not detected between groups, although successful horses presented a non-significant trend towards lower Fe concentrations (121.1 vs. 154.8 µg/L in RHB). A weak correlation was found between Fe and CK ($r = 0.520$). In conclusion, serum Fe concentrations did not aid in the diagnosis of acute muscle injury in endurance horses. Possible reasons are differences between systemic and localized inflammation, effect of hemoconcentration and/or exercise-induced hemolysis.

60

REFERENCE VALUE ADVISOR: FREEWARE TO HELP COMPUTE REFERENCE LIMITS. **A. Geffré[1], D. Concordet[2], J.P. Braun[1,2], C. Trumel[1].** [1]Département des Sciences Cliniques (Groupe de Recherche en Animaux de Compagnie) and [2]UMR 181 Physiopathologie et Toxicologie Expérimentales, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France.

Background: International recommendations to determine reference intervals have been recently updated (IFCC-CLSI, C28-A3) especially for small reference sample groups: robust method and Box-Cox transformation are now recommended. Unfortunately, these methods are not yet available with most software used for clinical laboratory data analysis. Objective: To make a set of macroinstructions for the widely available Excel software to calculate reference limits according to different methods. Results: For any series of data, this freeware: 1) calculates the reference limits (with 90% CI) by a nonparametric method (when $n \geq 120$) and by a parametric and robust method from native and Box-Cox transformed values; 2) tests the normality of distributions according to Anderson-Darling and outliers according to Tukey and to Dixon-Reed; 3) shows the distribution of values (dot-plot and histograms) and QQ plots for visual normality inspection; and 4) provides some observations according to C28-A3. Discussion and Conclusion: The main point in determination of reference intervals is the correct selection of as large a number of reference individuals as possible and analysis of specimens under controlled preanalytical and analytical conditions. Whatever computing tools are used, they cannot compensate for low numbers, poor methods, etc. This freeware offers the possibility to rapidly assess and compare results calculated by different methods, including methods that are not available currently, thus allowing selection of the most appropriate method, especially one that provides the CI of limits. This should be useful in veterinary clinical pathology where only small reference sample groups are available.

61

ANTIAGGREGANTS FOR PLATELET COUNTING IN FELINE BLOOD. **F. Granat[1], A. Geffré[1], J.P. Braun[1,2], C. Trumel[1].** [1]Département des Sciences Cliniques Groupe de Recherches Animaux de Compagnie and [2]UMR 181 Physiopathologie and Toxicologie Experimentales INRA, ENVT, Ecole Nationale Vétérinaire, Toulouse, France.

Background: Due to platelet clumping, platelet and white cell counts obtained with cell counters are often erroneous in feline blood. The use of EDTA or citrate alone as an anticoagulant for such samples is questionable. CTAD (citrate, theophylline,

adenosine and dipyridamole) is more efficient for feline samples and the antibiotic, kanamycin, limits the in-vitro aggregation in some human blood samples. Objectives: To compare platelet aggregation and platelet and WBC counts in standard EDTA-K3 tubes and in EDTA-K3 tubes supplemented with CTAD with or without kanamycine. Methods: 46 EDTA-K3 feline blood samples were added to CTAD and CTAD+kanamycin preparations. Aggregation was quantified on blood smears, and then platelet and WBC counts were performed on the 3 specimens with a Sysmex XT2000iV analyser. A smear estimation of the platelet count was also performed as a basis for comparison. Results: According to the degree of aggregation, specimens were divided into two groups: 11 and 35 samples had a high and low degree of aggregation, respectively. CTAD and CTAD+kanamycin significantly reduced platelet clumping, thus allowing more accurate platelet and white blood cell counts than EDTA alone in "aggregated" samples. Conclusion: The addition of CTAD with or without kanamycin to EDTA allows more accurate platelet and white blood cell counts in feline samples, especially when platelet clumping can be expected.

62

EVALUATION OF THE EFFECT OF THE COLLECTION REGIME ON RENAL URINARY BIOMARKERS. **M. Pinches, C. Betts, S. Bickerton, L. Burdett, N. Derbyshire, H. Jones, H. Caddick, M. Moores, S. Sourial.** Safety Assessment AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK.

Background: Numerous novel urinary biomarkers for rodents have recently become available in multiplex assay format. To minimise the impact on animal welfare, guidelines recommend the use of 6-hour (hr) timed urine collection. However, to date only 18-hr overnight urine collections have been used in evaluation studies. Objective: The aim of this study was to evaluate the influence of a shorter (6-hr vs 18-hr) timed urine collection on routine (total protein, glucose and NAG) and emerging renal urinary biomarkers (albumin, aGST, calbindin d28, GSTYB1, lipocalin, KIM-1, osteopontin, and RPA-1). Methods: Multiple groups of 10 male han-wistar rats were given a single dose of 0.1 mg, 1 mg or 2.5 mg cisplatin to induce renal injury. Urine was collected at multiple time points thereafter. At each time point, urine was initially collected by 6-hr-timed collection, followed by an 18-hr-timed collection. Results: Difference plots were constructed between 6-hr and 18-hr collections and percentage bias calculated for all samples. Pearson correlation coefficients with 95% confidence intervals were also calculated. Percentage bias between collections was calculated to be: KIM-1 (4.0), urinary total protein (6.2), albumin (9.0), lipocalin (15.6), osteopontin (17.3), calbindin (38.8), aGST (35.2), RPA-1 (41.2), urine glucose (46.3), GSTYb1 (46.4), and NAG (124). Pearson correlation coefficient values were > 0.83 for all biomarkers except urinary NAG and calbindin. Conclusions: There was correlation and limited bias between different urine collections in KIM-1, total protein, osteopontin, albumin and lipocalin. There was correlation but moderate bias between different urine collections in aGST, urine glucose, GSTYb1, and RPA-1.

63

COMPARISON OF THREE DIFFERENT METHODS OF URINE CANINE SEDIMENT PREPARATION FOR MICROSCOPIC ANALYSIS. **C. Layssol[1], A. Geffré[1], J.P. Braun[1,2], C. Trumel[1].** [1]Department of Clinical Sciences, Groupe de Recherche des Animaux de Compagnie and [2]UMR 181 Physiopathologie and Toxicologie Expérimentales INRA, Ecole Nationale Vétérinaire, Toulouse, France.

Background: Common recommendations for urine sediment preparation in small animal veterinary medicine are low speed (approximately 400 g) centrifugation for 5 minutes and large urine volume. The latter is frequently difficult to obtain in small dogs and cats. In addition, many veterinary practitioners only have a benchtop high speed/low volume/short time centrifuge. Objective: To compare three different methods of urine sediment preparation: low speed and large volume (LS/LV), low speed and small volume (LS/SV) and high speed and small volume (HS/SV). Methods: Three aliquots of 50 canine urine samples were processed as follows: 1) LS/LV: 5 ml at 400 g for 5 minutes with a ROTOFIX 32A centrifuge; 2) LS/SV: 1.5 ml at 250 g for 5 minutes with a ROTOFIX 32A centrifuge; and 3) HS/SV: 1.5 ml at 3900 g for 45 seconds with a high speed benchtop centrifuge STATSPIN. Microscopic examination was performed by the same person: red blood cells, white blood cells, epithelial cells, crystals and casts were counted in 10 fields. Means were compared by Student's paired *t*-test or Wilcoxon test (+Bonferroni's correction) according to the homogeneity of variances. Results: There was no significant difference for any paired results except for cast counts in LS/LV and HS/SV. Conclusion: For canine urine sediment preparation, when necessary or convenient high speed-short time sedimentation of a small volume of urine can be a valid substitute for the usual LS/LV procedure.

64

HEMATOLOGY AND PLASMA BIOCHEMISTRY REFERENCE VALUES IN THE FREE-LIVING BLACK VULTURE *(AEGYPIUS MONACHUS)* IN THE DADIA FOREST RESERVE, THRACE, GREECE. **M. Kritsepi- Konstantinou[1], A. Komnenou[2], I. Georgopoulou[3], A.-L. Thomas[2], D. Vasilakis[4], Th. Skartsi[4].** [1]Diagnostic Laboratory, [2]Clinic of Companion Animal Medicine, and [3]Clinic of Avian Medicine, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece; and [4]Dadia Project, WWF Greece, Athens, Greece.

The National Park in Dadia hosts some very important and rare species of raptors. Among all hosted raptors, the flag species of the park is the Black Vulture (*Aegipius monachus*), which is preserved in the forest, its last breeding colony in the Balkans and one of the few remaining in Europe. Knowledge of hematological and biochemical parameters in free-living birds is important for assessing and managing the remaining populations, particularly in the case of rare or endangered species. The goal of our study was to establish reference values and compare them among different age groups. Blood samples were collected from 74 apparently healthy, free-living *A. monachus*. A blood chemistry profile, including 17 parameters, was available for 73 of them while a complete blood count was obtained in 22 birds. Regarding their age, there were 26 nestlings, 32 immature and 16 adults. Nestlings had significantly lower hematocrit values compared with immature

($p = 0.007$) and adult birds ($p = 0.004$). There was a statistically significant rise in hemoglobin concentration and erythrocyte count from nestlings over immature to adult birds (p for trend 0.001 and 0.003 respectively). On the other hand, alkaline phosphatase, creatine phosphokinase and lactate dehydrogenase levels progressively decreased with increasing age (p for trend 0.002, 0.038 and 0.017, respectively). Adult birds had significantly lower eosinophil counts compared with nestlings ($p = 0.031$) and immature birds ($p = 0.037$). Conversely, total protein levels were significantly higher among adult birds, compared with nestlings ($p = 0.003$) and immature birds ($p = 0.0002$). The results of our study are the first reported in free-living Black Vultures in Greece.

65

STABILITY OF HEMATOLOGY ANALYTES IN FELINE BLOOD STORED FOR 2 DAYS AT ROOM TEMPERATURE. **N. Bourgès-Abella[1], F. Granat[1], A. Geffré[1], J.P. Braun[1,2], C. Trumel[1].** [1]Département des Sciences Cliniques Groupe de Recherches Animaux de Compagnie and [2]UMR 181 Physiopathologie and Toxicologie Experimentales INRA, ENVT, Ecole Nationale Vétérinaire, Toulouse, France.

Background: In-clinic hematology analyzers are not available in all veterinary practices. Thus, some analyses cannot be performed within a few hours of blood sampling. Objective: To compare results of automated blood analyses of feline blood stored for up to 2 days at room temperature (~20°C). Methods: 46 EDTA-K3 feline blood specimens were analyzed with a Sysmex XT2000iV analyser within one hour of sampling. They were then stored at room temperature. After 24 and 48 hours, specimens were gently mixed and reanalyzed. After performing ANOVA for paired samples and testing for homogeneity of variances, paired comparisons were performed by the Wilcoxon test with Bonferroni's correction. Results: ANOVA showed decreases (mean change in parentheses for 24 and 48 hours of storage, respectively) in RBC-impedance (0.15×10^{12}/L for both hours), MCHC (38.2 and 53.0 g/L), eosinophils (0.2 and 0.37×10^9/L), basophils (0.05 and 0.07×10^9/L), and increases in hemoglobin (0.9 and 1.2 g/L), hematocrit (0.05 and 0.08 L/L), MCV (6 and 9 fL), PLT-optical (23 and 53×10^9/L), RDW-SD (3.6 and 7.6 fL), and reticulocyte count (12.9 and 24.5×10^9/L). In specimens showing platelet clumping at t 0, PLT count was increased on the following days. Conclusion: Feline hematology parameters obtained on blood stored for up to 48 hours at room temperature and analyzed on the XT-2000iV were mainly stable except for some red blood cell parameters such as hematocrit, MCV and reticulocyte count.

66

STABILITY OF HEMATOLOGY ANALYTES IN CANINE BLOOD STORED FOR 2 DAYS AT ROOM TEMPERATURE. **N. Bourgès-Abella[1], P. Deshuilliers[1], A. Geffré[1], J.P. Braun[1,2], C. Trumel[1].** [1]Département des Sciences Cliniques Groupe de Recherches Animaux de Compagnie and [2]UMR 181 Physiopathologie and Toxicologie Experimentales INRA, ENVT, Ecole Nationale Vétérinaire, Toulouse, France.

Background: In-clinic hematology analyzers are not available in all veterinary practices. Thus, some analyses cannot be performed within a few hours of blood sampling. Objectives: To compare results of automated blood analyses in canine blood stored for up to 2 days at room temperature (~20°C). Methods: 42 EDTA-K3 canine blood specimens were analyzed with a Sysmex XT2000iV analyser within one hour of sampling. They were then stored at room temperature. After 24 and 48 hours, specimens were gently mixed and reanalyzed. After performing ANOVA for paired samples and testing for homogeneity of variances, paired comparisons were performed by the Wilcoxon test with Bonferroni's correction. Results: ANOVA showed decreases (mean changes in parentheses for 24 and 48 hours of storage, respectively) in RBC-impedance (0.3 and 0.5×10^{12}/L), MCHC (37.3 and 57.7 g/L), lymphocytes (0.11 and 0.25×10^9/L), monocytes (0.35 and 0.59×10^9/L), basophils (0.0015 and 0.0041×10^9/L), platelet-impedance (40.4 and 75.7×10^9/L) and platelet-optical (29.2 and 49.6×10^9/L) and increases in hemoglobin (1.0 and 1.13 g/L), hematocrit (0.052 and 0.087 L/L), MCV (8 and 14 fL), RDW-SD (4.3 and 7.9 fL), and reticulocytes (9.2 and 25.4×10^9/L). Conclusion: Canine hematology run on blood stored for up to 48 hours at room temperature and analyzed on the XT-2000iV analyser was mainly stable except for parameters such as hematocrit, MCV and reticulocyte count.