BRISTOL 2025
Congress of the ESVCP and ECVCP

27th Annual ESVCP & ECVCP Congress

1- 4 October 2025

European Society Veterinary Clinical Pathology

ECVCP

Congress Venue
The Watershed
1 Canons Road
Bristol
BS1 5TX

Gala Dinner Venue
Bristol Museum &
Art Gallery
Bristol
BS8 1RL

Wednesday 1 October 2025 (Residents Day) Chair: Kostas Papasouliotis & Marta Costa	
TIME	**CINEMA 3**
9:00 - 9:45	Equine Respiratory Cytology EMMA DICKEY
9:45 - 10:30	Cytology of Equine Joints, Eyes and CSF EMMA DICKEY
10:30 -11:15	Break
11:15 -12:00	Investigating Subacute Ruminal Acidosis in Dairy Herds GEORGE OIKONOMOU
12:00 -12:45	Downer Cows, Twisted Guts and Sick Calves - Can You Help the Farm Animal Vet? GEORGE OIKONOMOU
12:45 - 2:00	Lunch
2:00 - 2:45	Metabolic Challenges in Transition Dairy Cows: Energy, Protein and Calcium Homeostasis NEKTARIOS SIACHOS
2:45-3:30	Clinical Pathology in the Rabbit: A Case Based Approach RICHARD SAUNDERS
3:30 - 4:00	Break
4:00 - 4:45	Hormonal Disorders and Endocrinopathies in the Rabbit RICHARD SAUNDERS
4:45 - 5:30	Microbiology and Antimicrobial Selection in the Rabbit RICHARD SAUNDERS
6:00 - 8:00	**WELCOME RECEPTION (Watershed 2 & 3)**

Thursday 2 October 2025		
TIME	**CINEMA 1**	**CINEMA 3**
9:00 - 9:45	CHAIR: STEPHAN NEUMAN PLENARY LECTURE: AI in Veterinary Medicine: Ethics, Challenges ADELE WILLIAMS-XAVIER	
9:45 - 10:30	CHAIR: STEPHAN NEUMAN The In-Clinic Lab: Quality Assurance (Part 1) EMMA HOOIJBERG	CHAIR: MARTA COSTA The In-Clinic Lab: Measurement of Acute Phase Proteins EMMA DEWHURST IDEXX
10:30 - 11:15	Break	Break
11:15 - 12:00	CHAIR: NIKI SKELDON The In-Clinic Lab: Quality Assurance (Part 2) EMMA HOOIJBERG	CHAIR: MARTA COSTA Diagnostic Microbiology - Supporting Antimicrobial Stewardship: Updated Clinical Microbiology Methods and Guidelines SIAN FROSINI
12:00 - 12:45	CHAIR: NIKI SKELDON The In-Clinic Lab: Haematology Analysers ANDREAS MORITZ IDEXX	CHAIR: MARTA COSTA Diagnostic Microbiology - The clinical pathologist - microbiologist interface: how can we support each other?pathologist - microbiologist interface - how can we support each other? SIAN FROSINI
12:45 - 14:00	Lunch	Lunch
14:00 - 14:45	CHAIR: ALESSIA GIORDANO The In-Clinic Lab: Haematology Analysers ANDREAS MORITZ IDEXX	Canine Vector Borne Disease: Decision Making Based on POC Serological Testing MATHIOS MYLONAKIS
14:45 - 15:30	CHAIR: ALESSIA GIORDANO The In-Clinic Lab: Biochemistry Analysers JOSEP PASTOR	Clinicopathological Aspects of Canine Monocytic Ehrlichiosis (Ehrlichia Canis) MATHIOS MYLONAKIS
15:30 - 16:00	Break	Break
16:00 - 16:45	CHAIR: TIM WILLIAMS The in-Clinic Lab: Thyroid Hormones IOANNIS OIKONOMIDIS	CHAIR: BUTTY VILLIERS Advanced LAB diagnostics-Clinical Applications Mass Spectroscopy TOMMASO FURLANELLO
16:45 - 17:30	CHAIR: TIM WILLIAMS The in-Clinic Lab: Adrenal Hormones IOANNIS OIKONOMIDIS	CHAIR: BUTTY VILLIERS Advanced LAB diagnostics Immunocytochemistry NAZARE DA CUNHA
17:45 - 19:00		**ECVCP AGM**

Friday 3 October 2025		
TIME	**CINEMA 1**	**CINEMA 3**
08.30 - 9.00		Research Communications
9:00 - 9:45	CHAIR: KATE IRVINE Internal Medicine & Clinical Pathology Cases JORGE PENA-RAMOS SONIA SANCHEZ-REDONDO	Research Communications
9:45 - 10:30	CHAIR: KATE IRVINE Internal Medicine & Clinical Pathology Cases JORGE PENA-RAMOS SONIA SANCHEZ-REDONDO	Research Communications
10:30 - 11:15	Break	Break
11:15 - 12:00	CHAIR: FRANCESCO CIAN Diagnostic Imaging & Cytology Cases TOBIAS SCHWARZ CHARALAMPOS ATTIPA	Research Communications
12:00 - 12:45	CHAIR: FRANCESCO CIAN Diagnostic Imaging & Cytology Cases TOBIAS SCHWARZ CHARALAMPOS ATTIPA	Research Communications
12:45 - 14:00	Lunch	Lunch
14:00 - 14:45	CHAIR: STEFANIE KLENNER Biomarkers for Gastric and Pancreatic Diseases ED HALL	Research Communications
14:45 - 15:30	CHAIR: STEFANIE KLENNER Biomarkers for Intestinal Diseases ED HALL	Research Communications
15:30 - 16:00	Break	**END OF SESSION**
16:00 - 16:45	Panel Discussion - Clinical Pathologists Survey Recruit & Retain KATHY FREEMAN ZOE POLIZOPOULOU	
16:45 - 17:30	Panel Discussion - Clinical Pathologists Survey Recruit & Retain KATHY FREEMAN ZOE POLIZOPOULOU	
17:45 - 19:00		**ESVCP AGM**
20:00 - Late		**GALA DINNER**

Saturday 4 October 2025		
TIME	**CINEMA 1**	**CINEMA 3**
9:00 - 9:45	Mystery Clinical Cases Presentation (CHAIRS) MARIANA SERRA &MARTA COSTA	CHAIR: KOSTAS PAPASOULIOTIS Cytology of Skin Lumps & Bumps: Inflammatory or Neoplastic? ELPIDA SARVANI
9:45 - 10:30	Mystery Clinical Cases Presentation (CHAIRS) MARIANA SERRA &MARTA COSTA	CHAIR: KOSTAS PAPASOULIOTIS Haematology Analysers - Understanding the Plots & Graphs CLARE ROBERTS
10:30 - 11:15	Break	Break
11:15 - 12:00	CHAIR: SILVIA ROSSI Mystery Cytology Slides Discussion CARLO MASSERDOTTI	Clinical Biochemistry Results - Fact or Artefact? MATT GARLAND
12:00 -12:45	CHAIR: SILVIA ROSSI Mystery Cytology Slides Discussion CARLO MASSERDOTTI	Urine Analysis Results & Findings Fact or Artefact? MATT GARLAND
12:45 - 13:00	**END OF CONGRESS - FAREWELL**	

Friday 3 October 2025
Research Communications

8.30 - 8.45 Alex Currie - Canine serum procalcitonin concentrations in canine pyelonephritis.

8.45 - 9.00 Estelle Lua - Effects of different creatinine analytical methods on Urine Protein to Creatinine ratio variability in canines.

9.00 - 9.15 Ingo Schafer - Impact of infection intensity, antibody levels, and stays abroad on hematological and biochemical parameters as well as acute-phase proteins in 342 dogs with acute Babesia canis infections.

9.15 - 9.30 Lina Boukadda - DGGR-lipase for Canine Pancreatitis: Siemens-Atellica-930 liquid assay concords with IDEXX-Catalyst, dry-reagent, activity-assays.

9.30 - 9.45 Tim Williams - Analysis of serum amylase activity in dogs with portosystemic shunts.

9.45 - 10.00 Anne K H Krogh - C-reactive protein and Serum Amyloid A concentrations in serum samples from a large cohort of dogs – corresponding and discordant results.

10.00 - 10.15 Monika Keresztes - Application of the Gyrolab xPlore microfluidic platform for establishment of safety biomarkers: Lessons from ACTH and Beyond.

10.15 - 10.30 Belén Larrán Franco - Plasma trace element profiles in dogs with cancer: Associations with tumour type and clinical status.

11.15 - 11.30 Federico Bonsembiante - Relationship between inflamm-aging and myxomatous mitral valve disease (MMVD) in senior and geriatric dogs.

11.30 - 11.45 Enda O'Hagan - Novel, Inexpensive, Multicolour-Stick, DGGR-Lipase, Point-of-Care Test for Canine Pancreatitis.

Friday 3 October 2025
Research Communications

11.45 - 12.00 Michael Frill - Assessment of Oxidative Stress and Cellular Response in Progressive Feline Chronic Kidney Disease (CKD): TBARS and Glutathione Peroxidase Activity in Kidney Lysates.

12.00 - 12.15 Amelia Goddard - Scanning electron microscopy of the fibrin network of thrombi in dogs with Babesia rossi infection.

12.15 - 12.30 Liesl van Rooyen - The use of an indirect method of reference interval determination to assess age-related changes in selected measurands in adult Labrador retrievers.

12.30 - 12.45 Kevin Le Boedec - HARISS: Histogram Analyzer for Reference Intervals of Small Samples, a free web app to estimate reference intervals of small samples by automatic visual inspection of distribution histograms.

2.00 - 2.15 Felipe Reggeti - Bone Marrow Changes Mimicking Malignancy in Dogs with Phenobarbital-Induced Myelotoxicity.

2.15 - 2.30 Larissa Almeida - Canine Leukaemia: Retrospective study of 127 Irish cases.

2.30 - 2.45 Chiara Masci - Evaluation of Immature Platelet Fraction in the Sysmex XN-2000V in healthy and thrombocytopenic dogs.

2.45 - 3.00 Emma Strage - EDTA contamination in feline and canine sera.

3.00 - 3.15 Javier Martínez-Caro - Differentiation between canine large B-cell and T-cell lymphoma using the Sysmex XN-1000V: a diagnostic performance study.

3.15 - 3.30 Barbara Riond - Novel neutrophilic parameters of the Sysmex XN-1000V for the prediction of inflammation in dogs.

Saturday 4 October 2025

Mystery Clinical Cases

9.00 - 9.15
Peripheral Lymphadenomegaly In A Jack Russell Terrier
Argyrios Ginoudis

9.15 - 9.30
Cervical Swelling In A Dog
Ignacio Amarillo-Gómez

9.30 - 9.45
The Mystery Of The Vomiting Cat: A Granular Analysis Of Splenic And Hepatic Nodules
Theo Chenal

9.45 - 10.00
Urinary Cytological Twist
Marta Lemos

10.00 - 10.15
Unexpected Cytological Findings In A Feline Neck Mass
Sara Meazzi

10.15 - 10.30
Gingival Mass In A Dog
Maša Vilfan

Posters

Alessandra Gavazza - National Italian Guidelines On Canine And Feline Transfusion Medicine
Alessandra Gavazza - Evaluation Of Reticulocyte Hemoglobin Content In Dogs Affected By Neoplastic Disease
Alex Draper - Method Validation And Establishment Of Reference Intervals For Serum Protein Concentrations Analysed By Capillary Zone Electrophoresis, In German Warmblood Horses, Compared To Agarose Gel Electrophoresis'
Alexandra Dreanca - Retrospective Study Of Anemia In Dogs In Eastern Europe – Romania Using The Mindray Poc Analzyer With Irf
Angelica Stranieri - Alterations In The Serum Protein Electrophoretic Patterns In Dogs Following Administration Of An Iodinated Contrast Medium
Angelica Stranieri - Sysmex XN-1000V WDF Scattergram In A Dog With Hepatozoon Spp. Infection
Annemarie Baur-Kaufhold - Diagnosis Of Anti-Erythrocyte Antibodies In Equine Blood Samples:Direct Antiglobulin (DAT)-Test Versus Direct Immunofluorescence Flow Cytometry (DIF)
Argyrios Ginoudis - Evaluation Of The Utility Of Fragmented Red Blood Cells Automated Measurement For The Detection Of Schistocytosis In Dogs Using The ADVIA 2120i
Argyrios Ginoudis - Imprint Cytology In The Diagnosis Of Meningiomas In Dogs And Cats
Azalea Hani Othman - Evaluation Of The Hematological And Serum Biochemistry Profile Of Bornean Sun Bears (Helarctos Malayanus Euryspilus) In Matang Wildlife Centre And Bornean Sun Bear Conservation Centre, Malaysia
Carola Curcio - Reference Intervals For The Sysmex KX-21 Hematology And Sclavo Konelab 200 Analyzers In Sprague-Dawley Rats
Evelyn Kuhlmeier - Preliminary Results On The Evaluation Of Hematologic Parameters In Humboldt Penguins (Spheniscus Humboldti) Using The Sysmex XN-1000V Analyzer And The PLT-F Channel
Gabriele Ghisleni - Diagnostic Cytology Of Teleost Fish
Gabriele Rossi - Long-Term Endocrine And Biochemical Effects Of SGLT2i Treatment In Horses With Equine Metabolic Syndrome
Ingo Schafer - Apolipoprotein A-1 Does Not Appear To Be A Suitable Acute Phase Reaction Marker In Canine Babesiosis And Hemoplasmosis
Ingo Schafer - Clinical Suspicion Of In-Vivo Resistance Against Allopurinol In Five Dogs Infected With Leishmania Infantum Associated With A Reduction In Copy Numbers Of The S-Adenosylmethionine Synthetase (METK) Gene
Ingo Schafer - Cytokine Concentrations (IL-8, IL-10, MCP-1) In Dogs With Acute Babesia Canis Infections, Dogs With Hemotropic Mycoplasma Infections, And Clinically Healthy Dogs
Ioana Madelina Moraru - Preliminary Results On Leukocyte Differentiation In Rabbits Using The Mindray BC-60R Analyzer
Ioana Madelina Moraru - Validation Of Home-Use Urinary Screening Kits For Hematuria And Ph Detection In Cats
Ioana Sandu - Are cytology slides and blood smears reliable materials for molecular diagnosis in veterinary medicine?
Ioana Sandu - Evaluation Of Nucleic Acid Stability In Blood Smears For Retrospective RT-PCR
Ioana Sandu - Renal Mucormycosis Due To Cunninghamella Bertholletiae Infection In A Cat

Posters

Ivana Vanova - Serum Proteins And Effusions: Which Cut-Off Really Matters?
Ivana Vanova - Urinary ALP And GGT In Dogs With Neoplasia: Preliminary Data
Joana Fonseca - Ki-67 As A Prognostic Marker In Feline Lymphoma: Preliminary Findings Using Immunocytochemistry
Katrin Toerner - Molecular Genetic Characterization Of 84 Canine Tumours By Commercial Sequencing Panels **Kevin Le Boedec** - Reference Intervals From Small Samples: HARISS Versus Robust Versus Parametric Or Nonparametric Methods
Kristina Rehakova - Buffy Coat Basophils In Canine Patients With Neoplasia: Silent, But Not Mute.
Labrini Athanasiou - White Blood Cells And Platelets Alterations In Diarrheic Neonatal Lambs With Or Without Signs Of Bacteremia
Lucie Koudelkova - Basophils In Canine And Feline Buffy Coats: How Rare Do They Dare To Be?
Maria Giulia Ferrari - Cytologic Examina/On Of The Liver In Dogs With Non-Associative Immunemediated Hemolytic Anemia
Marilisa Novacco - Evaluation Of Automated Hematology Analysis Using Sysmex XN-1000V In Healthy And Diseased Long-Tailed Chinchillas (Chinchilla Lanigera)
Martina Baldin - Oxidative Stress Markers And Acute Phase Proteins In Dairy Calves Undergoing Different Vaccination Protocols Against Bovine Respiratory Disease
Myriam Defontis - Cytological Composition Of Bone Marrow In Athymic Nude RH-Foxn1rnu Rats
Nicolas Soetart - Assessment Of Plasma Homocysteine Levels In Canine Leishmaniosis
Raquel Pato - Verification Of The MAGLUMI 800 Chemiluminescence Immunoanalyzer For Measurement Of T4, TSH And Cortisol In Canine Plasma Samples
Reinhard Mischke - Haemostatic Abnormalities In Dogs Suffering From Haemangiosarcoma
Sabiha Zarin Tasnim Bristi - Accuracy Of Hemoglobin Instrumental Measurement In Little Owls (Athene Noctua)
Sabine Hammer - Lymphocyte Clonality Testing In Feline Intestinal Lympho-Plasmacellular Infiltration: Friend Or Foe?
Sabine Hammer - The European Canine Lymphoma Network (ECLN) – A Joint Initiative For Comparative Studies Of Neoplastic Diseases In Dogs
Samuel Demssie - Concurrent Rabies And Canine Distemper Infections In Endangered Ethiopian Wolves: Diagnostic And Conservation Challenges
Sandra Lapsina - Establishing Reference Intervals For T4, T3 And Reverse Triiodothyronine (Rt3) Via LC-MS/MS In Clinically Healthy Dogs
Sara Meazzi - Agreement Between SYSMEX XN-V And Hemocue201+ In Measuring Hemoglobin From Testudo Hermanni
Verónica Mato Martín - Comparison Of Manual And Automated Hematological Parameters In Hyacinth Macaws (Anodorhynchus Hyacinthinus) Using The Sysmex XN-1000V And The New PLT-F Channel
Virginia Bettoni - Comparison Of Biochemical And Electrophoretical Evaluation Of Serum Lipoproteins In Assessing Inflammation Or Oxidation In Dogs

Wednesday 1 October 2025

(Residents Day)

Equine Respiratory Cytology

Emma Dickey MS BVMS (Hons) DipACVP DipACVIM MRCVS

Equine respiratory washes are one of the most commonly submitted equine samples for cytology. Collection techniques include tracheal wash (via endoscopy and transtracheal aspirate) and bronchoalveolar lavage (usually performed blindly with catheter but can also be performed via bronchoscopy). Tracheal wash fluid is suitable for both cytology and culture but BAL fluid is typically only appropriate for cytology.
Tracheal wash samples contain mixed secretions from all lobes, so are a good screening test and are best for bacteriology. The 'tracheal puddle' however does not necessarily reflect the small airways and alveoli.
BAL samples when performed blindly, typically sample the smaller distal airways in the right middle lung lobe. It assumes diffuse disease so may not necessarily be abnormal with focal/multifocal pathology. Generally in most situations BAL samples are not suitable for culture.

	Tracheal Wash	Bronchoalveolar Lavage
Neutrophils %	<20	<5
Lymphocytes %	<10	20-50
Macrophages %	40-80	40-80
Eosinophils %	<2	<1
Mast cells %	<1	<2

Causes of neutrophilic airway inflammation in horses include Equine Asthma, Infectious disease, aspiration, inhalation of irritant substances, interstitial disease (eg Equine Multinodular Pulmonary Fibrosis) and neoplasia.
Equine Asthma is one of the most commonly diagnosed disease processes in equine respiratory cytology. Mild to moderate equine asthma (previously Inflammatory Airway Disease IAD) typically affects young to middle age, and presents with poor performance, mild coughing and no heaves. Severe equine asthma (previously RAO/COPD) usually affects older animals and presents with coughing, exercise intolerance and increased respiratory rate and effort at rest. Signs can be seasonal (pasture associated).

Dictyocaulus arnfieldi (Lungworm) infection typically produces marked eosinophilic inflammation. Although horses can be infected, disease is rarely patent, and usually occurs when there is co grazing with donkeys.
Equine Pulmonary Multinodular Fibrosis (EMPF) is a severe progressive and fibrosing interstitial lung disease of adult horses. Correlation with EHV-5 infection.
Causes of haemorrhage include Exercise Induced Pulmonary Haemorrhage (EIPH) as well as inhalation of blood due to epistaxis, bleeding from neoplasm within respiratory system, pneumonia/pulmonary abscessation, cardiovascular disease- atrial fibrillation possible risk factor for EIPH.
Rhodococcus equi is a cause of pneumonia in foals, typically weanlings, Classic history of endemic disease on farm, ill thrift, and 'comet tails' on thoracic ultrasound. Often however pneumonia in foals and weanlings is a mixed bag, commonly involving *Streptococcus equi zooepidemicus*.

References

2021 Equine Hematology, Cytology and Clinical Chemistry 2nd edition, Walton, Cowell and Valenciano

Equine Internal Medicine 2nd edition, Reed, Bayly and Sellon

ACVIM Consensus Statement 2016 30 (2) Inflammatory Airway Disease of Horses- A revised consensus statement

EVE 2013 25 (8) 393-397 Equine multinodular pulmonary fibrosis- Diagnosis and treatment P.A Wilkins

Equine Veterinary Education 2014l 26, (12) Management of chronic airway inflammation in the horse- A systematic review- Ivester and Couetil

Cytology of Equine Joints, Eyes and CSF

Emma Dickey MS BVMS (Hons) DipACVP DipACVIM

Synovial fluid is produced in tendon sheaths, bursae and joints. Up to 1-2ml can be aspirated from normal equine joints. Normal synovial fluid is colourless to light yellow and viscous.

	Normal	Degenerative	Inflammatory
Colour clarity	Colourless to yellow, clear	Colourless, clear	Whitish, turbid to opaque
Viscosity	High	Normal to decreased	Typically decreased
Cellularity cells/ul	<500-1000	<5000	>5000
Neutrophils	<10%	<10%	>10%

Degenerative joint disease includes osteoarthritis and Developmental Orthopaedic Disease (DOD). TNCC <5000/UL and usually mild increase in total protein. Comprises predominantly mononuclear cells (sometimes activated) with <10% neutrophils.

Inflammatory synovitis comprises septic, traumatic, chemical, immune mediated, eosinophilic and lymphocytic (villonodular synovitis). It can be a diagnostic challenge to distinguish between septic synovitis and chemical synovitis (joint flare) as they can have very similar clinical presentations and cytological patterns. It is very important to remember that while identification of intracellular bacteria is confirmatory for sepsis, absence of bacteria on cytology certainly does not exclude sepsis. Similarly a negative culture of joint fluid does not exclude sepsis.

Immune mediated synovitis is not as common as in small animals. It can be seen secondary to bacterial infections such as *Rhodococcus equi* in weanlings.

Corneal cytology preparations are primarily examined for the purposes of identifying bacterial and fungal keratitis when corneal ulceration is present, investigating the possibility of eosinophilic keratitis and also when neoplasia eg SCC is suspected.

Eosinophilic keratitis is thought to be an immune mediated hypersensitivity to environmental/parasitic allergens. Cases typically occur in the Summer and the classic presentation is a plaque like appearance at the medial or lateral canthus, and lesions can be bilateral. However there are a range of presentations, as there can be secondary bacterial and fungal infection. Typically cases of EK require long term treatment.

Equine CSF is an uncommon submission to external laboratories with the major rule out being bacterial meningitis, although certain viral infections are important to consider, especially EHV-1.

CSF can be collected in horses from the atlantooccipital (AO) or lumbosacral sites, with the former typically requiring general anaesthesia. Ultrasound guided CSF collection at C1-C2 is also described.

CSF Reference Intervals

	Adult Horses	Foals
Colour clarity	Colourless clear	Colourless, clear
Total protein	30-80mg/dL	Up to 100-120mg/dL
Cellularity cells/ul	<5	<5
Cytology	Predominantly lymphocytes	Predominantly lymphocytes

References
2018 Interpretation of Equine Laboratory Diagnostics Pusterla and Higgins
Equine Hematology, Cytology and Clinical Chemistry 2nd edition Walton, Cowell and Valenciano
2018 Textbook of Veterinary Diagnostic Radiology 7th Edition, Equine Metacarpophalangeal and metatarsophalangeal joint
In Practice June 2018, Volume 40, Issue 5 Eosinophilic keratitis in horses Hartley C
Equine Neurology 2008 Furr and Reed

Investigating sabacute/ subclinical ruminal acidosis in dairy herds.

Georgios Oikonomou DVM PhD FHEA FRCVS
Department of Livestock and One Health, Institute of Infection, Veterinary, and Ecological Sciences, University of Liverpool

Subacute ruminal acidosis (SARA), also referred to as subclinical ruminal acidosis, is a metabolic disorder in high-yielding dairy herds, arising from diets rich in rapidly fermentable carbohydrates and low in effective fibre. The condition is characterised by prolonged periods of depressed ruminal pH, leading to rumenitis, impaired fibre digestion, osmotic diarrhoea, and colonic acidosis. Unlike acute acidosis, which presents with severe systemic illness, SARA may manifest through more subtle but economically significant signs including reduced feed intake, loose faeces, undigested grains, decreased milk yield and butterfat, lameness (?), poor fertility, and increased disease incidence. The pathophysiology involves imbalances in rumen microbial populations driving excessive volatile fatty acid and lactic acid production, coupled with insufficient buffering or absorption. Diagnosis relies on herd-level observations, dietary assessment, and rumen fluid pH monitoring, with sampling recommended 2–4 hours post-feeding. In this talk we will also be discussing new diagnostic approaches that may allow more accurate herd level assessment. In the future, emerging tools such as microbiomics and metagenomics may also provide new insights into microbial dynamics underpinning SARA.

References:
E. Bramley, I. J. Lean, W. J. Fulkerson, M. A. Stevenson, A. R. Rabiee, and N. D. Costa. 2008. The Definition of Acidosis in Dairy Herds Predominantly Fed on Pasture and Concentrates. J. Dairy Sci. 91:308–321 doi:10.3168/jds.2006-601.

H. M. Golder and I. J. Lean. 2024. Invited review: Ruminal acidosis and its definition. A critical review. J. Dairy Sci. 107:10066–10098 https://doi.org/10.3168/jds.2024-24817.

Downer cows, twisted guts and sick calves; can you help the farm animal vet?

Georgios Oikonomou DVM PhD FHEA FRCVS
Department of Livestock and One Health, Institute of Infection, Veterinary, and Ecological Sciences, University of Liverpool

Farm animal vets are dealing with most of their cases while on farm and therefore diagnosis is often relying entirely on history and clinical examination. In this talk, we will discuss cases relevant to the farm animal veterinarian and how they are usually addressed on farm. Additionally, we will discuss how further diagnostic investigation could help the farm animal vet better address such cases.

References:
Peek S., T. Divers. Rebhun's Diseases of Dairy Cattle, 3rd Edition.

Trefz, A. Lorch, M. Feist, C Sauter-Louis, I Lorenz. Metabolic acidosis in neonatal calf diarrhea. Clinical findings and theoretical assessment of simple treatment protocol. J Vet Intern Med 2021. 26: 162-170

Metabolic challenges in transition dairy cows: energy, protein and calcium homeostasis

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SRUC School of Veterinary Medicine and Biosciences, Aberdeen, UK

Abstract

The transition period, spanning 3 to 4 weeks before and after calving, represents the most physiologically demanding phase in the life of a dairy cow. During this period, profound homeorhetic adaptations occur to support the onset of lactation, yet if in excess they expose cows to a high risk of metabolic disorders and infectious diseases. Approximately 75% of diseases arise within the first month postpartum, reflecting the huge impact maladaptation has on a cow's health and the herd's performance. This presentation attempts to synthesise current knowledge on three axes of transition adaptation: energy metabolism, protein metabolism and calcium homeostasis, while providing novel data to overlooked features of transition cow metabolism.

Transition is characterised by a status of negative energy balance. The abrupt rise in glucose demand for lactose synthesis, doubling by one week after calving, coincides with a lag in feed intake recovery compared to the peak of lactation. Cows rely more on hepatic gluconeogenesis from substrates to source glucose, while insulin resistance spares glucose for the mammary gland but promotes lipolysis. The consequent surge in non-esterified fatty acids fuels *β*-oxidation and ketogenesis, while excess re-esterification risks hepatic triglyceride accumulation. Over-conditioned cows face higher risk of ketosis, fatty liver and displaced abomasum. Lipid mobilisation is an adaptive mechanism, but its magnitude and persistence determine whether pathology ensues (Mann et al., 2022).

Alongside energy deficit, cows experience negative protein balance too. Skeletal muscle represents 60% of a cow's metabolically active tissues and serves as the main labile amino acid reservoir. Muscle proteolysis begins prepartum and continues through the first 3-4 postpartum weeks, with losses of 8–21 kg body protein estimated across this interval. Our work using repeated *longissimus dorsi* thickness measurements and serum creatinine confirms that muscle mobilisation is common and clinically relevant (Siachos et al., 2022). Greater loss was associated with increased odds of metritis, whereas higher prepartum muscle reserves favoured reproductive outcomes. Biomarkers such as 3-methylhistidine, indicative of myofibrillar protein breakdown, revealed both farm-level variation and links with health and fertility outcomes (Siachos et al., 2025).

Calcium homeostasis constitutes a third axis of metabolic adaptations. Calcium requirements rise more than sixfold at calving, overwhelming the capacity of the small extracellular pool. Even modest declines in blood calcium impair gastrointestinal motility, uterine contractility and immune competence, predisposing to transition diseases. Subclinical hypocalcaemia is highly prevalent but occurs in distinct temporal and severity-related patterns (Tsiamadis et al., 2021). Prevention strategies include negative DCAD close-to-calving diets and

phosphorus-binding approaches, each requiring careful implementation and monitoring (Martín-Tereso and Martens, 2014).

To summarise, transition cow biology is best understood not as isolated disorders but rather as interconnected adaptations in energy, protein and mineral metabolism. Muscle mobilisation adds an overlooked dimension to this framework beyond adipose tissue and calcium dynamics. By recognising these processes as well orchestrated yet fragile for the modern high-yielding cow, we can better design management and nutritional strategies that support adaptation while minimising the risk of maladaptation.

References

Mann, S., 2022. Symposium review: The role of adipose tissue in transition dairy cows: Current knowledge and future opportunities. Journal of Dairy Science, 105(4), pp.3687-3701.

Martín-Tereso, J. and Martens, H., 2014. Calcium and magnesium physiology and nutrition in relation to the prevention of milk fever and tetany (dietary management of macrominerals in preventing disease). Veterinary Clinics: Food Animal Practice, 30(3), pp.643-670.

Siachos, N., Tsiamadis, V., Oikonomou, G., Panousis, N., Banos, G., Sampsonidis, I., Kalogiannis, S., Arsenos, G. and Valergakis, G.E., 2024. Variation in protein metabolism biomarkers during the transition period and associations with health, colostrum quality, reproduction, and milk production traits in Holstein cows. Journal of Dairy Science, 107(6), pp.4056-4074.

Siachos, N., Oikonomou, G., Panousis, N., Tsiamadis, V., Banos, G., Arsenos, G. and Valergakis, G.E., 2022. Skeletal muscle and adipose tissue reserves and mobilisation in transition Holstein cows: Part 2 Association with postpartum health, reproductive performance and milk production. Animal, 16(9), p.100626.

Tsiamadis, V., Panousis, N., Siachos, N., Gelasakis, A.I., Banos, G., Kougioumtzis, A., Arsenos, G. and Valergakis, G.E., 2021. Subclinical hypocalcaemia follows specific time-related and severity patterns in post-partum Holstein cows. Animal, 15(1), p.100017.

Clinical pathology in the Rabbit: A case based approach (AKA "The Rabbit that didn't bark")

Richard Saunders BSc (Hons) BVSc FRSB CBiol DZooMed (Mammalian) DipECZM(ZHM) FRCVS
RCVS Specialist in Zoo and Wildlife Medicine (Mammalian)
European Specialist in Zoological Medicine (ZHM)

In this presentation we will discuss the obtaining of blood, and interpretation of haematological and serum biochemical parameters, using real life examples. We will go through normal and abnormal results in a number of rabbit cases, with only the names changed.

Loki is a 3 year old male neutered Dwarf Lop rabbit with sudden onset inappetence and abdominal pain. On obtaining blood, a number of haematological and biochemical abnormalities are seen. The successful management of his case depends on a decision: to go to surgery or not. The owners are prepared for you to do anything to save Loki, but do not want him operated on if he doesn't need it.

Hedwig is a 2 year old neutered female, one of a pair of mixed breed rabbits with sudden onset inappetence and abdominal pain. On obtaining blood, a number of haematological and biochemical abnormalities are seen, including severe anaemia. The successful management of her case depends on a decision: to go to surgery or not. The owners are prepared for surgery, but only if there is an excellent long term prognosis for her.

Domino is a 5 year old neutered mixed breed male rabbit, presented to your colleague, with a gradual reduction in appetite, and weight loss. They have taken radiographs and a blood sample, and diagnosed neoplasia to the owner, recommending euthanasia. Are they right?

Qixote is a 3 year old entire male rabbit with a head tilt, in whom we determine whether this is more likely to be *E cuniculi* or aural disease, from blood tests.

Hormonal disorders and endocrinopathies in the Rabbit

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RCVS Specialist in Zoo and Wildlife Medicine (Mammalian)
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Abstract

When to look for them, how to diagnose them, and how to treat them, we look at hormonal disorders and endocrinopathies in the rabbit.

As much about what we DON'T see in this species, and how to differentiate ovarian remnant syndrome from adrenal disease, we look at this relatively uncommon class of conditions in the rabbit.

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Microbiology and antimicrobial selection in the Rabbit

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Abstract

Worried about AMR? Worried about going off licence?

This presentation discusses both (valid) concerns, and how to please the RCVS, the VMD, Global anti-AMR campaigns, and your patients.

We will be looking at what antibiotics are safe, let alone legal, in rabbits, and when to go off licence, off Cascade, or completely off-piste.

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Thursday 2 October 2025

Cinema 1

PLENARY LECTURE: AI in Veterinary Medicine: Ethics, Challenges

Speaker: Adele Williams-Xavier
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Abstract

Artificial intelligence (AI) is rapidly transforming veterinary clinical pathology, offering unprecedented opportunities to enhance diagnostic accuracy, workflow efficiency, and patient care. This presentation provides a comprehensive update on the current state and future potential of AI applications specifically relevant to veterinary clinical pathologists.

Beginning with an accessible introduction to AI fundamentals, real-world applications currently available in veterinary practice will be explored, including automated cell identification in hematology, urine sediment analysis, and fecal parasite detection. Examples will show how these technologies can augment point-of-care diagnostics and support clinical decision-making.

The presentation addresses critical considerations for responsible AI implementation, including the importance of robust training datasets, understanding the "AI chasm" between research performance and clinical reality, and recognising potential sources of bias that can impact diagnostic accuracy. Factors to be examined include data distribution, laboratory variations, and patient population differences that can affect AI performance, emphasising the essential role of clinical pathologists in AI validation and quality assurance.

Practical guidance will be provided on evaluating AI tools for laboratory implementation, including performance metrics, validation approaches, and integration with existing workflows. The session concludes with a balanced perspective on the future of AI in veterinary pathology, acknowledging both the tremendous potential and current limitations, while emphasising that AI should augment rather than replace clinical expertise.

This presentation equips veterinary clinical pathologists with the foundational knowledge needed to start critically evaluating, implementing, and optimising AI technologies.

Quality Assurance in Veterinary In-Clinic Laboratories: Overview of Current Practices and QA for the Pre- and Post-Analytical Phases

Emma Hooijberg

This presentation series aims to present a summary of the current state of laboratory QA practices in veterinary clinics, highlights elements of a QA system most important for in-clinic laboratories, and suggests an approach that clinical pathologists can use to assist veterinarians in designing a QA system. This first presentation will focus on general principles of a QA system and the pre-analytical and post-analytical phases of testing.

The vast majority of veterinary practices in the developed world now have in-clinic laboratories, with most clinics equipped with a chemistry analyser, haematology analyser, and microscopy facilities. These allow for rapid diagnostic testing, but also introduce new responsibilities for ensuring quality. Unlike commercial reference laboratories, where comprehensive QA/QC systems are standard, published information and informal survey data collected by the presenter from the UK and South Africa, indicate that in-clinic laboratories often have less structured approaches. Many practitioners are likely uninformed and uncertain about best practices. This can have direct implications for patient safety and clinical decision-making.

Implementing QA in practice requires planning, personnel, and documentation. A designated quality manager, ideally a veterinarian supported by a nurse or technician, should oversee QA activities. Development of a quality manual, encompassing SOPs, maintenance logs, error records, and corrective action plans, provides the framework for a sustainable system. Ongoing training and competency assessment for all staff using the laboratory are essential.

Approximately 75% of laboratory errors occur during the pre-analytical phase, including incorrect labelling, inadequate sample volume, haemolysis, and improper patient preparation. To mitigate these risks, clinics should adopt standard operating procedures (SOPs) covering test selection, specimen collection, handling, and transport. Key Quality Indicators (KQIs), such as rates of haemolysis or misidentified samples, provide objective metrics for monitoring and improvement.

The post-analytical phase, responsible for an estimated 15% of errors, is also critical. Common issues include transcription mistakes, reporting delays, and failure to recognise implausible results or analytical interferences. Verification against clinical presentation and timely communication of critical results are vital components of effective QA in this stage.

While the establishment of QA systems can be resource-intensive for veterinary practices, they are essential for maximising the value of in-clinic diagnostics. By focusing on pre- and post-analytical quality, practitioners can reduce error, improve patient outcomes, and build greater confidence in in-house testing

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Quality Assurance in Veterinary In-Clinic Laboratories: QA for the Analytical Phase

Emma Hooijberg

This second presentation will focus on the analytical phase of testing. The analytical phase typically contributes the lowest proportion of errors in commercial laboratories, but the frequency of analytical error in in-clinic laboratories is not well documented. Information from publications and from the SRUC Capital EQA Scheme suggests that in-clinic analyser performance varies considerably, and structured quality assurance (QA) systems are often lacking. Addressing these gaps requires attention to instrument validation, internal quality control (IQC), and external quality assurance (EQA).

The key quality goal in this phase is that results accurately reflect the analyte's true value within the patient. This is defined by Total Allowable Error (TEa), the maximum error permissible without compromising clinical decision-making. Observed total error (TEobs) should remain below TEa, but studies show this is not always achieved across common point-of-care instruments. For example, in one study, calcium measurements often met TEa, whereas analytes such as ALP, creatinine, and electrolytes frequently exceeded acceptable error limits. Analytical performance for haematology analysers is variable, with high TEobs for PLT and WBC differential counts.

Individual analyser performance can be variable and verification of analyser performance is therefore essential. While full validation is impractical in practice, inter-assay precision (CV) and bias should be measured and compared to published TEa values. Applications like the **BSAVA Lab Equipment Verification Help Tool** provide a practical resource for clinics, offering built-in calculations for CV, bias, TEobs, and sigma metrics, and comparison with available TEa standards. This enables practitioners to assess analyser performance more objectively and consistently.

Internal QC provides a critical safeguard. Manufacturer-supplied control materials should be run regularly, ideally at multiple concentrations, with results assessed using defined QC rules. Alternative approaches like repeat-patient testing or patient-based population analysis QC (e.g., moving averages), may be more cost-effective but may have limited availability or be too complex to set up for in-clinic QC.

External QA complements IQC by comparing results with a peer group, identifying bias, and monitoring long-term performance. Veterinary-specific schemes are available in some regions, and comparative testing with reference laboratories can also highlight clinically important discrepancies.

In summary, while the analytical phase may generate fewer errors than other parts of the laboratory cycle, reliable in-clinic testing depends on structured QA. Verification, IQC, and EQA are essential to ensure that analytical results remain fit for clinical decision-making and patient

care. As clinical pathologists, we have the necessary expertise and access to resources to assist our colleagues in practice with designing and implementing a QA system.

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The In-Clinic Lab: Haematology Analysers

Andreas Moritz

The evaluation of blood is an integral part of the routine diagnosis of healthy and diseased companion animals. The packed cell volume (PCV), or as in humans the total hemoglobin concentration, and the microscopic examination of a blood smear have been used since the beginning of veterinary care and still form the cornerstones of clinical hematology. Veterinary in-house hematology analyzers are, in our days, critical tools in veterinary medicine, enabling practitioners to perform a complete blood count (CBC) within their clinics. These analyzers provide rapid results, allowing veterinarians to make timely diagnostic decisions and implement appropriate treatment plans. Besides speed and efficiency, a user-friendly interface with intuitive software and ease of operation, accuracy is most important. For most analyzers, evaluation data are published comparing results with either reference analyzers (e.g., ADVIA 120/2120, Sysmex XT/XN series, both with veterinary software settings), packed cell volume (PCV), and manual blood smear evaluation, including manual differential blood and reticulocyte count. There are no instruments, not even the big laboratory analyzers, providing 100% accurate results. It is therefore important that users know about the strengths and weaknesses of their particular instrument, which are closely related to the technologies used. Besides a robust quality management system for automated hematology analyzers, including maintenance and calibration, and if available, quality control material, operator training is crucial for generating high-quality results. For most analyzers with impedance technology, best practice would be that if no technical or morphological flags are shown, total cell counts (except feline platelets) can be accepted, and a blood smear (semi-quantitatively) can be used for differential cell count. Instruments with advanced laser technology and graphical reports (dot plots) provide the option to verify results, particularly cell morphological aspects. Results with no technical or morphological flags and regular (normal) cell morphology could be accepted in most cases. If misclassification is noted, blood smear evaluation is needed. Crucial to success is good user training in reading the dot plots. Conclusion Veterinary in-house hematology analyzers are essential tools in modern veterinary practice. They offer rapid, accurate, and comprehensive diagnostic capabilities, enabling veterinarians to provide timely and effective care. Despite the initial investment and maintenance requirements, the benefits of these analyzers in terms of improved patient outcomes and enhanced diagnostic capabilities make them a valuable addition to any veterinary clinic. The variety of available instruments ensures that clinics of all sizes and specializations can find a suitable analyzer to meet their needs.

The In-Clinic Lab: Biochemistry analysers

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The use of "Point of Care" (POC) analyzers in veterinary clinics and hospitals is becoming increasingly common. These types of analyzers have both advantages and disadvantages that may limit their use. The most important advantages include the speed at which results are obtained, allowing for quicker clinical decision-making. Another benefit is the increased possibility of more constant patient monitoring. They can minimize pre-analytical errors and artifacts caused by sample transport and prevent sample degradation since the analysis is immediate.

Among the disadvantages, it is worth noting that these analyzers often have lower precision and accuracy in their results, which can lead to certain biases compared to reference methods. There are also economic considerations for the clinic or hospital, as costs must include maintenance and depreciation of the equipment. Since these devices are used by many people, there is a tendency to have less specialized operators, increasing the likelihood of e rrors in the analyses caused by the personnel using the equipment. Sometimes, the results are not always interchangeable with those from reference laboratories. Calibration by untrained personnel can lead to inconsistent results. Staff dedication to the equipment is lower than in a reference center, making it harder to detect equipment errors. Additionally, many centers do not follow quality control procedures to verify proper functioning.

In the marked exits several types of in clínic biochemistry Point-of-Care (POC) analyzers, that can be group in:

1. General Biochemistry analyzers: These devices analyze multiple biochemical parameters in serum or plasma such as the Heska Element DC, IDEXX Catalyst analyzers, VetScan VS2, Fuji, Nova, StatSensor(hand Device), Bionote Vcheck, Eurolyser RL etc most of them use dry biochemistry.
2. Portable Blood Glucose Meters (PBGMs) such as AlphaTrak, AlphaTrak 2, and AccuTell, used for rapid measurement of blood glucose.
3. Blood Gas and Electrolyte Analyzers: these analyzers measure pH, partial pressures of carbon dioxide (PaCO2) and oxygen (PaO2), hematocrit (HCT), and electrolytes including sodium, potassium, magnesium and chloride. Some may also measure urea nitrogen and creatinine. Examples of devices: i-STAT analyzer, NOVA Stat Profile Critical Care Xpress (CCX), and Enterprise Point-of-Care (EPOC) analyzers.
4. Cardiac Biomarkers System such as Cardiac Troponin I (cTnI) Device (i-STAT analyzer) or N-terminal pro-brain natriuretic peptide (NT-proBNP) assess myocardial stress or distension. Device Example: Vcheck canine NT-proBNP Test kit utilizing Vcheck 200 or Vcheck 2400 instruments.
5. Inflammation Markers: Indicators of systemic inflammation. Canine C-reactive protein (cCRP): An acute-phase protein that increases with inflammation. Device Example: VetChroma canine-specific POCT assay. Serum Amyloid A (SAA): A sensitive biomarker for detecting inflammation in cats. Device Examples: Fuji Dri-Chem Immuno AU CARTRIDGE vf-SAA and CUBE-VET analyzer.

6. Hormone Testing: Specific hormone measurements for endocrine disorders.
7. Ketone Monitoring: For the detection and management of ketosis. Ketones (Acetoacetate, Acetone, 3-β-hydroxybutyrate): Device Examples: Nitroprusside reagent test strips (for urine and plasma) and 3-β-hydroxybutyrate (3-HB) ketone meters (e.g., Precision Xtra).

POC tests aim to provide rapid results, facilitating quicker diagnostic and treatment decisions in a clinical setting, however, all analyzers must be evaluated for performance and validated for the intended species and sample volume requirements. Also, POC analyzer results are not directly interchangeable with those from reference laboratories or even between different POC models due to methodological variations and inherent bias. For this reason, it is recommended to establish specific reference intervals for each analyzer and species as well as harmonization of results that can help adjust POC results to make them comparable with those from commercial laboratories, facilitating the use of common reference intervals and decision thresholds.

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The in-clinic lab: thyroid and adrenal hormones

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Introduction

The diagnosis of endocrine disorders of the thyroid and adrenal glands is challenging. Hormone assays and dynamic endocrine tests are essential diagnostic tools, but their interpretation must never be done in isolation. Instead, results must be carefully interpreted taking into consideration the animal's signalment, clinical history, physical examination findings, and results of routine clinicopathological testing.

Three fundamental principles underpin correct endocrinopathies diagnosis: i) understanding indications and limitations of each assay or dynamic test; ii) utilising appropriate and validated for the examined species assays with rigorous internal and external quality control; and iii) knowing how to interpret the results within the appropriate clinical context.

This review highlights current diagnostic strategies for the most relevant thyroid and adrenal endocrinopathies in small animal practice: canine hypothyroidism, feline hyperthyroidism, canine Cushing's syndrome and canine hypoadrenocorticism.

Canine hypothyroidism

The total thyroxine (tT4) concentration is the preferred initial screening tool. Historically measured by radioimmunoassay (RIA), tT4 is now typically measured using chemiluminescent or enzyme immunoassays, which eliminate issues associated with radioactive isotopes while maintaining reliability. However, tT4 measurement using in-clinic ELISA-based analysers has been shown to produce inconsistent results, raising concerns about its reliability. A low tT4 demonstrates excellent sensitivity for diagnosing canine hypothyroidism. Yet, specificity is limited: low tT4 may also reflect non-thyroidal illness syndrome (NTIS), drug effects (notably glucocorticoids or phenobarbital), or breed-related normal variation (e.g. sighthounds). Importantly, falsely elevated concentration can occur in the presence of anti-T4 autoantibodies (T4AA). Therefore, a diagnosis of hypothyroidism cannot be made solely on the basis of reduced tT4 concentration.

Measurement of thyroid-stimulating hormone (TSH), typically via a canine-specific chemiluminescent immunoassay, provides useful complementary information. The combination of low tT4 and elevated TSH has high specificity for primary hypothyroidism, essentially confirming the diagnosis in the appropriate clinical context. However, an important proportion of hypothyroid dogs has TSH concentrations within reference interval (sensitivity 63-82%). Thus, a TSH within the reference interval does not exclude hypothyroidism.

To distinguish hypothyroidism from NTIS in dogs with low tT4 and non-elevated TSH, measurement of free T4 (fT4) is indicated. While immunoassays are widely available, equilibrium dialysis (fT4ed) is the gold standard and should be preferred, as immunoassays are optimised for human samples and show inferior performance to fT4ed in dog and cats. A low fT4ed is more

specific than low tT4, although severe non-thyroidal disease can also reduce fT4ed. Conversely, fT4 is less sensitive than tT4 and may remain within the reference intervals in early hypothyroidism. Importantly, fT4ed is unaffected by T4AA and is thus highly useful when autoantibody interference is suspected.

The TSH stimulation test is considered the reference standard for hypothyroidism but is seldom performed in practice due to the prohibitive cost of recombinant human TSH. The TRH stimulation test has been studied but adds little diagnostic value and has interpretive limitations.

Autoantibody testing, particularly thyroglobulin autoantibodies (TgAA), which are usually measured with the use of ELISA, is useful in identifying immune-mediated thyroiditis. TgAA are present in 8-55% of hypothyroid dogs and in a subset of euthyroid dogs.

Feline hyperthyroidism

In cats, elevated tT4 is typically diagnostic for hyperthyroidism, with 91% sensitivity and 100% specificity. However, early or mild disease, concurrent NTIS, or drug therapy can result in tT4 within the reference interval. In such cases, further evaluation is required.

Measurement of fT4ed provides enhanced sensitivity (98.5%) but reduced specificity (93%) due to occasional elevation in NTIS. Thus, fT4ed should not replace tT4 as the primary screening test but is recommended when tT4 concentration is high-normal and clinical suspicion persists.

TSH measurement is rarely employed in cats, but when performed, undetectable concentrations (<0.03 ng/mL) strongly support hyperthyroidism (98% sensitivity, 70% specificity).

When tT4 and fT4ed remain inconclusive, the T3 suppression test can be utilised. Lack of appropriate suppression confirms hyperthyroidism, though borderline results must be interpreted cautiously. TRH or TSH stimulation tests are not recommended in cats due to poor discriminatory power.

Canine Cushing's syndrome

The diagnosis of Cushing's syndrome relies primarily on two dynamic assays, low-dose dexamethasone suppression test (LDDST) and ACTH stimulation test (ACTHst). The sensitivity of LDDST is high (85-97%), but specificity is moderate (44-73%). The ACTH stimulation test is generally less sensitive (80-92% for pituitary-dependent Cushing's syndrome and 0-63% for functional adrenocortical tumour) but more specific (59-93%). When iatrogenic Cushing's syndrome is suspected, ACTHst is the only choice for confirmation. In both tests, cortisol is measured either three times (LDDST) or twice (ACTHst). Immunoassays such as chemiluminescent methods are most commonly used for the measurement of cortisol, though ELISA and RIA remain alternatives. In-clinic ELISA analysers exist, but they characterised by inferior performance.

The UCCR was traditionally used as an exclusion test for Cushing's syndrome; however, according to more recent studies, it appears to have an overall moderate accuracy for the diagnosis of Cushing's syndrome in dogs (sensitive of 80-86% and specificity of 63-71%).

Canine hypoadrenocorticism

In dogs, basal cortisol is a valuable exclusionary test. A basal cortisol concentration >55 nmol/l essentially rules out hypoadrenocorticism. However, a low basal cortisol must always be followed by an ACTH stimulation test, the gold standard for diagnosis.

The cortisol-to-ACTH ratio has been proposed as a highly accurate diagnostic test distinguishing dogs with hypoadrenocorticism from both healthy controls and systemically ill dogs, though it is less widely adopted and possibly needs further study.

Although, urine corticoid-to-creatinine ratio (UCCR) was traditionally used for the exclusion of canine Cushing's syndrome, it has been recently suggested as a very accurate test for the diagnosis of canine hypoadrenocorticism.

Suggested literature

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Thursday 2 October 2025

Cinema 3

The In-Clinic Lab: Measurement of acute phase proteins

Emma Dewhurst MA VetMB DipECVCP FRCPath MCRVS
EBVS Recognised Specialist in Clinical Pathology

The measurement of acute phase proteins (APPs) within a referral veterinary laboratory has been available for many years, more recently measurement of some APPs in-clinic has become available. This talk will cover some generalities about acute phase protein measurement in domestic species available in-clinic.

When discussing acute phase proteins these include fibrinogen, C-reactive protein (CRP), Serum Amyloid A (SAA), haptoglobin and α1-Acid-Glycoprotein (AGP).

For the dog, CRP measurement is available in clinic. The IDEXX Catalyst CRP has been externally validated and found acceptable, and its clinical utility will be discussed further in the presentation. Assessment of APPs is often initiated as part of the investigation for suspected inflammatory disease. Although not APPs other parameters which can be useful which may be available in clinic are a full haematology to include RETIC-HGB and a leukogram, albumin and globulin assessment and these will be covered further in the presentation.

RETIC-HGB may be useful as part of a laboratory investigation of inflammation in the dog and cat. Decreased RETIC-HGB may be considered an indicator of iron restriction, this may include iron restriction due to reduced iron availability secondary to inflammation, but also in cases of true iron deficiency. Further assessment for the cause of a low RETC-HGB can include red cell morphology eg MCV, MCHC, presence of microcytic and hypochromic red cells and also presence of a neutrophilia with or without a left shift which may aid in further differentiation of likely cause.

In clinic tests are available for SAA in the cat, for which there are peer reviewed publications. Elevation in SAA is a sensitive test for presence of inflammation, but poorly specific at differentiating different inflammatory conditions. A referral laboratory ELISA for the measurement of AGP in the cat has been recently validated. AGP. Elevation in AGP has been considered a sensitive but not specific test for the diagnosis of FIP. More recently AGP has been seen to be a useful marker of response to treatment for FIP which will be discussed further.

SAA is considered a marker of acute inflammation in the horse, elevations in SAA in the horse are seen in many inflammatory, infectious and neoplastic processes, so SAA may be considered a sensitive but not specific marker for pathology in the horse. SAA is also useful as its elevation may occur earlier in the course of disease than fibrinogen. SAA may be measured stall side using, eg Stablelab-Zoetis, which has been the subject of peer-reviewed publication.

In summary, APPs can be a useful part of the diagnostic investigation of disease, there are though few validated assays available for in clinic use in the dog and cat. Across all publications accessed it is noted that results for the APP analysed should not be compared between methods, eg in clinic and then referral laboratory, if serial monitoring is being considered then the same test methodology should be employed across all time points.

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Diagnostic microbiology – Supporting antimicrobial stewardship: updated clinical microbiology methods and guidelines

Sian Frosini

Robust bacteriology consists of two key parts: accurate bacterial identification and informative antimicrobial susceptibility testing (AST), but laboratories across Europe use very divergent methods to achieve these aims (Koritnik *et al.,* 2024). Rapid changes in the technologies used with bacteriology laboratories in veterinary medicine, as they catch up to human medicine, highlights the importance of laboratories providing bacteriology services considering the limitations of any methods they choose to use.

Where bacterial identification traditionally relies on slow, biochemical techniques which group or sometimes presume the identity of similar pathogens, modern techniques such as MALDI-TOF provide rapid (<15 minute) and specific identification of bacterial species. This can result in reporting of rare species that may have not been previously reported with their correct nomenclature, and a 'translation' of this terminology should be considered when reporting, to avoid clinician confusion (van Belkum *et al.,* 2017). However, it is important to ensure laboratories retain benchtop fundamentals, as MALDI-TOF identification is hampered by its own database, and un-identifiable potential pathogens should undergo Gram staining and biochemical testing to suggest their identity. Furthermore, metagenomic approaches to culture and susceptibility testing vastly increase sensitivity of pathogen detection beyond traditional culture techniques but requires careful interpretation prior to sending results to submitting veterinarians. These developments have further extended the possibilities in the microbiology laboratory to allow isolate serotyping and multi-locus sequence typing to track and link related pathogens of key public health or infection prevention and control (IPC) relevance.

AST is also a rapidly changing field, with disk diffusion slowly being phased out for Minimum Inhibitory Concentration (MIC) assessment. Interpretative criteria ('clinical breakpoints' [CBPs]), the choice of which depends on bacterial identification, infection type and host species, then allow clinicians the nuance to interpret MICs to compare antimicrobial efficacy, and predict impact of amended dosing, for example. However, as clinicians expect to gain more insights from a microbiology report, this leaves the laboratory in a quandary: how to deal with missing CBPs? How to report intrinsic resistance without increasing concern about 'multidrug' resistance? Which CBPs are best to extrapolate for a rabbit? When a new CBP appears, how do we put this into practice? (Feßler *et al.,* 2023). Furthermore, molecular assays designed to rapidly identify significant AMR genes must be carefully interpreted, especially if there is discordance between that and the final phenotypic results (Yee *et al.,* 2021).

On the other side of the coin, veterinarians are increasingly aware of the need for careful stewardship as clinical guidelines are launched, such as the 2025 release of canine pyoderma guidelines (Loeffler *et al.,* 2025). These always cover diagnosis and antimicrobial treatment options, amongst other aspects of disease, including recommendation of appropriate first-line antimicrobials. The laboratory should consider alignment of reporting with those guidelines, prioritising the opportunity of a laboratory report to steer the veterinary practitioner to available guidance where appropriate.

Understanding the newer, more complex landscape of veterinary microbiology is a challenge, but imperative to support stewardship efforts, preserve antimicrobial efficacy, and guide clinical decision-making.

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Diagnostic Microbiology - The clinical pathologist - microbiologist interface: how can we support each other?

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Clinical microbiology has emerged as a newly recognised veterinary specialty. The European College of Veterinary Microbiology (ECVM) was provisionally formed as an EBVS specialist college in 2016 and has grown to be home to more than 70 diplomats and 30 residents across 18 countries. Although in its infancy, these developments highlight the importance of promoting diagnostic stewardship. As clinical freedom becomes threatened by increasing rates of antimicrobial resistance, varying restrictions on antimicrobial use globally, and increasing expectations for successful veterinary care in challenging clinical cases, microbiology reports are in the spotlight.

However, there are limited numbers of clinical microbiologists, and it is often the clinical pathologist who represents the first or only port-of-call to advise on appropriate sample submission to support bacteriological culture. Discussing specimen handling guidance with microbiology specialists preserves the accuracy and reliability of microbiology reports (Franklin-Gould *et al.,* 2025). It is important to understand the best types of samples to minimise environmental or commensal contamination; how to store these, especially where delays are expected prior to processing; and what information is most useful to the microbiology team when interpreting bacterial growth (Cole *et al.,* 2025). As susceptibility testing should be done only on clinically significant isolates, the laboratory needs to reduce the 'background noise' of commensal microbes unrelated to the disease process, and specimens of poor quality or those that have not been stored appropriately should be rejected.

Cytology can support interpretation of culture, however this raises challenges when cytology and microbiology results are mismatched (Clement *et al.,* 2015), or when organisms are suspected that present a challenge for culture. These pathogens, be they hazard groups that cannot be handled, difficult or impossible to culture through routine methods, or requiring molecular identification tools, need to be highlighted to the microbiology team as soon as possible to ensure timely reporting.

Furthermore, it is important to assess that the microbiology laboratory offers reports with the most accurate, significant and clinically relevant interpretation from submitted specimens. Animal-specific proficiency testing schemes (e.g. VETQAS®) are offered to ensure that technical processes meet third party accreditation quality standards. Labs may be familiar with quality assurance (QA) schemes to support ELISA or other immunological assays. However, these systems also include broader 'microbiology' QA where test samples are provided alongside clinical vignettes to assess laboratory performance.

Similarly, submission of data supporting surveillance efforts for antimicrobial resistance (AMR) and specific pathogens (e.g. *Salmonella*) is an important contribution of the laboratory. Across Europe, only 40-50% of microbiology laboratories self-reported contributing to AMR surveillance but were keen to see guidelines targeted towards laboratory methodology (Koritnik *et al.,* 2024). This requires integration of clinical microbiologists to provide support to the laboratory. A recent US-based article has called for clinical microbiologists to provide directorship within human microbiology laboratories (Samuel *et al.,* 2021). It is hoped that the ECVM will support these aims

within veterinary medicine by promoting training programmes and guideline development. Clinical pathologists and microbiologists must work together to deliver these evidence-based and standardised approaches to bacteriology.

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CANINE VECTOR-BORNE DISEASES: DECISION MAKING BASED ON POINT OF CARE SEROLOGICAL TESTING

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Overview of point-of-care serological testing for canine vector borne diseases

Point-of-care (POC) assays represent a fast, user-friendly and affordable method for veterinarians to screen dogs for exposure to (e.g., *Anaplasma* spp. or *Ehrlichia* spp.) or infection by (e.g., *Dirofilaria immitis*) vector-borne pathogens (VBP). They are mostly based on enzyme-linked immunosorbent (ELISA) or immunochromatography (IC) assays, which detect antibodies (or l antigens) to one or multiple VBP. For the correct interpretation of the POC assay results, the clinicians must always 1) perform the POC test in strict compliance with the manufacturer's instructions, 2) be aware of what is the target of the POC test (i.e., antibody or antigen), as well as their kinetics over time, 3) recognize that no single POC assay alone is sufficient for the definitive diagnosis of a vector-borne disease (VBD) and therefore, there must be compatibility between the POC result and the historical, clinical and laboratory profile of the dog being tested, and 4) be aware of the test's diagnostic performance (i.e., sensitivity and specificity). The results obtained from these assays are mostly qualitative (positive or negative), and semiquantitative in some, as opposed to the quantitative laboratory-based assays.

POC serological testing in common canine vector-borne diseases

Canine monocytic ehrlichiosis (*Ehrlichia canis*)

The clinical course of *E. canis* infection can be divided into acute (2-4 weeks), subclinical (several months to years) and chronic phases. Indirect fluorescent antibody testing is considered the "gold standard" for the detection and titration of anti-*E. canis* antibodies. Antibodies (IgG) develop 7-35 days post-infection. Numerous POC immunoassays (ELISA or IC tests) are commercially available for *E. canis* IgG antibody testing. In general, these tests have been found to be of high diagnostic specificity (approaching 100%); sensitivity is high in IgG titers $\geq$1/320, but substantially lower in titers <1/320; therefore, a relatively low sensitivity may be anticipated in acutely infected dogs, in which clinical signs and hematological abnormalities may precede detectable seroconversion. Furthermore, due to the prolonged subclinical phase and the persistent seropositivity following drug-mediated or self-eradication of the infection, the clinicians should be aware that seroreactivity to *E. canis*, does not confirm that the clinical manifestations upon presentation are due to *E. canis* infection.

Anaplasmosis (*Anaplasma phagocytophilum, A. platys*)

Canine anaplasmosis is caused by two distinct VBP, including *Anaplasma phagocytophilum* (granulocytic anaplasmosis), and *A. platys* (thrombocytic anaplasmosis or infectious cyclic thrombocytopenia). Although both are considered mostly as acute diseases, persistent infections have been documented in dogs. Dogs experimentally infected with *A. phagocytophilum* or *A. platys* seroconvert between 8-13 days post-infection. A limited number of POC assays are commercially available for *Anaplasma* spp. IgG antibody testing. Compared to IFA, these assays have a variable sensitivity ranging from 13-85% (*A. phagocytophilum*) and from 33-83% (*A. platys*), while they typically cannot differentiate between antibodies against *A.*

phagocytophilum or *A. platys*. The presence of specific antibodies against *Anaplasma* spp only indicates exposure to the organisms and should not be used alone to make a diagnosis without taking into consideration clinical and laboratory abnormalities.

Leishmaniosis (*Leishmania infantum*)

Canine leishmaniosis (CanL) is caused mainly by the species *L. infantum*. Only a subset (5-10%) of the naturally infected dogs develop clinical disease, while most of them remain subclinically infected for years or for life. Dogs that are resistant to the development of clinical disease mount low and sometimes intermittent and borderline antibody titers. Clinical disease along with typically high antibody titers may occur after a variable subclinical period ranging from 3 months to several years. When a qualitative POC assay is positive, along with compatible clinical and clinicopathologic findings, they are considered sufficient to support the diagnosis of CanL. However, for the post-treatment monitoring a quantitative assay is preferred. Most POC tests employ recombinant antigens for the detection of *Leishmania*-specific IgG antibodies. Overall, the diagnostic sensitivity and specificity of POC assays is satisfactory for the diagnosis of CanL (clinically sick dogs), whereas subclinically infected dogs or dogs with early clinical disease may often be seronegative or have low antibody levels. In regions where multiple *Leishmania* species and/or *Trypanosoma* spp. co-exist, serological cross-reactions may occur, while a seropositive result may occasionally detect antibodies elicited by prior anti-*Leishmania* vaccination.

Dirofilariosis (*Dirofilaria immitis*)

Heartworm infection (*Dirofilaria immitis*) in dogs has been diagnosed around the globe. In the clinical setting, antigen testing is the preferable diagnostic method for examining either healthy dogs or dogs suspected of heartworm infection and should be performed annually (in tandem with microfilariae testing). A reasonable first serological testing for *D. immitis* antigens is at 7 months of age. Although several heartworm antigen tests identify most infections consisting of at least one mature female worm and are nearly 100% specific, sensitivity among different POC tests can vary in dogs infected with very small numbers of female worms, while all tests are expected to be negative in case of male-only worm infection. The earliest detection of antigen is approximately 5 months post infection (typically antigenemia precedes microfilaremia). A positive heartworm antigen test mostly, but not invariably, indicates the presence of heartworm infection, especially in dogs with clinical suspicion of the disease. The amount of circulating antigen has an imprecise relationship with the adult worm burden and therefore, the graded test reaction provided by some ELISA assays is of limited clinical utility. A negative antigen test result does not definitely confirm that an animal is free of heartworm infection. False-negative results occur most commonly when infections are light, female worms are immature and male-only worms are present. Uncommonly, antigen-antibody complexes may interfere with antigen testing, resulting in false-negative tests, in dogs with manifestations suggestive of heartworm disease. In the latter instance, heating serum may release blocked antigen and result in more positive test results. Some parasitic infections (e.g. *D. repens*, *Angiostrongylus vasorum*, *Spirocerca lupi*) may rarely cross-react with *D. immitis*, especially following heat treatment of the serum. Heartworm antigen testing is also the most reliable method to confirm the efficacy of adulticidal therapy and should be performed 9 months following the last dose of melarsomine. In instances of noncompliance or changing the type of heartworm preventive, it is important to determine the heartworm status of the dog.

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CLINICOPATHOLOGIC ASPECTS OF CANINE MONOCYTIC EHRLICHIOSIS (*EHRLICHIA CANIS*)

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Introduction

Ehrlichia canis, a gram-negative, obligate intracellular, tick-transmitted bacterium is currently recognized as the principal cause of canine monocytic ehrlichiosis (CME) worldwide. The course of natural *E. canis* infection can be divided into acute, subclinical and chronic phases. Clinical recovery is the typical outcome of acutely infected dogs, entering the subclinical phase, during which they show no or minimal clinical signs and/or mild clinicopathologic abnormalities. An unpredictable proportion of dogs may progress to the chronic phase, typically characterized by bone marrow (BM) aplasia, peripheral blood bi- or pancytopenia and high mortality. From a clinical perspective, the terms "non-myelosuppressive" and "myelosuppressive" CME, may better reflect the clinical severity of the disease, regardless of its time progression. Fever, lethargy, anorexia, lymphadenomegaly, splenomegaly, mucosal pallor and ocular abnormalities are common clinical manifestations. Bleeding diathesis, the clinical hallmark of the disease, is associated with impaired primary hemostasis due to thrombocytopenia, thrombocytopathy and mild vasculitis.

Clinicopathologic aspects of CME

Thrombocytopenia is the most common hematologic abnormality in CME, appearing in more than 80% of dogs, but CME should not be ruled out based on a normal platelet count alone. A typically non-regenerative anemia, leukopenia, neutropenia and lymphopenia or mild lymphocytosis are additional abnormalities. A $CD8^+$ T-cell expansion (which may rarely be clonal) with an occasional granular cytologic phenotype, and an inverted CD4/CD8 ratio, may occur in chronically infected dogs, mimicking emerging chronic lymphocytic leukemia. It is not clear how long after treatment for *E. canis* the CD4/CD8 ratio may be normalized. Thus, in endemic areas, CME should be a top differential in dogs exhibiting persistent mild-to-moderate lymphocytosis (up to 25,000/μl) especially when lymphocytes are of granular phenotype. Aplastic pancytopenia is the typical feature in myelosuppressive CME and the latter is a leading cause of canine pancytopenia in endemic areas.

Hyperproteinemia, hyperglobulinemia, hypoalbuminemia and mild to moderately elevated ALP and ALT activities are common biochemical abnormalities in CME. Hyperglobulinemia appears on serum electrophoresis as a polyclonal or rarely monoclonal gammopathy pattern. Historically, the incidence of monoclonal gammopathies associated with CME (or other infectious diseases) may have been overestimated, because serum protein electrophoresis cannot reliably differentiate between a restricted polyclonal gammopathy from a true monoclonal gammopathy. In a recent study, 37 dogs seropositive to *E. canis* (9 were co-seropositive to *A. phagocytophilum*), which demonstrated abnormal globulin fractions in agarose gel electrophoresis, were further refined by immunofixation. Only one (2.7%) dog had an electrophoretic/immunofixation pattern suggestive of a monoclonal gammopathy. Increased creatinine concentration may occur, and in a recent retrospective study, the relative risk of chronic kidney disease in dogs seropositive to *Ehrlichia* spp. was found to be increased. Transient

proteinuria of variable severity has been reported in dogs with experimental acute CME and histological examination indicated a minimal-change glomerulopathy rather than immune-complex glomerulonephritis.

Microscopic visualization of *Ehrlichia* spp. morulae in the cytoplasm of monocytes, macrophages, lymphocytes and rarely granulocytes, in buffy coat and less frequently lymph node, BM, spleen, liver and cerebrospinal fluid smears, is helpful in establishing a definitive diagnosis of acute CME, sometimes even prior to seroconversion. On the other hand, cytology is a labor-intensive task even in the acute phase of the disease (less than 1% infected mononuclear cells), requires a well-trained technician or clinical pathologist and it is notoriously insensitive in the subclinical and chronic CME.

Bone marrow cytology is useful in differentiating the non-myelosuppressive from the myelosuppressive CME, or to rule out other hematological syndromes causing pancytopenia (e.g., myelophthisis). Although BM core biopsy is superior to cytology in appreciating the BM cellularity, review of at least 4 BM cytology smears of sufficient quality correlates well with core biopsy in assessing BM cellularity in CME. While in the acute CME BM appears to be normocellular to hypercellular, in the chronic CME a marked reduction of hematopoietic tissue is noticed, and usually consists of adipocytes, endothelial and stromal cells and a mild-to-moderate increase in mature mast cell and/or plasma cells, which should not be confused with mast cell tumor or multiple myeloma, respectively.

Serological and polymerase chain reaction (PCR)-based diagnosis of CME

Serology is currently the main diagnostic method for the confirmation of exposure to *E. canis*. Indirect fluorescent antibody (IFA) testing is considered the "gold standard" for the detection and titration of anti-*E. canis* antibodies, although quantitative enzyme-linked immunosorbent assays (ELISA) are also applied. Antibodies develop 7-35 days post-infection, and they do not reliably correlate with the current carrier status, the duration of infection, or the presence and severity of clinical disease. Importantly, in acutely-infected dogs, clinical signs and hematological abnormalities may precede seroconversion. Due to the prolonged subclinical phase and the persistent seropositivity following drug-mediated or self-eradication of the infection, the clinicians should be aware that seroreactivity to *E. canis*, especially in an endemic area, does not confirm that the clinical manifestations upon presentation are due to *E. canis* infection. The specificity of serology is also affected by the cross-reactivity that occurs among the same (i.e. *E. canis*, *E. chaffeensis* and *E. ewingii*), or, less likely, closely-related (i.e. *A. phagocytophilum*) genogroup species.

Polymerase chain reaction (PCR) may overcome several diagnostic limitations of serology (confirmation of exposure versus current infection) and cytology (low diagnostic sensitivity). It is a highly sensitive method for the early detection (usually 4-10 days post-inoculation), molecular characterization and quantification (real-time PCR) of the ehrlichial organisms. In addition, PCR is more useful than serology for the documentation of concurrent infections with different ehrlichial species and the post-treatment monitoring. Successful amplification of *Ehrlichia* DNA may be accomplished from several tissues, including whole blood, BM, spleen, lymph nodes, liver, kidney, lung, and cerebrospinal fluid.

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Advanced LAB diagnostics-Clinical applications: Mass Spectroscopy

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Mass spectrometry (MS) is a powerful analytical tool with a wide range of applications, including clinical laboratory medicine. This technique involves bombarding vaporized organic molecules with high-energy electrons, producing fragment ions of different masses that are then separated, measured, and quantified[1]. MS offers high analytical specificity, minimal matrix effects, and insensitivity to interference from heterophile antibodies. Its versatility enables the measurement of almost all analytes, as well as the simultaneous measurement of multiple biomarkers from a single sample[2].

Liquid Chromatography-MS (LC-MS) has become the gold standard in many clinical applications, combining the rapid separation capabilities of LC with the sensitive detection of MS. This synergy enables immediate and highly sensitive detection and quantification[3]. In human medicine, MS has revolutionized clinical endocrinology by improving the accuracy and specificity of hormone measurements. Mass analysers can be combined to perform tandem mass spectrometry (MS/MS), which is a relatively simple concept — two mass analyses occur in series, often with a fragmentation step in between. One approach to MS/MS is to combine two or more identical types of mass analysers in series, such as a triple quadrupole analyser (LC-MS/MS)[1]. This technology has established gold standards for hormone analysis, including steroid hormone measurements[2], thyroid function tests, and vitamin D metabolite quantification[4]. We believe that MS has opened a new window in the clinical laboratory with its remarkable sensitivity and specificity, which is often not produced by other analytical techniques. Although MS is still underutilized in various clinical settings, it has the potential to extend the current capabilities of disease detection with its high level of accuracy, precision, and reproducibility.

Mass spectrometry (MS) is a powerful analytical technique, yet its widespread implementation in clinical laboratories remains limited due to several practical constraints. Most MS instrumentation is designed for broad applications—including food safety, environmental toxicology, and industrial quality control—rather than for clinical diagnostics. It is important to note that commercial kits—such as those commonly used for cortisol determination via chemiluminescence—are extremely rare in the context of mass spectrometry. Unlike immunoassay platforms, which offer ready-to-use diagnostic kits, MS-based testing typically lacks standardized commercial solutions. As a result, clinical laboratories must develop diagnostic applications from the ground up, adapting general-purpose equipment to meet stringent medical standards. This process involves not only customizing analytical protocols but also overcoming significant chemical, biological, and infrastructural challenges to ensure accuracy, reproducibility, and clinical relevance. The development of LC-MS/MS assays is particularly complex, requiring extensive method validation, specialized reagents, and highly trained personnel. Moreover, most diagnostic MS workflows are executed in-house, with laboratory staff responsible for preparing critical components such as calibration standards and mobile phases[2]. At the Veterinary Laboratory San Marco, we have over 15 years of experience in the development, validation, and clinical application of LC-MS/MS assays. In this presentation,

we will share our published work on endocrine and toxicological testing, with particular emphasis on urinary cortisol quantification[5].

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Advanced LAB Diagnostics – Immunocytochemistry

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Immunocytochemistry (ICC) is a laboratory technique used to identify cellular antigens in cytological samples. It works by using specific antibodies that bind to these antigens, which are then tagged with a chromogen for visualization under a light microscope. By this way, "families" of different cells that are morphologically indistinguishable can be classified on the microscope, enhancing the accuracy of cytological diagnoses.

Applications and Advantages:
The primary application of ICC is the classification and subtyping of tumors. However, its utility extends beyond this, providing valuable information for determining prognosis, identifying etiologic agents and serving as a marker for cell growth and differentiation. Compared to other immunophenotyping methods like immunohistochemistry (IHC) and flow cytometry, ICC offers several key advantages. It allows for superior antigen preservation, the simultaneous assessment of both the immunostaining pattern and the cell's morphology, and requires only minimally invasive sampling, It can even be performed on the same slides where the morphological diagnosis has already been made, without the need for a new sample. These benefits make it a particularly attractive option in veterinary practice.

Methodological Considerations:
The success of ICC is highly dependent on proper specimen handling and processing. While it is most commonly performed on fine-needle aspirates, it can also be used with various exfoliated cells from effusions or other liquid samples. Samples can be prepared as smears, cytospins, cell blocks, or liquid-based cytology slides. A significant advancement in the field is the ability to perform ICC on slides that have already been stained and coverslipped, which makes the technique more accessible and efficient. This has notably increased its applicability. Furthermore, the introduction of automated methodologies has dramatically improved standardization and consistency, reducing the high error rates associated with manual techniques and leading to more reliable results with a lower percentage of inconclusive findings. For cases with limited sample availability, techniques like dual-staining with two different chromogens or tissue-transfer methods allow for multiple analyses from a single specimen.

Antibody Panels and Interpretation:
The interpretation of ICC results requires careful consideration. Because most cellular markers are not perfectly specific, pathologists must utilize a panel of two or more antibodies and interpret the findings in conjunction with routine cytological staining. It is crucial to remember that ICC is a tool for further characterization, not for confirming the malignancy of a lesion. For example, it is used to subtype a lymphoma (e.g., B-cell vs. T-cell), not to differentiate it from reactive lymphadenopathy. The ever-expanding list of available antibodies, with varying

sensitivities and specificities, continues to broaden the diagnostic capabilities of ICC in veterinary medicine:

- Epithelial neoplasia: CK AE1/AE3, CK7, CK14, CK18, CK20.
- Histiocytic neoplasia: CD1, CD11, CD18, CD204, MHCII and IBA-1
- Lymphoid neoplasia: CD3, CD4, CD8, CD20, CD21, CD79a, PAX-5.
- Melanocytic neoplasia: Melan-A, SOX-10
- Mesenchymal neoplasia: S-100, vimentin, desmin, factor VIII, CD31, myoglobin.
- Proliferation marker: Ki-67

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Friday 3 October 2025

Cinema 1

Adrenal cytology – To FNA or not to FNA?

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Introduction

Adrenal masses are increasingly detected in small animals due to the widespread use of abdominal ultrasound and advanced imaging modalities. These lesions can be benign and non-functional, but a proportion represent malignant neoplasms or hormone-producing tumours. The role of fine-needle aspiration (FNA) in their evaluation remains controversial, with ongoing debate between perceived risk, diagnostic yield, and impact on clinical management. This presentation intends to explore the utility, limitations, and safety of adrenal FNA in dogs, offering a dual perspective from internal medicine and clinical pathology.

Prevalence of adrenal masses

Studies indicate that incidental adrenal gland masses are not uncommon. *Baum* et al. (2016) reported a 9.3% prevalence in 270 dogs undergoing abdominal CT, with an increased prevalence (15.9%) in dogs scanned for suspected neoplasia. *Cook* et al. (2014) found a 4% prevalence in 3,748 dogs via ultrasound, noting a 30% chance of malignancy if the mass was ≥20 mm. These findings highlight that adrenal masses found by chance should still be carefully assessed, as they are relatively common and can sometimes be malignant.

Evidence review

Survey-based studies suggest adrenal FNA is performed infrequently: fewer than one-third of radiology specialists report routinely undertaking it, primarily due to concerns regarding complications, particularly hypertensive crises in dogs with pheochromocytomas.

The potential benefits of adrenal cytology include distinguishing cortical from medullary origin, identifying metastatic or infiltrative disease, and supporting the diagnosis of benign nonfunctional lesions. Overall accuracy ranges from 80-90%, with excellent inter-pathologist agreement for well-sampled cases.

Complications, while uncommon, do occur. Most are transient and self-limiting (e.g. haemorrhage, arrhythmias).

Clinical perspective

From an internal medicine standpoint, adrenal FNA is not required when imaging and hormonal evaluation confirm functionality (e.g. cortisol or aldosterone-producing tumour, pheochromocytoma), or when imaging features strongly suggest malignancy (e.g. size >20 mm, vascular invasion, diffuse mineralisation). In these scenarios, adrenalectomy is typically indicated.

Cytology can also play a pivotal role in the diagnosis of adrenal neuroendocrine tumours (most commonly catecholamine-producing phaeochromocytomas); alpha-adrenergic receptor blockers or antagonists, such as phenoxybenzamine, are not uncommonly used pre-surgically in

dogs with phaeochromocytoma, and therefore the correct identification of a phaeochromocytoma prior to adrenalectomy can impact on the presurgical management of the case.

A minority of dogs with adrenal tumours can present with two different tumours in one adrenal gland (e.g., phaeochromocytoma and cortical adenoma) which both can be hormone-producing tumours. Moreover, dogs can present with tumours affecting both adrenal glands (e.g., bilateral phaeochromocytoma, or benign non-functional mass in one adrenal and functional adrenal tumour on the other, among other possible combinations). Cytology can add determining the nature of each adrenal tumour when planning adrenalectomy.

Cytology may add diagnostic value in small, non-functional, or equivocal lesions, where distinguishing benign from malignant disease could alter case management. This approach mirrors human medicine, where FNA is reserved for inconclusive cases after imaging and endocrine testing, particularly when the results could change patient management, such as confirming infection or metastatic disease.

Take-home messages

- Adrenal incidentalomas are relatively common, particularly in older dogs.
- Adrenal FNA is safe in most cases, but not risk-free.
- Accuracy is good but not absolute; malignancy cannot always be confirmed or excluded.
- FNA is most valuable in small, non-functional, or indeterminate lesions where results could alter management.

Feline infectious peritonitis – internal medicine and clinical pathology working together

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Introduction

Feline infectious peritonitis (FIP) once considered a uniformly fatal disease; the landscape of its management has been revolutionised by the advent of effective antiviral treatments. Early diagnosis is now essential, although this remains a challenge, requiring close collaboration between clinicians and clinical pathologists to integrate clinical presentation, cytology, fluid analysis, and molecular diagnostics.

Specific pathophysiological findings

Common findings in cats with FIP include body cavity effusions, lymphadenomegaly, and hyperbilirubinaemia. Other less commonly recognised syndromes associated with FIP are gastrointestinal ileus and myocarditis.

Diagnostics

Cytology remains pivotal: pyogranulomatous inflammation in effusions or tissues strongly supports FIP in the appropriate context. PCR and immunostaining for FCoV antigen improve diagnostic confidence.

Treatment and monitoring

Antiviral nucleoside analogues (GS-441524 and its prodrug remdesivir) are now standard of care, with survival rates of 80–90%. Recent evidence suggests that shorter treatment courses (6 weeks) may be sufficient. Molnupiravir and protease inhibitors (e.g. GC-376 and nirmatrelvir) represent promising alternatives.

Haematological, biochemical, and urinary abnormalities associated with these antiviral medications are increasingly being more recognised.

Supportive treatment may include prokinetics, diuretics for effusion-associated heart failure, and general supportive care. Early initiation of antivirals improves outcomes, and treatment is often started prior to molecular confirmation.

Laboratory monitoring of treatment response is increasingly important. Acute phase proteins provide objective measures, with AGP showing greater consistency for long-term monitoring.

Challenges

A growing concern is the empirical use of antivirals without adequate diagnostics, often sourced from unregulated suppliers, risking treatment of non-FIP cases and the development of viral resistance. Subtherapeutic dosing, inadequate duration, or poor absorption further complicate outcomes.

Take-home messages

- FIP is now a treatable disease, with high survival when antivirals are used appropriately.
- Early diagnosis is more essential than ever: Cytology, fluid analysis, and molecular diagnostics can be game changers.
- APPs provide useful monitoring tools.

- Early treatment initiation improves prognosis but must be balanced with diagnostic certainty.
- Responsible use of antivirals is essential to preserve long-term efficacy.

Diagnostic Imaging & Clinical Pathology of Dogs and Cats

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Diagnostic imaging (DI) and clinical pathology (CP) play complementary roles in the diagnostic work-up of dogs and cats. Whilst DI provides structural and functional insight, CP establishes the biological pathology of lesions through minimally invasive techniques such as fine-needle aspiration biopsy (FNAB) or analysis of effusions.

Thoracic investigation encompasses radiography, ultrasound, MRI, and CT, each with distinct advantages. Radiography remains a cost-effective screening tool; ultrasound is valuable for soft tissue assessment and for guiding sampling procedures; MRI is primarily reserved for brain and spinal cord evaluation. CT, however, is unrivalled in the assessment of the lungs, mediastinum, and pleura. CT permits sensitive detection of small pulmonary nodules, metastatic disease, and infiltrative lung pathology. Examples include idiopathic pulmonary fibrosis in terriers, eosinophilic bronchopneumopathy, pneumonia, septic emboli, and mycobacterial infections. In addition to diagnosis, CT aids surgical planning, particularly when considering lung lobe resection.

Mediastinal pathology illustrates the need for integrated approaches. Imaging can suggest differential diagnoses such as cysts, lipomas, but there is overlapping for many pathological processes such as paragangliomas, lymphoma, thymoma, or ectopic thyroid/parathyroid carcinoma. Cytology remains critical to distinguish the previous. A case of spindle-cell thymoma with multiple epithelial cysts highlighted the difficulty of correlating imaging features with histopathology. Pleural disease provides another example of DI and CP complementarity. CT can provide an initial discrimination between effusion types on the basis of attenuation values, but cytology is necessary for a definitive classification. Chylous effusions, exudates, and hemorrhagic effusions can be distinguished by cytological features that imaging alone cannot resolve.

Abdominal investigation has expanded considerably since the early 2000s. CT now allows comprehensive evaluation of all abdominal organs, with contrast protocols enabling assessment of perfusion, vascular supply, and extracellular leakage. In the liver, CT features help differentiate nodular hyperplasia from hepatocellular carcinoma (HCC), the most common primary hepatic tumour in dogs. HCC typically presents as a large (>9.8 cm), heterogeneously enhancing mass with low portal phase attenuation. Cytology in conjunction with the activity of hepatic enzymes and the imagining can favour a specific underlying hepatic pathology. Hemangiosarcoma demonstrates aggressive behaviour and characteristic CT signs (“halo” and “SPLASH”).

Us clinical pathologists can benefit from the CT findings of pancreas, that is often a challenging organ to acquire good quality cytology samples. Acute pancreatitis produces marked swelling

and fat stranding on CT, with cytology of peritoneal fluid revealing neutrophilic exudates. Neoplastic processes such as adenocarcinoma and insulinoma can be suggested by imaging but require cytological and immunohistochemical confirmation. A particular focus is the recognition of insulin-like growth factor II (IGF-II) secreting pancreatic neuroendocrine tumours. These rare lesions mimic insulinomas clinically but are hypoinsulinaemic; immunohistochemistry demonstrating IGF-II positivity is diagnostic and prognostically relevant.

Adrenal evaluation benefits from CT's ability to define size, density, vascular invasion, and metastatic spread. Cytology and immunohistochemistry distinguish cortical carcinomas from pheochromocytomas. A unique case of feline adrenocortical carcinoma with myxoid differentiation underscores the diagnostic importance of combined imaging and pathology.

Take-home message: DI defines lesion morphology, distribution, and intervention feasibility, while CP can confirm pathological behaviour. Their integration provides a more complete diagnostic picture, enhances clinicopathological correlation, and ensures clinically meaningful reporting.

BIOMARKERS FOR GASTRIC AND PANCREATIC DISEASES

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Biomarkers are naturally occurring molecules found in blood or other bodily fluids that can be measured objectively to indicate a pathological process or normality. Some can be used to aid the diagnosis of and/or indicate the prognosis in gastric and pancreatic diseases and, potentially, enable monitoring of disease progression or treatment response.

There are no pathognomonic haematological or serum biochemical changes in gastric or pancreatic diseases, although they may support the diagnosis. Serum pepsinogen A, and gastric lipase activity may be markers for gastric pathology. Inflammatory markers (e.g. serum calprotectin) may also indicate gastric pathology.

In pancreatic diseases, enzymes are released from damaged tissue and can be assayed in the blood. However, results do not always correlate with disease severity or prognosis, and the utility of these tests in chronic pancreatitis is unknown. False positives due to benign pancreatic hyperenzymaemia occur in humans without AP and may do so in animals.

Ultimately, the definitive diagnosis of AP can only be made by the histological examination of either multiple pancreatic biopsies or the whole pancreas *post mortem*. Consequently, the claimed sensitivity and specificity of biomarkers has only been assessed in patients with either severe or fatal AP where the pancreas has been sampled, and, therefore, their reliability to diagnose milder cases is uncertain.

The trypsin-like immunoreactivity (TLI) test is highly sensitive and specific for the diagnosis of exocrine pancreatic insufficiency.[1] It has poor sensitivity for the diagnosis of acute pancreatitis (AP) in dogs although may be better in cats.

Serum amylase and lipase activities are the traditional markers for AP although both are affected by renal insufficiency. However, amylase-like activity is also present in the intestinal brush border, and lipase activity is not totally pancreas-specific with significant amounts of gastric and hepatic lipase. A four-fold increase in lipase activity (with 1,2 diglyceride or triolein as substrate) in dogs, has similar sensitivity and specificity to pancreas-specific lipase assays.

Species-specific assays of pancreatic lipase immunoreactivity (PLI) only measure pancreas-derived lipase molecules, with the Spec cPL and fPL claimed to have the best sensitivity and specificity for identifying AP.[2] SNAP PL tests have a lower cut-off and so are more sensitive, increasing the false positive rates. However, if negative, they virtually rule out AP.

It is claimed that assay of lipase activity using 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester (DGGR) as the substrate is pancreas-specific. Yet, DGGR activity is present in dogs with EPI so this assay cannot be totally specific. However, there is a reasonable correlation between cPLI and DGGR lipase.[3,4] The advantages of the DGGR lipase assay are

that it can be run in-house and is much cheaper than the Spec cPL. As both tests have similar sensitivity and specificity, and neither is perfect, the DGGR lipase test is most useful in practice as a screening test that can be followed up with a PLI test. A new methodology (*Catalyst® PL*) for measuring DGGR lipase activity in-house appears to be pancreas-specific and correlates well with the Spec cPL.[5]

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BIOMARKERS FOR INTESTINAL DISEASES

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Biomarkers can aid the diagnosis of and/or indicate the prognosis in intestinal diseases and enable monitoring of disease progression or treatment response.[1] The minimum database can help identify intestinal bleeding or protein-losing enteropathies. Fecal identification of parasites and parvovirus are commonly performed but the value of stool culture is limited. The dysbiosis index (DI) is a validated method that assesses the relative abundance of key fecal bacteria giving a numerical score for the degree of dysbiosis.[2]

FUNCTIONAL BIOMARKERS

Folate, cobalamin and methylmalonic acid (MMA)

Other than the minimum database and DI, the biomarkers most clinically useful and readily available to practitioners are serum cobalamin and folate as they not only identify intestinal dysfunction, but also any need for supplementation.[3] Subnormal cobalamin concentration is not a perfect marker of functional cobalamin deficiency, and concurrent assay of MMA would be ideal if available. The clinical importance of subnormal folate and any benefit of supplementation is yet to be elucidated.

INFLAMMATORY BIOMARKERS [4]

Neutrophil:Lymphocyte ratio (NRL)

A change in the NLR is a totally non-specific indicator of disease but can be altered in chronic inflammatory enteropathies (CIEs).

C-reactive protein (CRP)

This is a sensitive but non-specific serological marker of inflammation. It may suggest intestinal inflammation if it is increased in conjunction with intestinal signs but it does not indicate the cause or its type.

Calprotectin

Calprotectin is a protein dimer (S100A8/A9) from the S100 calcium-binding protein family. It is mainly expressed by neutrophils but also by activated macrophages and epithelial cells and serum calprotectin concentrations are also increased in CIEs. The presence of calprotectin in feces is a better marker for intestinal inflammation.

Calgranulin C

Calgranulin C (S100A12) is another molecule in the S100 protein family primarily released from activated neutrophils. Measurement of fecal calgranulin C is a marker of CIEs.

DAMAGE BIOMARKERS

Alpha1 proteinase inhibitor (a1PI)

A serum antiproteinase, a_1PI, is found in feces in protein-losing enteropathies (PLEa) and is a surrogate marker for albumin loss. [5] The test alone is most useful in detecting a PLE during early, subclinical disease, particularly when screening at risk breeds.

METABOLIC BIOMARKERS

Vitamin D

Hypovitaminosis D can occur in chronic enteropathies and is a negative prognostic factor in PLEs.

Others

N-Methylhistamine, 3-bromotyrosine, and urinary nitrosonaphthol are potential markers of histamine, eosinophil and intestinal bacterial metabolism resectively,

IMMUNOLOGICAL BIOMARKERS

Faecal immunoglobulin A (IgA)

Whilst absolute IgA deficiency in people predisposes them to intestinal infections, absolute IgA deficiency has not been proven in dogs.

Anti-gliadin antibodies

Antibodies to tissue transglutaminase cross-react with gliadin, the immunogenic component of wheat and are markers of gluten sensitivity.

Perinuclear anti-neutrophilic cytoplasmic antibody (pANCA)

This autoantibody stains material around a neutrophil nucleus and is found in some inflammatory enteropathies in dogs.

GENOMIC BIOMARKERS

Single nucleotide polymorphisms (SNPs) can help explain differences in susceptibility to chronic inflammatory enteropathies (CIEs) between individuals. DNA tests are available for inherited cobalamin deficiency and congenital megaesophagus in German shepherds.

MicroRNA (miR)

Circulating miR-20b is a potential biomarker for distinguishing intestinal neoplasia from CIE in dogs.

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EUROPEAN VETERINARY CLINICAL PATHOLOGISTS WORKPLACE WELLBEING SURVEY

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Veterinary medicine has received attention over the last several years regarding the importance of workplace wellbeing. There is increasing awareness of the 'human factors' and workplace wellbeing and their importance in recruiting and retaining veterinarians. However, to the authors' knowledge no surveys have examined these issues within the specialty of veterinary clinical pathology.

Thirty-two questions were sent to 152 member of the European Society of Veterinary Clinical Pathology and 30 residents, with 92 and 13 responses, respectively (75% response rate for ESVCP members and 43% response rate for residents). Only 47% of ESVCP members strongly agreed that they had high job satisfaction, with 34% indicating that they had experienced burnout on one or more occasions. Twenty-three percent strongly agreed that their organization was chronically understaffed due to inability to recruit and retain clinical pathologists. Only 22% strongly agreed that their caseload was reasonable. Only 14% indicated that their organizations had clear expectations for performance goals. Only 10% strongly agreed that their organization was pro-active in promoting mental health and job satisfaction. Only 21% strongly agreed that their organization made use of their variety of interests and competencies. The top four activities in which ESVCP members indicated that they would like to be involved, but did not have the opportunity to do so were participation in research and development, assay/instrument/method validation, QC evaluation and teaching residents. Hours of working in order to adequately deal with the caseload was perceived to be be too long (> 8 hours/working day) and pay was perceived to be inferior to that of other European Board of Veterinary Specialists. High demand for cytology work resulting in exclusion of variety in jb tasks was indicated to be disheartening. A lack of recognition and appreciation of veterinary clinical pathology as an important veterinary specialty was emphasized.

Overall, the survey results indicate a need improvements in staffing and workload, increased opportunities professional development, increased diversity of tasks, better management, improved compensation and recognition, and increased opportunities to participate in student and client education.

They highlight the need for difficult conversations regarding case load, job tasks and hours worked between clinical pathologist s and their employers. Increased variety and fulfillment in the work place needs to be addressed, with more opportunities for collaboration with clinicians and other departments and time for followup and continued learning.

These themes reflect the desire for a more balanced workload, more opportunities for professional growth, better compensation, and improved work environments for veterinary clinical pathologists, as well as a need for additional client and student education, research opportunities and professional recognition.

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Friday 3 October 2025

Cinema 3

Research Communications

Canine serum procalcitonin concentrations in canine pyelonephritis

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Background. Serum procalcitonin (PCT) concentrations are a biomarker for the identification of human urinary tract infections[1]. PCT ELISAs are validated for use in dogs[2,3], although some aspects of assay validation (sample quality and freeze-thaw effects) have not been evaluated.

Objectives. Undertake additional validation of a PCT ELISA and investigate the utility of serum PCT concentrations as a diagnostic biomarker in canine pyelonephritis.

Methods. Bilirubin and haemoglobin interference studies were performed by sample spiking and lipaemia interference studies by lipid separation. Samples were subjected to three freeze-thaw cycles. Dogs with pyelonephritis were identified via a positive bacterial urine culture and physical examination or imaging findings consistent with pyelonephritis. Control dogs had no history of urinary disease or systemic co-morbidities, normal urinalysis including a non-active sediment and negative urinary culture. Data are presented as median [minimum–maximum] and comparisons between groups were made using the Mann Whitney U test.

Results. All interference studies recorded recovery percentages within 80–120%. In freeze- thaw studies, 1/5 samples recorded a PCT concentration outside the 80–120% recovery percentage (67.8%), after three freeze-thaw cycles. Serum PCT concentrations of dogs with pyelonephritis were higher than in controls (128.5 [38.0–330.4] pg/mL, n=7, vs. 42.5 [7.8– 190.3], n=8; P=0.029).

Conclusions. Serum PCT concentrations are higher in dogs with pyelonephritis than controls, although serum PCT concentrations are likely to be poorly sensitive for canine pyelonephritis. The PCT ELISA is not subjected to interference by haemoglobin, bilirubin or lipaemia. Multiple freeze-thaw cycles may decrease serum PCT concentrations.

Keywords: Infection, Interference study, Pyelonephritis

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Effects of different creatinine analytical methods on Urine Protein to Creatinine ratio variability in canines

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Background. Proteinuria measured as the Urine Protein to Creatinine Ratio (UPCR) is an early diagnostic marker and prognostic indicator in dogs with chronic kidney disease (CKD). Two laboratory assays, the modified Jaffe and enzymatic reaction, are available to measure urine creatinine but variability in measurement and their resultant UPCR variability have yet to be assessed.

Objectives. To assess the UPCR variability using two different creatinine assays and the possible effect on CKD sub-staging of these canine patients.

Material and Methods. 76 urine samples were collected from canine patients presented to a teaching hospital. These samples were collected irrespective of their breed, age, sex as well as underlying diseases. Complete urinalysis was performed, and the same urine samples were analysed using the two techniques for urine creatinine measurement.

Results. There was no significant difference in urine creatinine concentration between the two methods ($p=0.627$). The resultant calculation of UPCR showed no significant differences when both methods of urine creatinine measurement were compared ($p= 0.789$). The concordance in classifying the dogs as proteinuric, borderline proteinuric and non-proteinuric, was very good, $\kappa= 0.990$ ($p <0.001$). Both constant and proportional bias were identified when using the Passing-Bablok regression analysis, with Jaffe method over-estimating urine creatinine at higher urine creatinine concentrations. Despite this, the clinical impact was negligible given the strong agreement between the two methods.

Conclusion. Both methods of urine creatinine measurement can be used to classify proteinuria.

Keywords: *Proteinuria, Urine Protein to Creatinine Ratio, Chronic Kidney Disease, Urine Creatinine, Canine*

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Impact of infection intensity, antibody levels, and stays abroad on hematological and biochemical parameters as well as acute-phase proteins in 342 dogs with acute *Babesia canis* infections

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Background. *Babesia* (*B.*) *canis* infections are of rising importance in Germany.
Objectives. Aims of the retrospective study were to analyze hematological/biochemical parameters as well as acute-phase proteins in dogs with acute *B. canis* infections, and to determine the impact of infection intensity, antibody levels, and stays abroad.
Material and Methods. Dogs in Germany tested PCR-positive for *B. canis* and negative for *Anaplasma phagocytophilum* from January 2018 to Dezember 2024 were included, if data on hematocrit, leukocytes, and platelets were available. A hematological scoring (HES) was performed according to severities of hematological abnormalities. Results of biochemical and CRP analysis, *Babesia* antibody determination and pathogen quantification were included, if available.
Results. History of stays abroad was known for 191/342 dogs (55.8%; no stays abroad 113/191 (59.2%), imported 55/191 (28.8%), travel 23/191 (12.0%)). The most common clinicopathologic findings were increased CRP (87.4%), thrombocytopenia (85.1%), anemia (78.7%), and hyperbilirubinemia (74.2%). Dogs without stays abroad showed significantly higher HES, CRP, and infection intensity, but lower serum antibody levels compared to imported dogs (P<0.001 each). Positive correlations were found between CRP and infection intensity (ρ=0.444), CRP and HES (ρ=0.406), as well as infection intensity and HES (ρ=0.348), while negative correlations were observed between antibody levels and infection intensity (ρ=-0.666), as well as antibody levels and HES (ρ=-0.652) (P<0.001 each).
Conclusion. Approximately 60% of the dogs had no stays abroad, representing autochthonous infections, and were predominantly immunologically naïve. Most imported dogs had positive antibody levels. Dogs with high antibody levels showed less severe clinicopathological alterations and lower pathogen concentrations, explained by protective antibody activity.

Keywords: canine babesiosis, acute phase reaction, serology, PCR, pathogen quantification

DGGR-lipase for Canine Pancreatitis: Siemens-Atellica-930 liquid assay concords with IDEXX-Catalyst, dry-reagent, activity-assays

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Background. DGGR-lipase is validated for diagnosing canine pancreatitis.[1] It is enzyme-activity-based, equally-effective as immunoassay, but inexpensive and rapid. IDEXX added DGGR, dry-reagent activity-assay (PL) to its Catalyst-analyzer, in addition to its standard lipase assay (Lipa). Siemens-Atellica DGGR, liquid-reagent activity-assay (SA-DGGR) was previously validated.[2]
Objectives. Evaluate effectiveness of PL, Lipa and amylase activity-assays compared to SA-DGGR assay. Determine correlation with other standard chemistry biomarkers affected in pancreatitis.
Material and Methods. 35 canine cases were analysed for PL, Lipa and amylase on IDEXX-Catalyst, and for SA-DGGR on Siemens-Atellica-930 (with ALP, ALT and triglycerides). Diagnostic concordances and linear regressions (log values) were determined. All $p<0.05$.
Results. At diagnostic cutoffs for PL, Lipa and amylase, respectively, of >400U/L, >1800U/L, >1500U/L, results (+/-) were 97/86/74% concordant with SA-DGGR (cutoff >80U/L). Correlations to SA-DGGR were (r=0.94/0.86) higher, and distances from regression lines (F=253/93) lower for PL than Lipa. Compared to SA-DGGR, PL had more false positives (11% >200U/L); Lipa had more false negatives (14%). Correlations to SA-DGGR were lower for amylase (r=0.71), ALT (r=0.60), ALP (r=0.55) and triglyceride (r=0.51).
Conclusion. IDEXX and Siemens DGGR assays showed nearly-perfect concordance at diagnostic cut-off >400/>80U/L, respectively. IDEXX-DGGR had ~10% false positives for cut-off >200U/L. Lipa had ~15% false negatives. For other parameters, only amylase had good correlation, although poor concordance. Pancreatitis correlated with hepatocellular and hepatobiliary pathology, and hypertriglyceridemia.

Keywords: Siemens-Atellica, DGGR-lipase, pancreatitis, canine, IDEXX

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Analysis of serum amylase activity in dogs with portosystemic shunts

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Background: Results of a previous study (Thomson and Williams, JVDI 2022) identified a potential contribution of hepatic-derived amylase to serum amylase activity. In addition, humans with functional liver impairment have reduced serum amylase activity.

Objectives: To investigate if serum amylase activity is reduced in animals with portosystemic shunts (PSS).

Materials and methods: Dogs in a single referral centre with bile acid (BA) stimulation test results available were identified. Dogs were excluded if post stimulation BA concentrations (PSBA) were below pre stimulation BA concentrations. Dogs with azotaemia, elevated serum DGGR lipase activity, or a clinical history of pancreatitis or recent treatment with steroids were excluded. Dogs with PSBA <22.5 µmol/L were included in the control group and dogs with PSBA >100 µmol/L were included in the PSS group. Diagnosis of PSS by imaging was also confirmed. Control data were used to derive a non-parametric reference interval for serum amylase activity. Data are presented as median [minimum-maximum] and comparisons between groups were made using the Mann Whitney U test.

Results: Serum amylase activity was significantly lower in the PSS group compared to control group (395 [135 – 668] IU/L, n=13, vs. 708 [320 -1451] IU/L, n=104; P<0.0001). No significant difference was observed in serum DGGR lipase activity between the control and the PSS groups (P=0.872). 46% of dogs with PSS had serum amylase activity below the calculated reference interval (352 – 1395 IU/L).

Conclusion: Low serum amylase activity could be utilised as an additional biomarker for PSS in non-azotaemic dogs without pancreatitis.

C-reactive protein and Serum Amyloid A concentrations in serum samples from a large cohort of dogs – corresponding and discordant results

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Background. C-reactive protein (CRP) and Serum Amyloid A (SAA) are both canine major acute phase proteins with different physiological roles[1,2]. While CRP activates the complement system, SAA is an apolipoprotein. The two proteins' distinct pathophysiological processes may influence their response to various diseases.

Objectives. The objective of this study was to evaluate circulatory CRP and SAA concentrations in dogs with different diseases, evaluate the correlation between the two proteins, and identify discordant results.
Material and Methods. The study included canine serum samples submitted to the laboratory for analysis for CRP measurement. In addition, SAA analyses were performed. Samples were analyzed using the canine CRP Immunoassay (Gentian, Norway) and VET-SAA (Eiken, Japan) at an Atellica CH Analyzer (Siemens Healthineers, Germany). Each dog was categorized into one of 11 affected organ categories.
Results. 451 measurements of pairwise CRP and SAA analyses were included. Concentrations of CRP and SAA in Gastrointestinal diseases ((median;range;mg/L)CRP:14.9;0-242; SAA:1.8; 0.1-2257, n=113) and other diseases (CRP:17.7;0-290; SAA:2.6;0.1-1959, n=73) were significantly different ($p<0.0001$) compared to neurological diseases (CRP:3.8;0-37.3; SAA: 0.8;0.1-8.9, n=61). A moderately strong positive correlation was found between CRP and SAA ($r=0.68$;$p<0.0001$) with discordant results in 40 samples (9%); primarily in dogs with immune mediated diseases (SAA>CRP) or infected with angiostrongylosis (SAA<CRP).
Conclusion. The study revealed differences in CRP and SAA concentrations. SAA demonstrated a wider range of concentrations across most organs categories compared to CRP, potentially making it more effective in capturing the spectrum of inflammatory responses in various diseases. Nine percent of samples showed discordant results.

Keywords: Acute phase proteins, canine, CRP, SAA

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Application of the Gyrolab xPlore microfluidic platform for establishment of safety biomarkers: Lessons from ACTH and Beyond

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Keywords: biomarker, immunoassay, ACTH assay, nonclinical, Gyrolab xPlore

Background: Biomarker analysis plays a critical role in the early phases of drug development and safety evaluation of new drug candidates. However, biomarker analysis is often challenged by the limited availability of species-specific assays and limited availability of sample volumes. Antibody-based assays remain a significant method for biomarker quantification, typically employed as conventional two-step sandwich colorimetric ELISAs.

Objectives: This study aims to demonstrate the application of the Gyrolab xPlore microfluidic platform for the development of safety biomarker assays, with a focus on ACTH and other challenging analytes. The goal is to establish robust, low-volume immunoassays suitable for nonclinical toxicology settings.

Methods: A collaborative effort between Boehringer Ingelheim and Gyros Protein Technologies was undertaken to develop and validate immunoassays using the Gyrolab xPlore system. The platform utilizes nanoliter-scale sample volumes in a microfluidic compact disk format. Assay development steps included optimization, troubleshooting (e.g., carryover mitigation), and fit-for-purpose validation, particularly for ACTH in rat species. Additional strategies were explored for analytes lacking species-specific antibodies.

Results The Gyrolab xPlore system enabled successful development of an ACTH assay with sample volumes as low as 3.5–8 µL. Challenges related to ACTH's high isoelectric point were addressed through tailored troubleshooting strategies. Broader assay development efforts yielded insights into competition assay formats and alternative approaches for challenging safety biomarkers, demonstrating the platform's flexibility and efficiency.

Conclusion: The Gyrolab xPlore platform offers a transformative solution for biomarker assay development in nonclinical toxicology, enabling low-volume, high-throughput immunoassays. Lessons learned from ACTH and other biomarkers highlight the system's potential to overcome common assay development challenges and support safety biomarker research.

Plasma trace element profiles in dogs with cancer: Associations with tumour type and clinical status

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Background. Trace elements are essential for physiological processes and have been associated with cancer development, progression and prognosis in human medicine[1,2].
Objectives. To characterise the plasma mineral profile of dogs with cancer and explore associations between elements and clinical findings.
Material and Methods. A retrospective case-control study including 204 dogs: 164 with cancer (44 mammary, 28 mast cell, 27 soft tissue sarcoma, 27 intracranial, 21 lymphoma, and 17 hepatic tumours) and 40 healthy controls. Plasma concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se and Zn were measured using inductively coupled plasma mass spectrometry. Clinical findings assessed included apathy, dysorexia, metastasis and biochemical alterations. Statistical significance was defined as $p < 0.05$.
Results: Dogs with cancer had higher Cu, Mn, Ni, Pb, Se and Zn than controls. Element profiles varied by tumour type with the hepatic group showing the greatest alterations. Principal component analysis allowed differentiation of each tumour group from controls. Higher Cu and Mn and lower Se were associated with clinical symptoms and distant metastasis. Dogs with increased BUN had higher Cu levels. Patients with increased ALT activity had higher Mn and Se concentrations, while elevated ALP activity was linked to higher Cu, Mn and Se levels.
Conclusions. Plasma trace element profiles in dogs with cancer showed distinct patterns linked to tumour type and clinical features. Their close association with inflammatory and metabolic alterations supports their potential as biomarkers for staging, prognosis, and monitoring in veterinary oncology, mirroring findings in human medicine.
Keywords: trace elements, oncology, biomarkers.

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Relationship between inflamm-aging and myxomatous mitral valve disease (MMVD) in senior and geriatric dogs

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Background: Aged dogs are more prone for degenerative diseases and exhibit immune-system alterations, a phenomenon known as "inflamm-aging." Myxomatous mitral valve disease (MMVD) commonly causes congestive heart failure in older dogs, with its progression being influenced by inflammatory mediators and cytokines

Objective: To assess whether inflamm-aging contributes to the development and progression of MMVD.

Material and methods: 149 dogs were enrolled and categorized by MMVD stage (healthy, pre-clinical: B1 and B2, clinical: C+D) and age (adult, senior, geriatric). The dogs were also grouped by combined classification (age+MMVD class). Comprehensive analyses included hematobiochemical exams, serum protein electrophoresis (SPE), oxidative-stress markers [paraoxonase-1 (PON-1), advanced oxidized protein product (AOPP)], cytokines: TNF-α, IL-1β, IL-6, leptin, ghrelin, and heat-shock protein 70 (HSP70).

Results: Clinical geriatric dogs had higher leukocytes and neutrophils (P=0.006, P=0.005). Monocytes, neutrophil/lymphocyte ratio (N/Lr) and monocyte/lymphocyte ratio (M/Lr) were elevated in clinical geriatric and senior dogs (P<0.001, P=0.01, P=0.01). SPE showed lower albumin percentages (P=0.01) and higher α2-globulin and β1-globulin percentages in pre-clinical and clinical geriatric dogs (P=0.02, P=0.01). PON-1 activity was reduced in clinical geriatric dogs and healthy adult dogs (P=0.045). No differences for AOPP were observed. WBC, neutrophils, N/Lr were more influenced by the severity of MMVD while albumin, α2-globulin and β1-globulin by the age. Monocytes and M/Lr were affected by both. In clinical dogs, regardless of age, leptin concentration was lower and HSP70 concentration higher.

Conclusion: Clinical geriatric dogs exhibit increased inflammatory markers. Variations in some inflammatory parameters are more associated with the severity of MMVD, others with age.

Keywords: oxidative stress, inflammation, aging, MMVD

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Novel, Inexpensive, Multicolour-Stick, DGGR-Lipase, Point-of-Care Test for Canine Pancreatitis

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Background. DGGR-lipase is validated for the diagnosis of canine pancreatitis, however an inexpensive point-of-care test is unavailable.
Objectives. Develop an inexpensive, DGGR-lipase, point-of-care test for canine pancreatitis.
Material and Methods. DGGR-lipase reagents for our Siemens Atellica-CH930 chemistry analyzer were used. 320 uL of Reagent 1 and 12 uL of plasma were incubated for 4 minutes in a 1-ml-cuvette at 37°C. The reaction was initiated by adding 160 uL Reagent 2 containing DGGR. At 8 minutes, reaction-mixture colour was dermined by matching against that on a "multicolour stick" with 6 different colours corresponding to different enzyme activities and severities of pancreatitis: 1) yellow, 0 U/L; 2) orange, <80 U/L, upper limit (UL) of normal; 3) pink, <160 U/L, UL mild pancreatitis; 4) light-red, 320 U/L, moderate pancreatitis; 5) dark-red, 500 U/L, UL moderate pancreatitis; 6) purple, <1000U/L marked pancreatitis . DGGR is orange in Reagent 2 and converted to red-purple methylresorufin that absorbs light at 518 nm. Colour was quantified using a smartphone colorimeter app, and a spectrophotometer (Fisher, Helios).
Results. The progressive colour change through yellow, orange, pink, light-to-dark red, purple correlated, respectively, to Smartphone measures of green (155, 120, 100, 70, 50, 35) and spectrophotometric measures at 581 nm (0.30, 0.45, 0.60, 0.78, 1.12, 1.25)
Conclusion. DGGR-lipase can be inexpensively, rapidly, and semi-quantitatively assayed by comparison of reaction colour with the colour on a multicolour-stick, to determine severity of pancreatitis. A colourimetric, smartphone app or spectrophotometer can quantify DGGR lipase activity.

Keywords: DGGR, pancreatic lipase, point-of-care

Assessment of Oxidative Stress and Cellular Response in Progressive Feline Chronic Kidney Disease (CKD): TBARS and Glutathione Peroxidase Activity in Kidney Lysates

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Background. Feline CKD is typified by tubulointerstitial inflammation and fibrosis. [1] Local hypoxia [2, 3] results in generation of **reactive oxygen species (ROS), causing lipid peroxidation and cell injury [4].** G**lutathione peroxidase (GPx), an antioxidant that neutralises radicals through catalytic removal of H_2O_2 [5], is evaluated here in the feline CKD kidney.**
Objectives. Quantify GPx activity and lipid peroxidation in kidney tissue from cats with stable versus progressive CKD and controls.
Material and Methods. PBS-EDTA lysates were generated from post-mortem kidneys (100mg/mL PBS EDTA) from non-azotaemic cats (> 9 years) and those with stable and progressive CKD (n=7/group). TBARS assay (Invitrogen) evaluated lipid peroxidation and ransel (Randox) assay GPx activity. Values were normalised to protein concentration, and CD10 (a tubular cell marker) data from Western Blotting semi-quantification. Non-parametric descriptive data are presented (median [25th, 75th percentile]) with Kruskall-Wallis and Dunn's correction for group comparisons ($p<0.05$).
Results. There was no significant difference in [TBARS] or GPx activity when normalised to protein between groups ($p=0.15$ and $p=0.46$). However, normalised to CD10, GPx was significantly higher in progressive (8.82U/mg [6.21, 10.97]) compared to stable (2.07U/mg [1.72, 2.83], $p=0.03$) and control cats (2.17U/mg [1.18, 2.55], $p=0.011$). When normalised to CD10, [TBARS] were highest in progressive cats (12.07µmol/g [9.42, 43.66]) compared to stable (6.82µmol/g [4.29, 7.84]) and controls (4.24µmol/g [3.24, 4.73]) but did not reach significance.
Conclusion. When normalised to tubular cell content, progressive cats trended towards greater degrees of lipid peroxidation as a marker of oxidative stress with significantly higher GPx antioxidant activity evident.

Keywords. Feline; Progressive CKD; TBARS; Glutathione Peroxidase

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Scanning electron microscopy of the fibrin network of thrombi in dogs with *Babesia rossi* infection

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Background. Despite severe thrombocytopenia, dogs with *Babesia rossi* infection do not exhibit a bleeding phenotype. Instead, a consumptive coagulopathy with microthrombi formation has been described.
Objectives. This pilot study aimed to assess fibrin network ultrastructure in dogs with babesiosis, using electron microscopy.
Materials and Methods. Citrated samples were collected from six dogs with *B. rossi* infection and two healthy controls. Thromboelastography was used to induce in vitro thrombus formation and clots were collected at initiation, amplification, maximum clot strength and fibrinolysis. Fibrin networks within clots were examined with scanning electron microscopy.
Results. Thrombi from control dogs at clot initiation showed fibrin bundles, platelet-fibrin aggregates, and areas of thick fibres connecting to aggregates. As thrombus formation progressed, the network increased in density with fibrin sponge formation (mesh-like structure) becoming apparent. Thrombi from babesiosis cases consisted of netted, highly branched thin fibres; some areas of matted fibre bundles. As thrombus formation progressed, babesiosis cases showed an increase in matted fibre bundles, often obscuring the thin fibre network, with areas of platelet-fibrin aggregates and fibrin sponge being observed.
Conclusion. The fibrin network in babesiosis cases showed marked abnormalities, paralleling those seen in human inflammatory conditions. The predominance of thin, denser, more compact and highly branched fibres, as well as progression to thick, matted fibre bundles has been reported to increase resistance to fibrinolysis, clot rigidity and reduced deformability, resulting in more occlusive clots. This may contribute to the coagulopathy and microthrombi formation, resulting in multiple organ dysfunction, in dogs with *B. rossi* infection.

Key words: *Babesia rossi*, fibrin, fibre bundles, microthrombi

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The use of an indirect method of reference interval determination to assess age-related changes in selected measurands in adult Labrador retrievers

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Background. The use of statistical algorithms for estimating indirect reference intervals (RI) from real-world veterinary patient data has recently been explored. Their utility to characterize age-dependent changes in various measurand concentrations in dogs has not been described.

Objectives. Characterization of expected age-dependent changes for two measurands in adult Labrador retrievers using age-specific indirect RI.

Materials and Methods. Following extraction from a mixed patient database from IDEXX UK over a six-year period, non-age-restricted and age-specific indirect RI with associated confidence intervals (CI) were estimated for Labradors aged 1-8 years for serum alkaline phosphatase (ALP) and total white blood cell count (WBC) using the RefineR algorithm. Total and age-specific direct RI for Labradors were also calculated from a healthy blood donor database from the same laboratory and time period.

Results. For ALP and WBC, direct and indirect non-age-restricted RI had similar lower and upper reference limits. For both direct and indirect age-specific RI for ALP, the lower reference limit remained similar across age groups, with an age-dependent increase observed for the upper reference limit. For both direct and indirect age-specific RI for WBC, the lower and upper reference limits showed gradual age-dependent decreases.

Conclusion. The indirect RI model confirmed an age dependency for serum ALP activity and WBC, consistent with the direct method and reported changes in Labradors. This supports its potential as a practical and inexpensive approach for assessing age-specific RI. Further studies should explore its application to other age groups, including paediatric and geriatric populations, and to breed-specific RI.

Keywords: Indirect reference intervals, real-world patient data, age-related changes, haematology, clinical chemistry.

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HARISS: Histogram Analyzer for Reference Intervals of Small Samples, a free web app to estimate reference intervals of small samples by automatic visual inspection of distribution histograms

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Background. Small sample sizes often lead to inaccurate reference interval (RI) estimates.[1] Visual inspection of distribution histograms (VADH) can guide statistical method selection, yet human subjectivity influences its effectiveness.[2]

Objectives. This study sought to create a machine learning model capable of performing VADH on data from limited samples.

Material and Methods. A training dataset comprising 45,000 distribution histograms, derived from samples of 20 to 40 subjects, was generated from simulated Gaussian, lognormal, and left-skewed populations. A Convolutional Neural Network (CNN) was trained to classify these histograms by VADH, predicting the original population distribution. Its performance was then evaluated against 900 human-labeled histograms from a previous study[2] (samples of 20 to 60) and compared to the Shapiro-Wilk test's ability to identify population distributions. A web app was built to facilitate CNN usage and 95% RI estimation with 90% confidence intervals (CI) via bootstrapping.

Results. The CNN model accurately predicted original population distributions by VADH in 84.0% (95% CI: 83.7–84.4) of training samples and 89.8% (95% CI: 87.8–91.8) of test samples. In contrast, the Shapiro-Wilk test achieved 65.0% (95% CI: 61.8–68.1) and 72.3% (95% CI: 69.3–75.2) accuracy on the test set, using P-value thresholds of 0.05 and 0.2, respectively.[3] The HARISS web application was successfully launched and can be accessed at: https://hariss.streamlit.app/.

Conclusions. The CNN model proved effective for VADH and may improve RI estimation precision via the HARISS web application, though careful reference individual selection and preanalytical and analytical considerations remain crucial.

Keywords: *Artificial Intelligence, Convolutional Neural Network, Data distribution, Machine learning, Reference range*

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2. Coisnon C et al, 2021, Vet Clin Pathol, 427–441
3. Le Boedec K, 2019, Vet Clin Pathol, 335–346

Bone Marrow Changes Mimicking Malignancy in Dogs with Phenobarbital-Induced Myelotoxicity

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Case 1: A 3.5-year-old dog diagnosed with Tier 1 idiopathic epilepsy was treated with phenobarbital. The dog was otherwise healthy with unremarkable baseline bloodwork. Serum phenobarbital concentrations were therapeutic and seizures were controlled. However, two weeks later, the dog developed acute lethargy, fever, and hyporexia. Follow-up diagnostics revealed marked neutropenia progressing to pancytopenia, severe proteinuria, and a positive anti-nuclear antibody (ANA) titre. Sternal bone marrow (BM) aspirates showed granulocytic hyperplasia consistent with ineffective granulopoiesis. A follow up BM aspirate revealed 40% early myeloid precursors and severely restricted neutrophilic maturation, raising concerns for acute myeloid leukemia (AML). Flow cytometry indicated a predominantly monocytic origin of the immature cells. Cytopenias resolved two months after phenobarbital withdrawal. Case 2: A 5-year-old spayed female mixed-breed dog with suspected idiopathic epilepsy was started on phenobarbital. Two months later, the dog developed lethargy, anorexia, pyrexia, and leukopenia due to neutropenia progressing to pancytopenia. Sternal BM aspirates revealed a hypercellular marrow (90%) with up to 50% blasts, restricted granulocytic maturation, and erythroid hypoplasia. A humeral core biopsy confirmed similar findings. Despite concerns for AML, an idiosyncratic drug reaction to phenobarbital was favored. Hematologic parameters completely normalized on a CBC performed three months after drug discontinuation. These cases underscore the importance of a thorough clinical history and ongoing CBC monitoring when interpreting bone marrow findings, as recovery from drug-induced myelotoxicity may mimic hematologic malignancy.

Canine Leukaemia: Retrospective study of 127 Irish cases

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Background. Canine lymphocytic leukaemia has not been well-studied in Ireland
Objectives. To determine prevalent types and distinguishing features.
Material and Methods. 127 canine cases over 14 years analysed: myeloid versus lymphocytic, acute versus chronic, subtype of myeloid, presence of lymphoma. Advia 2120 haemograms and gender were studied. Myeloid leukaemias confirmed by bone marrow aspirate or immunophenotyping. Analysis by ANOVA and t-test; data as mean±SEM; counts as $x10^9$/L; $p<0.05$.
Results. Lymphocytic leukaemia occurred in cases with (25, LSA) and without (82, LL) lymphoma. 35 leukaemia were: acute (ALL) with large lymphocytes; 20 chronic (CLL) with small lymphocytes. Of 20 myeloid cases (ML), 3 were chronic, 12 myelomonocytic (M4) or monocytic (M5), 2 myeloblastic, and 1 erythroid. For LL/ML/LSA, lympocytes varied across groups ($x10^9$/L) 81±10/18±5/54±14; neutrophils were highest in ML 11±2/53±14/13±2 as were eosinophils (0.24±0.05/1.1±0.5/0.23±0.1) and monocytes (4.9±1.1/10.3±2.0/2.8±1.2); large-unstained-cells (LUCs) were high in LSA 14±4/5.3±1.8/8.9±7.5. LUCs were 9x higher in ALL than CLL. All cases were anaemic, especially ML (Hct 0.27±0.02 versus 0.32±0.01 L/L). Females comprised 76% ML but only 36% LSA and 49% in LL.
Conclusion. 84% leukaemias were lymphocytic of which 23% were associated with lymphoma and 19% were chronic. 85% ML were acute, mostly M4 and M5. There was no gender difference for LL but surprisingly, 76% ML and 36% LSA were female. ML cases had much lower lymphocytes counts but much higher counts of other leukocytes. LUCs were higher in LSA versus LL and ML, and in ALL versus CLL and ML.
Keywords: myeloid leukaemia, lymphocytic leukemia; lymphoma

Evaluation of Immature Platelet Fraction in the Sysmex XN-2000V in healthy and thrombocytopenic dogs

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Background. The Immature Platelet Fraction (IPF) measures the proportion of newly released platelets and serves as an indicator of bone marrow activity. Using a specific fluorescent channel, the Sysmex XN-2000V analyzer can quantify both total platelet counts and IPF.

Objectives. This study aimed to assess whether there was a statistically significant difference in IPF values between thrombocytopenic and healthy dogs, and to evaluate the potential of IPF as a parameter of bone marrow function.

Material and Methods. Fifty CBCs with corresponding blood smears, were evaluated from thrombocytopenic dogs and compared with fifty from healthy controls. Platelet counts were measured using three different methods: optical (O), impedance (I) and fluorescence (F). The absolute IPF, IPF%, Mean Platelet Volume (MPV) and Platelet Large Cell Ratio (P-LCR%) were compared.

Results. Median (Me) platelet values in thrombocytopenic dogs for PLT-O, PLT-I and PLT-F were 60, 30 and 73 (10^3/µL), respectively. Absolute IPF and IPF% were significantly higher in thrombocytopenic dogs compared to healthy controls (Me 10.5 vs 6.4; p=0.0327 and Me 13.5% vs 2.2%; p<0.0001, respectively). P-LCR% was also increased (Me 40.0% vs 33.9%; p=0.0071) whereas MPV did not differ significantly (Me 11.30 fl vs 10.80 fl; p=0.1729).

Conclusion. Thrombocytopenic dogs showed elevated levels of both absolute IPF and IPF% compared to controls, indicating an active bone marrow response. An increase in P-LCR% was observed, but it was not significant enough to affect the MPV. IPF appears to be a useful non-invasive parameter of thrombopoietic activity, suitable for diagnostic evaluation and monitoring.

Keywords: dog, Sysmex XN-V analyzer, Immature Platelet Fraction, thrombocytopenia, MPV

Reference:
Perez-Ecija A, Martinez C, Fernandez-Castañer J, Mendoza FJ. 2024 May-Jun;38(3): J Vet Intern Med 1512-1519
Jornet-Rius O, Mesalles-Naranjo M, Pastor J. 2023 Vet Clin Pathol Sep;52(3):433-442.

EDTA contamination in feline and canine sera

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Background: EDTA (K2/K3-EDTA) contamination of serum during sampling can lead to life-threatening incorrect interpretation of test results and erroneous clinical decisions. To minimize risk of contamination, serum samples should be collected prior to EDTA samples which might not always be the case in the clinical setting.

Objectives: To develop and validate a method for analysis of EDTA in canine and feline sera and evaluate the effect of EDTA contamination on measured calcium, magnesium, zinc, iron and potassium concentrations.

Material and Methods: A method based on copper-PAN was developed for DxC 700 AU (Beckman Coulter). A 5-pointcalibration curve (0-0.4 mM) was made from sera spiked with K2-EDTA. Linearity, coefficient of variation (CV) and limit of quantification (LOQ) were evaluated. Effect of K2-EDTA was investigated by analysing samples spiked with different K2-EDTA concentrations and results compared to total error allowable (TEa).

Results: In both cats and dogs, intra-, inter and total assay CV was <13.1%. LOQ was set to 0.03 mM. Recovery upon dilution was 75-118%. K2-EDTA caused a dose-dependent increase in potassium and decrease in iron, zinc, calcium and magnesium. The most profound effect was seen on iron and zinc. In samples spiked with K2-EDTA, TEa was exceeded, even though the analytes remained within reference interval.

Conclusions: Small sample contaminations of K2-EDTA will have clinically significant effects on specific test results. To discover these effects the sample EDTA concentration must be determined. The developed automated EDTA assay gives a simple and robust way to identify EDTA contaminated sera.

Keywords: Hyperkalemia, hypocalcemia, copper-PAN, EDTA contamination

Differentiation between canine large B-cell and T-cell lymphoma using the Sysmex XN-1000V: a diagnostic performance study

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Background. Canine lymphoma is a common hematopoietic neoplasm. The immunophenotype is one of the major prognostic factors and may influence treatment recommendations.[1,2]
Objectives. To assess the diagnostic performance of the Sysmex XN-1000V WDF scattergram to differentiate canine large B-cell and T-cell lymphoma, using the percentage of highly fluorescent cells (%HFC) and WDF scattergram evaluation.
Material and Methods. A retrospective study was conducted on data from 103 cases of cytologically diagnosed canine large cell lymphoma. Cases had concurrent lymph node aspirate cell suspensions in saline that were analyzed using the Sysmex XN-1000V and multiparametric flow cytometry (FC) for lymphoma classification as B or T-cell.
Results. Large B-cell lymphomas (n=86) showed significantly higher %HFC compared to large T-cell lymphomas (n=17), with a median (IQR) of 50% (36–84) vs. 9.7% (3.9–19), respectively. The ROC analysis showed an AUC of 0.93, with an optimal cutoff of <24.15%HFC for identifying T-cell lymphoma, achieving 88.2% sensitivity, 87.2% specificity, 57.69% PPV, and 97.40% NPV. The following data is expressed as 'overall-percentage-agreement (kappa value)'. Using the previous cutoff, the agreement between the %HFC classification and FC was 88.24% (κ=0.76). Regarding the WDF scattergram evaluation, the intra- and inter-observer agreement were 86.27% (κ=0.71) and 67.65% (κ=0.55), respectively. Agreement between the WDF scattergram evaluation and FC was 77.45% (κ=0.55), and improved to 90.63% (κ=0.74) when just the confident cases were used.
Conclusion. A preliminary approach to the phenotype of canine large cell lymphoma can be made using either the visual inspection of the WDF scattergram or the %HFC.

Keywords: dog, fluorescence, flow cytometry, hematology analyzer, lymphoproliferative, phenotype.

Reference:
1. Valli VE, Bienzle D, Meuten DJ. Tumors of the Hemolymphatic System. In: Tumors in Domestic Animals. 5th ed. John Wiley & Sons; 2016:203-321.
2. Childress MO, Avery A, Behling-Kelly E, et al. Diagnosis and Classification of Primary Nodal Lymphomas in Dogs: A Consensus of the Oncology-Pathology Working Group. Vet Comp Oncol. 2025;0:1-15.

Novel neutrophilic parameters of the Sysmex XN-1000V for the prediction of inflammation in dogs

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Background: In dogs, early diagnosis of systemic inflammation is important. In human medicine, the intensive care infection score (ICIS) offers a faster, cost-effective alternative using advanced hematological parameters. While ICIS is not available for veterinary use, some components (e.g., neutrophil side fluorescent light) can be measured using analyzers like the Sysmex XN-1000V.

Objectives: This study aimed to establish a control group of healthy dogs for the novel parameters neutrophil side fluorescent light (NE-SFL), neutrophil side scattered light (NE-SSC), and neutrophil forward scattered light (NE-FSC) and assess their utility in detecting inflammation in diseases such as sepsis, pyometra, steroid-responsive meningitis-arteritis (SRMA), and idiopathic epilepsy.

Material and Methods: 21 healthy dogs were used as control group, 84 diseased dogs were retrospectively grouped in non-inflammatory disease (idiopathic epilepsy) and inflammatory diseases (SRMA, pyometra, sepsis).

Results: In healthy dogs, minimum and maximum for NE-SFL, NE-SSC, and NE-FSC were 38.2–42.7 channel value (ch), 92.6–106.6 ch, and 38.7–53.4 ch, respectively. Compared to controls, NE-SFL levels were significantly elevated in sepsis, pyometra, and SRMA, while NE-SSC was only elevated in sepsis and pyometra and NE-FSC only in sepsis. No increases were observed in idiopathic epilepsy. Manual gating of the white blood cell differential scattergram was necessary in samples showing high neutrophil toxicity and the presence of bands.

Conclusion: NE-SFL and NE-SSC, obtainable from routine complete blood cell count, may serve as accessible markers for inflammation in dogs. Further research is needed to validate their broader diagnostic use.

Keywords: canine, inflammation, marker, neutrophilic parameters, Sysmex XN V

Saturday 4 October 2025

Cinema 1

Mystery Clinical Cases

Peripheral lymphadenomegaly in a Jack Russell Terrier

Contributors
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Specimen
Peripheral lymph node aspirates (cytology smears, RPMI 1640-preserved specimens), lung aspirates, EDTA whole blood.

Signalment
Dog, Jack Russell Terrier, 13 years old, male neutered.

History
Referred for investigation of generalized lymphadenomegaly that was noticed two weeks prior to admission.

Clinical findings
The dog was bright, alert, and responsive but exhibited generalized severe lymphadenomegaly and hepatosplenomegaly. A complete blood count (ADVIA® 2120 Hematology System, Siemens Healthineers, Malvern, PA, USA) revealed moderate thrombocytopenia which was confirmed with a blood smear examination where no platelet clumps were seen (Table 1). Serum biochemistry was unremarkable. A serological point-of-care ELISA test (SNAP Leish 4Dx test, IDEXX Laboratories Inc., Westbrook, ME, USA) for *Leishmania*

infantum, *Ehrlichia* spp. and *Anaplasma* spp. antibodies and *Dirofilaria immitis* antigen was negative.

Fine-needle aspirates were obtained from the submandibular, prescapular, axillary, and popliteal lymph nodes and submitted for cytological evaluation (Figures 1-6).

Extensive soft tissue diffuse opacities were identified in the left lung on plain radiographs. Ultrasound-guided aspiration of the pulmonary lesions was performed and submitted for cytological evaluation (Figures 7-10). Abdominal ultrasonography indicated severe enlargement of hepatic lymph nodes. Moderate mesenteric lymphadenopathy, and mild gastric and internal iliac lymphadenomegaly was also noted. The spleen exhibited moderate splenomegaly with diffuse hypoechogenicity.

The thoracic CT scan revealed severe enlargement of the sternal, tracheobronchial, and mediastinal lymph nodes. Consolidation was noted in the caudal part of the left cranial lung lobe, with alveolar-pattern infiltrates and extensive areas of complete loss of aeration (Figure 11).

Questions

1. What is your cytologic description of the lymph node and pulmonary aspirates?
2. What are the main differential diagnoses based upon cytologic examination?
3. Which are the next steps in the diagnostic investigation?

Table 1. Complete Blood Count (ADVIA® 2120 Hematology System, Siemens Healthineers, Malvern, PA, USA)

Parameter	*Value*	*Reference Interval*
Red blood cells (x10^9/µL)	**5.41**	**5.50-8.50**
Hemoglobin (g/dL)	14.2	12.0-18.0
Hematocrit (%)	42.2	37.1-55.0
Mean corpuscular volume (fL)	**78.1**	**60.6-77.0**
Mean Corpuscular Hemoglobin Concentration (g/dL)	33.6	31.0-36.2
Red Cell Distribution Width (%)	13.7	11.9-14.5
Reticulocytes (x10^3/µL)	99.1	10.0-110.0
White Blood Cells (x10^3/µL)	11.6	6.0-17.0
Neutrophils (x10^3/µL)	7.1	3.9-8.0
Lymphocytes (x10^3/µL)	3.5	1.3-4.1
Monocytes (x10^3/µL)	0.6	0.2-1.1
Eosinophils (x10^3/µL)	0.29	0.00-0.60

Basophils (x10^3/µL)	0.03	0.00-0.10
Large Unstained Cells (x10^3/µL)	0.06	0.00-0.30
Platelets (x10^3/µL)	**117**	**200-500**
Mean Platelet Volume (fL)	**11.6**	**5.4-9.2**

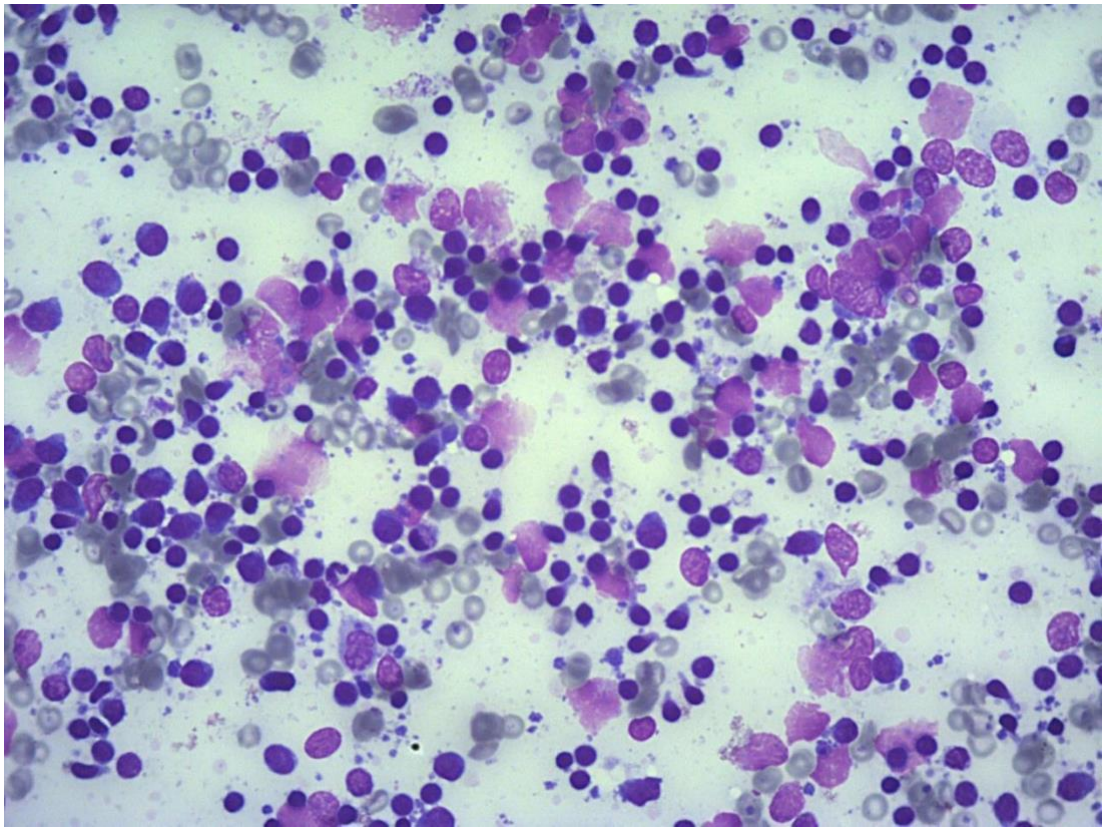

Figure 1. Fine needle aspirate of the right submandibular lymph node (x400, Giemsa)

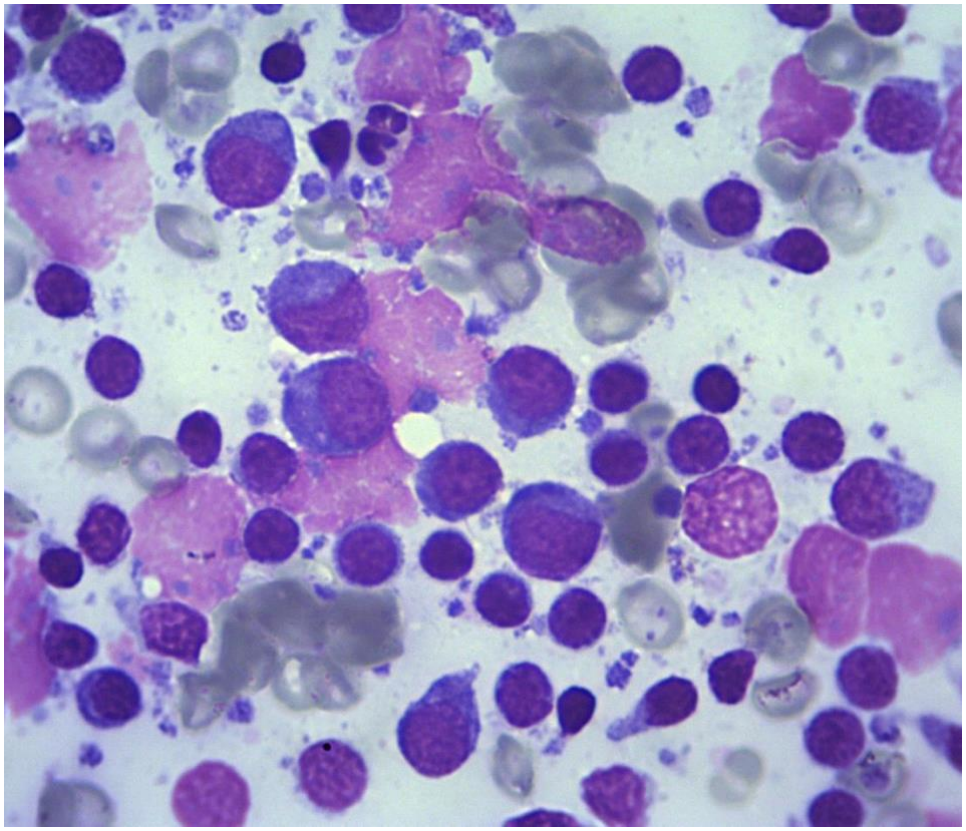

Figure 2. Fine needle aspirate of the right submandibular lymph node (x1000, Giemsa)

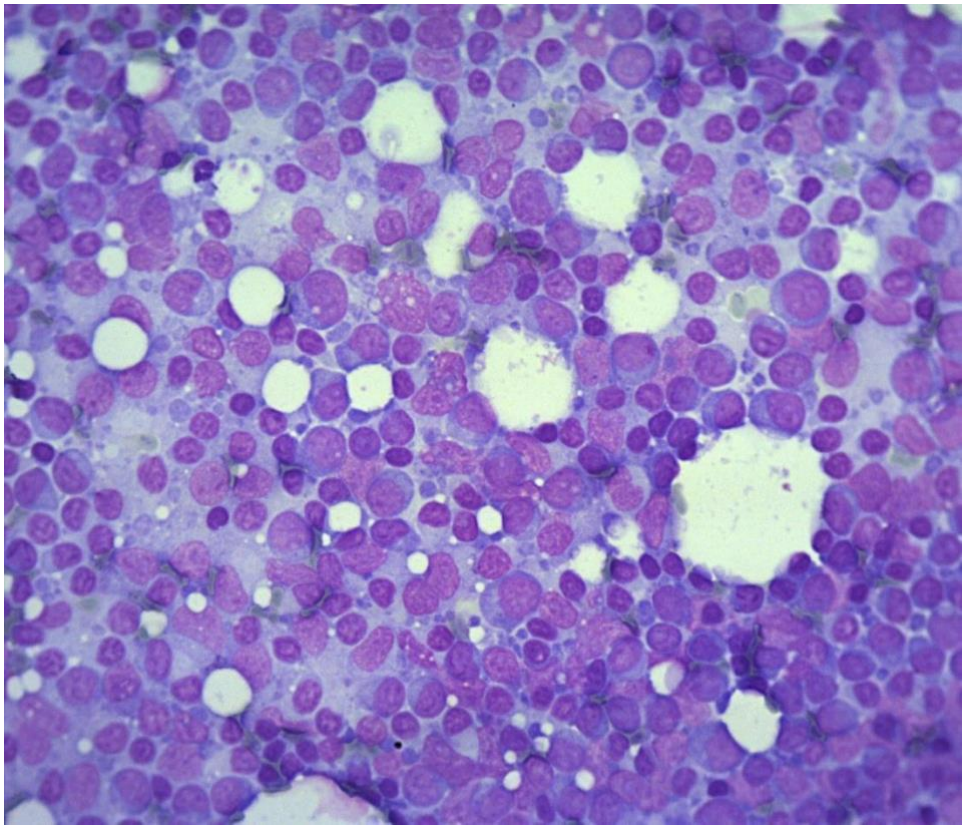

Figure 3. Fine needle aspirate of the left popliteal lymph node (x400, Giemsa)

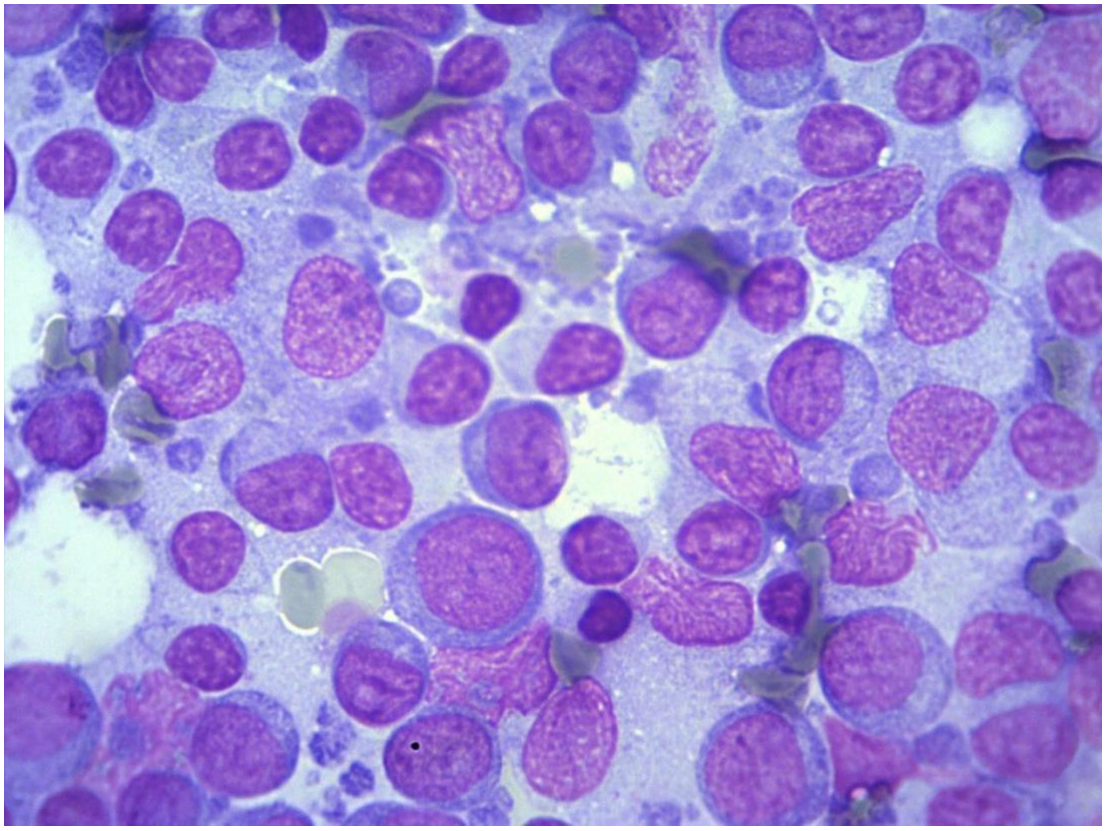

Figure 4. Fine needle aspirate of the left popliteal lymph node (x1000, Giemsa)

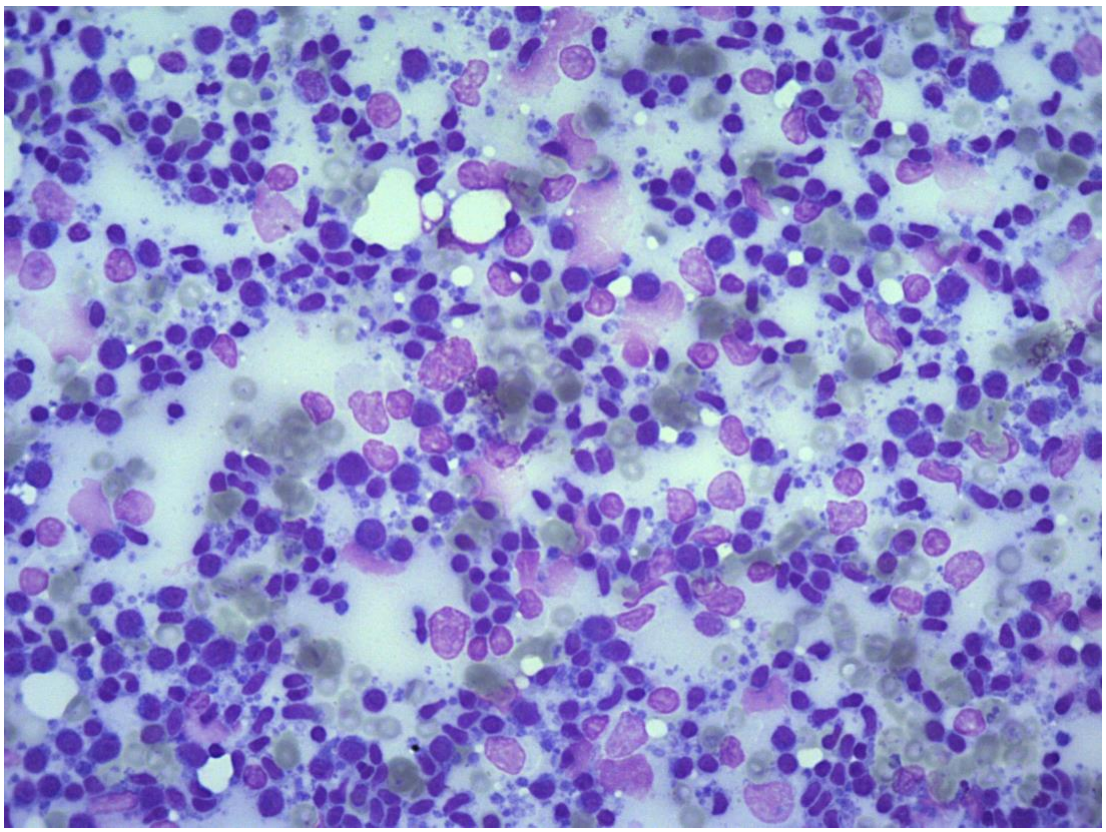

Figure 5. Fine needle aspirate of the right popliteal lymph node (x400, Giemsa)

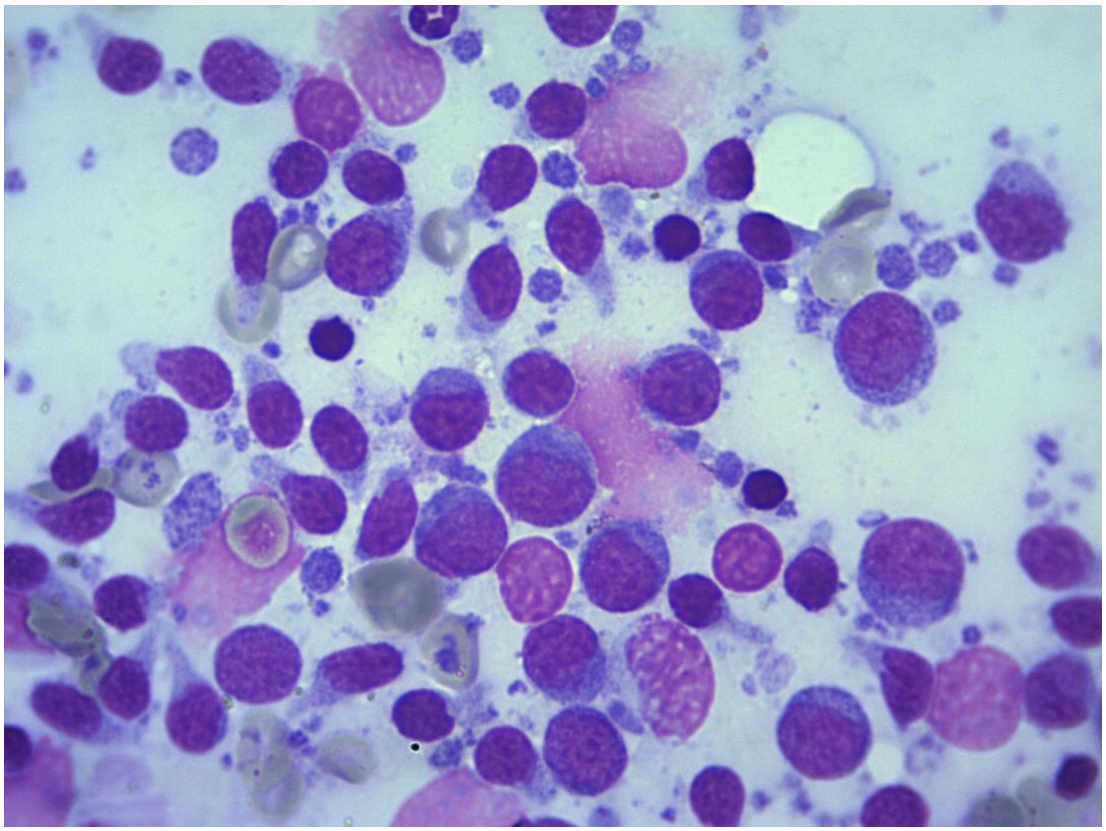

Figure 6. Fine needle aspirate of the right popliteal lymph node (x1000, Giemsa)

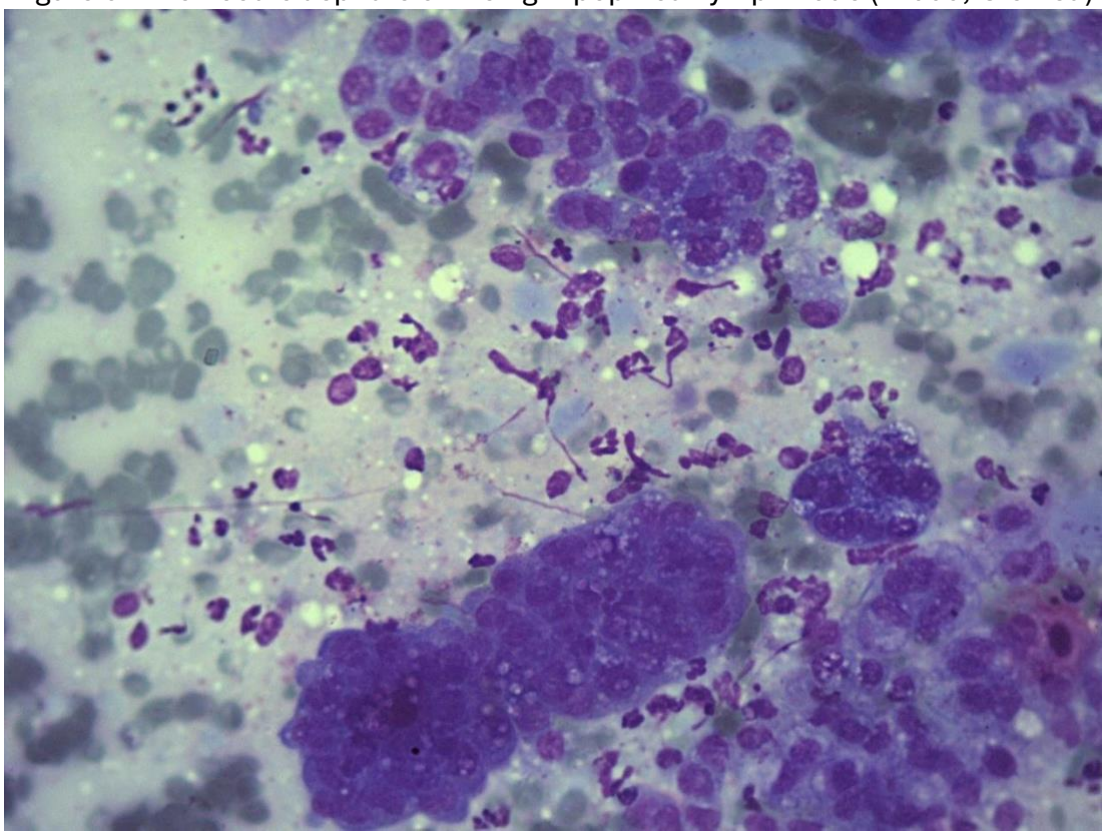

Figure 7. Fine needle aspirate of the pulmonary lesions (x400, Giemsa)

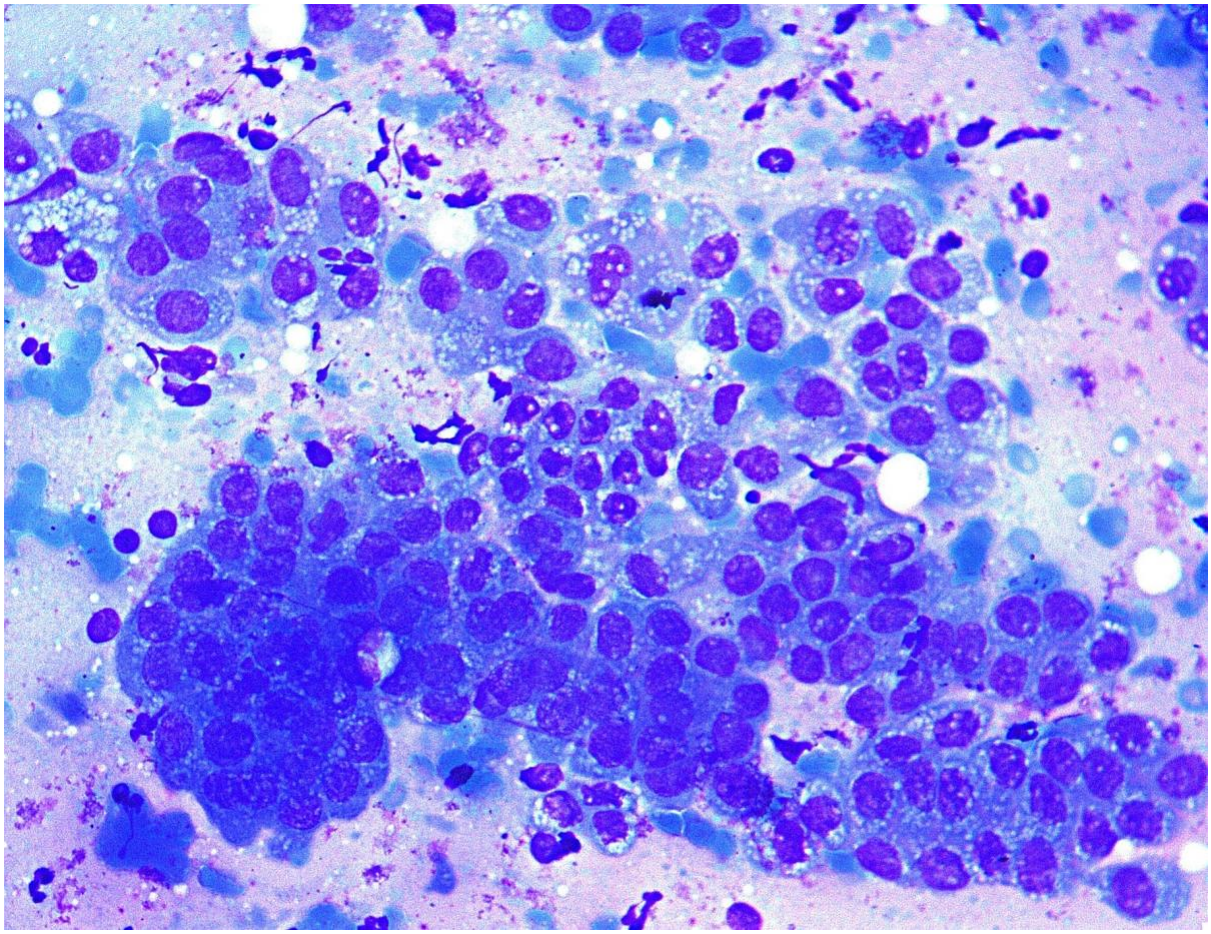

Figure 8. Fine needle aspirate of the pulmonary lesions (x400, Giemsa)

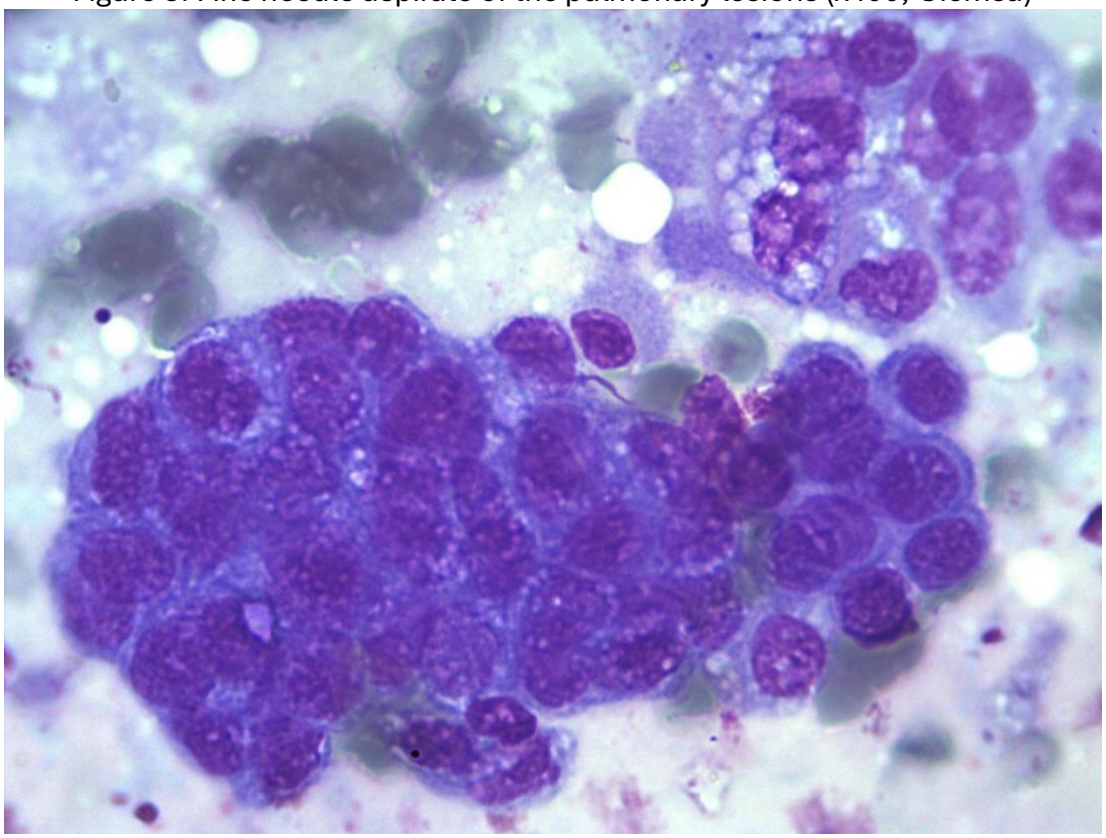

Figure 9. Fine needle aspirate of the pulmonary lesions (x1000, Giemsa)

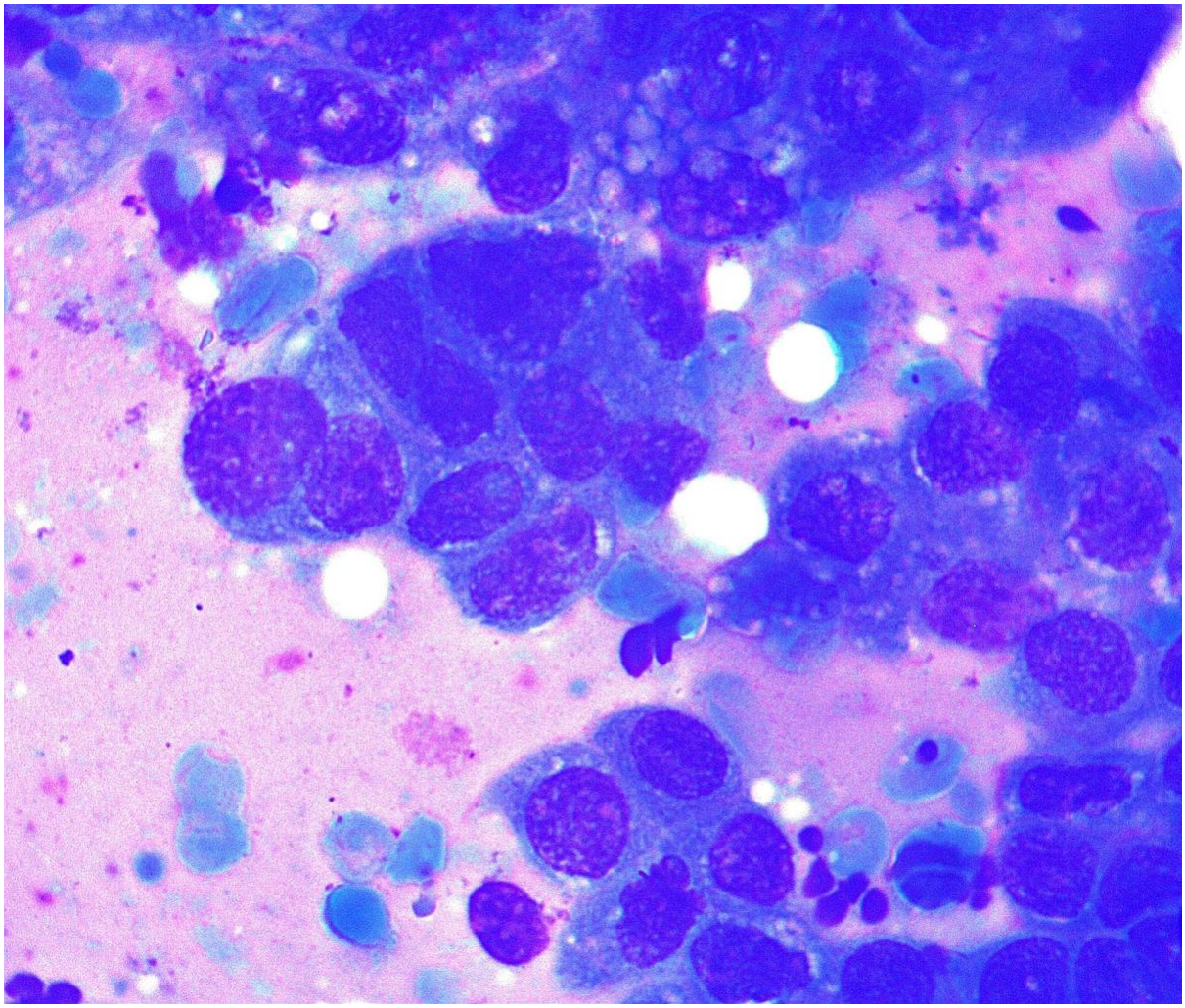

Figure 10. Fine needle aspirate of the pulmonary lesions (x1000, Giemsa)

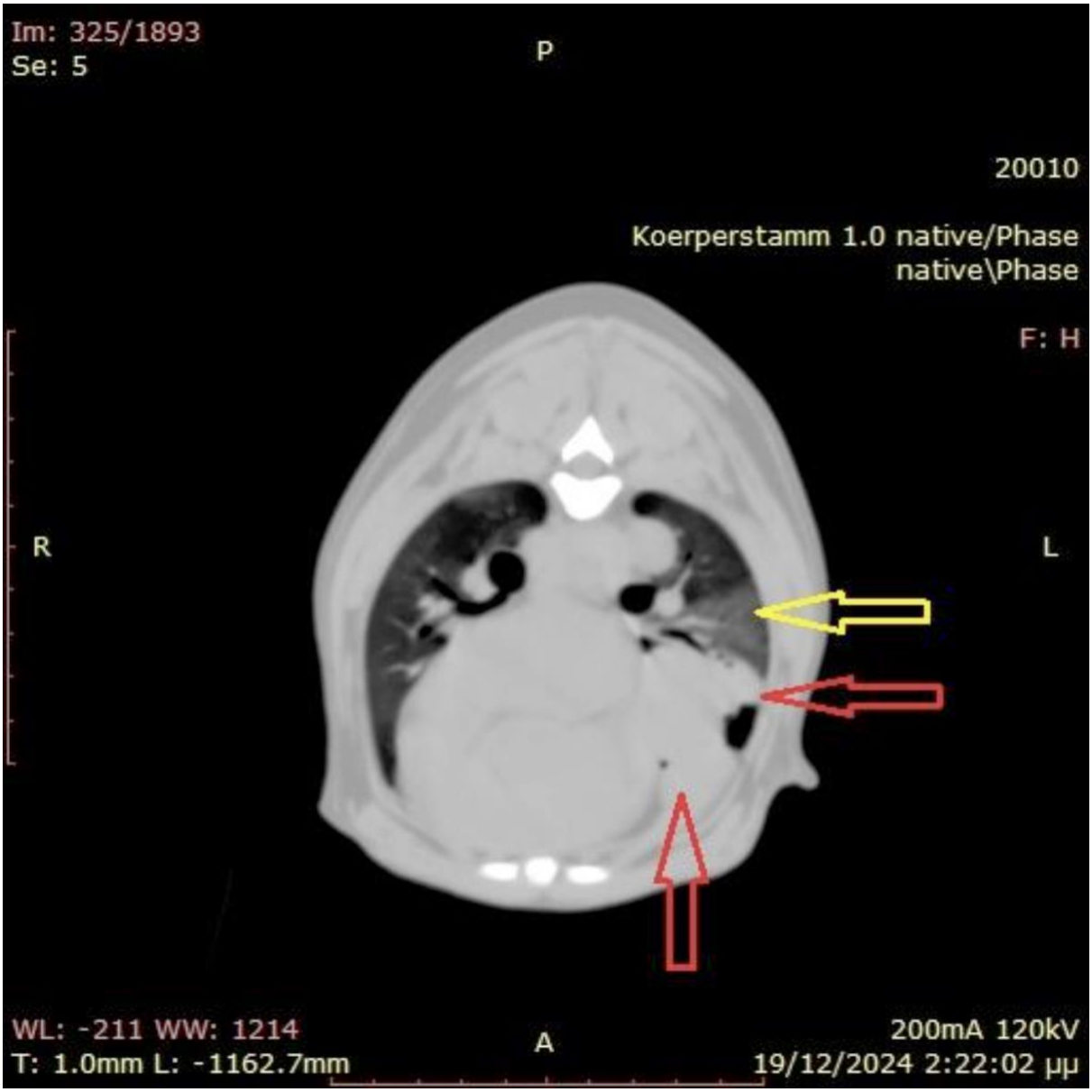

Figure 11. Consolidation of the caudal part of the left cranial lung lobe, with alveolar-pattern infiltrates and extensive areas of complete loss of aeration (red arrows). Associated ground-glass opacities are also noted (yellow arrow).

Cervical swelling in a dog

Contributors
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Specimen
Fine needle aspirate biopsy of the right cervical area.

Signalment
10-year-old male hunting dog.

History
The dog presented for swelling of the right cranial cervical area.

Clinical findings
Initial clinical examination revealed cervical swelling and pain upon palpation of the right retropharyngeal lymph node area. The rest of the general physical examination, hematology and serum biochemistry did not reveal any alterations.
Fine needle aspiration of the cervical region was performed, and samples were submitted to the laboratory for cytologic examination (Figures 1-2).

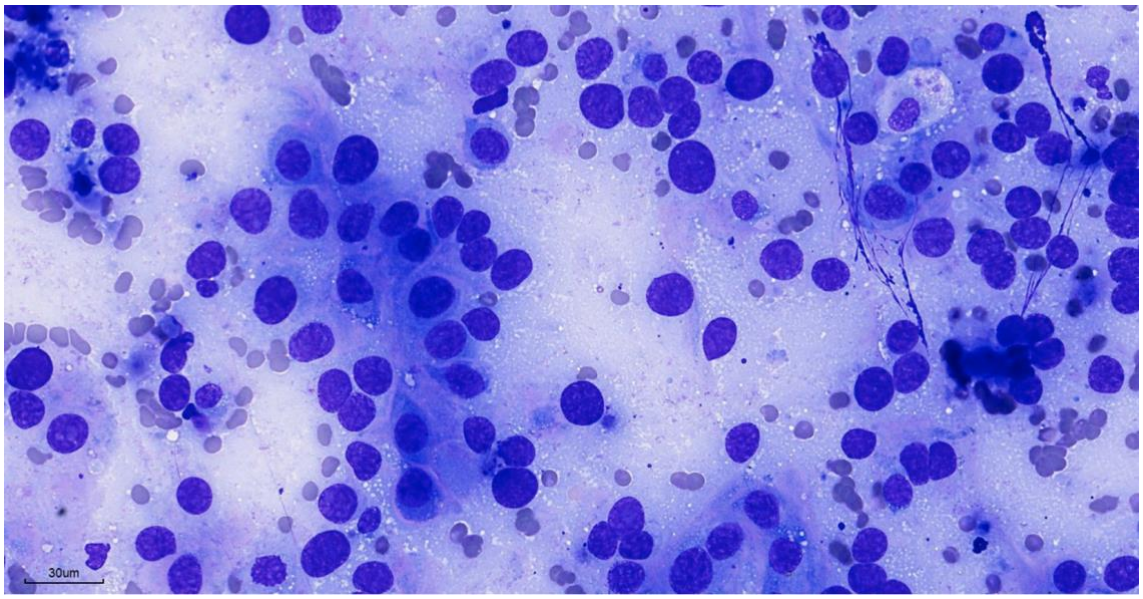

Figure 1. Fine needle aspirate of the cervical region (Modified Wright Giemsa) (100x)

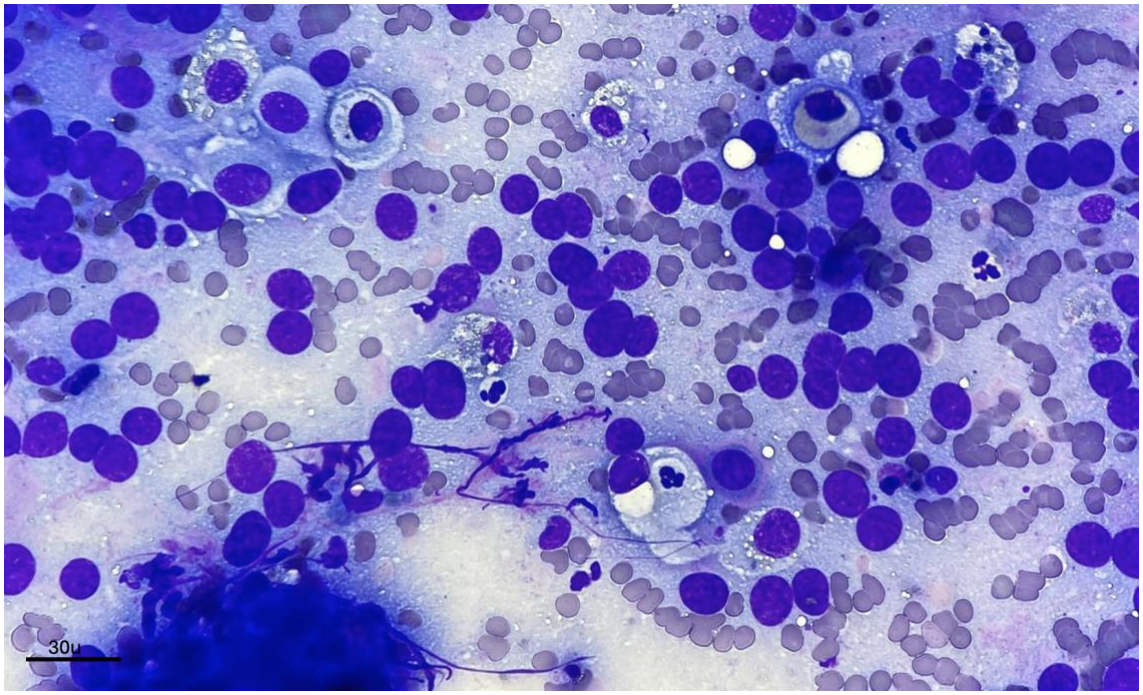

Figure 2. Fine needle aspirate of the cervical region, (Modified Wright Giemsa) (100x)

Questions

1. Based on the initial cytological findings, what are the main differential diagnoses for the observed cell population?
2. Considering the cytological interpretation and the location of the lesion, what would be the next recommended diagnostic steps to further investigate this case?

The mystery of the vomiting cat:
A granular analysis of splenic and hepatic nodules

Contributors

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Specimen
Cytology of splenic and hepatic nodules

Signalment
3-year-old spayed female European shorthair cat

History
The cat was presented to the emergency unit at the veterinary teaching hospital of Toulouse, France, with a two-day history of anorexia and two episodes of vomiting.

Clinical findings
Clinical examination was unremarkable. A biochemistry panel revealed mild hyperproteinemia (93g/L [57-89]) and hyperglobulinemia (63 g/L [28-51]). A CBC showed eosinopenia ($0.03x10^9$/L [0.17-1.57]).
Blood gas analysis revealed mild hypokalemia (3.2 mmol/L [3.5-4.8]), ionized hypocalcemia (1.09 mmol/L [1.10-1.33]) and moderate hypochloremia (95.6 mmol/L [116.0-126.0])
Thoracic X-ray was unremarkable. Abdominal ultrasound revealed multiple hepatic nodules (<20mm), a splenic nodule (17mm) and a round, nonobstructive intestinal mass (6mm), suggestive of multiple neoplastic processes.
The cat was sedated and fine needle aspiration of the splenic and hepatic nodules was performed. Samples were submitted to the laboratory for cytological interpretation (Figures 1 and 2).

Follow-up
Splenectomy was performed and the splenic nodule was submitted for histopathological analysis.
The cat showed clinical improvement following splenectomy; however, occasional vomiting persisted. A follow-up ultrasound performed 2-month after splenectomy revealed persistence of the previously observed hepatic nodules and a new 20mm nodule in the right medial liver lobe, a

mild enlargement of the intestinal mass (6x7x9mm), and a hypertrophy of the ileo-caecal lymph node. A CBC revealed a moderate leukocytosis ($22.7x10^9$/L [4.0-15.2]) and lymphocytosis ($13.6x10^9$/L [1.2-10.2]) with a moderate number of reactive lymphocytes and very rare granulated cells (Figure 3).

Figure 1. Cytology of the splenic nodule. May-Grunwald-Giemsa, original magnification x10 and x100 oil objectives, respectively

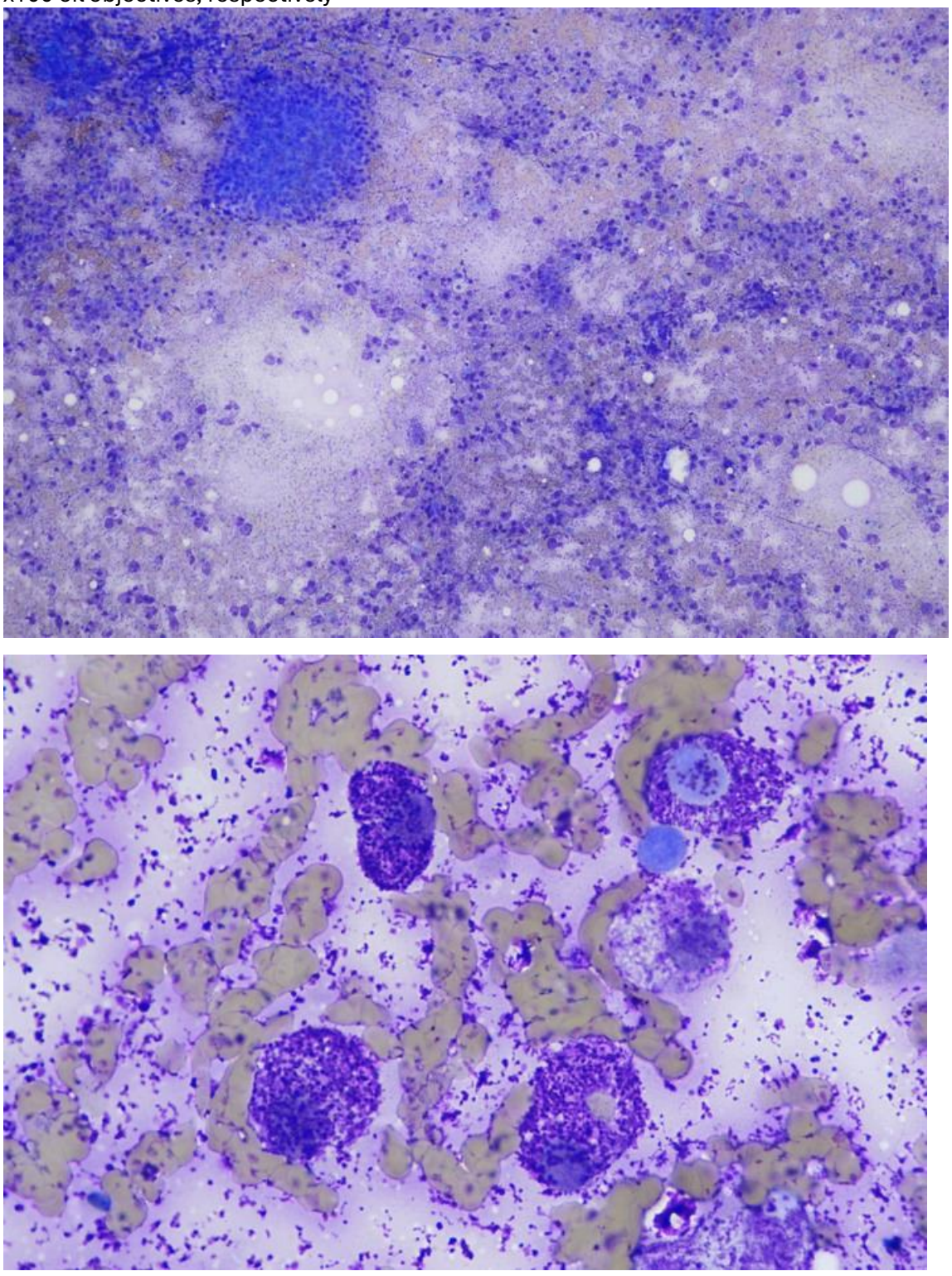


Figure 2: Cytology of one hepatic nodule. May-Grünwald-Giemsa, original magnification x20 and x100 oil objectives, respectively

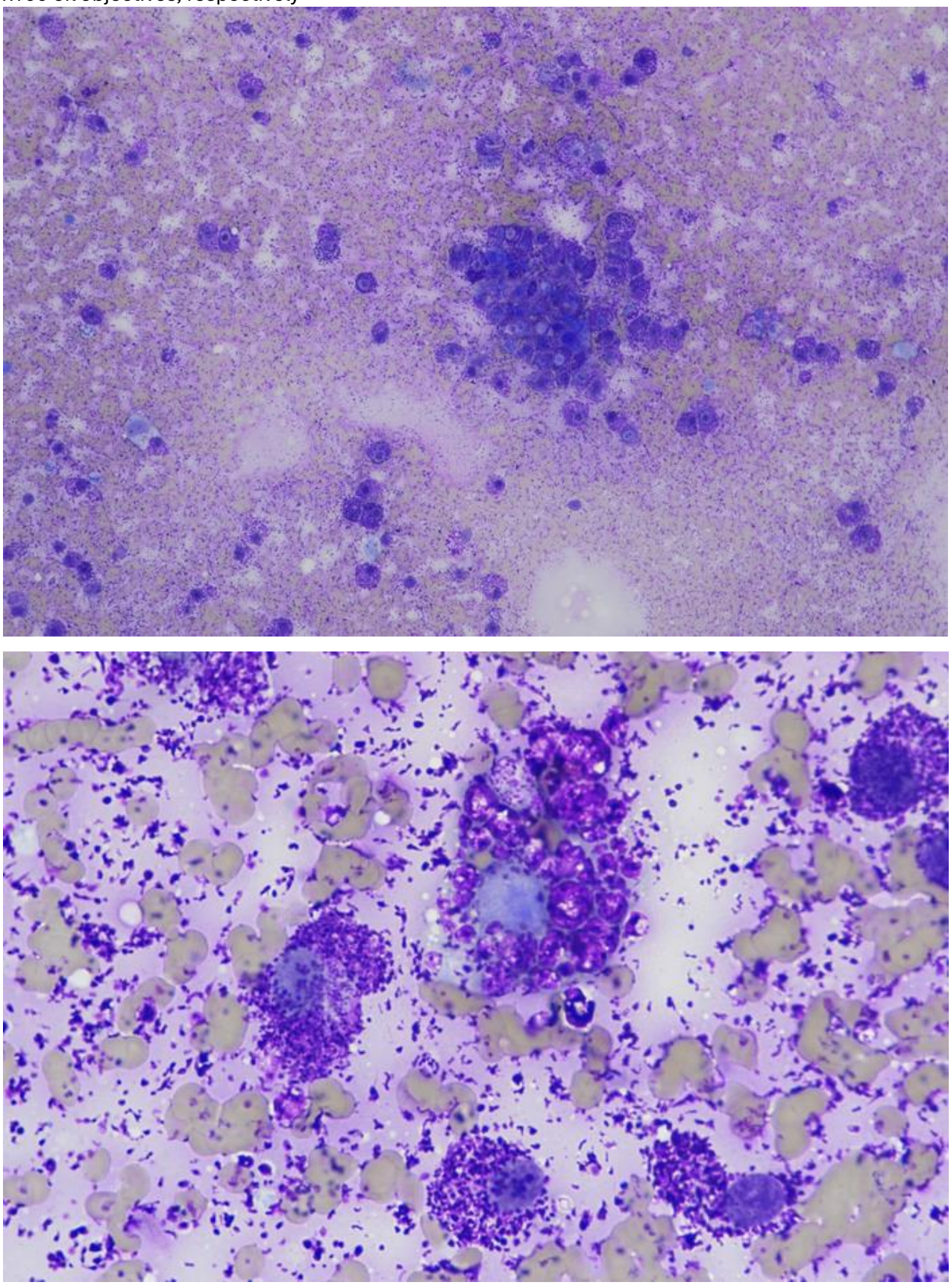
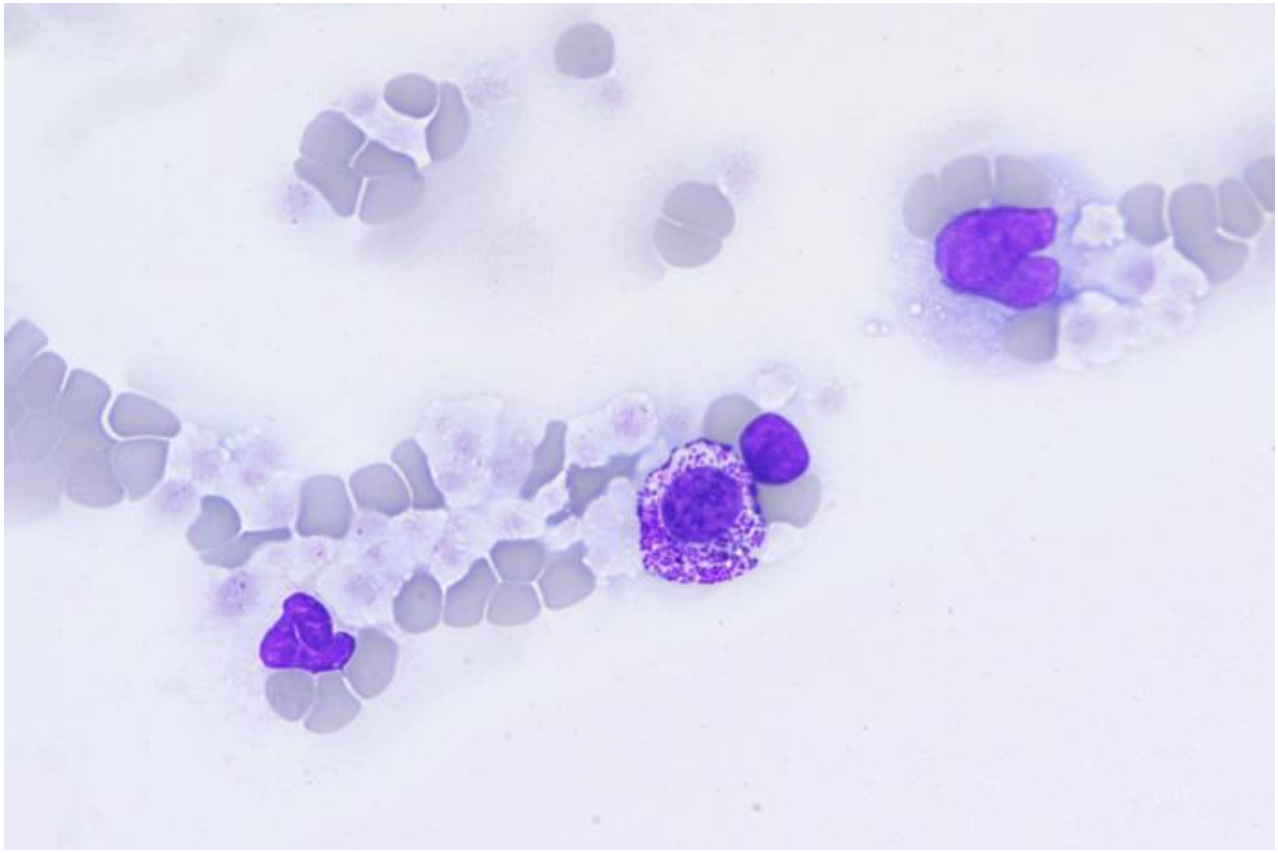


Figure 3. Peripheral blood smear. May-Grünwald-Giemsa, original magnification x100 oil objective



Questions

1/ How would you describe the cytological samples (Figures 1 and 2)? What is the most probable diagnosis?

2/ What would you recommend to confirm the diagnosis?

3/ Identify the granulated cells on peripheral blood smear (Figure 3) and give the differential diagnosis.

Urinary cytological twist

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Specimens
Whole blood-EDTA; heparin-plasma; serum; urine; urinary cytology obtained through cystocentesis; cytocentrifuge preparation; liver and spleen fine needle aspirates (FNA).

Signalment
Eleven-year-old male neutered Rottweiler mixed breed dog.

History
The dog presented to the emergency service at the Hospital Clínic Veterinari (HCV) of the Universitat Autònoma de Barcelona (UAB), with a four-day history of apathy, anorexia, and polyuria/polydipsia.
Two weeks prior to the current episode, the dog developed nonspecific clinical signs such as vocalization. At that time, hematological analyses were performed by the referring veterinarian (data not available), and, according to the owners, there were no abnormalities. Aside from a corneal ulcer, the physical examination was unremarkable. A nonsteroidal anti-inflammatory drug (NSAID), carprofen, was prescribed to address presumed pain-related signs, along with topical ophthalmic treatment. Clinical signs of pain resolved during the 8-day NSAID course, however, due to unsatisfactory improvement of the corneal ulcer, the dog was referred to the ophthalmology service at the HCV. A corneal ulcer with detached edges was diagnosed, with cytological findings revealing a scarce number of neutrophils with intra- and extracellular cocci. The area was debrided, and ocular treatment with drops was prescribed. After returning home from this consultation, the dog vomited once, followed by additional episodes throughout the night. From that point onwards, he became anorexic.

Clinical findings

On physical examination, the dog presented mentally alert, but markedly apathetic, with pink to slightly congestive mucous membranes, tachycardia (100 bpm), and showed generalized discomfort on abdominal palpation.

Upon admission to the emergency service, an abdominal point-of-care ultrasound was performed, as well as blood and urine analyses in the in-hospital laboratory: a complete blood cell count (ProCyte Dx Hematology Analyzer) with blood smear review, a complete biochemistry panel (Catalyst Dx Chemistry Analyzer and NOVA Stat Profile Prime Plus® Critical Care Blood Gas Analyzer), urinalysis (IDEXX VetLab UA Analyzer), and urine cytology. The latest was subsequently reviewed at the Clinical Pathology Laboratory of the UAB.

Table 1 - Hematology results for the EDTA-blood specimen performed on the ProCyte Dx. Bolded values are outside the reference interval.

Parameter (units)	**Result**		**Reference interval**
RBC (x10^{12}/L)	7.49		5.65 - 8.87
HCT (%)	46.4		37.3 - 61.7
Hgb (g/dL)	16.7		13.1 - 20.5
MCV (fL)	61.9		61.6 - 73.5
MCH (pg)	22.3		21.2 - 25.9
MCHC (g/dL)	36		32 - 37.9
RET (x10^{9}/L)	45.7		10 - 110
RET-He (pg)	**21.7**		**22.3 - 29.6**
WBC (x10^{9}/L)	6.38		5.05 - 16.76
	Automated Count	**Manual Count**	
Neutrophils (x10^{9}/L)	3.91	4.85	2.95 - 11.64
Lymphocytes (x10^{9}/L)	1.40	**0.73**	1.05 - 5.10
Monocytes (x10^{9}/L)	0.83	0.73	0.16 - 1.12
Eosinophils (x10^{9}/L)	0.20	0.06	0.06 - 1.23
Basophils (x10^{9}/L)	0.04	0	0 - 0.10
PLT (x10^{9}/L)	**129**	Adequate (platelet clumping)	**148 - 484**

Table 2 - Biochemistry results for the Heparin-plasma specimen performed on the Catalyst Dx and NOVA. Bolded values are outside the reference interval.[a] Parameter obtained with NOVA analyzer.

Parameter (units)	Result	Reference Interval
Glucose (mg/dL)	94	70 - 143
Creatinine (mg/dL)	**2.8**	**0.5 - 1.8**
BUN (mg/dL)	**51**	**7 - 27**
Phosphorus (mg/dL)	6	2.5 - 6.8
Calcium (mg/dL)	**14.9**	**7.9 - 12**
Sodium (mmol/L)	152	144 - 160
Potassium (mmol/L)	4.1	3.5 - 5.8
Chloride (mmol/L)	110	109 - 122
Total Protein (g/dL)	**8.3**	**5.2 - 8.2**
Albumin (g/dL)	3.5	2.2 - 3.9
Globulins (g/dL)	**4.8**	**2.5 - 4.5**
ALT (U/L)	82	10 - 125
ALP ((U/L)	76	23 - 212
GGT (U/L)	0	0 - 11
Cholesterol (mg/dL)	156	110 - 320
Free calcium (fCa)[a] (mmol/L)	**1.74**	**1.25 - 1.5**

Table 3 - Urinalysis results for the urine specimen performed on the IDEXX VetLab UA Analyzer. Bolded values are considered abnormal.

Parameter (units)	Result
Color	Straw
Turbidity	Very Cloudy
Specific gravity	1.030
pH	8
Protein	**3+**
Glucose	**1+**
Ketones	Negative

Blood/ Hemoglobin	**4+**
Bilirubin	Negative
Urobilinogen	Normal
Leukocyte esterase	2+

Urine cytological preparations were obtained by cystocentesis, centrifuged and stained with Wright-Giemsa, using Hematek 2000, for cytologic examination.

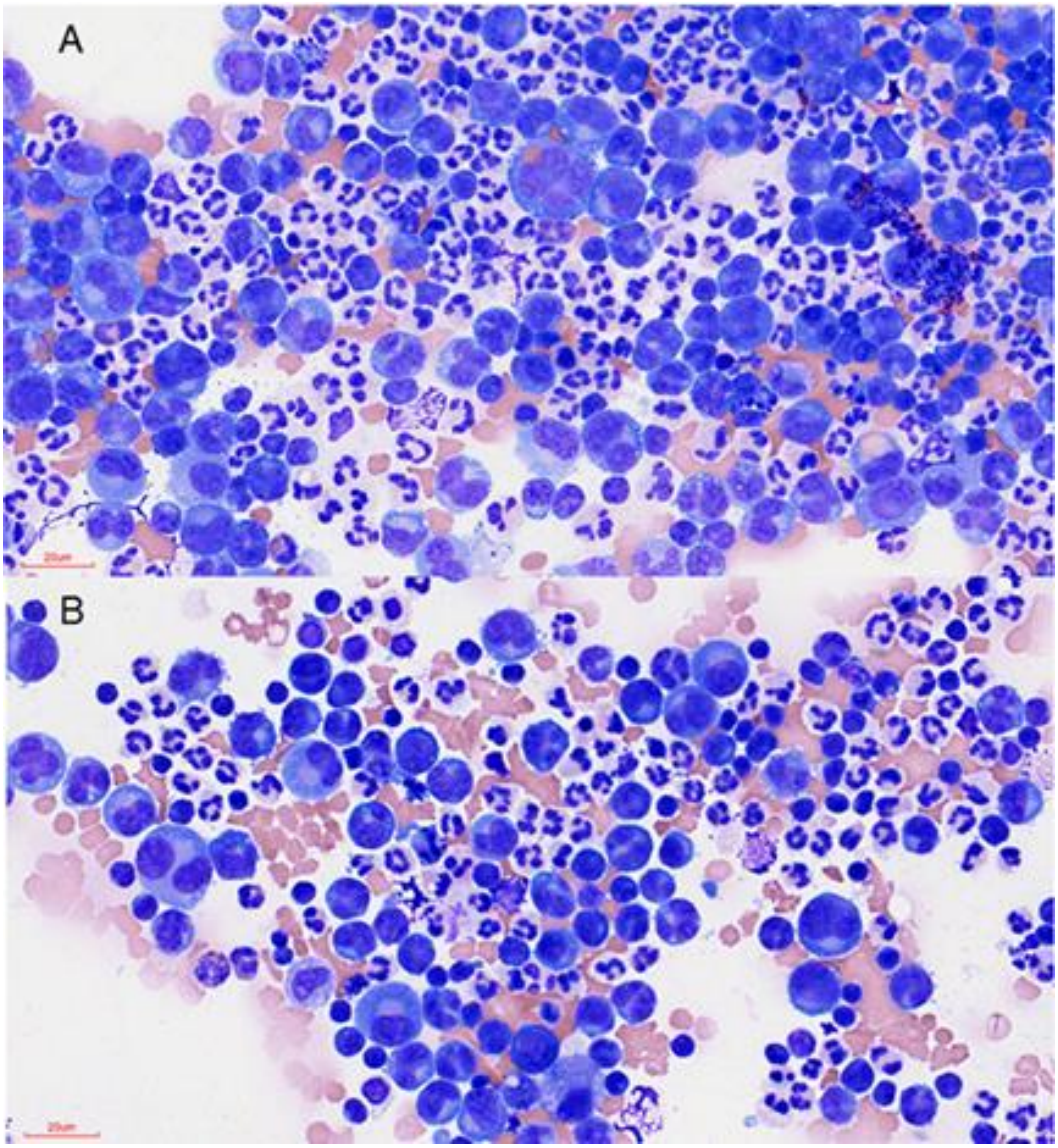

Figure 1 – A and B: urine cytological preparations of the dog from this report. Wright-Giemsa stain, images acquired using a digital slide scanner (Motic EasyScan One, MoticEurope SLU, Barcelona, Spain)

Questions

1. Considering the laboratory findings, what are your main differential diagnoses?
2. What other diagnostic tests would you consider performing?

Unexpected Cytological Findings in a Feline Neck Mass

Contributors
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Specimen
Fine needle aspiration of a subcutaneous mass located on the right side of the neck, close to the trachea, firm, approximately 1 cm diameter

Signalment
Alessia, Cat, Domestic shortair (DHS), female spayed, 5 years old,

History
Alessia is an indoor - outdoor cat (access to a private garden), living with another cat and regularly vaccinated. It was asymptomatic except for the presence of a small firm mass located on the right side of the neck, near the trachea.

Clinical findings

Fine needle aspirate (FNA) of the subcutaneous neck mass. Staining: May – Grünwald Giemsa.

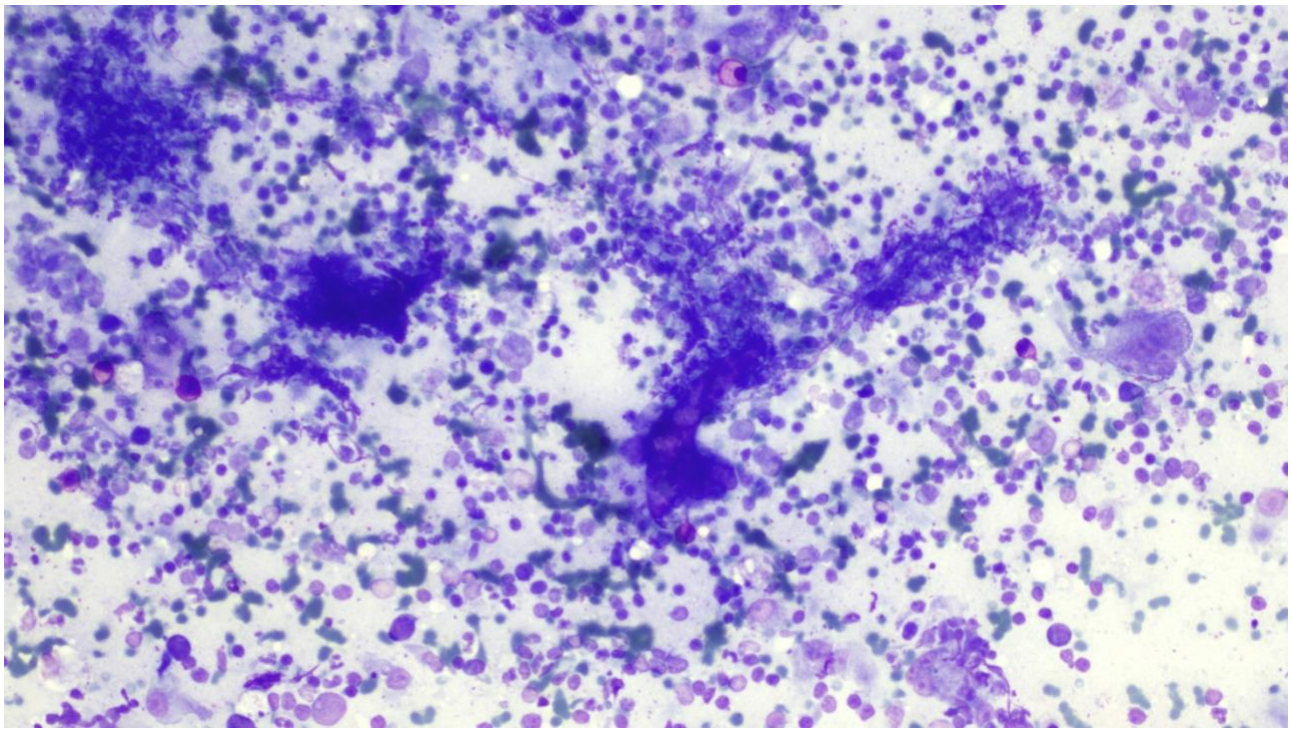

Figure 1. 20x Objective

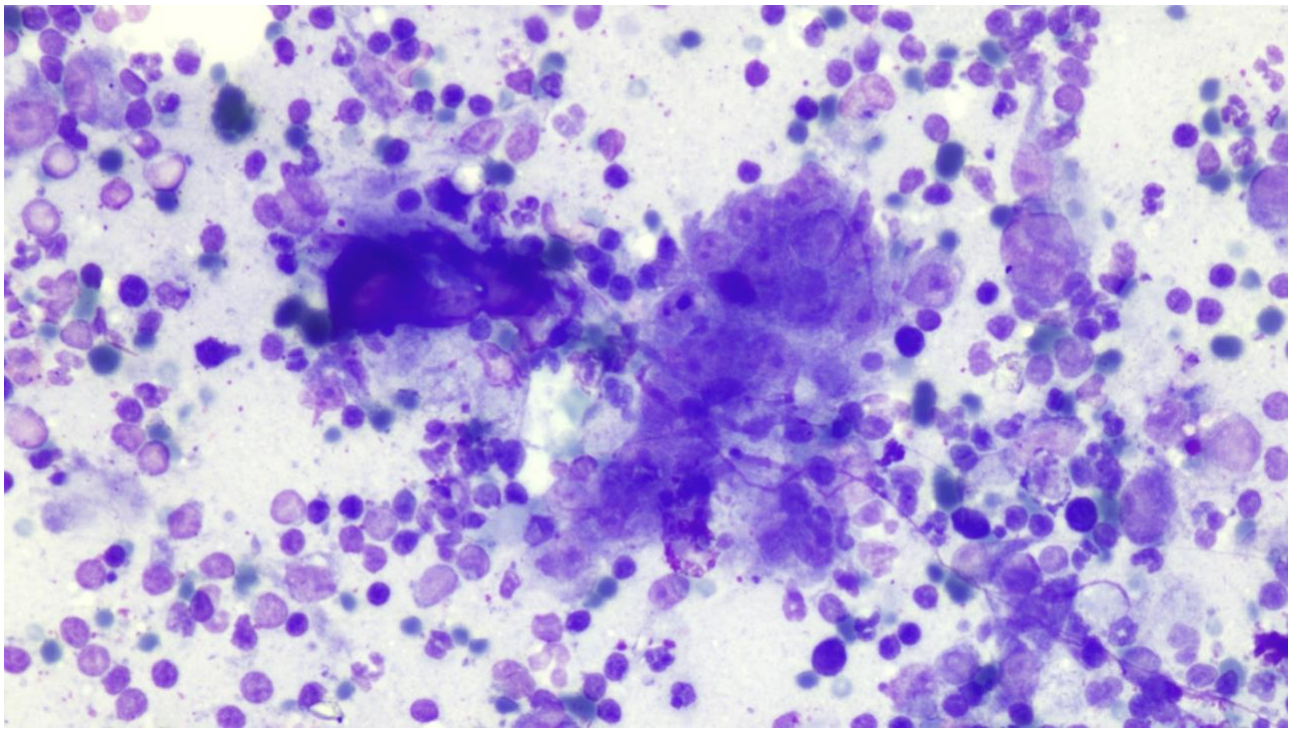

Figure 2. 40x Objective

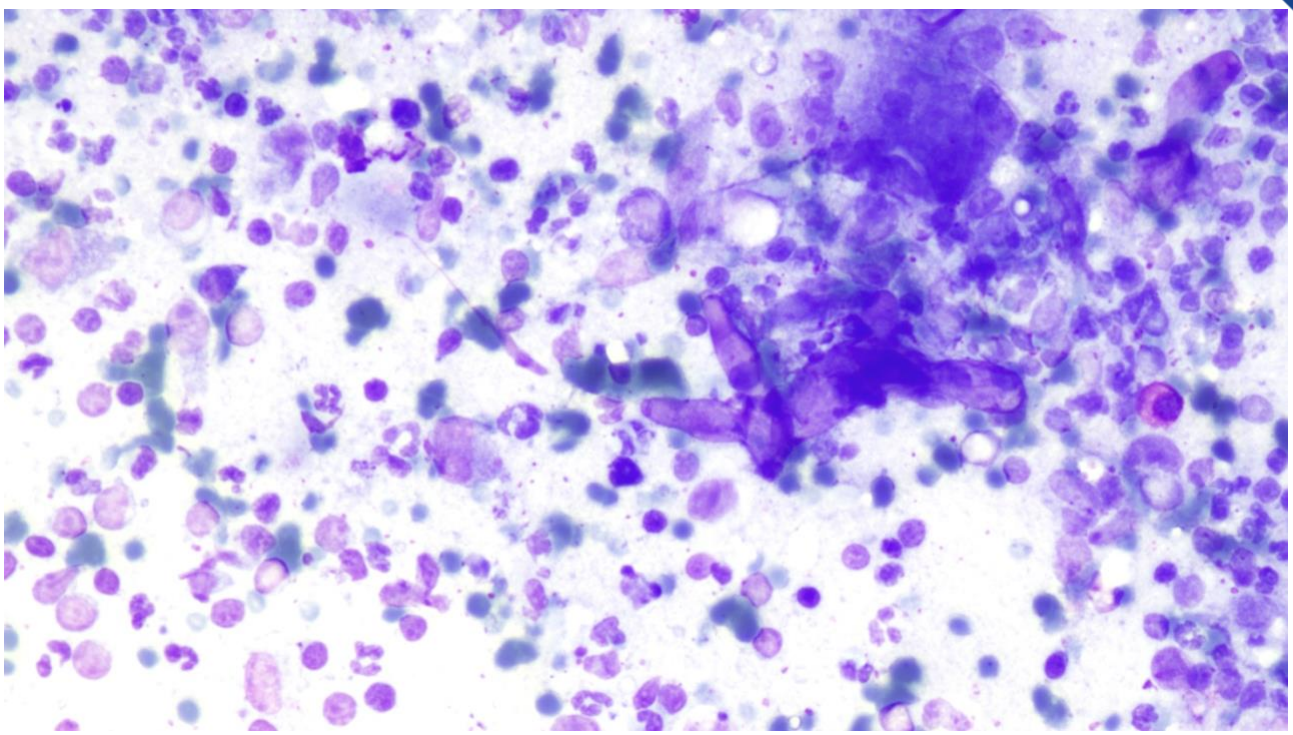

Figure 3. 40x Objective

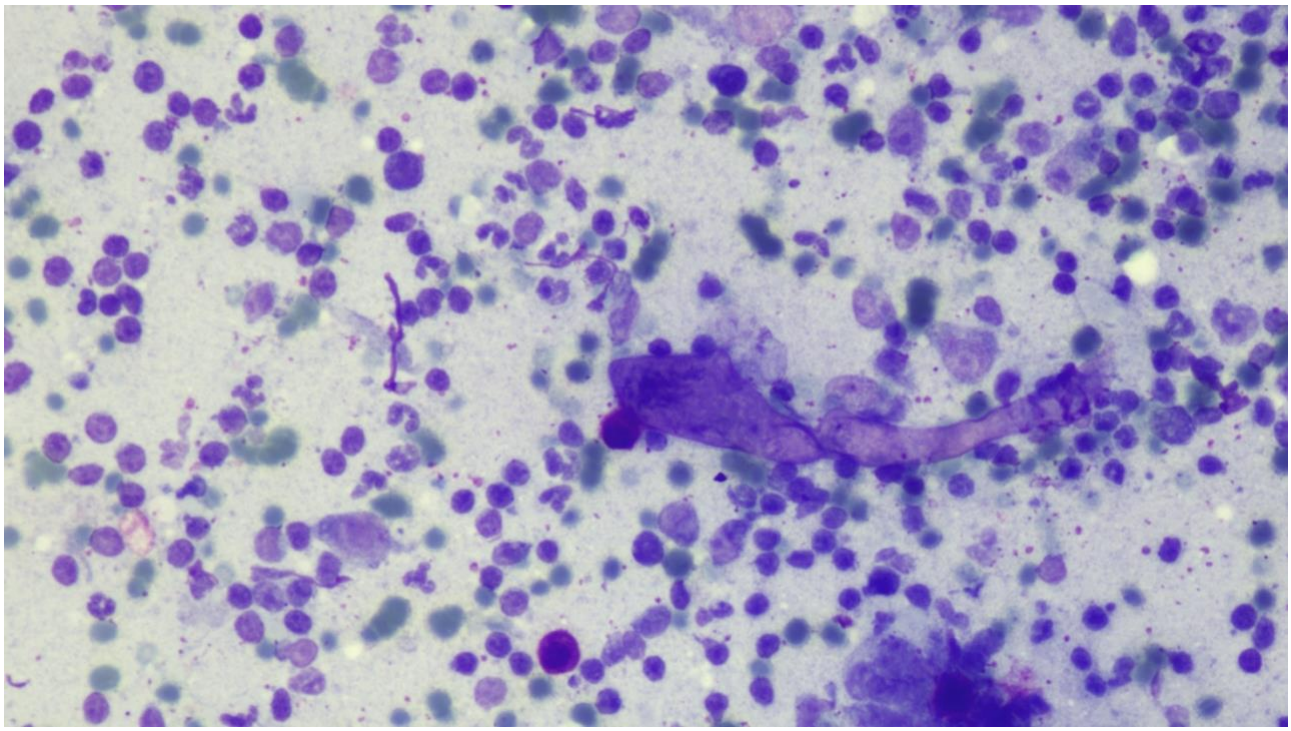

Figure 4. 40x Objective

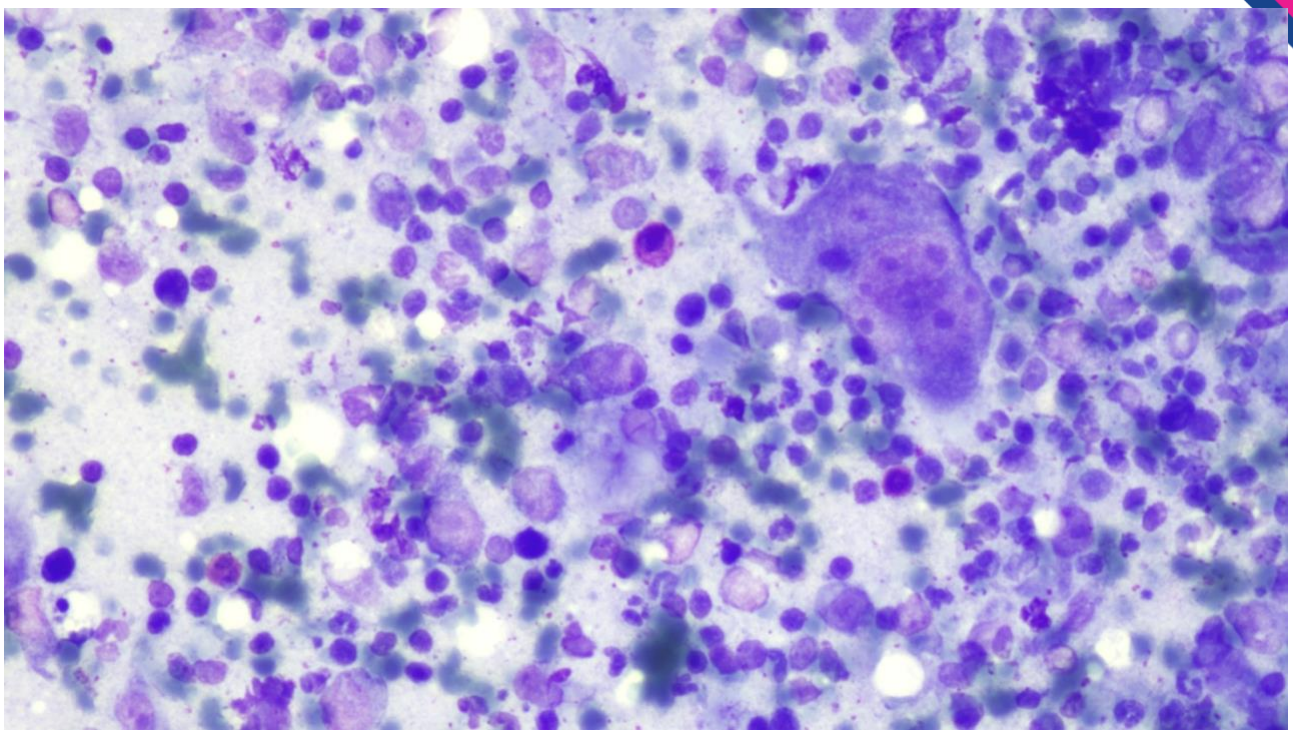

Figure 5. 40x Objective

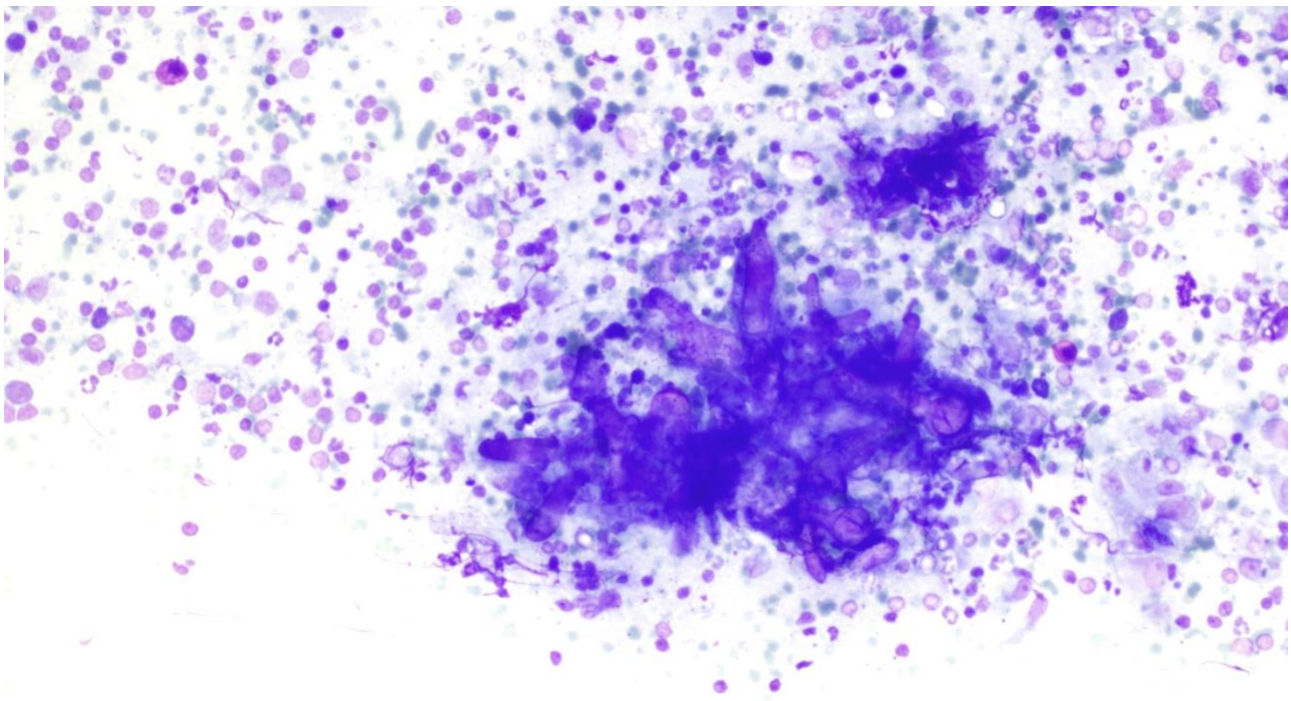

Figure 6. 40x Objective

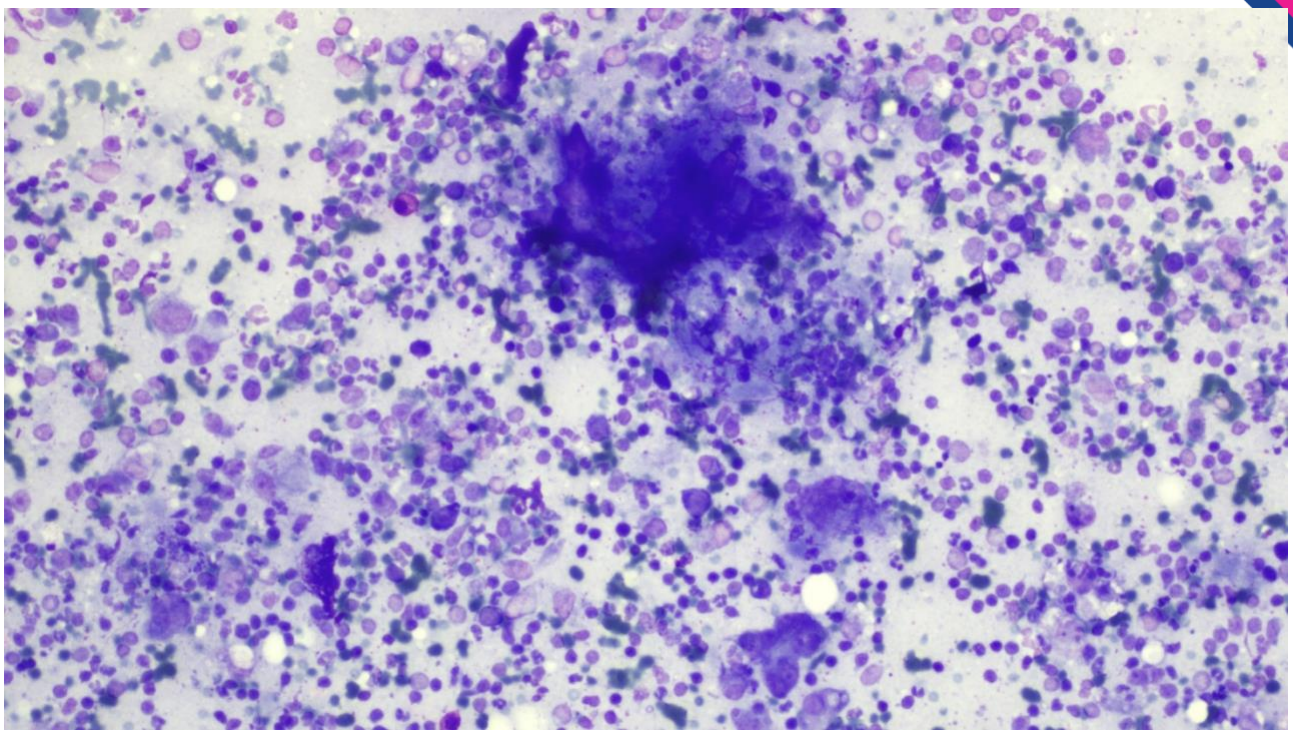

Figure 7. 20x Objective

Questions

What is your cyological interpretation and diagnosis?

Which further analysis would you recommend?

Gingival mass in a dog

Contributors

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Specimen
Fine needle aspirate cytology of an ulcerated mass at the mucogingival junction.

Signalment
7-year-old male neutered Labrador retriever dog

History
A dog was referred to the Royal (Dick) School of Veterinary Studies, University of Edinburgh, for investigation of right hind limb lameness.

Clinical findings

Clinical examination with the Dick Vet General Practice (DVGP) prior to orthopaedic referral revealed a 2 cm diameter, pink to purple, irregular, multilobulated oral mass arising from the mucogingival junction adjacent to the maxillary incisors. The mass appeared pedunculated and was mobile on palpation. There were no clinical signs of involvement of underlying structures such as bone or deeper soft tissue. As no images of the lesion in situ were available, the appearance of the mass after submission and fixation for histopathology is shown in Figure 4. Fine needle aspirates (FNA) of the gingival mass were submitted for cytologic examination.

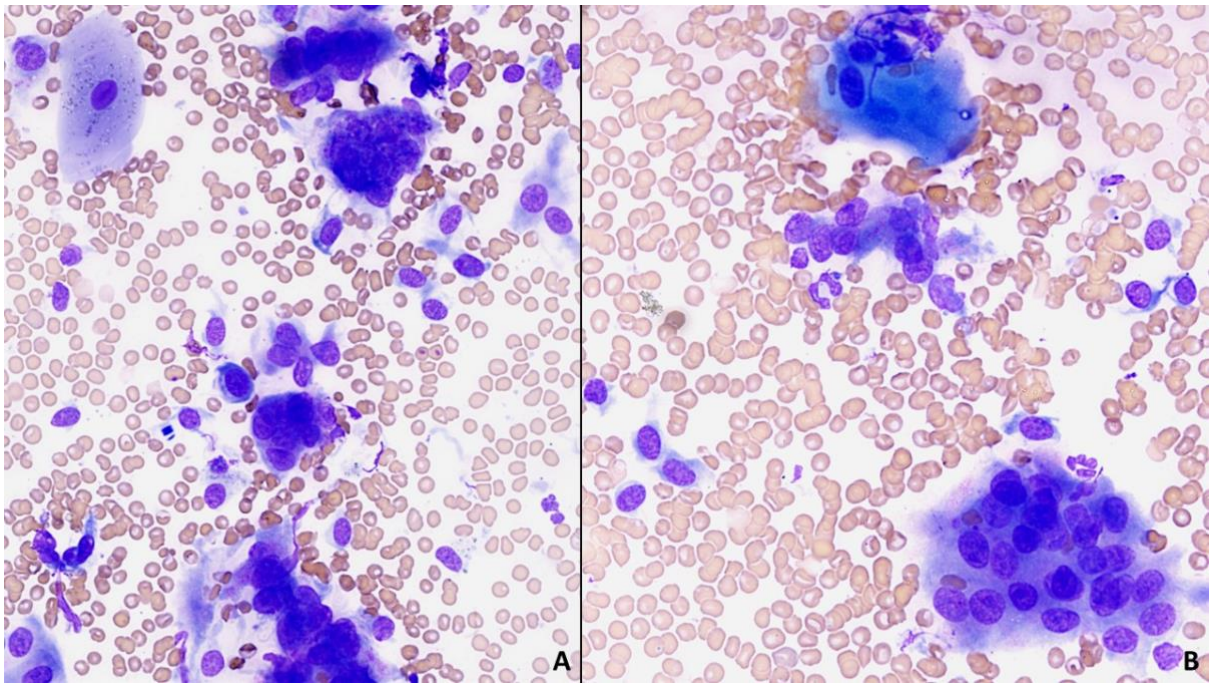

Figure 3 *Photomicrographs from a FNA of a gingival mass in a dog. (A) May-Grünwald-Giemsa stain, 200× magnification, (B) May-Grünwald-Giemsa stain, 400× magnification.*

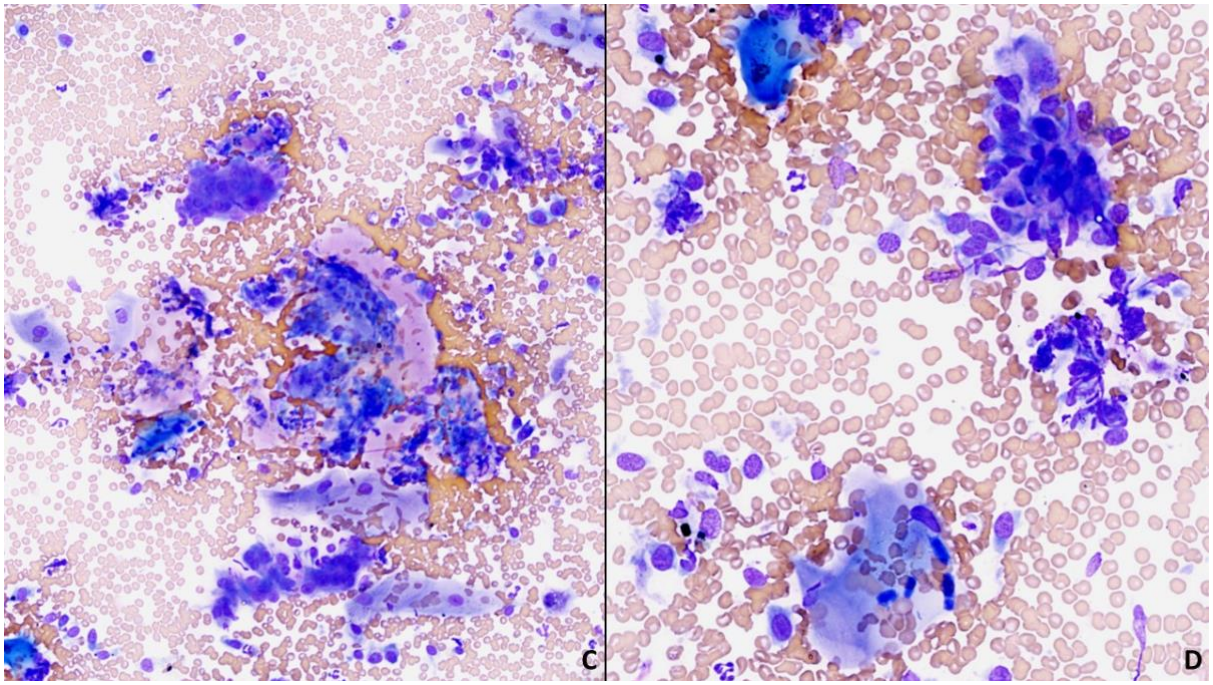

Figure 4 *Photomicrographs of a FNA from a gingival mass in a dog. (C) May-Grünwald-Giemsa stain, 100× magnification, (D) May-Grünwald-Giemsa stain, 200x magnification.*

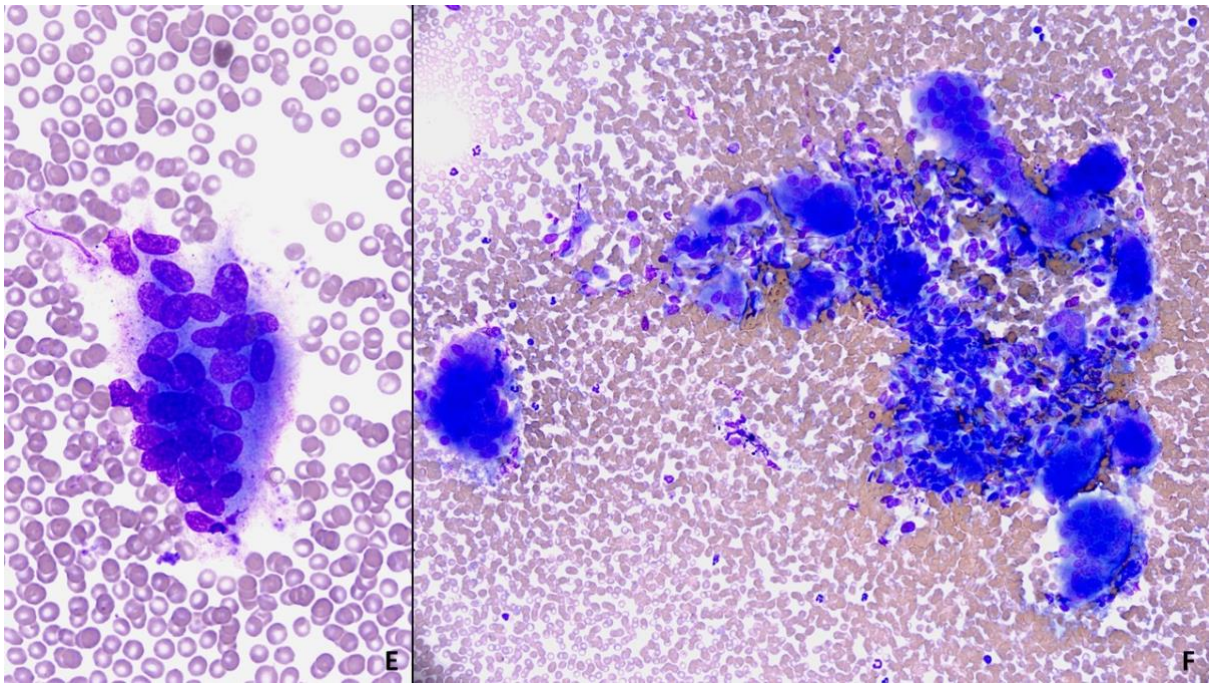

Figure 5 *Photomicrographs of a FNA from a gingival mass in a dog. (E) May-Grünwald-Giemsa stain, 400× magnification, (F) May-Grünwald-Giemsa stain, 200× magnification.*

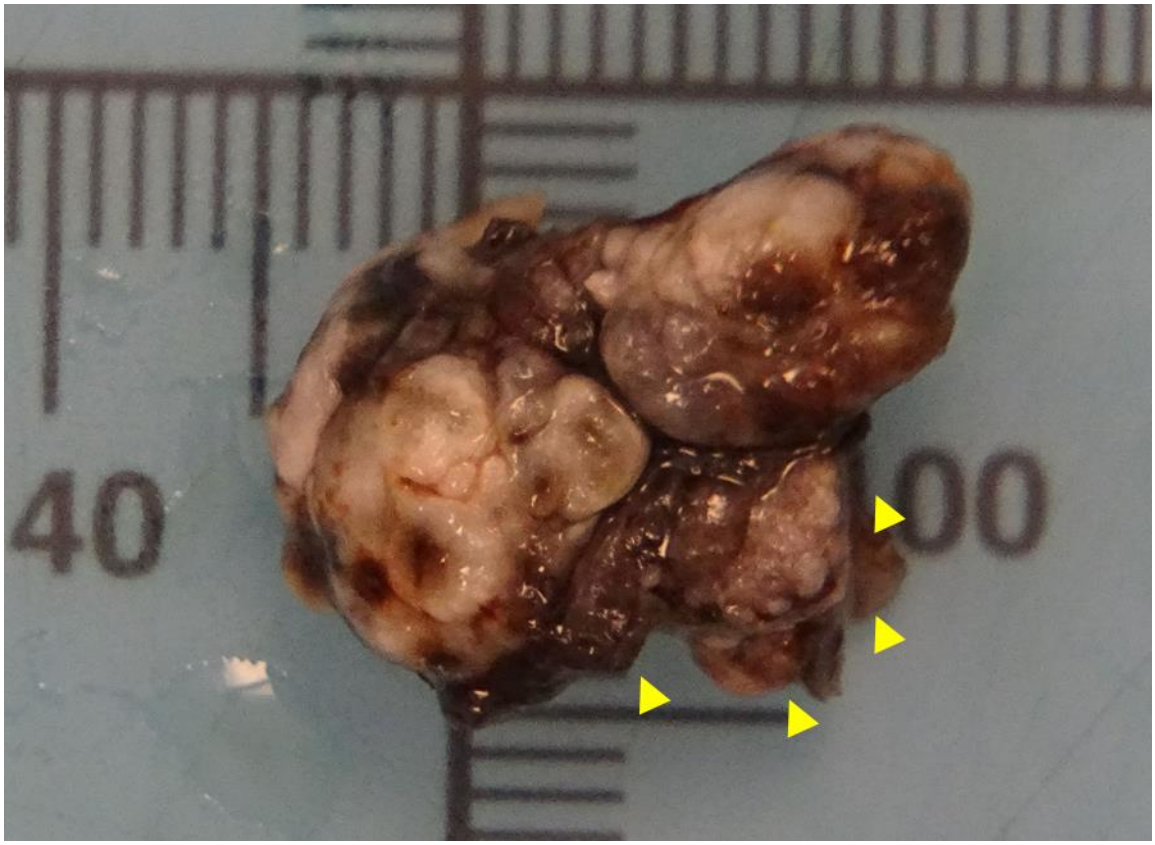

Figure 6 *Gross photograph of the lesion, post formalin fixation. A raised, multilobular mass of multifocally tan to dark brown tissue measuring approximately 1.5cm x 1.5cm x 1.5cm was removed surgically from the mucogingival junction adjacent to the maxillary incisors. The deep surgical margin is indicated by the yellow arrowheads.*

Questions

1. What is the main differential diagnosis based on the cytologic findings and lesion location?

2. Which of the following best describes biological behaviour of this growth?
    a. Malignant and locally invasive
    b. Reactive and benign
    c. Metastatic potential with bony destruction
    d. Indolent neoplasm with high reoccurrence rate

Saturday 4 October 2025

Cinema 3

Cytology of Skin Lumps and Bumps in Practice: Differentiating Inflammation from Neoplasia

Elpida Sarvani
DVM, DipACVP (Clinical Pathology), DipECVCP, MRCVS American, European & RCVS Specialist in Veterinary Clinical Pathology

Cutaneous and subcutaneous masses are a frequent presentation in small animal practice. Cytology provides a rapid, minimally invasive, and cost-effective first-line diagnostic tool, with veterinary nurses playing an essential role in sample preparation, preliminary assessment, and communication. Optimal results depend on appropriate needle choice, sufficient sample collection, gentle smear technique, rapid drying, and correct staining. Veterinary nurses are central to these steps, ensuring sample quality while contributing to early interpretation and recognising the limitations of cytology.

Differentiating inflammatory from neoplastic processes can be challenging, particularly when reactive or mixed populations are present. This presentation reviews the cytologic features of inflammation and neoplasia, highlights common pitfalls, and touches on mast cell tumour cytological grading, relevant to veterinary nurses supporting in-house diagnostics. Neoplasia may also co-exist with inflammation, as tumours can trigger responses through necrosis, cytokine release, ulceration, secondary infection, or irritant materials. Importantly, there are also non-inflammatory non-neoplastic lesions (e.g. hamartomas, cysts, hyperplasia).

Inflammation is recognised by the predominance of inflammatory cells, often mixed populations, sometimes accompanied by infectious agents or foreign material. Subtypes such as neutrophilic, macrophagic, eosinophilic, and lymphocytic inflammation are briefly discussed. Morphological features such as histiocytic responses or reactive changes that can mimic malignancy are also addressed.

Neoplasia typically presents as a more homogeneous cell population, sometimes with criteria of malignancy such as anisocytosis, anisokaryosis, prominent nucleoli, and abnormal mitoses. Distinguishing epithelial, mesenchymal, and round cell tumours on cytology is valuable, and some common examples are illustrated. Mast cell tumours are amongst neoplasms that are often diagnosed cytologically, but clinical behaviour
can be variable. Histopathology remains the gold standard for grading and prognostication, with the Kiupel 2-tier system currently recommended. Proliferation markers and molecular tools (Ki-67, AgNOR, PCNA, c-KIT mutations) can refine prognosis. Although cytology cannot replace histology — as it does not evaluate architecture, margins, or distinguish subcutaneous from cutaneous origin — it can support risk assessment. Several studies have explored cytologic grading of MCTs, including descriptions based on atypical features and granulation (Camus et al. 2016), a cytologic atypia score incorporating nuclear and cytoplasmic changes with mitoses

(Scarpa et al. 2016), and more recent work applying numeric thresholds and evaluating tumour microenvironment (Paes et al. 2022). All three showed correlation with prognosis. Ancillary approaches, including Ki-67 and AgNOR on cytologic samples, as well as digital image analysis, are under investigation.

Case-based polling with real examples is used to aid in differentiating inflammatory from neoplastic lesions. These cases highlight the importance of integrating cytologic findings with clinical information and lesion behaviour.

Conclusion:

Cytology is a valuable screening tool for cutaneous and subcutaneous masses and, in some cases, can also be diagnostic. It supports clinical decision-making and client communication, and veterinary nurses play a vital role in its use. Despite limitations, cytology remains an essential, practical, and impactful part of small animal practice.

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Haematology, understanding the plots and graphs.

Clare Roberts

Haematology is an important but often not fully interpreted aspect of the CBC. The majority of analysers produce either histograms or scatterplots (dotplots) to illustrate their values and it is these graphs that require further understanding. As the analyser technician there is a responsibility to validate the results but also be able to flag abnormalities. In a high pressured environment being able to understand the graphs gives the technician a rapid method for bringing results to the attention of the vet and therefore highlighting the need for blood film examination. Through understanding the analysers' methodology, impedance or flow cytometry and the plotted information, size, frequency, granulation or fluorescence more insight into the patient's results is gained. Recognising normal plots from abnormal and knowing the influence of analytical error enables the technician to optimise the instrument. Cell pathology changes during disease and a fundamental knowledge of the expected changes enables interpretation of where curves rise and fall on histograms or by the shape and location of cell clouds on scatterplots. Impedance analysers produce histograms which are limited in their information giving an overall view of each cell population size and distribution, but in disease there will be changes in peak height and location or abnormal troughs each requiring blood film examination. Scatterplots additionally give insight into the cell morphology and by recognising cloud pattern changes, suspicion for a number of conditions e.g. Inflammation, Lymphoma, IMHA, Splenic bleeds can be raised. By studying all graphs and noting deviations from normal plots, even if the analyser has reported values within reference ranges, there are grounds for a blood film examination. In practice it is not generally possible to perform a smear with every sample but analysis of the graphs allows technicians to highlight those patients whose samples need further investigation. The graphs cannot be used for diagnosis but accurate interpretation will assist the vet towards it.

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Clinical Biochemistry Results - Fact or Artefact?

Matt Garland

Veterinary clinical biochemistry plays a pivotal role in diagnosing and monitoring animal health, yet its reliability is frequently challenged by analytical interferences. These interferences—primarily haemolysis, icterus, and lipemia—can significantly distort biochemical parameters, leading to misinterpretation and potentially inappropriate clinical decisions. In veterinary settings, where sample quality control may be less standardized than in human medicine, understanding and mitigating these interferences is essential.

Haemolysis, caused by red blood cell rupture during or after sample collection, releases intracellular contents that can falsely elevate analytes such as potassium, creatine kinase and AST. Icterus, resulting from elevated bilirubin levels, can interfere with photometric assays due to its strong absorbance properties. Lipemia, often due to postprandial sampling or metabolic disorders, scatters light and affects spectrophotometric measurements, particularly of enzymes and electrolytes.

This presentation explores the mechanisms by which these interferences affect biochemical assays, highlights age-specific considerations—especially in canine and feline samples—and discusses strategies for detection and correction. Studies demonstrate that even mild haemolysis or lipemia can exceed acceptable bias thresholds for many measurands, underscoring the need for rigorous preanalytical protocols.

Ultimately, improving awareness and management of biochemical interferences is critical for advancing veterinary diagnostics, ensuring animal welfare, and optimizing laboratory efficiency.

Urine Analysis Results & Findings Fact or Artefact?

Matt Garland

Urine analysis is a cornerstone of veterinary diagnostics, offering valuable insights into renal function, metabolic status, and systemic health in companion animals. However, the accuracy of urinalysis in canine and feline patients is frequently compromised by preanalytical and analytical artefacts. These artefacts—ranging from sample contamination and improper storage to interference from endogenous substances—can lead to misinterpretation and diagnostic errors.

Common artefacts include cellular degradation due to delayed processing, which can obscure sediment findings such as casts or epithelial cells. Bacterial overgrowth from unpreserved samples may mimic infection, while crystal formation during refrigeration can be mistaken for pathological crystalluria. Additionally, pigment interference from hematuria, bilirubinuria, or myoglobinuria can distort dipstick results, particularly for protein, blood, and bilirubin. In feline samples, concentrated urine and high specific gravity may exacerbate false positives, especially in protein estimation.

Technical factors such as centrifugation speed, reagent strip variability, and observer subjectivity further contribute to inconsistencies. Artefacts are especially prevalent in samples collected via free-catch methods, where contamination from hair, debris, or genital secretions is common. Age-related physiological differences also influence urine composition, with juvenile animals exhibiting variable pH and lower concentrating ability, complicating interpretation.

This presentation reviews the most frequent artefacts encountered in canine and feline urinalysis, explores their underlying mechanisms, and outlines strategies for minimization. Emphasis is placed on standardizing collection techniques, timely sample processing, and integrating confirmatory tests such as microscopy and biochemical assays. By recognizing and mitigating artefacts, veterinary professionals can enhance diagnostic reliability, reduce unnecessary interventions, and improve patient outcomes.

Posters

National Italian Guidelines on Canine and Feline Transfusion Medicine

Alessandra Gavazza[1], Chiara Agnoli[2], Marta Vascellari[3], Daniela Proverbio[4], Eva Spada[4], Maria Teresa Antognoni[5], George Lubas[6]

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[4]Veterinary Transfusion Research Laboratory (REVLab), Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Lodi (LO), Italy
[3]Canine and Feline Blood Bank, Department of Veterinary Medicine, University of Perugia, Italy
[6]Colombo Veterinary Clinic, VetPartners Italy, Lido di Camaiore (LU), Italy

Background. The use of whole blood and blood components is a well-established worldwide procedure in both human and veterinary practice. Differently from the human counterpart, no recognised official veterinary regulation is available in the European Countries (EC).
Objectives. Aim of this study was to identify standard analytical protocols for canine and feline blood donor selection, along with procedures for high quality production and storage of whole blood and blood components, to be adopted as an official guideline by the Italian Ministry of Health (IMH).
Material and Methods. An Italian committee of veterinary specialists was designated, and a consensus process was carried out taking in account a comprehensive human and veterinary literature review. A list of analyses and standard operative procedures considering the risk/benefit ratio and as well as the quality standards for blood products were defined.
Results. The laboratory tests for candidate blood donor screening included: blood typing and count, serum biochemical and coagulation profile, and urinalysis; serological and/or biomolecular investigations against blood borne pathogens considering the epidemiological framework and the donor lifestyle. Quality requirements as free Hb and microbiological evaluation to validate blood products were also established.
Conclusion. These guidelines issued by the IMH represent the first example of a national official regulation in EC about this topic. Considering the ongoing evolution and improvements of veterinary transfusion medicine and the movements of dogs and cats across EC, coherent official EC regulations would be desirable through the establishment of an international expert panel.

Keywords: blood components, cat, dog, guidelines, national, transfusion medicine

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Evaluation of reticulocyte hemoglobin content in dogs affected by neoplastic disease

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Background. Reticulocyte hemoglobin (RETIC-HGB) is a parameter provided by hematology analyzer that has recently been shown to be valuable for the early diagnosis of iron deficiency anemia. Furthermore, in human medicine it has been proposed that reticulocyte hemoglobin content may represent an easy diagnostic tool for monitoring iron deficiency anemia in patients with neoplastic disease undergoing chemotherapy.
Objectives. To assess the correlation between total iron and RETIC-HGB and to evaluate the relationship between RETIC-HGB and erythroid parameters in dogs affected by various types of neoplasia.
Materials and Methods. 153 CBCs were performed in a different group of dogs (43 not anemic/RETIC-HGB normal; 50 not anemic/RETIC-HGB reduced; 60 anemic) to assess the correlation with total serum iron. Also, 354 CBCs were performed by ProCyte DX (IDEXX™) from 121 dogs with neoplastic disease: 31 epithelial, 58 discrete round cell, 19 mesenchymal and 13 other.
Results. RETIC-HGB shows a significant correlation with iron levels in the overall study population ($p < 0.0001$) as well as in the subgroup of anemic subjects ($p=0.0004$). Reduced RETIC-HGB was observed in 38.54% of CBCs from patients with neoplastic disease. A correlation was observed between RETIC-HGB and RBC ($r=0.13$; $p=0.0195$), Hct ($r=0.23$; $p=0.0001$), Hb ($r=0.28$; $p<0.0001$), MCV ($r=0.41$; $p<0.0001$), MCH ($r=0.69$; $p<0.0001$), MCHC ($r=0.39$; $p<0.0001$), RDW ($r=-0.24$; $p<0.0001$).
Conclusions. The correlation between RETIC-HGB in neoplastic subjects is stronger against erythrocyte indices which are related to iron content and especially to MCH. RETIC-HGB can be considered a marker for the early diagnosis of iron-deficiency erythropoiesis in anemic subjects and is a useful tool for pre-chemotherapy assessment.

Keywords: Reticulocyte hemoglobin, dog, iron deficiency, anemia, neoplastic disease.

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Method validation and establishment of reference intervals for serum protein concentrations analysed by capillary zone electrophoresis, in German Warmblood horses, compared to agarose gel electrophoresis.

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Background: Serum protein electrophoresis is a common methodology within equine clinical pathology for diagnostic and research purposes. Recently agarose gel electrophoresis (AGE) has been superseded by capillary zone electrophoresis (CZE) due to performance and accuracy benefits. Canine and feline CZE reference intervals (RIs), and comparison studies to AGE, have been performed[1]. However, the equivalent equine studies are lacking, limiting CZE use in horses.

Objectives:
1) To establish RIs for CZE in adult warmblood horses
2) To assess the relationship between CZE to AGE results in both normal and pathological horse samples, and to assess the ability of CZE to detect abnormalities identified by AGE.

Methods: Stored, leftover serum samples from 55 adult warmblood horses, including 43 healthy and 12 diseased animals, submitted to the reference laboratory, were analysed using automated systems for AGE (Sebia Hydrasys 2) and CZE (Sebia Capillarys). CZE RIs were calculated based on healthy animals' results. Descriptive statistics and Bland–Altman and Passing–Bablok statistical analyses were used to compare results.

Results: The RIs established for the CZE technique overlapped with AGE RIs at every fraction. Albumin, alpha-1, and gamma protein fraction values obtained by AGE and CZE were significantly different from each other ($P<0.05$), and systemic and proportional biases were detected.

Conclusions: In general, CZE is equivalent to AGE when method-specific RIs and knowledge of unique albumin patterns (CZE) are utilised for interpretation. This datum supports the clinical extension of CZE use to horses and establishes new RIs for CZE protein fractions in adult warmblood horses.

Keywords: *capillary zone electrophoresis/CZE, equine, serum proteins, agarose gel electrophoresis/AGE.*

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RETROSPECTIVE STUDY OF ANEMIA IN DOGS IN EASTERN EUROPE – ROMANIA USING THE MINDRAY POC ANALZYER WITH IRF

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Background. Anemia is a common reason for consultation in canine patients, often associated with various systemic conditions. However, in Eastern Europe, specific epidemiological data remain limited.

Objectives. This is a retrospective analysis, which aims to characterize the types of anemia observed in dogs, estimate their prevalence and evaluate the contribution of new reticulocyte indices in the diagnosis.

M**aterials and Methods.** The study was conducted between August 2024-April 2025. Out of 421 dogs admitted to the veterinary university clinic in Cluj-Napoca, 164 cases of anemia were analyzed. Each case was classified according to descriptive, morphological and regenerative criteria, supplemented by the study of markers such as IRF and RHE, obtained using the Mindray BC-60R Vet analyzer.

Results. The prevalence of anemia in the study population was 39%. Non-regenerative anemia was predominant (75%), mainly normocytic (85%) and normochromic (96%). The main causes identified were chronic inflammation (27%), trauma (20%) and vector-borne infections (19%). IRF was increased in 33% of regenerative anemias and in 2% of non-regenerative anemias, early on before any increase in the number of reticulocytes, while a decreased RHE was observed in 15% of cases, sometimes before any decrease in MCHC.

Conclusion. These results highlight the importance of a complete hematological assessment, including the interpretation of reticulocyte indices such as IRF and RHE, for a refined diagnostic approach (1). Their systematic integration could improve the early detection and characterization of canine anemia (2), particularly in regions with high infectious pressure.

Keywords: Anemia, IRF, RHE, Mindray BC-60R, Eastern Europe

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Alterations in the serum protein electrophoretic patterns in dogs following administration of an iodinated contrast medium

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Background. Capillary zone electrophoresis (CZE) is widely used for serum protein electrophoresis (SPE) in veterinary medicine.[1] In people, the administration of iodinated contrast media (ICM) causes the appearance of additional peaks on CZE, sometimes leading to diagnostic errors.[2] No reports on the occurrence of this finding in veterinary medicine are available.
Objectives. To evaluate if the administration of ICM induces additional peaks on CZE in dogs.
Material and Methods. Eighty-four serum samples from 21 dogs undergoing computed tomography were collected before (T0), and 5 (T1), 15 (T2), 60 (T3) minutes after the administration of 2 mL/kg Visipaque 320 (Iodixanol), and used to perform CZE and agarose gel electrophoresis (AGE). The presence of additional peaks was visually evaluated, and relative and absolute values of electrophoretic fractions were statistically compared between each time points.
Results. On CZE, but not in AGE, all dogs had visible additional α2 peaks in the 3 samplings after Visipaque administration. Variations were >10% at T1 and T2. Both relative and absolute α2-globulin concentrations were significantly higher ($P<0.001$) at T1 and T2 and, consequently, the concentration of the other fractions significantly decreased. This induced the appearance of an inflammatory pattern in 9/21 dogs that had non-inflammatory electrophoretograms at T0 and of a more evident inflammatory pattern in the other 12/21 dogs.
Conclusion. In dogs, Iodixanol administration causes the appearance of visible α2 peaks and increased α2 concentrations on CZE, but not on AGE. Performing CZE within 60 minutes after ICM administration could lead to wrongly overdiagnose inflammation.

Keywords: *Dogs, serum protein electrophoresis, capillary zone electrophoresis, iodixanol, inflammation*

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Sysmex XN-1000V WDF scattergram in a dog with *Hepatozoon spp.* infection

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Background. A 7-year-old, spayed female, English setter was referred to the Veterinary Teaching Hospital of the University of Milan for history of multiple simultaneous cutaneous mast cell tumors. A CBC on the Sysmex XN-1000V analyzer was performed and, despite the absence of any numerical alterations or flag, an additional cluster was present. This was located between those of neutrophils, lymphocytes and eosinophils and partially identified as these three types of cells.
Objectives. To describe this peculiar Syxmex XN-1000V finding.
Material and Methods. A blood smear was prepared and stained with May Grunwald-Giemsa, and several neutrophils containing Hepatozoon spp. gamonts were identified. A differential count including infected neutrophils (INs) was performed on 250 cells. A manual gating of the WDF scattergram including the 5 leukocyte classes as well as the INs was also performed to enumerate these cells and compare them with the regating results.
Results. The original instrumental differential count revealed 52,1% neutrophils, 30,8% lymphocytes, 5,2% monocytes, 11,9% eosinophils and 0,1% basophils. The manual gating of the WDF scattergram revealed 50,5% neutrophils, 7,8% INs, 23,9% lymphocytes, 6,7% monocytes, 9,2% eosinophils and 0% basophils. The manual differential count revealed 52,8% neutrophils, 8,0% INs, 27,2% lymphocytes, 5,9% monocytes, 7,1% eosinophils and 0% basophils.
Conclusion. The blood smear examination and the regating of the WDF Sysmex scattergram yelded almost identical results, supporting the hypotesis that the additional cluster consisted of neutrophils infected by Hepatozoon spp. This report confirms the importance of blood smear examination, also in the absence of flags from the instrument.

Keywords: *Sysmex, scattergram, blood smear, Hepatozoon spp*

Diagnosis of anti-erythrocyte antibodies in equine blood samples: direct antiglobulin (DAT)-test versus direct immunofluorescence flow cytometry (DIF)

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Background. To diagnose immune-mediated haemolytic anaemia (IMHA) in horses can be challenging.[1] So far, the DAT ("Coombs test") is used as gold standard for anti-erythrocyte-antibody diagnostics. However, due to its low specificity[2], it appears to be of limited suitability in horses.
Objectives. We hypothesize that DIF[3] is more suitable to diagnose IMHA in horses compared to DAT.
Material and Methods. One hundred and twenty-one leftover samples of clinically healthy horses of different breeds and sexes without changes in blood count and clinical chemistry laboratory parameters and five patients with clinically and laboratory diagnosed IMHA and matching laboratory parameters, that were sent to a routine diagnostic laboratory, were included in the study. Additionally, DAT (Rabbit-anti-horse IgG (IgG)) and DIF (Attune NxT acoustic focusing cytometer (Invitrogen, Karlsruhe, Germany); Anti-horse IgG, Anti-Horse IgG $F(ab')_2$ ($F(ab')_2$), was performed according to standard procedures.
Results. Using DIF, 100% of the horses were correctly classified as positive (IgG:81,18 ± 32,40 SD; $F(ab')_2$:92,28 ± 9,97 SD) or negative (IgG:3,38 ± 4,55 SD; $F(ab')_2$:0,17 ± 0,29).
In the DAT, 57% (n=72) were true negatives, 38.9% (n=49) were false positives, 3.2% (n=4) were true positives and 0.8% (n=1) were false negatives.
Conclusion. Results of the DIF were encouraging for the diagnosis of equine IMHA. It is therefore considered as suitable replacement for the DAT, especially because the low sensitivity and specificity of the DAT could be confirmed[2]. To calculate the sensitivity and specificity of the DIF, a higher sample number of animals with anti-erythrocytic antibodies is required.

Keywords: anti-RBC, Coombs, direct immunofluorescence, flow cytometry

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Evaluation Of The Utility Of Fragmented Red Blood Cells Automated Measurement For The Detection Of Schistocytosis In Dogs Using The ADVIA 2120i

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Background. Schistocytes, or fragmented red blood cells (RBCs), are markers of mechanical erythrocyte damage. They are rarely seen in healthy dogs but may increase in conditions such as disseminated intravascular coagulation, vasculitis, portosystemic shunts, and vascular neoplasms (e.g., hemangiosarcoma). Detection is performed via blood smear examination and typically reported semi-quantitatively. The Siemens ADVIA 2120i hematology analyzer provides a semiquantitative estimation of RBC fragmentation through the "RBC FRAG" flag. While this flag is used in human medicine to exclude schistocytosis, its diagnostic utility in dogs remains unknown.

Objectives. To evaluate the utility of the "RBC FRAG" flag from the ADVIA 2120i analyzer for detecting schistocytosis in dogs.

Material and Methods. A retrospective review of the digital archive of the Diagnostic Laboratory, School of Veterinary Medicine, Aristotle University of Thessaloniki, was performed. Dogs with a reported "RBC FRAG" flag on their complete blood count and/or recorded schistocytosis on blood smear were included. The association between analyzer flags and smear results was assessed in 312 cases using Spearman's rank correlation and the chi-square test for independence.

Results. Of the 312 cases, 50 had schistocytosis on smear. The "RBC FRAG" flag appeared in 268 cases but identified schistocytosis in only 6 of the 50 smear-positive dogs. A strong negative correlation was found between flag values and smear scores (Spearman's ρ=–0.93, $p<0.001$). Chi-square analysis confirmed a significant discordance ($\chi2=268.9$, $p<0.001$).
Conclusion. The ADVIA 2120i "RBC FRAG" flag does not reliably detect or exclude schistocytosis in dogs and should not replace smear evaluation.

Keywords: ADVIA 2120i, blood smear, canine, schistocytes.

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Imprint Cytology in the Diagnosis of Meningiomas in Dogs and Cats

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Background. Meningiomas are the most common brain and spinal cord tumors, and their diagnosis is based on imaging, cytology, and histopathology. Cytology is a cost-effective, easy, rapid, and efficient examination for differentiating inflammation from neoplasia, even intraoperatively. In human medicine, imprints exhibit better morphological details than squash smears, by avoiding crushing artifacts, thick layering, and difficulties in smearing, and are particularly useful when tissue availability is limited.

Objectives. To retrospectively review cytological features of imprint cytology and to evaluate its diagnostic utility in canine and feline meningiomas.

Material and Methods. Twelve cases (six dogs, six cats) of histopathologically confirmed meningiomas were assessed. Cytological features (cellularity, cellular distribution, cellular morphology, presence of whorling, psammoma bodies, inflammatory infiltrate) and diagnosis were recorded.

Results. 11/12 cases were cytologically intepreted as meningiomas, while in 1/12 meningioma was listed in differentials. High cellularity and thin-layering was observed in 58.3% and 75% of cases, respectively. Most smears demonstrated individual cells (91.7 %) with a wispy cytoplasmic appearance, clusters (58.3%), and a whorling arrangement (58.3%). Elongated nuclei were seen in all cases, while round nuclei appeared in 58.3% cases. Nuclear folding (33.3%) and intranuclear pseudoinclusions (8.3%) were noted. Anisokaryosis was evaluated as mild (33.3%), moderate (41.7%), and marked (25.0%). Inflammatory infiltrates were predominantly lymphocytic (66.7%). Background features included amorphous eosinophilic material (58.3%), psammoma bodies (25.0%), collagen fibers (25.0%), and neuropils (16.7%).

Conclusion. Imprints are valuable in the diagnostic investigation of meningiomas. Moreover, when a limited amount of tissue is available the sample could be preserved for further examinations.

Keywords: imprint, impression smear, meningioma

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EVALUATION OF THE HEMATOLOGICAL AND SERUM BIOCHEMISTRY PROFILE OF BORNEAN SUN BEARS (*Helarctos malayanus euryspilus*) IN MATANG WILDLIFE CENTRE AND BORNEAN SUN BEAR CONSERVATION CENTRE, MALAYSIA

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Background. The Bornean sun bear (*Helarctos malayanus euryspilus)* is the smallest Ursid species and is listed under the red list of the International Union for Conservation Nature. The goal of conserving and rehabilitating this species in Borneo requires many efforts including routine health assessment using clinicopathological parameters. However, due to lack of research on the species, evaluation of the clinicopathological values is still required especially in understanding its nutritional rehabilitation needs.

Objectives. The objective of this study is to evaluate the hematology and serum biochemistry (SBC) parameters of the Bornean sun bear kept in semi-captivity management. **Material and Methods.** Blood samples of Bornean sun bears from Matang Wildlife Centre (n=16) and Bornean Sun Bear Conservation Centre (n=43) were taken during the annual health check program. Analysis of the complete hematology, comprehensive SBC, and morphological assessment of the red (RBC) and white blood cells (WBC) were done. **Results.** In general, the younger age groups have higher WBC numbers compared with mature adults. The SBC values were higher in older animals compared to the younger counterparts, particularly lipase, meanwhile males have higher SBC values when compared with females. The morphology of both RBC and WBC of Bornean sun bear revealed similar to the domestic canine species, although the eosinophilic granules are smaller than of canine.

Conclusion. The hematology and SBC of Bornean sun bears are affected by different sex and age groups. Assessment of different demographic characteristics of the species is crucial in developing establishing reference for future health assessments for the success rehabilitation and release purposes.

Keywords: *Bornean Sun Bear, Helarctos malayanus euryspilus, conservation, wildlife*

Reference Intervals For The Sysmex KX-21 Hematology And Sclavo Konelab 200 Analyzers In Sprague-Dawley Rats

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Background: Analyzer-specific reference intervals are lacking for *Sprague-Dawley* rats for the Sysmex KX-21 and Sclavo Konelab 200i analyzers but are essential for assessment of hematologic and biochemical parameters in preclinical toxicological studies to determine test article-related effects.

Objectives: The objective of this study is to establish analyzer-specific reference intervals in healthy control *Sprague-Dawley* rats according to the guidelines of the American Society of Veterinary Clinical Pathology. Furthermore, effects of age and sex on the respective parameters should be evaluated.

Materials and methods: Blood samples from 1033 healthy *Sprague-Dawley* rats (n = 367 females, n = 666 males) of two different age groups (n = 380 rats >12 weeks old, n = 653 rats <12 weeks old) were included in the study. Non-parametric 95% reference intervals were calculated.

Results: Significant differences were found between age groups and sexes for all parameters, except for platelet count, sodium, and potassium by sex, and hemoglobin and urea by age (Wilcoxon-Mann-Whitney, *P* <.05). Males had higher leukocyte, erythrocyte, hemoglobin, and hematocrit concentrations than females. Females had higher albumin, total protein, calcium, creatinine, and urea concentrations than males.

Conclusion: Partitioning of reference intervals is important in *Sprague-Dawley* rats to accurately assess data in preclinical safety studies.

Keywords: *Sprague-Dawley, reference interval, hematology, biochemistry, rat*

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Preliminary Results On The Evaluation Of Hematologic Parameters In Humboldt Penguins (Spheniscus Humboldti) Using The Sysmex XN-1000V Analyzer And The PLT-F Channel

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Background. In non-mammalian species, hematology is time-consuming and performed using manual methods. The PLT-F channel in the Sysmex XN-1000V analyzer offers potential for automated analysis in avian species.

Objectives. The aim of this study was to evaluate the PLT-F channel of the Sysmex XN-1000V analyser in Humboldt penguins and develop a standardized gating strategy to simplify white blood cell (WBC) count Additionally, the study compared Sysmex analysis of hemoglobin concentration and hematocrit with manual methods.

Material and Methods. Seventeen samples of Lithium-heparinized blood from clinically healthy adult Humboldt penguins were analyzed with the Sysmex XN-1000V to evaluate agreement to manual methods for hematocrit (PCV), hemoglobin (cyanmethemoglobin method) and WBC counts (Natt-Herricks stain, improved Neubauer hemocytometer). **Results**. Preliminary results showed that Sysmex hematocrit values correlated with manual measurements (r = 0.94) with mean bias of 3.8 % (95% CI: 2.9 to 4.8), indicating constant systematic bias. Hemoglobin correlated (r = 0.71) with manual methods with very little bias of 0.08 g/dL (95% CI: – 0.70 to 0.85); four samples with high lipemic index were excluded due to interference in hemoglobin measurement. The created PLT-F-gate for WBC enumeration correlated with manual WBC counts (r = 0.88), with very little bias of 0.36 ×109/L (95% CI: -1.35 to 2.07).

Conclusion. Sysmex hematocrit and hemoglobin measurement can be routinely used in Humboldt Penguins if lipemic samples are excluded. The individual PLT-F-gate for Humboldt Penguins shows promising results compared to manual methods. Additional samples need to be added to strength the findings and calculate reference intervals.

Keywords: Humboldt Penguins, Hematology, Sysmex XN-1000V, PLT-F, Gating

Diagnostic cytology of teleost fish

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Background. Cytology is commonly used as a diagnostic tool in veterinary medicine. It be considered as a part of clinical settings in teleost (bony) fish medicine.
Objective. This study aims to provide a general bony fish normal cytology overview and regarding the most frequent lesions in the various tissues.
Material and Methods. Cytological samples from normal tissues were obtained by scraping or impression smears. Cytological samples from lesions were collected during surgery or necropsy by mass aspiration or by scraping and imprint preparation. Cytological samples were air-dried and stained with May-Grűnwald Giemsa stain. Histologic diagnoses were used to confirm the cytological diagnosis.
Results. The main cytologic features of both normal and pathological tissues are described and compared. The most common cytologic preparation is of samples from the gills, skin and muscle lesions of the fish for parasite identification. Skin spindle cell tumors were the most frequently diagnosed tumors by cytology.
Conclusions. Our results confirmed cytology as a reliable and useful diagnostic procedure for the evaluation of lesions in teleost fish.

Keywords: cytology, teleost fish, normal cytology, inflammation, parasite, neoplasia.

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Long-term endocrine and biochemical effects of SGLT2i treatment in horses with equine metabolic syndrome

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Background: Hyperinsulinemia is the central mechanism for laminitis development in horses with equine metabolic syndrome (EMS). Sodium-glucose co-transporter 2 inhibitors (SGLT2i) like ertugliflozin and dapagliflozin have been shown to reduce [insulin] short-term; however, their long-term effects on [insulin] and biochemical parameters have not been characterised.
Objectives: To document endocrine and biochemical effects during long-term use of ertugliflozin and dapagliflozin in EMS horses.
Material and Methods: A retrospective review of clinical records was conducted on horses treated with dapagliflozin or ertugliflozin between 2021 and 2025. Wilcoxon matched-pairs tests compared within-horse changes at 1 year (330–390 days) and 2 years (690–750 days) relative to baseline values.
Results: 197 horses received treatment (ertugliflozin n=109; dapagliflozin n=88). Median age was 16 years (IQR: 11, 19), with ponies predominating (66.6%). At 1 year, 42 horses remained on treatment (ertugliflozin n=30; dapagliflozin n=12). Significant ($p<0.05$) within-horse changes [median (IQR)] included: serum [insulin] (uIU/L) reduced from 264 (165, 300) to 34 (16, 93) ($p<0.0001$); [triglyceride] (mmol/L) increased from 0.61 (0.5, 0.9) to 0.92 (0.55, 1.7) ($p=0.005$); [GGT] (U/L) reduced from 29 (21.8, 36.3) to 22 (17, 31) ($p=0.02$). No significant difference in [GLDH] ($p=0.21$) or [creatinine] ($p=0.34$). At 2 years (n=12) (ertugliflozin n=7; dapagliflozin n=5), [insulin] reduced from 291 (214, 648) to 67.5 (14.4, 158) ($p=0.001$), with no significant changes in [triglyceride] ($p=0.31$), [GGT] ($p=0.06$), or [creatinine] ($p=0.18$). Median [bile acids] at 1-2yrs (n=18) was 7 µmol/L (RI: 0–20).
Conclusion: Long-term SGLT2i use effectively lowered [insulin] without adverse biochemical effects in horses with EMS.

Keywords: hyperinsulinemia, laminitis, SGLT2 inhibitor, ertugliflozin, dapagliflozin

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Apolipoprotein A-1 does not appear to be a suitable acute phase reaction marker in canine babesiosis and hemoplasmosis

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Background. Acute *Babesia canis* infections most often cause a marked acute phase response (APR) influencing lipoprotein metabolism. The significance of apolipoprotein A-1 (ApoA-1), a major protein and key determinant of high-density lipoprotein (HDL) formation, as a negative acute phase protein is questionable.

Hypothesis. Dogs with acute *B. canis* infection and marked APR show altered lipoprotein concentrations with impact on ApoA-1.

Material and Methods. Observational cross-sectional study. Eighty-four dogs were classified in four study groups (SGs): (I) 23 dogs with acute *B. canis* infection tested PCR positive, (II) 26 dogs with high antibody levels against *Babesia* spp., (III), 17 dogs with acute hemotrophic *Mycoplasma* infections, (IV) 18 clinically healthy dogs. Complete blood count, total protein, albumin, globulins, triglycerides, cholesterol, and CRP were compared between the SGs. Correlation analysis was performed.

Results. ApoA-1 concentrations did not differ significantly between SGs but were decreased in SG I compared to SG II-IV. Significantly different concentrations in CRP and triglycerides indicated APR in dogs with acute *B. canis* infection. ApoA-1 concentrations showed a moderate negative correlation with cholesterol, and moderate positive correlation with eosinophils. No statistical significance was found when comparing PCR-positive dogs to dogs serologically positive for *Babesia* spp., dogs with hemotrophic *Mycoplasma* infections, and clinically healthy dogs.

Conclusion. APR was observed in dogs with acute *B. canis* infections with significantly elevated triglycerides and CRP, but the value of ApoA-1 as a negative acute phase protein is questionable. No evidence for APR was observed in *Babesia* spp. seropositive dogs and those with hemotrophic *Mycoplasma* infection.

Keywords: acute phase protein, *Babesia canis*, hemotrophic *Mycoplasma*, PCR, serology

Clinical suspicion of in-vivo resistance against allopurinol in five dogs infected with *Leishmania infantum* associated with a reduction in copy numbers of the *S*-adenosylmethionine synthetase (*METK*) gene

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Background. Allopurinol is the drug of choice for long-term management of canine leishmaniasis. A copy number variation (CNV) <3 of the *S*-adenosylmethionine synthetase (*METK*) gene was related to resistance against allopurinol in-vitro. Knowledge regarding clinical significance in-vivo is still limited.

Objectives. To quantify *METK* CNVs in dogs with clinical relapse and suspicion of resistance against allopurinol.

Material and Methods. *METK* CNVs were quantified by ddPCR out of EDTA blood and/or bone marrow. Three dogs lived in Germany previously imported from Spain, Bulgaria, and Greece. The other dogs were in Southern France and Israel.

Results. One dog presented with polyarthritis was recently diagnosed with leishmaniasis and *METK* CNVs were tested before starting any treatment. Leishmaniasis was already diagnosed in the other four dogs, all four treated with allopurinol for 2 (n=2), 3.5, and 7 years. All five dogs tested PCR positive for *Leishmania infantum*. Relapse of leishmaniasis was confirmed by reoccurrence of clinical signs and results of bloodwork (anemia n=5, thrombocytopenia n=3, hyperproteinemia/hyperglobulinemia n=4, azotemia n=2, elevated c-reactive protein n=4). A CNV <3 was detected in all five dogs.

Conclusion. Indications for quantifying *METK* CNVs in dogs include checking for resistance prior to treatment of diagnosed leishmaniasis and in dogs with clinical relapse while under treatment with allopurinol. In all five dogs the clinical suspicion for resistance was in accordance with the quantification of *METK* CNVs <3. Early recognition of resistance is crucial for successful management of leishmaniasis in dogs. *Leishmania infantum* strains resistant against allopurinol represent a great risk for infection of naïve dogs, cats, and humans.

Keywords: canine leishmaniasis, genetics, relapse

Cytokine concentrations (IL-8, IL-10, MCP-1) in dogs with acute *Babesia canis* infections, dogs with hemotropic *Mycoplasma* infections, and clinically healthy dogs

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Background. The knowledge regarding pathogenesis in acute *Babesia* (*B.*) *canis* infections and acute hemotropic *Mycoplasma* infections is limited. Interleukin (IL)-8, IL-10, and Monocyte Chemotactic Protein (MCP)-1 were associated with disease severity in canine *B. rossi* infections, and IL-8 and MCP-1 with *B. canis* infections. Significant immunological reactions are expected in acute infectious diseases compared with healthy ones.
Objectives. To investigate IL-8, IL-10, and MCP-1 concentrations in dogs with acute babesiosis and hemoplasmosis compared to healthy dogs.
Material and Methods. Sixty-seven dogs were included in the prospective study; 31 tested positive for *B. canis* by PCR (SG I), 17 tested positive for hemotropic *Mycoplasma* by PCR (SG II), and 19 healthy dogs (SG III). IL-8, IL-10, and MCP-1 concentrations in serum were measured by the MILLIPLEX® Canine Cytokine Magnetic Bead Panel (Merck, Germany). Coinfections with other vector-borne pathogens and comorbidities were ruled out.
Results. Lower IL-8 concentrations were found in SG I compared with SG II (P=0.002) and SG III (P=0.008). Higher IL-10 concentrations were seen in SG I compared with SG II and III (P<0.001 each), as well as higher MCP-1 concentrations (SG I-II P=0.001, SG I-III P<0.001). No statistically significant changes were observed for IL-8, IL-10 (P=1.000 each), and MCP-1 (P=0.762) when comparing SG II with SG III.
Conclusion. *Babesia canis* infections result in a mixed, statistically different cytokine response, while hemoplasmosis showed no significant difference compared to healthy dogs. Targets for novel therapeutic strategies in canine babesiosis may be identified.

Keywords: canine babesiosis, hemoplasmosis, immunology

Preliminary results on leukocyte differentiation in rabbits using the Mindray BC-60R analyzer

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Background. The number of pet rabbits has increased in recent years, which has made them more prevalent in veterinary practices and increased the demand for dedicated services[1].
Objectives. To evaluate the performance of the Mindray BC-60R hematology analyzer in differentiating leukocytes types in rabbits.
Material and Methods. Blood sample form 17 rabbits were processed between September 2024 and May 2025. Complete blood count (CBC) was performed on Mindray BC-60 R hematology analyzer and manual differential count was carried out on May-Grunwald-Giemsa and Diff Quick stained blood smears. Spearman's rank correlation, Passing-Bablok regression analysis, and Bland-Altman plots were used to assess agreement and accuracy. $P<0.05$ was considered statistically significant.
Results. Plots were investigated for outliers and removed from data set. After their exclusion, heterophiles and lymphocytes presented an excellent correlation ($r=0,97$ and $r=0,96$; $p<0,01$), a fair correlation for monocytes ($r=0,7$; $p<0,01$) and eosinophils correlated poorly ($r=0,33$; $p>0,05$). A proportional bias was observed for monocyte count. Further investigation of the outliers revealed that heterophiles with toxic changes were underrecognized and misclassified by the analyzer, often being counted as monocytes or basophils. Moreover, myeloid precursor were characterized as lymphocytes.
Conclusion. The Mindray BC-60R analyzer showed good overall accuracy for leukocyte differentiation in rabbits, except in cases involving heterophils toxic changes and myeloblasts. Evaluation of blood smear in rabbits is important to confirm analyzer's result.

Keywords: Mindray BC-60R, method comparison, rabbit, toxic changes

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Validation of home-use urinary screening kits for hematuria and pH detection in cats

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Background. Early detection of hematuria and changes in urinary pH is essential, as it allows timely intervention before serious health problems develop.
Objectives. Validation of home-use urinary kits for detecting hematuria and pH changes in cats.
Material and Methods. Urine samples from 59 cats (healthy and diseased) were analyzed with dipstick strips and with the two kits where hematuria and pH were assessed at 5 minutes (T0), 2 (T1), 4 (T2), and 24 hours (T3). Sensitivity, specificity, and diagnostic accuracy of the kits were determined, and Kruskal-Wallis and Mann-Whitney tests were performed, with significance set at $p<0.05$.
Results. Sensitivity and diagnostic accuracy for hematuria kit were ≤50% and <70%, respectively and specificity was 100% in in all the time points. The kit was not able to detect mild hematuria (1+ or 2+). At T0 and T1, samples with 4+ hematuria had higher scores than those with lower hematuria ($P<0.001$), however this difference disappeared at T2 and T3. The pH determination kit showed sensitivity >90%, specificity >75% and diagnostic accuracy >80% in all the time points for the identification of alkaline pH. It also gave different results in samples with physiologic pH, alkaline pH and acidic pH at all time points ($P<0.001$) and no significant differences were seen in the pH scores at the different time points.
Conclusion. The urinary kit for the identification of hematuria is quite accurate solely in patients with severe hematuria, only when evaluated shortly after urination. Alkaline pH detection remained accurate even after 24 hours.

Keywords: urinalysis, screening, hematuria, pH, validation

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Are cytology slides and blood smears reliable materials for molecular diagnosis in veterinary medicine?

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Background. Air-dried cytology slides offer stable, high-quality DNA for molecular applications. In human[1] and veterinary medicine[2], they support techniques like PCR and PARR, enabling fast and accurate diagnosis of infections and neoplasia.
Objectives. Assess the suitability of cytological specimens from patients infected with vector-borne pathogens through qPCR, by analyzing parasite load, host DNA content, and temporal performance metrics.
Materials and methods. qPCR was performed on material recovered from blood smears, lymph nodes, skin lesions cytology slides, and EDTA whole blood from two dogs with leishmaniosis and ehrlichiosis. Internal controls were included. Cycle threshold (Ct) values were used to estimate parasite load and sample cellularity.
Results. In the *Leishmania*-infected dog, qPCR returned positive results for all samples. Wholeblood (Ct = 18.66) and lymph node (Ct = 17.31) samples indicated high parasite loads, while the skin sample showed a moderate load (Ct = 27.85). Higher Ct values for the internal control in skin (Ct = 24.51) compared to blood and lymph node (Ct ~14.7) point out lower cellularity. In the *Ehrlichia*-infected dog, EDTA whole-blood parasite load (200µl) was approximately tenfold higher than in the material recovered from cytology slides.
Conclusions. Recovered material from cytology slides with vector-borne pathogens is a reliable sample type for PCR, allowing confirmation or exclusion of cytologically suspected pathogens, and serves as an alternative when fresh samples are unavailable
Keywords. vector-borne pathogens, *Leishmania spp.*, qPCR, glass slides, *Ehrlichia canis*

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Evaluation of nucleic acid stability in blood smears for retrospective molecular analysis

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Background. In retrospective studies, stained cytologic slides are frequently the only specimens available. To establish their utility in molecular diagnostics, it is essential to validate the workflows for nucleic acid extraction and purification, as well as to confirm the long-term stability of the nucleic acids[1-2].
Objectives. Assess nucleic acid stability on blood smear slides stored at room temperature for qPCR analysis.
Materials and methods. Sixteen MGG-stained blood smears from an *Anaplasma phagocytophilum*-positive dog were stored at room temperature and analyzed weekly in duplicates over four weeks period. Biological material was rehydrated, harvested, and nucleic acids extracted. qPCR targeting pathogen (FAM), inhibition control (HEX), and β-actin housekeeping gene (Cy5) was employed for monitoring stability.
Results. Cycle threshold (Ct) values in HEX channel (mean=23.9; SD=0.09) confirmed the absence of PCR inhibition across all samples. Detection of pathogen over four weeks indicated adequate stability of nucleic acids on stained slides stored at room temperature. Cy5 channel, targeting β-actin, demonstrated minimal degradation of overall genetic material. Minor variability in Ct values was attributed to inconsistent recovery of sample material during the scraping process.
Conclusions. MGG-stained slides retain amplifiable nucleic acids at room temperature for at least four weeks, supporting their use in retrospective PCR-based diagnostics.
Keywords. hematology, *Anaplasma phagocytophilum*, qPCR, slide stability

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Renal mucormycosis due to *Cunninghamella bertholletiae* infection in a cat

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Background. *Cunninghamella bertholletiae (CB)* is a rare fungus from the *Mucorales* order, found worldwide[1]. In humans, it mainly affects immunocompromised individuals, particularly those on chemotherapy or steroids, with infection occurring primarily via the respiratory tract[1-2]. CB is not known to infect animals other than cetaceans[3].
Objectives. To describe a clinical case of a cat diagnosed with renal mucormycosis.
Materials and methods. An 8-year-old spayed female DSH cat with a history of asthma was presented to a veterinary facility with a history of chronic vomiting and anorexia. Diagnostic evaluations included CBC, serum biochemistry, SNAP Feline Triple viral screening, radiographs, abdominal ultrasound (US), ultrasound-guided fine-needle aspiration (US-FNA), and histopathology. Molecular analysis of formalin-fixed, paraffin-embedded tissue (FFPET) was performed at Robert Koch Institute, Germany.
Results. CBC revealed non-regenerative, moderate anemia, mild leukocytosis with left-shift neutrophilia and eosinophilia. Serum biochemistry showed azotemia and hyperglobulinemia. US examination revealed an enlarged right kidney with irregular margins, heterogeneous cortex, and a dilated pyelocaliceal system. US-FNA of the kidney revealed pyogranulomatous inflammation with fungal elements exhibiting broad, sparsely septate hyphae with irregular walls and right-angle branching. Histopathological evaluation confirmed the cytological diagnosis, and PCR from FFPET identified CB as the causative agent.
Conclusions. This case report is the first to identify a renal mucomycosis caused by *CB* in a cat.
Keywords. fungal, kidney, mucormycosis, *Cunninghamella bertholletiae,* zygomycetes

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Serum proteins and effusions: which cut-off really matters?

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Background. Hypoalbuminemia is traditionally associated with risk of low-protein transudate (LPT). While the role of other proteins in maintaining oncotic pressure is acknowledged, it remains underexplored.
Objectives. This retrospective study aimed to evaluate the incidence of LPT in hypoalbuminemic dogs and compare the predictive value (AUC) of albumin, globulins, and total protein (TP) for LPT formation.
Material and methods. Dogs (07/2022–02/2025) with albumin <20 g/l and diagnostic imaging were included. Based on albumin levels, dogs were classified as having mild (15.0–19.9 g/l), moderate (13.0–14.9 g/l), or severe (<12.9 g/l) hypoalbuminemia. Presence and type of effusion were recorded. ROC analysis determined optimal cut-off values for TP, albumin, and globulins.
Results. A total of 182 dogs were identified as having mild (n=156), moderate (n=18), or severe (n=8) hypoalbuminemia. Effusion was detected in 87 dogs (46%): 40% with mild, 61% with moderate, and 63% with severe hypoalbuminemia. In 28/87 dogs, fluid volume was insufficient for analysis. LPT was found in 3%, 22%, and 38% of dogs with mild, moderate, and severe hypoalbuminemia, respectively. If the combined severe and moderate groups, dogs with LPT had median albumin of 13.0 g/l and globulins of 14.9 g/l, compared to 14.0 g/l and 30.9 g/l in dogs without effusion. AUC values (all groups) were 0.527 (TP), 0.811 (albumin), and 0.931 (globulins). Globulins cut-off ≤25.2 g/l had 100% sensitivity and 75% specificity for LPT prediction.
Conclusion. In hypoalbuminemic dogs, globulin concentration ≤25.2 g/l is strongly associated with development of LPT.

Keywords: *effusion, transudate, globulins, albumin*

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Urinary ALP and GGT in dogs with neoplasia: preliminary data

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Background. Various mechanisms may contribute to tubular injury in dogs with neoplasia or those undergoing antineoplastic treatment.
Objectives. This study aimed to evaluate urinary gamma-glutamyltransferase (uGGT) and alkaline phosphatase (uALP) in dogs with neoplasia before, during, and after oncologic therapy.
Material and Methods. Urine samples were collected from three groups of dogs: dogs with neoplasia before treatment (NT, n = 19), dogs in complete remission without treatment (R, n = 25), and treated dogs (T, n = 33). Group T was further divided into dogs in remission (T-R, n=13) and dogs without remission (T-nR, n=20). Urinary GGT and ALP were measured and normalized to urinary creatinine concentration (uGGT/uCr and uALP/uCr). Comparisons were made using the Mann-Whitney U test.
Results. Median uALP/uCr values were as follows: NT (0.78 U/g creatinine), R (0.67 U/g), T (0.42 U/g), T-R (0.47 U/g), and T-nR (0.40 U/g), with no significant differences among groups. For uGGT/uCr, the values were: NT (46.7 U/g), R (30.1 U/g), T (47.6 U/g), T-R (28.6 U/g), and T-nR (56.0 U/g). Dogs in remission (R) had significantly lower uGGT/uCr than NT ($p = 0.007$) and T ($p = 0.02$) groups. A significant difference was also observed between R and T-nR ($p = 0.03$), but not between R and T-R ($p = 0.08$).
Conclusion. Dogs with neoplasia prior to or during treatment, particularly those not in remission, may have slightly elevated uGGT/uCr values compared to dogs in complete remission without therapy.

Keywords: urine, uGGT, uALP, neoplasia, dog

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Ki-67 as a Prognostic Marker in Feline Lymphoma: Preliminary Findings Using Immunocytochemistry

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Background: Ki-67 is a nuclear protein expressed during cell proliferation, commonly investigated as prognostic marker in canine and human lymphomas, and to a lesser extent in feline.

Objectives: To evaluate whether Ki67%, assessed by immunocytochemistry (ICC), is associated with clinicopathological features and prognosis in feline lymphoma.

Material and methods: Multi-institutional cytological samples of feline lymphoma were retrieved. Cases were included if had clinical data available. Ki-67% was assessed by ICC, both as a continuous and categorical variable. Associations between Ki67% and investigated variables were evaluated using the Kruskal-Wallis test. Lymphoma-specific survival (LSS) was analysed in chemotherapy treated cases using Kaplan–Meier analysis and log-rank test. Associations were evaluated in the entire cohort and within cell-size subgroups (small, intermediate, large).

Results: One hundred and twenty-five cases of feline lymphoma were analysed (12 small cell, 18 intermediate cell, 3 large granular, and 91 large cell lymphomas). Histological data was available in 35 cases. Forty-five cases were treated with chemotherapy and included in LSS. Ki-67% showed no significant association with FIV/FeLV status ($p = 0.169$), treatment response ($p = 0.299$), cell size ($p = 0.265$), age ($p = 0.979$), or histological grade ($p = 0.216$). However, Ki-67% differed significantly between anatomical locations ($p = 0.006$) and clinical stage ($p = 0.001$). No significant association was found between Ki-67% and LSS ($p = 0.62$).

Conclusion: Ki-67 does not appear to be a reliable standalone prognostic marker in feline lymphoma. However, significant variation by location and stage support further studies exploring location-specific cut-offs to clarify its clinical significance.

Keywords: Cat, Prognostic marker, Cell proliferation, Survival

Molecular genetic characterization of 84 canine tumours by commercial sequencing panels

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Background. Molecular genetic detection of tumour mutations is used, among others, for individualised therapy in human oncology, but is regarded as new in veterinary medicine.[1-4]
Objectives. This study aims to assess the results of sequencing panels from canine tumour samples in relation to potential treatment benefits.
Material and Methods. Samples from 84 canine tumours (19x cytology, 65x FFPE-tissue) were sent to the USA for molecular genetic characterisation utilising Searchlight DNA®, Anivive (n=69) or Canine CGP®, VetOmics (n=15). Eleven cases were excluded due to failed DNA isolation (6x insufficient sample material, 3x decalcified tissue, 2x non-identified reasons). Included were 26 different tumours with spindle cell sarcomas (n=9), anal sac carcinomas (n=7), hemangiosarcomas (n=6), mammary carcinomas (n=6), and mast cell tumours (n=6) being most common. Detected mutations and corresponding therapy recommendations were analysed retrospectively.
Results. A total of 86 distinct mutations were reported (up to 40 mutations/tumour). The most frequent mutations were located in the genes TP53, ATM, ATRX, FLCN, KMTD2, CDKN2B, ERF1, FANCA, PTEN, and MEN1. In 78% of the tumours, up to 13 therapeutically relevant mutations were identified. Based on the molecular genetic profiles, 29 different therapeutic agents (olaparib, sirolism, platinum-based chemotherapies,a.o.) were recommended. In 19 cases, no therapeutically targetable mutations could be detected.
Conclusion. A comprehensive mutation analysis can unveil new opportunities for personalised therapy in specific tumour types if relevant mutations are present. While limited data regarding dosage, efficacy, risks, and treatment outcomes for these agents is available for dogs, further validation is necessary through experienced veterinary oncologists.

Keywords: dog, mutation analysis, oncogenetics, personalised tumour therapy, chemotherapy

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Reference intervals from small samples: HARISS versus robust versus parametric or nonparametric methods

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Background. The current veterinary guidelines recommend using the robust method (RM),or alternatively the parametric or nonparametric method (PNPM) depending on the sample data's distribution, to build RI from small samples.[1] A web application (HARISS) uses visual inspection of distribution histograms to more sophisticatedly adjust the statistical method based on the small sample data distribution (Gaussian, lognormal, or left-skewed).[2] HARISS's decision rules led to more accurate RI based on a simulation study, which assumed Gaussian, lognormal, or left-skewed population distribution.[3]
Objectives. This study aims to compare HARISS, RM, and PNPM in terms of RI accuracy when small samples are extracted from populations with various distributions.
Material and Methods. Samples of n = 40, 50, or 60 values were randomly selected 50 times from four simulated populations of 5,000 values per distribution. Seven distributions were simulated: Gaussian, Student, lognormal, right-skewed, left-skewed, bimodal, and irregular. HARISS, RM, and PNPM were used to build RI, and accuracy was compared using repeated-measures ANOVA.
Results. Overall, HARISS led to significantly more accurate RI lower and upper limit estimations ($P<0.001$), independent of the population distribution and sample size. However, the accuracy of the three methods evaluated varied with the population distribution. HARISS was significantly more accurate than RM to build RI from Gaussian and Student population samples, and significantly more accurate than PNPM to construct RI from skewed population samples.
Conclusions. Overall, HARISS led to the most accurate RI limit estimations from small samples extracted from the seven population distributions simulated in this study.

Keywords: *Artificial Intelligence, Convolutional Neural Network, Data distribution, ASVCP, Reference range*

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Buffy coat basophils in canine patients with neoplasia: silent, but not mute.

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Background. Basophilia is a rare haematological abnormality in dogs, recently associated with neoplasia in 32% of cases, particularly haematopoietic malignancies. In human oncology, basophils exhibit diverse roles depending on tumour type. Buffy coat preparations have previously proven useful in identifying feline mast cell tumours (MCTs).
Objectives. This pilot study prospectively evaluated basophil counts in buffy coat smears from dogs diagnosed with neoplasia.
Material and Methods. For each case, 4–6 buffy coat smears were prepared from residual EDTA-anticoagulated blood samples. The highest quality slide was selected for Wright staining and evaluated at 400×/1000× magnification. Basophils were identified based on the presence of prominent purple to deeply basophilic granules. Neoplastic conditions included MCTs, lymphoproliferative disorders (LDs) and mammary gland tumours (MGTs). All MCTs and MGTs were confirmed by histopathology.
Results. Twenty-eight dogs were included: 8 with MCTs, 9 with LDs, and 11 with MGTs. No statistically significant differences in the number of basophils per buffy coat were observed among the groups. Median basophil counts for MCTs, LDs and MGTs were 6.5 (range 0–3080), 2 (range 0–17) and 1 (range 0–239), respectively. The four highest basophil counts were observed in cases of visceral MCT, grade II MCTs and one dog with two invasive mammary carcinomas.
Conclusion. Canine basophil counts in buffy coat smears did not significantly differ among the neoplastic groups. However, the highest values were recorded in MCTs, particularly those with aggressive clinical features, suggesting a potential association warranting further investigation.

Keywords. *buffy coat, basophilia, cancer, mastocytoma, dog*

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White Blood Cells and Platelets Alterations in Diarrheic Neonatal Lambs with or without Signs of Bacteremia

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Introduction:
Neonatal diarrhea in lambs, commonly attributed to *Cryptosporidium spp.*, rotavirus, and *Escherichia coli*, is frequently complicated by secondary intestinal overgrowth of *E. coli*, often leading to bacteremia. However, data on white blood cell (WBC) and platelet (PLT) changesduring diarrhea, and their value as bacteremia indicators are limited.
Objective:
To investigate WBC and PLT alterations during neonatal diarrhea and assess their potential utility as biomarkers of bacteremia.
Materials and Methods:
Sixty-seven diarrheic neonatal lambs, clinically monitored from birth to 20 days, werecategorized based on clinical signs into bacteremic (DB; n=23) and non-bacteremic (D; n=44) groups. Blood samples for complete blood count were collected on day 2, at 48-hour intervals from diarrhea onset (Drh-1, Drh-3, Drh-5), at 24 hours post-recovery, and on day 20.
Results:
In group D, WBC and lymphocyte counts increased significantly from day 2 to Dhr-1, followed by a significant WBC and neutrophil decline by Drh-3 ($P<0.05$). Group DB showed similar but not significant WBC and neutrophil trends ($P>0.05$). PLT counts increased progressively in both groups, with significantly elevated values by day 20 ($P<0.05$). PLTs also increased significantly from day 2 to Drh-1 in both groups. Importantly, between Drh-1 and Drh-3, PLTs rose significantly in bacteremic lambs ($P<0.05$), but not in non-bacteremic counterparts.
Conclusions:
Neonatal diarrhea is linked to early WBC and lymphocyte elevation, neutrophil drop on Drh-3and sustained thrombocytosis. A marked PLT rise within the first three days of diarrhea may serve as an early hematological indicator of bacteremia, supporting its use in clinical decision-making.

Basophils in canine and feline buffy coats: how rare do they dare to be?

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Background. Basophils are the rarest population of blood leukocytes and their accuratequantif ication remains challenging, even with advanced hematology analyzer software. Recently, amo ng canine patients with basophilia, Chihuahuas appeared overrepresented. Buffy coat smears have previously proven useful for detecting canine and feline mast cellsand may aid in detectio n of basophilia.
Objectives. This prospective study evaluated basophil counts in buffy coat smears fromhealthy non-Chihuahua dogs (NCH), Chihuahuas with non-neoplastic conditions (CH), and healthy cats (HC).
Material and Methods. EDTA-anticoagulated blood samples were collected from 10 NCH, 10 CH, and 10 HC. For each animal, 4–6 buffy coat smears were prepared, and the highestquality slide was selected for staining (Wright's stain for dogs; Hemacolor for cat s). Smearswere examined under 400×/1000× magnification.Canine basophils were identified by prominent purple to deeply basophilic granules within a lavender cytoplasm; feline basophilsby their characteristic uniformly lavender granules.
Results. Buffy coat basophils showed a statistically significant difference among the groups (p = 0.0004), with median basophil counts per buffy coat of 1 (range 0–6) and 88 (range 59–379) in NCH and HC, respectively (p = 0.0003). No significant difference was observedbetween NCH and CH groups (p = 0.5498).
Conclusion. Feline buffy coat samples contained 10- to 100-fold more basophils than caninesamples, suggesting both a physiological interspecies differen ce and potentially lowerstaining affinity of canine basophils. Chihuahuas with non-neoplastic conditions did not show elevated basophil counts compared to the canine controls.

Keywords. *buffy coat, basophilic, healthy, dog, cat*

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Cytologic examina/on of the liver in dogs with non-associative immune- mediated hemolytic anemia

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Background. The cytologic examinaJon of the liver (CEL) is proposed in the diagnostic workout of dogs with immune-mediated hemolytic anemia (IMHA) to rule out triggering factors.
Objective. This study aimed to describe the changes in CEL and their possible association with clinicopathological data in dogs with non-associative IMHA (naIMHA).

Materials and Methods. The medical records of dogs diagnosed with naIMHA (IMHA ACVIM consensus statement) were reviewed (2014-2025). Inclusion criteria included abdominal ultrasound and CEL upon admission. Dogs treated with steroids before admission or with known hepatic diseases were excluded. CEL was performed by a board-certified clinical pathologist; cholestasis and hepatocellular damage (HD) were graded. Data were analyzed with nonparametric statistics and reported as median and range.

Results. Thirty-two dogs were enrolled. Cholestasis was idenJfied in 21/32 dogs (65.6%), and HD in 26/31 (81.2%). Dogs with cholestasis had significantly higher serum bilirubin concentraJons (BILC; 2.07 mg/dL; 0.46–59.02) compared to those without cholestasis (0.5 mg/dL; 0.18–2.07; P<.0001). Increased BILC was associated with the severity of cholestasis (P<.0001). ROC curve analysis showed an AUC of 0.88 (95% CI: 0.76–1.00) with a BILC cut-off value of 0.79 mg/dL, providing 81% sensiJvity and 81.8% specificity for detecJng cholestasis. The hematocrit values were not significantly different between dogs with and without cholestasis, or between those with and without hepaJc damage.

Conclusions. HD and cholestasis are common findings in dogs with naIMHA. Cholestasis is strongly associated with increased BILC, suggesJng that factors beyond the severity of hemolysis should be considered when interpreJng BILC in dogs with naIMHA.

Keywords: IMHA, cholestasis, hyperbilirubinemia, cytology, dog

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Evaluation of automated hematology analysis using Sysmex XN-1000V in healthy and diseased long-tailed chinchillas (Chinchilla lanigera)

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Background The long-tailed chinchilla is a hystricomorph rodent from South America. Hematologic features of chinchillas are often reported to be similar to those of other rodents, and neutrophils are frequently described as hyposegmented.

Objectives: Evaluate the morphological characteristics of scattergrams (White blood cell Differential (WDF) and White cell Nucleated count and Red blood cell (WNR) channels) on Sysmex XN- 1000V in healthy and diseased long-tailed chinchillas with particular emphasis on cases of left shift and toxic changes.

Material and methods: A retrospective evaluation of blood smears and automated hematology results, including scattergrams from clinical cases presented between 2018 and 2025, was performed. Animals were categorized as healthy, moderately or severely diseased based on the clinical findings.

Results: 29 chinchillas were included. The predominant leukocytes in healthy chinchillas are neutrophils, and no hyposegmentation is noted. Severely sick animals showed higher total leukocyte counts and band neutrophils compared to healthy and moderately ill animals. Morphological features, such as giant cells and bizarre nuclear morphology, are noted only in severely ill animals. In healthy chinchillas, leukocyte subpopulations are not correctly classified in the WDF channel. A manual re-gate is needed for the correct classification of leukocyte subpopulations. The WNR channel correctly identified basophils and nucleated red blood cells in healthy and diseased chinchillas.

Conclusion: The preliminary results of this study suggest that manual re-gating may be necessary for classifying white blood cell subpopulations, including those in healthy animals. A manual white blood cell differential remains mandatory in this species.

Keywords: Chinchillas, Hematology, Sysmex XN-1000V, WDF, WNR, toxic changes

Oxidative stress markers and acute phase proteins in dairy calves undergoing different vaccination protocols against Bovine Respiratory Disease

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Background. Bovine Respiratory Disease (BRD) represents a major health and economic concern in dairy cattle production.

Objectives. To evaluate the effect of different BRD vaccination protocols on oxidative stress markers and acute phase proteins response in dairy calves.

Materials and Methods. Three groups of calves were enrolled: control (CTR, n = 9), intranasal vaccine (INT, n = 25), and combined intranasal plus parenteral vaccine (VAC, n = 15). Serum samples were collected at T0 (10±2 days of life, dof), T1 (17±2 dof), T2 (31±2 dof for CTR/INT; 38±2 dof for VAC), and T3 (45±2 dof for CTR/INT; 52±2 dof for VAC). INT and VAC calves received the intranasal vaccine at T0, while VAC calves also received parenteral vaccination at T1 and a booster at T2. Serum Biological Antioxidant Potential (BAP), derivatives of Reactive Oxygen Metabolites (dROMs), Paraoxonase-1 (PON-1), and Serum Amyloid A (SAA) were measured. Oxidative Status index (OSi) was calculated as dROMs/BAP*100. Lung Lesion Score (LLS) assessed health status at T0 (LLS<10.5 = Healthy; LLS≥10.5 = Diseased). A mixed model included the fixed effects of group, time, health status, and their interactions. Post-hoc comparisons among least square means were adjusted with Bonferroni correction.

Results. PON-1 increased over time ($p<0.001$), likely reflecting hepatic maturation. SAA showed a group*time*health interaction ($p=0.03$), but levels remained clinically irrelevant. BAP decreased after the first intranasal dose in vaccinated animals (time and group*time effects) ($p<0.001$). dROMs and OSi showed no significant changes, but levels were lower in non-vaccinated animals.

Conclusion. Intranasal vaccination may consume antioxidant potential. PON-1 increase likely reflects liver development.

Keywords: dairy cattle; acute phase proteins; oxidative stress; vaccine; vaccination protocol

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Cytological composition of bone marrow in Athymic Nude RH-FoxN1^{mu} rats

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Background. Long-term pharmacology and safety evaluation of innovative human-derived cell therapy products require the use of immunocompromised animals to avoid immune-mediated destruction of injected human-derived cells. Bone marrow smear microscopic examination is an essential part of assessment of the hematopoietic system. There is no available published data regarding the cellular composition of bone marrow in athymic RH-FoxN1^{rnu} rats.
Objectives. The purpose of this study was to evaluate the cytological composition of the bone marrow of nude rats and to compare it with published data from non-athymic rats.
Materials and Methods.
28 clinically healthy RH-FoxN1^{rnu} rats (11 males, 17 females) provided by Envigo RMS, Netherlands, aged 9.5 months, were included in this study. Bone marrow smears were prepared after longitudinal incision of the proximal femoral bone. Complete differential cell count (approximately 300 cells) and morphological assessment at high power magnification were performed. Myeloid to erythroid (M:E) ratios and the percentage of each individual cell types were calculated.
Results. M:E ratios ranged from 0.66 to 3.16 (mean: 1.62). Mean percentage of lymphocytes was 11.96%. Mean percentage of eosinophilic cells (all stages) was 6.27%. Morphological findings were similar to those observed in Sprague-Dawley and Wistar rats.
Conclusion. When compared with other rat strains commonly used in toxicological studies, we observed an increase in eosinophils (all stages) and a decrease in lymphocytes. Theseresults provide preliminary data of cytological bone marrow findings in RH-FoxN1^{rnu} rats.

Keywords.
Nude rat, toxicology, bone marrow, hematotoxicity

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Assessment of plasma homocysteine levels in Canine Leishmaniosis

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Background. Clinical signs and laboratory findings are essential for diagnosing and staging canine Leishmaniosis (CanL). Recent studies indicate that dogs with leishmaniosis have significantly lower serum cobalamin concentrations than healthy dogs. Since homocysteine (Hcy) requires cobalamin for metabolism, its levels increase with cellular cobalamin deficiency

Objectives. This study evaluates plasma Hcy concentrations in dogs with CanL at various clinical stages and correlates Hcy levels with other disease features during follow-ups.

Results. We analyzed 67 samples from 26 dogs with confirmed CanL, including 12 females (7 spayed) and 14 males (6 castrated), aged 1.2 to 10.2 years. Results are presented as median [range]. Hcy levels were significantly lower in dogs with Leishvet stage 2 or higher (10.1 [1.9- 47.9] vs. 15.4 [5-47.9] µmol/L, p=0.024). Hcy was also lower with C-reactive protein >10 mg/L (9.6 [2.1-40.2] vs. 16.3 [1.9-47.9], p=0.006), haptoglobin >2 g/L (11.1 [1.9-47.9] vs. 17.0 [5- 39.4], p=0.014), and albumin/globulins ratio <0.5 (12.3 [2.1-42.4] vs. 14.9 [1.9-47.9], p=0.041). Plasma Hcy did not correlate with cobalamin concentration ($\rho_{Spearman}$=-0.05, p=0.7), and cobalamin levels showed no significant differences according to the tested criteria.

Conclusion. Decreased homocysteinemia was associated with CanL severity markers and was independent of cobalamin concentration. Parasite spoliation may contribute to its cysteine synthesis, enhancing antioxidant capacities and virulence. Further research is needed to evaluate the implications of decreased Hcy levels for the host.

Keywords: *Leishmania infantum, homocysteine, cobalamin, oxidative stress*

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Verification of the MAGLUMI 800 chemiluminescenceimmunoanalyzer for measurement of T4, TSH and cortisol in canine plasma samples

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Background. The Maglumi 800 (Snibe Diagnostic) immunoanalyzer has been introduced as analytical platform for endocrine assays in small animal practice. Following the discontinuation of the Immulite® 1000 (SIEMENS Healthcare Diagnostics), there is a need for validated and reliable alternatives to ensure accurate measurement of hormones such as total thyroxine (T4), thyroid-stimulating hormone (TSH), and cortisol. Testing the analytical performance of new platforms is essential to maintain diagnostic confidence and support clinical decision-making in veterinary endocrinology.

Objectives. To verify the Maglumi 800 performance for T4, TSH and cortisol measurements in canine plasma samples.

Material and methods. Canine plasma samples, comprising individual patient samples across a wide concentration range and pooled samples at clinical decision limits (1), were used to verify the Maglumi 800 platform. Verification included method comparison with the Immulite® 1000 analyzer, precision and detection limit studies, following ASVCP guidelines (2). The reagents assessed were Canine Total T4, Canine TSH and Cortisol (Maglumi®).

Conclusion. The Maglumi 800 immunoanalyzer showed acceptable analytical performance. However, a systematic error was observed when compared to the Immulite® 1000. Therefore, the platform is suitable for measurement of T4, TSH and cortisol in canine samples, provided that reference values are recalculated.

Keywords: *Verification, T4, TSH, Cortisol, Chemiluminescence, Maglumi 800*

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Haemostatic abnormalities in dogs suffering from haemangiosarcoma

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Background. Haemangiosarcoma in dogs is frequently associated with haemostaticabnormalities, but systematic studies are rare (1).
Objectives. To define haemostatic abnormalities in dogs with haemangiosarcoma regarding a wide range of haemostatic tests.
Patients. 30 dogs were included with histologically confirmed tumour in the spleen (n=18), in spleen and liver (n=10) or liver (n=2).In9 cases additional tumour locations were found, 22 dogs were suffering from haemoperitoneum.
Material and Methods. Platelet count was measured automatically. Group tests andindividual coagulation factor activities were measured coagulometrically.
Fibrinogen concentration was measured using a kinetic photometer assay. Chromogenic tests were usedfor antithrombin, protein C, plasminogen, alpha2-plasmin inhibitor, and soluble fibrin.Fibrin(ogen) degradation products (FDPs) were measured with a latex agglutination test. Results were compared with a control group of healthy dogs using the Mann-Whitney U test.
Results. Two third of patients showed reduced platelet counts and abnormalities of theplasmatic coagulation were even more frequently present. Fibrinogen was decreased in 13 (43%) and increased in 3 (10%) patients. Except factor IX, all individual factor activities weredecreased compared to the control group. Activities of inhibitors antithrombin (median: 67%, reference range: 81–116 %) and protein C (median: 60%, reference range: 68–139 %) were also significantly reduced. Concentrations of FDPs and soluble fibrin were increased in 28 (93%) or 26 (87%) of the patients, respectively.
Conclusion. The results underline the importance of haemostatic changes in dogs withhaemangiosarcoma. Nearly all patients revealed laboratory diagnostic indications ofdisseminated intravascular coagulation and hyperfibrinolysis.

Keywords: *DIC, hyperfibrinolysis, soluble fibrin, blood coagulation*

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Accuracy of hemoglobin instrumental measurement in little owls (*Athene noctua)*

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Background. Automated hematology in birds is challenging due to nucleated erythrocytes, which can affect accuracy of blood parameters. Nevertheless, reliable hemoglobin values are essential for health and physiology assessment in wild birds. In most animal species, hemoglobin concentration is expected to be approximately one-third of the packed cell volume (PCV).
Objectives. To assess the accuracy of hemoglobin measurement in little owls using two instruments and compare them the hemoglobin estimated obtained from PCV.
Material and Methods. Heparinized blood from 59 little owls hosted in a rescue center was collected for health monitoring and analyzed within 24 hours. Hemoglobin concentration was determined using the Hemocue201+ portable hemoglobinometer and the Sysmex XN-1000Vanalyzer, which also provides hematocrit. PCV was determined by microcentrifugation. Linearity under dilution (LUD), method comparison and correlation between measured and estimatedhemoglobin were evaluated.
Results. LUD showed strong correlation between expected and obtained hemoglobin values forboth instruments ($r>0.87$ for each dilution). Bland-Altman analysis revealed a significant negative bias (-3.7; $p < 0.0001$) with Hemocue201+ yielding consistently higher values than Sysmex XN-1000V. Despite a strong correlation between the two methods ($r = 0.855$), Passing & Bablokregression indicated both constant and proportional errors (intercept 1.302, 95% CI: 0.01-2.42;slope 0.61, 95% CI: 0.53 – 0.73). Sysmex XN-1000V hematocrit did not differ from PCV.
Conclusion. Hemocue201+ appears more accurate than Sysmex XN-1000V in measuring hemoglobin in little owls, possibly due to avian erythrocyte resistance to lysis. However, estimating hemoglobin as one-third of the Sysmex XN-1000V hematocrit is a viable alternative.

Keywords: *Hemoglobin, Little owls, Sysmex XN-V1000, Hemocue201+*

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Lymphocyte clonality testing in feline intestinal lympho-plasmacellular infiltration: friend or foe?

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Background. Lymphoplasmacytic enteropathy and alimentary small cell lymphoma are most common gastrointestinal diseases in cats. Clonality testing (PARR) detects clonal B- or T-lymphocyte populations.
Objectives. In this retrospective study on feline chronic enteropathy, PARR was added to the diagnostic work-up. We asked if analyzing the CDR3 repertoire of the B- and T-cell receptors can be seen as a suitable stand-alone method.
Material and Methods. Based on histopathological findings of mild or moderate lymphoplasmacytic intestinal infiltration, 34 archived small intestine feline patient samples were analyzed. Samples were assayed by clonality testing, together with immunohistochemistry (CD20, CD3), and histopathological examination.
Results. None of the 34 samples subjected to clonality testing showed B-cell clonality, whereas in 23 cat patients, T-cell clonality was detected. In a lymphoplasmacyticinflammation, the infiltration is usually confined to the *Lamina propria mucosae* and whereas in lymphoma, cells often infiltrate beyond the mucosa. Mild to moderate lymphoplasmacytic infiltration of the *Tela submucosa* was considered as potential indication for early lymphoma, hence explaining the high number of monoclonal PARR results. In 15 out of 34 cases, mild lymphoplasmacytic infiltration was confined to the *Lamina propria mucosae* and 80 % of these samples showed a 50:50 ratio of T- and B-cells.
Conclusion. This study underlines that PARR can be used as adjunct diagnostic approach in feline patients with chronic enteropathy. However, this technique is far from being a stand-alone tool and the obtained results must be interpreted in the context with the patient's history, clinical status and histopathological examination.

Keywords: feline, chronic enteropathy, alimentary small cell lymphoma, lymphoplasmacytic infiltration, PARR, clonality testing

Concurrent Rabies and Canine Distemper Infections in Endangered Ethiopian Wolves: Diagnostic and Conservation Challenges

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Background:
Ethiopian wolves (Canis simensis) are the most endangered wild canid species globally, with populations increasingly threatened by infectious diseases such as rabies virus (RABV) and canine distemper virus (CDV). This report documents a rare concurrent outbreak of both pathogens in a vulnerable highland population.

Case Description:
In March 2019, an Ethiopian wolf carcass was discovered in the Bale Mountains, Ethiopia. Several individuals in the population had shown neurological symptoms, respiratory distress, and acute mortality. Field necropsy, sample collection, and laboratory diagnostics confirmed the presence of both RABV and CDV. Techniques used included RT-PCR, ELISA, and immunohistochemistry. Histopathology revealed Negri bodies and eosinophilic inclusions, confirming dual infection.

Discussion:
The simultaneous detection of rabies and distemper viruses in this endangered species presents serious diagnostic and conservation challenges. Harsh field conditions complicated sample handling, but coordinated efforts between veterinary professionals and diagnostic laboratories allowed for timely disease confirmation and response. This case emphasizes the critical role of clinical pathology and field diagnostics in managing wildlife health emergencies.

Conclusion:
This outbreak underscores the need for ongoing disease surveillance and strategic vaccination of wildlife populations. Effective conservation depends on strong collaboration between field veterinarians, laboratories, and wildlife protection authorities to reduce the impact of emerging infectious diseases.

Keywords: Ethiopian wolf, rabies, canine distemper, wildlife disease, diagnostics, conservation

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Establishing reference intervals for T4, T3 and reverse triiodothyronine (rT3) via LC-MS/MS in clinically healthy dogs

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Background. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) enables highly sensitive, accurate and precise simultaneous hormone measurements, thus becoming the gold standard in endocrine diagnostics[1]. To the authors` knowledge no LC-MS/MS-based reference intervals have been published for canine thyroxine (T4), triiodothyronine (T3) and reverse triiodothyronine (rT3).
Objectives. The objectives of this study were to establish LC-MS/MS-based reference intervals for T4, T3 and yet little explored rT3 in clinically healthy dogs, and to compare LC-MS/MS with routine chemiluminescence assays (CLIA) for T4 and T3 concentration measurements.
Material and Methods. Left-over serum samples from 97 clinically healthy dogs with unremarkable laboratory screening results were analyzed by LC-MS/MS (Xevo TQ-XS, Waters Eschborn, Germany). Reference intervals were calculated according to the guidelines of the American Society of Veterinary Clinical Pathology using “Reference Value Advisor” software (Biostatistiques). T4 and T3 results by LC-MS/MS and CLIA (T4: Immulite 2000 XPi, Siemens, Germany; T3: Advia Centaur XPT, Siemens, Germany) were compared.
Results. The following reference intervals were calculated: 13.77 – 59.46 nmol/l for T4, 0.777 – 2.196 nmol/l for T3 and 0.167 – 0.819 nmol/l for rT3. The average intra- and inter-assay-precision CVs (%) for T4, T3 and rT3 were 2.88, 7.52, 3.60 and 5.64, 6.17, 7.00,respectively. Pearson correlation coefficients (r) for T4 and T3 measurements by CLIA and LC-MS/MS were 0.90 and 0.71, respectively.
Conclusion. LC-MS/MS provides accurate measurements for T4, T3, and rT3, and shows strong correlation with CLIA for T4 in canine serum. The correlation was only moderate for T3 showing need for further investigation.
Keywords: *dog, T4, T3, rT3, reference interval, LC-MS/MS*

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Agreement between SYSMEX XN-V and Hemocue201+ in measuring hemoglobinfrom *Testudo hermanni*

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Background.
Hermann's tortoise (*Testudo hermanni*) is widespread across Mediterranean Europe. Hematological analyses are pivotal for health assessment in this species, where clinical signs are often subtle. The recently validated Sysmex XN-V hematology analyzer enables rapid blood cellcounts in this species, potentially improving diagnostic turnaround time. However, there are no information about the accuracy of hemoglobin measurement.

Objectives.
To assess theagreement between *Testudo hermanni* hemoglobin measurementobtained using Sysmex XN-V and the point – of – care hemoglobinometer Hemocue201+, already used for other mammalian and non – mammalian species.

Material and Methods.
Heparinized whole blood from 150 tortoises was collected for clinical monitoring purposes. All the samples were analyzed within 24 hours. Hemoglobin concentrationwas determined using Sysmex XN-V, which employs a cyanide-free sodium lauryl sulphatedetection method, and Hemocue201+, which uses sodium deoxycholate to lyse the erythrocytes.

Results.
The Bland – Altman difference plot revealed a statistically significant negative bias (BIAS: -0.75; $p < 0.0001$) with Hemocue201+ results consistently higher. The Passing & Bablok regression analysis indicated both constant and proportional errors (intercept 0.35, 95% CI: 0.19 – 0.58; slope 0.79, 95% CI: 0.75 – 0.82). A moderate correlation was found between the two methods ($r = 0.68$).

Conclusion.
Based on the assumption that hemoglobin values should approximate one – third of the packed cell volume, hemoglobin measured using Hemocue201+ gave the best results. The discrepancy between methods may stem from reduced erythrocyte lysis efficiency or differentstain specificity of the Sysmex XN-V to non – mammalian cells, possibly due to their differentphysico – chemical properties.

Keywords: *Hemocue201+, Hemoglobin, Sysmex XN-V, Tortoises*

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Comparison Of Manual And Automated Hematological Parameters In Hyacinth Macaws (Anodorhynchus Hyacinthinus) Using The Sysmex XN-1000V And The New PLT-F Channel

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Background. The Sysmex XN-1000V implemented a new channel (PLT-F) to quantify platelets and immature platelet fraction in mammals. Its application in birds appears promising. The hyacinth macaw (*Anodorhynchus hyacinthinus*), a vulnerable species, benefits from hematological monitoring under captivity. Manual hematological methods remain the standard in birds but are time-consuming; implementing an automated approach would represent a valuable advancement.

Objectives. To create a gating strategy for white blood cells (WBC) using the PLT-F channel, and to evaluate the performance of the Sysmex XN-1000V in measuring hemoglobin concentration (HGB) and hematocrit (HCT) in *A. hyacinthinus*, comparing results with manual methods.

Methods. Twenty-eight samples were obtained from 16 healthy adult hyacinth macaws. Manual methods included microhematocrit centrifugation, cyanmethemoglobin-based HGB quantification, and WBC counts using Natt-Herricks staining solution and improved Neubauer hemocytometer. Automated analysis was performed using Sysmex XN-1000V (3.07–00).
Results. Preliminary results showed that Sysmex HTC values correlated with manual measurements (r = 0.972) with a mean bias of 2.06% (95% CI: –0.50 to 5.62), indicating a systematic proportional bias. HGB values showed moderate correlation (r = 0.762) with a negative bias of –1.71 g/dL (95% CI: –4.83 to 1.40); three samples were excluded due to low volume. WBC counts using the PLT-F gate correlated moderately with manual counts (r = 0.772) with a mean bias of –0.801 ×109/L (95% CI: –6.77 to 5.17), also indicating a proportional bias.

Conclusion. Preliminary results support the use of Sysmex XN-1000V for automated hematological assessment in *A. hyacinthinus*, with clinically acceptable agreement to manual methods.

Keywords. Sysmex XN-1000V, PLT-F channel, avian hematology, hyacinth macaw, leukocyte count, gating strategy

Comparison of biochemical and electrophoretical evaluation of serum lipoproteins in assessing inflammation or oxidation in dogs

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Background. Lipid metabolism, oxidative phenomena, and inflammation are related to each other. Lipoproteins can be measured spectrophotometrically or using lipoprotein electrophoresis. Information on analytical or diagnostic performances of spectrophotometrical measurement in dogs is lacking.
Objectives. The aims of this study were: to validate in dogs two biochemical methods that measure serum HDL and LDL and to compare the results with those of lipoprotein electrophoresis; to assess the changes of HDL and LDL in different clinical conditions, with special emphasis on inflammation or oxidation, assessed by measuring CRP and PON-1, respectively.
Material and Methods. Intra- and inter-assay imprecision, linearity and storage effects of biochemical measurement of HDL and LDL were evaluated. Then, 80 samples classified in different disease groups were analyzed biochemically and electrophoretically.
Results. The two biochemical methods are precise and accurate, do not suffer from storage, and overlap to each other for HDL but not for LDL, likely due to a different analytical principle. The electrophoretic results do not correlate with the biochemical ones. Dogs with entero-hepatic diseases or inflammation had the lowest biochemical HDL and LDL concentrations but also low total cholesterol, total proteins, CRP and PON-1, suggesting that lipoprotein changes depend mainly on the decreased lipids. Conversely, the electrophoretic percentage of HDL decreases in dogs with inflammation and negatively correlates with CRP.
Conclusion. Biochemical measurement of HDL and LDL is precise and accurate, and it may be useful to investigate changes in canine lipid metabolism, while the electrophoretic separation is more appropriate to study and evaluate inflammation.

Keywords: *inflammation, oxidation, lipoproteins, dogs, biochemistry, electrophoresis*

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