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Welcome

Dear colleagues
Dear participants

We are pleased to welcome you in Athens for the 20th ESVCP-ECVCP meeting.

It is hard to believe that we have reached the milestone of 20 years regarding the organization of ESVCP scientific meetings.

What started as a one-day event two decades ago has now grown to a full four-day conference with a variety of scientific topics covering all aspects of Clinical Pathology.

In 2018, and nearly 10 years after our last meeting in Greece, the main topics of this event focus on Infectious Diseases and Toxicologic Clinical Pathology.

Infectious diseases are of significant importance to most clinical pathologists, who are confronted with their diagnosis almost on a daily basis.

The pre-congress day is dedicated to Toxicologic Clinical Pathology, which remains an intriguing area of our specialty and an integral part of training in Clinical Pathology.

As in every meeting, the Case Session and the Mystery Slide Session will offer new challenging and intriguing cases for all of us to solve.

Any scientific program would be incomplete without attractive accompanying social events. We believe that the magnificent venue location, in the heart of the historical center of Athens, will ensure that everyone will have a great time in Greece.

Enjoy your stay in Athens and have a great conference!

The local organizing committee

Zoe Polizopoulou
Labrina Athanasiou
Stratos Papakonstantinou
Organizers

European College
of Veterinary Clinical Pathology (ECVCP)

European Society
of Veterinary Clinical Pathology (ESVCP)
Our Sponsors

The ESVCP and ECVCP wish to express our sincere gratitude to our direct sponsors, without whom a meeting such as this could not be contemplated.

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Association for Comparative Clinical Pathology

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IDEXX BioAnalytics

EXHIBITOR

Tridelta Development Ltd.
Venue, Local Area and Transport

DIVANI PALACE ACROPOLIS

The 20th ESVCP-ECVCP will be held right by the Athenian Acropolis, at Divani Palace Acropolis in Athens, Greece. The Divani Palace Acropolis provides easy access to all areas both within and outside the city centre as it is very close to Athens Metro “Acropolis” station (200m).

http://www.20esvcp-ecvcp2018.gr/venue

LOCAL AREA

Within the historical area of Athens, this impressive deluxe hotel is in walking distance from the Acropolis museum, the ancient theatres of Herodes Atticus and Dionysos, Philopappou Hill, the Arch of Hadrian, the Temple of Olympios Zeus and Plaka (the oldest section of Athens and the most popular and picturesque area in Athens).

VISITING ATHENS

Athens is the historical capital of Europe, with a long history, dating from the first settlement in the Neolithic age. Athens is considered one of Europe’s safest capitals; its transportation network is user friendly; there are numerous museums and archaeological sites and hundreds of restaurants to satisfy every taste. Surrounded by a lining of stunning seas and mountains, Athens is filled with gems just waiting to be discovered. Located at the crossroads of three continents, the capital of Greece with an overall population of close to four million has often been the hub of many cultures. Characterized by a culture and people that are welcoming and hospitable, every visitor just feels at home. Athens is an ideal congress destination, combining state-of-the-art infrastructure, excellent conference facilities and easy access from all over the world with world-class cultural attractions, modern amenities, diverse entertainment and natural beauty.

Useful Links:

http://www.thisisathens.org/
http://www.athensconventionbureau.gr/
http://imanathenian.com/
TRANSPORT

Athens International airport “Eleftherios Venizelos” is approximately 32km from the hotel. Easy private or public transportation is available.

Taxi: A taxi from the airport to the city center costs a flat rate of 38€ from 5:00 a.m. to midnight and 50€ from midnight to 5:00 a.m. Confirm with driver before starting the journey and ask a receipt.

Metro: Take Metro line 3 (Blue Line/ Airport- Douk. Plakentias-Agia Marina), which connects the Athens airport with the city center. Trains run every 30 minutes, 7 days a week, from 6:30 a.m. to 11:30 p.m.

The trip from the airport to Syntagma Station (Athens center) lasts 40 minutes, where you change from the blue line to red line (Anthoupoli- Elliniko) and get off at the first stop (Acropolis Metro Station).

The ticket costs 10 euros for one-way and is valid for 70 minutes after its validation.

24-hour express bus: All buses leave passengers at the Departures Level and depart from the Arrivals Level, between Exits 4 and 5. The bus for the conference venue is X95: Syntagma – Airport - Syntagma

All buses run 24 hours a day, 7 days a week with frequency varying according to day, time and season. The bus route X95 takes approximately 40 minutes to Syntagma. You will then board the metro (red line) and get off at ACROPOLIS station. The hotel is about a 6 minutes walk.

The ticket costs 6 euros for one-way.

https://www.athenstransport.com/english/
SOCIAL PROGRAMME

Social Programme

WELCOME RECEPTION

The official opening of the Conference will take place at the beautiful terrace of the Divani Palace Acropolis with its stunning views of the Acropolis.

Date and Time: Wednesday, October 17th at 18:30
Location: Divani Palace Acropolis
Free entrance for all conference participants

GALA DINNER

With its tables set since 1975 in the shadow of the holy rock of the Acropolis and staying loyal to the traditional tastes of the the Greek and Mediterranean cuisine, Strofi remains one of the most historic restaurants in Athens.

Date and Time: Friday, October 19th at 20.00
Location: Strofi Restaurant
Entrance: 50€/person

GUIDED TOUR

The conference will take place in the area of the Acropolis, where most important sightseeing monuments of the capital of Greece are to be found there: The Acropolis Hill, the Ancient Forum, the New Acropolis Museum, Herod Atticus Theatre and the Dionysos Areopagitis pedestrian walk. They all preserve and highlight the grand classicality of Athens.

A guided tour is scheduled for any participant who would like to walk around this area and learn more about its history!

Date & Time: Thursday, 18th of October at 18.00
Duration: 2-hours walking tour
Meeting point: Divani Palace Acropolis
Admission: 20 euros/person*
*includes tour guide, entrance to the Acropolis museum & local delicacy
Programme Grid

**PRE-CONGRESS DAY | WEDNESDAY 17TH OCTOBER**

**ARISTOTELE B**

<table>
<thead>
<tr>
<th>TIME</th>
<th>TOPIC</th>
<th>SPEAKER(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00-12:00</td>
<td><strong>Part 1</strong></td>
<td>Interpretation of pre-clinical, toxicity study findings</td>
</tr>
<tr>
<td></td>
<td><strong>Part 2</strong></td>
<td>Morphologic, haematotoxicity findings in pre-clinical toxicity studies</td>
</tr>
<tr>
<td>12:00-13:00</td>
<td>Lunch Break</td>
<td></td>
</tr>
<tr>
<td>13:00-16:00</td>
<td><strong>Part 2</strong></td>
<td>Morphologic, haematotoxicity findings in pre-clinical toxicity studies</td>
</tr>
<tr>
<td>15:00-16:30</td>
<td>ECVCP Executive Board and Committee Chairs meeting</td>
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</table>

**Evening welcome reception follows Pre-Congress programme**
Our Welcome reception is at the roof garden of Divani Palace Acropolis, the conference venue.
Time: 18:30
<table>
<thead>
<tr>
<th>TIME</th>
<th>ERECTHION HALL</th>
<th>ARISTOTLE B HALL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Free Communications</td>
<td></td>
</tr>
<tr>
<td>9:00-10:00</td>
<td>Canine and Feline Babesiosis</td>
<td>900 Soetart</td>
</tr>
<tr>
<td></td>
<td><em>L. Solano-Gallego, G. Baneth</em></td>
<td>915 Schwartz</td>
</tr>
<tr>
<td>10:00-10:45</td>
<td>Canine and Feline Relapsing Fever Borreliosis</td>
<td>930 Lilliehöök</td>
</tr>
<tr>
<td></td>
<td><em>G. Baneth</em></td>
<td>945 Stirn</td>
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<td></td>
<td></td>
<td>1000 Neo</td>
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<td></td>
<td></td>
<td>1015 Medić</td>
</tr>
<tr>
<td>10:45-11:15</td>
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</tr>
<tr>
<td></td>
<td>Coffee Break</td>
<td></td>
</tr>
<tr>
<td>11:15-12:00</td>
<td>Clinicopathologic aspects of canine monocytic ehrlichiosis (<em>Ehrlichia canis</em>): diagnostic implications <em>M. Mylonakis</em></td>
<td>Haematotoxicity <em>P. Cotton</em></td>
</tr>
<tr>
<td>12:00-12:45</td>
<td>Canine Rickettsiosis</td>
<td>In Vitro to In Vivo Predictive Toxicology: Myelo-, Hepato- and Cardio toxicity <em>J. Harding, P. O’Brien</em></td>
</tr>
<tr>
<td></td>
<td><em>L. Solano-Gallego</em></td>
<td></td>
</tr>
<tr>
<td>12:45-14:15</td>
<td></td>
<td><strong>Lunch Break</strong></td>
</tr>
<tr>
<td>14:15-15:15</td>
<td>Canine and Feline Leishmaniosis</td>
<td>Regulatory validation</td>
</tr>
<tr>
<td></td>
<td><em>L. Solano-Gallego, G. Baneth</em></td>
<td><em>M. Burgess-Wilson, I. Roman</em></td>
</tr>
<tr>
<td>15:15-15:45</td>
<td></td>
<td><strong>Coffee Break</strong></td>
</tr>
<tr>
<td>15:45-16:30</td>
<td>Hereditary immunodeficiencies and infectious diseases <em>U. Giger</em></td>
<td>Immunotoxicology <em>B. Finney</em></td>
</tr>
<tr>
<td>16:45-18:00</td>
<td>ECVCP AGM</td>
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### FRIDAY 19TH OCTOBER

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<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td><strong>Free Communications</strong></td>
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<tr>
<td>9:00-9:45</td>
<td>Canine Angiostrongylosis</td>
<td>900 Mylonakis</td>
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<tr>
<td></td>
<td><em>S. Sotiraki</em></td>
<td>915 Kean</td>
</tr>
<tr>
<td>9:45-10:30</td>
<td>Bovine Respiratory Disease and BVD Diagnosis</td>
<td>930 Solano-Gallego</td>
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<tr>
<td></td>
<td><em>P. Burr</em></td>
<td>945 Attipa</td>
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<tr>
<td></td>
<td></td>
<td>1000 Melendez Lazo</td>
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<tr>
<td>10:30-11:00</td>
<td><strong>Coffee Break – Poster Session</strong></td>
<td></td>
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<tr>
<td>11:00-11:45</td>
<td>Mycobacterial infections</td>
<td>1100 Martinez</td>
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<tr>
<td></td>
<td><em>P. Burr</em></td>
<td>1115 Strage</td>
</tr>
<tr>
<td>11:45-12:30</td>
<td>Canine Dirofilariosis</td>
<td>1130 Soetart</td>
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<tr>
<td></td>
<td><em>S. Sotiraki</em></td>
<td>1145 Goddard</td>
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<tr>
<td></td>
<td></td>
<td>1200 Hooijberg</td>
</tr>
<tr>
<td>12:30-14:00</td>
<td><strong>Lunch Break</strong></td>
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</tr>
<tr>
<td>14:00-14:45</td>
<td>Feline Infectious Peritonitis</td>
<td></td>
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<tr>
<td></td>
<td><em>A. Giordano</em></td>
<td></td>
</tr>
<tr>
<td>14:45-15:30</td>
<td>Cytology diagnosis in cutaneous skin infections</td>
<td><em>R. Farmaki</em></td>
</tr>
<tr>
<td>15:30-16:00</td>
<td><strong>Coffee Break – Poster Session</strong></td>
<td></td>
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<tr>
<td>16:00-16:45</td>
<td>Feline Haemoplasmosis</td>
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<td></td>
<td><em>K. Papasouliotis</em></td>
<td></td>
</tr>
<tr>
<td>17:00-18:00</td>
<td><strong>ESVCP AGM</strong></td>
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**Congress Gala Dinner: Strofi Restaurant**  
Address: 25, Rovertou Galli str.  
Time: 20:30
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<tr>
<th>TIME</th>
<th>ERECTHION HALL</th>
<th>ARISTOTLE B HALL</th>
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<tbody>
<tr>
<td>9:00</td>
<td>Case Session</td>
<td>Free Communications</td>
</tr>
<tr>
<td>9:05</td>
<td>Case 1</td>
<td>Approach to diagnosis of defects in erythrocyte metabolism including a case of Heinz body anemia in a horse J. Harvey</td>
</tr>
<tr>
<td>10:00</td>
<td>Case 2</td>
<td>Splenic aspirates from a dog with azotemia I. Oikonomidis</td>
</tr>
<tr>
<td>10:15</td>
<td>Case 3</td>
<td>Aqueous humor from a dog H. Ferreira</td>
</tr>
<tr>
<td>10:30</td>
<td>Case 4</td>
<td>Coelomic fluid from a chicken K. Irvine</td>
</tr>
<tr>
<td>10:45</td>
<td>Case 5</td>
<td>Pericardial fluid from a cat S. Evans</td>
</tr>
<tr>
<td>11:00</td>
<td>Case 6</td>
<td>Sysmex scattergram in a cat T. Lavabre</td>
</tr>
<tr>
<td>11:15</td>
<td>Case 7</td>
<td>Peripheral nucleated red blood cells in a cat L. Magna</td>
</tr>
<tr>
<td>11:30</td>
<td>Case 8</td>
<td>Mandibular lymph node enlargement and lymphocytosis in a Shih tzu S. Bernardi</td>
</tr>
<tr>
<td>11:45</td>
<td>Case 9</td>
<td>Large subcutaneous tumour in a roe deer A. Penrose</td>
</tr>
<tr>
<td>12:00-12:30</td>
<td>Light Lunch</td>
<td></td>
</tr>
<tr>
<td>12:30-14:30</td>
<td>Mystery Slide Session</td>
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</tbody>
</table>
OUR SPEAKERS

Gad Baneth

DipECVCP, Professor of Veterinary Parasitology and Infectious Diseases, The Rybak-Pearson Chair in Veterinary Medicine, School of Veterinary Medicine, Hebrew University, Israel

Dr. Baneth graduated from the Hebrew University Koret School of Veterinary Medicine in Israel in 1990. He did a Small Animal internship and residency at the Hebrew University until 1994 followed by a fellowship in Internal Medicine and Infectious Diseases Research at the College of Veterinary Medicine, North Carolina State University during 1994 and 1995. He received a PhD in veterinary parasitology from the Hebrew University in 2000. Prof. Baneth served as the head of the Small Animal Internal Medicine Department at the Hebrew University Veterinary Teaching Hospital. He is a diplomate of the European College of Veterinary Clinical Pathology (ECVCP), an associate member of the European Veterinary Parasitology College (EVPC) and an editorial advisory board member for the Journal Veterinary Parasitology since 2006. He is the chairman of the World Small Animal Veterinary Association (WSAVA) Scientific Advisory Committee (SAC), the vice president of the LeishVet group for standardization of the diagnosis, treatment and prevention of canine leishmanioasis and a member of Board of Directors, Israel Society for Parasitology, Protozoology and Tropical Diseases.

His research interests focus on the pathogenesis, diagnosis and treatment of veterinary and zoonotic vector-borne infectious diseases including leishmaniosis, relapsing fever borreliosis, canine ehrlichiosis, babesiosis, hepatozooonosis, trypanosomiasis and dirofilariasis. Prof. Baneth is involved in the study of zoonotic and veterinary diseases in the Mediterranean Basin, Uzbekistan, Ethiopia, Southern Europe and South. He is the author of more than 190 scientific publications and book chapters. Prof. Baneth served as an advisor to the European Food Safety Authority (EFSA) on leishmaniosis. He is currently the director of the Koret School of Veterinary Medicine at the Hebrew University in Israel.

Michael Burgess-Wilson

Hematology, Principal Scientist, Envigo, UK

Michael, PhD, DMMLM was born in England, trained in Nottingham as a Medical Laboratory Scientist in the disciplines of Haematology, Blood Transfusion and Blood Coagulation and studied for the Fellowship Examination in Haematology. In 1978 joined the University Department of Medicine researching Coagulation and Platelet behaviour. In 1986 managed the Coagulation Laboratory in the University Hospital Nottingham. Between 1990 and 1995 worked as Research Scientist for Baxter Dade in Switzerland developing coagulation assays.

Between 1995 and 1999 worked as Research Scientist for J&J, Milpitas California developing a coagulation POCT device. In 1999 moved back the UK as Haematology Laboratory Manager at Northampton General Hospital. In 2010 moved to the CRO Huntingdon Life Sciences as Haematology Team Leader, and presently Haematology Principal Scientist at Envigo (formerly HLS). Joined the ACCP in 2010 and presently serving on the committee as membership secretary. Have supported ACCP conferences and given blood and bone marrow microscopy training courses and lectured at the Toxicology Course at the University of Surrey and ACCP meetings.
Paul Burr

Director, Biobest Laboratories Ltd, Edinburgh, UK

Paul is the Director of Biobest Laboratories Ltd, a veterinary laboratory based just outside Edinburgh with specialist expertise in virology, infectious disease and cell culture. He completed his veterinary degree at Edinburgh in 1992 and after a spell in mixed practice in Warwickshire returned to Scotland to complete a PhD in virology and molecular biology at Glasgow Vet School. He joined Biobest in 1999 and is actively involved in managing all aspects of Biobest’s testing services of both companion and farm animals.

Peter Cotton

Clinical Pathology Laboratory Manager, AstraZeneca, UK

Honours degree in Biology at Salford University graduating in 1979. Medical Laboratory Scientific Officer in Clinical Pathology at an NHS Hospital with a senior position from 1986 specialising in Haematology. In 1989 I joined Zeneca Toxicology Laboratory as Head of Haematology with responsibility for the running of the laboratory, data interpretation and reporting out of all pre-clinical toxicology studies on agrochemicals. In 1998 I became Deputy Clinical Pathologist and Haematology Specialist at AstraZeneca Pharmaceuticals initiating the Investigative Flow Cytometry laboratory. In 2001 became a Senior Scientist within Clinical Pathology with responsibility for the resource of the department to support Clinical and pre-Clinical studies and also responsible for the data interpretation in pre-clinical toxicology studies. In 2005 to date I obtained the position of Clinical Pathology Laboratory Manager at AstraZeneca with specialist knowledge of toxicological Clinical Pathology.

Rania Farmaki

DipECVD, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece

Rania Farmaki has graduated from the Veterinary School of University of Thessaloniki, Greece in 2001. After graduation she continued with further education by pursuing one - year internship (2001-2002) in Medicine at the Companion Animal Clinic (Veterinary school, University of Thessalonik) and by completing a PhD thesis (October 2002 - December 2006) on canine atopic dermatitis at the same Veterinary school. During that period, she was a volunteer scientific collaborator at the Dermatology Unit of Companion Animal Clinic, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece with clinical, teaching and researching activity. After completion of a 3-year formal residency in the same Veterinary School and successful examination in 2011 she became a Diplomate of the ECVD. She has worked as an adjunct lecturer in Medicine at the Clinic of Medicine, Faculty of Veterinary Medicine, Karditsa, University of Thessaly, Greece from 2008 until 2011. From 2012 until now she has been assigned to lead the Dermatology Unit of the Companion Animal Clinic, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece where she has clinical, teaching and researching activity. Apart from teaching she is providing referral dermatology service in Plakentia Veterinary Clinic, Athens. Rania Farmaki is a founding member and a member of the executive board of Hellenic Society of Veterinary Dermatology. She has given many continuing educational lectures in
congresses, seminars and meetings to veterinarians.

Brenda Finney

Biological Sciences Department Principal Scientist, Sequani, UK

Brenda did her PhD at Cardiff University in Biomedical Sciences, focusing on the role of calcium and the extracellular calcium sensing receptor in lung development. This was followed by a post-doc in The Platelet Group at the University of Birmingham where she characterised the developmental phenotype of transgenic mouse lines used for platelet signalling investigations. During this position she moved into performing in vivo microscopy of platelet activation and aggregation. She joined Sequani in 2013 and worked with the Pathology Sciences team before becoming part of the Biomarkers group in 2014. In 2017 she became the Principal Scientist for the Biological Sciences department and now provides scientific oversight for Clinical Pathology, In Vitro Toxicology and Histology. She has experience in a wide range of analysis techniques, but has in the past few years been focusing on the validation and use of immunoassays and immunophenotyping within a GLP-registered setting. She became involved with the ACCP in 2016, is an associate editor for the journal Comparative Clinical Pathology, and is also a member of the BTS.

Prof. Dr. Urs Giger

DipECVIM-CA, DipECVCP, DipACVIM-SA, University of Pennsylvania, School of Veterinary Medicine, Section Medical Genetics (PennGen), Philadelphia, USA

Urs Giger received his veterinary degree from the University of Zürich, Switzerland, where he also pursued his doctoral thesis on the surgical repair of hip dysplasia, initial clinical training in small animal medicine and surgery and postgraduate research work. After moving to the United States, he completed a residency in small animal internal medicine at the University of Florida and then joined the faculty of the School of Veterinary Medicine at the University of Pennsylvania in Philadelphia where he has the endowed Charlotte Newton Sheppard Professor of Medicine chair. He has a secondary professorship in small animal internal medicine at the University of Zürich as well as professorship in hematology at the Medical School University of Pennsylvania. He is a diplomate of the American and European College of the Veterinary Internal Medicine, as well as a diplomate of the European College of Clinical Pathology. He headed the Transfusion and Hematology Center and Pediatrics and Genetics Clinic, and is the director of the Metabolic Genetics and the DNA Genetic Disease Testing (PennGen) Laboratories. His clinical and research expertise and interests are in hereditary and hematologic disorders and are reflected in over 250 original research publications as well as many more reviews and scientific abstracts. He is also chairing the World Small Animal Veterinary Association (WSAVA) Hereditary Disease Committee. Among other awards, he was the recipient of a Transfusion Medicine Academic Award and Shannon Award from the National Institutes of Health, the 2002 WSAVA International Scientific Lifetime Achievement Award, the 2007 International Bourgelat Award from...
the BSAVA and the 2015 AVMA Excellence in Feline Research Award for outstanding clinical research in feline medicine. He is a frequently invited speaker at national and international conferences.

Alessia Giordano

DipECVCP, Associate Professor, Department of Veterinary Science and Public Health, University of Milan, Italy

Prof. Alessia Giordano received her DVM degree and her PhD in Animal Pathology and Veterinary Hygiene at the University of Milan where she works since 2001, currently as Associate Professor at the Department of Veterinary Medicine. Since 2006 she is board-certified by the European College of Veterinary Clinical Pathology (ECVCP) being chief of the Exam Committee from 2011 to 2016. Since 2017 she is the chief of the Diagnostic Labs at the Veterinary Teaching Hospital of the University of Milan where she’s especially involved in the Clinical Pathology service. Her main research interests include: diagnostic clinical pathology, feline infectious peritonitis, acute phase proteins and dysproteinemias, biomarkers of inflammation, method validation. Invited speaker at different national and international scientific meeting, Prof. Giordano is co-author of several scientific publications in peer-reviewed international journals.

Jo Harding

DipECVCP, Associate Professor, Department of Veterinary Science and Public Health, University of Milan, Italy

Following completion of my BSc Hons degree in Biomedical Technology from Sheffield Hallam University, I worked in the multi-disciplinary Clinical Pathology laboratory at Sanofi for 9 years, ultimately as Laboratory Head. During this time I completed a MSc degree in Biomedical Sciences at the University of Northumbria, and became a member of the ACCP, eventually joining the committee as Secretary. In 2004, I left the lab and took a role as study director with responsibility for General Toxicology studies at Covance, whilst maintaining an interest and specialism in clinical pathology data interpretation, and choice of biomarkers on Toxicology studies. More recently, I have taken a new position as a Project Toxicologist at AstraZeneca. Throughout my career I have maintained my association with the ACCP and I am a strong supporter of the training, education, and information-sharing philosophy of the association.
### Mathios Mylonakis

**Associate Professor, School of Veterinary, Faculty of Health Sciences, Aristotle University Thessaloniki, Greece**

Dr. Mathios Mylonakis graduated from the School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki (SVM-AUTh), Greece, in 1993. In the same institution, he completed a one-year internship (1994) in small animal internal medicine and a PhD thesis (2001) involving the diagnosis of canine monocytic ehrlichiosis. He has also spent five years in a private small animal Clinic. Dr. Mylonakis holds a faculty position in the SVM-AUTh since 2004, where he is currently an Associate Professor of Small Animal Internal Medicine. As part of his sabbatical, he has joined the Clinical Pathology Laboratory, Veterinary Medical Teaching Hospital, University of California-Davis, USA (March-July 2007), and the Medical Oncology Clinic, University of California-Davis, USA (March-June 2016). His clinical and research interests include clinical hematology and oncology, diagnostic cytology, and vector borne diseases of the dog and cat.

### Peter O’Brien

**DipECVCP, School of Veterinary Medicine, University College Dublin, Ireland**

Peter O’Brien, PhD, DVSc, Diplomate ECVCP was born in Toronto Canada of Irish and English parents. He got veterinary training in Canada, PhD at Minnesota, and certification in vetclnpath at Ontario’s Vet College. After 7 years here, he moved into tox and headed toxclnpath at P&G-Cincinnati, then SmithKline-Beecham-Welwyn, then Pfizer-Sandwich. After 11 years in tox, he returned to academia where he serves as Clinical Pathologist at Dublin Vet School. Peter is past president of ESVCP, ECVCP and EBVS. In 2008, he established ADL, for specialised (tox) clinpath testing. His >100 research papers focus on cardiac, muscle, liver and pancreatic biomarkers. He attended ACCP meetings from the 1997 ACH Morpeth conference. He joined ACCA then ACCP committees since 1998 and is currently Scientific Secretary. His role has been with conference management and education for all the above organisations.

### Kostas Papasouliotis

**DipECVCP, Langford Vets Hon. Senior Lecturer, Bristol Veterinary School, University of Bristol, UK**

Kostas Papasouliotis graduated from the Aristotle University of Thessaloniki, Greece in 1989. He has completed a PhD, a residency in Feline Gastroenterology, a residency in Clinical Pathology and has been a Lecturer and a Senior Lecturer in Clinical Pathology at the Bristol Veterinary School for more than 20 years. Kostas has published more than 100 scientific papers, research abstracts, professional articles and book chapters and he lectures regularly in the UK and Europe. He is an EBVS® European Specialist in Veterinary Clinical Pathology and the Secretary of ECVCP. Currently, he is a Senior Clinical Pathologist at IDEXX Laboratories, UK and an Honorary Senior Lecturer at the Bristol Vet School where he is the director of the Clinical Pathology residency programme.
Ian Roman

Head of Clinical Pathology and Diagnostics Department, Glaxo SmithKline, UK

My career started in the NHS in 1983 where I spent time in all laboratory disciplines, ranging from Haematology, Blood Transfusion, Immunology, Clinical Chemistry, Microbiology and Histology. I then specialised in Haematology and Transfusion Science and studied for my Fellowship in Immunology. In 1989 I joined Glaxo Haematology department where I studied for my MSc in Haematology and Transfusion Science. After the Glaxo Welcome merger Haematology and Clinical Chemistry merged and I joined the combined Clinical Pathology investigative group specialising in flow cytometry. When Glaxo and SmithKline Beecham merged I stayed in Clinical Pathology with responsibility for Haematology. I’m now head of the Clinical Pathology and Diagnostics Department of Glaxo Smithkline UK. I was part of the ACH from 1990 prior to the merger with the ACCA to become the ACCP. I eventually joined the committee of the ACCP and have been part of the group who lecture on the Toxicology MSc course at the University of Surrey as well as part of the team who run the data interpretation course of the ACCP.

Laia Solano-Gallego

DipECVCP, Senior Researcher, Facultad de Veterinaria, Universitat Autonoma, Barcelona, Spain

She received her DVM degree and PhD from the Autonomous University of Barcelona (UAB) in 1996 and 2001, respectively. Her PhD concerned the epidemiology, immunology and diagnosis of canine leishmaniosis. She did a post-doctoral clinical research in vector borne diseases of dogs and cats at the School of Veterinary Medicine of the North Carolina State University (USA) during 2002-2003. She did a small animal rotating internship at the Veterinary Teaching Hospital of the Purdue University (USA) during 2003-2004. She worked at the private hospital and laboratory of San Marco (Padova, Italy) during 2004-2007 where she performed clinicodiagnostics activity and clinical research in clinical pathology and internal medicine with predominant interest in vector borne diseases of dogs and cats. She worked as a lecturer in Veterinary Clinical Pathology at the Royal Veterinary College (RVC) of the University of London during 2007-2011. Currently, she works as a professora agregada interina at the School of Veterinary Medicine of the UAB. She is Diplomate of European College of Veterinary Clinical Pathology (ECVCP) since 2006. She has presented oral communications in international and national congresses and she is the author of scientific papers published in peer-reviewed international journals on leishmaniosis, arthropod borne diseases of dogs and cats including babesiosis, rickettsiosis, ehrlichiosis, anaplasmosis and bartonellosis and veterinary clinical pathology.
Smaragda Sotiraki

DipECVCP, Senior Researcher Veterinary Research Institute, Greek Agricultural Organization “Demeter”, Thessaloniki, Greece

Dr Sotiraki is a senior researcher and leads a research group on Parasitology and Parasitic diseases at the Veterinary Research Institute and focuses her R&D activities on epidemiology of parasitic infections, integrated disease management (including antiparasitic treatments and alternatives solutions) and spread of parasitic zoonoses. She holds a Degree in Veterinary Medicine and a Doctorate in Veterinary Parasitology (1991), both from the Aristotle University of Thessaloniki, Greece and conducted post-doctoral research at the Royal Agricultural & Veterinary University in Denmark between 2002 and 2004. She is also an EBVS Specialist in Veterinary Parasitology, de facto member of the European College for Veterinary Parasitology which at the moment serves as the Secretary of the Board (2018-2021). Dr Sotiraki has over 20 years R&D and teaching experience in parasitology and health management and he has produced over sixty peer-reviewed scientific publications in well recognised journals with a noteworthy number of citations. She has significant managerial experience in research projects. Overall, she has participated in more than 20 (coordinating most of them) major R&D programmes at National and International level, under Structural Funds and FP schemes like the FP6-MCTN “HealthHay”, the FP7-CP “LowInputBreeds” and as a Chair the FA0805 COST Action “CAPARA” and vice Chair of the COST Action “COMBAR”. She had also active participation in a significant number of international scientific conferences and is member at European level in scientific teams producing research policy. Moreover, she has important teaching experience at graduate (by teaching in Veterinary and Agricultural Universities) and post-graduate level (by supervising several PhD students).
Main Sessions

Canine and Feline Relapsing Fever Borreliosis, G. Baneth

Regulatory Validation, M. Burgess- Wilson, I. Roman

Bovine Respiratory Disease and BVD Diagnosis, P. Burr

Mycobacterial infections, P. Burr

Haematotoxicity, P. Cotton

Interpretation of pre- clinical toxicity study findings, P. Cotton, I. Roman, J. Harding, P. O’ Brien

Cytology diagnosis in cutaneous skin infections, R. Farmaki

Immunotoxicology, B. Finney

Canine and feline blood types, crossmatches and transfusion reactions, U. Giger

Hereditary immunodeficiencies and infectious diseases, U. Giger

Feline Infectious Peritonitis, A. Giordano

In Vitro to In Vivo Predictive Toxicology: Myelo-, Hepato- and Cardiotoxicity, J. Harding, P. O’ Brien

Clinicopathologic aspects of canine monocytic ehrlichiosis (Ehrlichia canis): diagnostic implications, M. Mylonakis

Feline Haemoplasmosis, K. Papasouliotis

Morphologic, haematotoxicity findings in pre-clinical toxicity studies, C. Smith, M. Burgess- Wilson

Canine Rickettsiosis, L. Solano-Gallego

Canine and Feline Babesiosis, L. Solano- Gallego, G. Baneth

Canine and Feline Leishmaniosis, L. Solano- Gallego, G. Baneth

Canine Angiostrongylosis, S. Sotiraki

Canine Dirofilariosis, S. Sotiraki
CANINE AND FELINE RELAPSING FEVER BORRELIOSIS

Gad Baneth, DVM, PhD. Dipl. ECVCP
Koret School of Veterinary Medicine, Hebrew University, Israel

Introduction
Relapsing fever is an infectious disease caused by arthropod-borne spirochetes of the genus *Borrelia* (1-3). The disease is characterized by recurrent episodes of fever borrelemia (4). The RF borrelioses include louse-borne relapsing fever caused by *Borrelia recurrentis* and tick-borne endemic relapsing fever transmitted by argasid soft ticks and caused by several *Borrelia* spp. such as *Borrelia crocidurae*, *Borrelia coriaceae*, *Borrelia duttoni*, *Borrelia hermsii*, *Borrelia hispanica* and *Borrelia persica*. Human infection with *B. persica* is transmitted by the soft tick *Ornithodoros tholozani* and has been reported from Iran, Israel, Egypt, India, and Central Asia (4). Dogs have been reported to be infected with *B. turicatae*, *B. hermsii* and *B. persica* while no report of feline relapsing fever borreliosis has been published prior to this study (5-9).

Case series
During 2003-2015, five cats and five dogs from Israel were presented for veterinary care and detected with the presence of *Borrelia* sp. in blood by observation of blood smear microscopy. The causative infective agent in these animals was identified as *B. persica* and characterized by PCR from blood and sequencing of parts of the flagellin (*flab*), 16S rRNA and glycerophosphodiester phosphodiesterase (*GlpQ*) genes. All animals were infected with *B. persica* genetically identical to the causative agent of human relapsing fever. Phylogenetic analysis indicated that DNA sequences from these cats and dogs clustered together with *B. persica* genotypes I and II from humans and *O. tholozani* ticks and distinctly from other RF *Borrelia* spp. The main clinical findings in cats included lethargy, anorexia, anemia in 5/5 cats and thrombocytopenia in 4/5. All dogs were lethargic and anorectic, 4/5 were febrile and anemic and 3/5 were thrombocytopenic. Three dogs were co-infected with *Babesia* spp. The animals were all treated with antibiotics and the survival rate of both dogs and cats was 80%. The cat and dog that succumbed to disease died one day after the initiation of antibiotic treatment, while survival in the others was followed by the rapid disappearance of spirochetemia (10).

Conclusions
This is the first report of disease due to *B. persica* infection in cats and the first case series in dogs (10). Infection was associated with anemia and thrombocytopenia. Fever was more frequently observed in dogs than cats. Domestic canines and felines suffer from clinical disease due to *B. persica* infection and other relapsing fever spirochetes and may also serve as sentinels for human infection.

References


REGULATORY VALIDATION
Dr. Michael Burgess-Wilson, ENVIGO, UK
Dr. Ian Roman, GSK, UK

REGULATORY LANDSCAPE
The importance of the past, present and future regulatory landscape is discussed. The pharmaceutical industry (Pharma) and Contract Research Organisations (CRO) have seen, over the past 10-20 years a considerable increase in range and detail of regulations surrounding the provision of diagnostic test results. It is anticipated that this will accelerate and will more and more involve groups that presently have not been subject to these levels of regulation. The latest example is the new Data Integrity Requirements, which requires careful selection of new instrumentation considering the computerised systems provided with this equipment.

WHAT CAN YOU DO?
The central message of this presentation is to alert the delegates to this environment and to give some suggestions as to how to prepare. It is explained how to provide the information that the regulators require. The importance of knowing the regulations and where to find them is discussed, and how to understand what work is required and who can give support, for example the ESVCP and the Association for Comparative Clinical Pathology (ACCP). The importance of the opportunity in a meeting like this one to develop a network with people who can help and support you in the planning of your instrument and assay validations.

WHAT IS VALIDATION
All the elements of validation are listed, and each element is described briefly, for example; Lower Limit of Quantification (LLOQ) is the lowest accurate and reproducible measurement of the analyte, which is not to be confused with the Lower Limit of Detection (LOD) which is the lowest measurable value that can be distinguished from the background. It is explained that it is beyond the scope of this presentation to fully describe and discuss each element, this could take several days. It is stressed, however, that before any attempt is made to ‘validate’ a method or system a good knowledge of all these elements is necessary. A further example is given in regarding the general misunderstanding of terms used is in respect to ‘sensitivity’ and ‘specificity’. The fact that there are different types, for example ‘Clinical’ and ‘Analytical’ is explained, emphasising the need for the clear understanding of the terms. It is often the case that in a project team planning a validation there are many different understandings of the terms used.

PLANNING VALIDATION
The critical importance of planning validation is explained, and the skills needed to plan correctly. It is important to understand there is no document or white paper that tells you exactly what to do in any particular validation. Planning involves detailing in each specific case the minimum work required to provide the right results. When planning a good understanding of the analyte and measurement system is very important and this will be discussed in more detail in the section on Challenges and Problems. However more important is to understand what the purpose of the testing is and match this the regulatory framework.
CHALLENGES AND PROBLEMS
The presentation continued with several examples of challenges and issues that the authors have had personal experience of. These sorts of examples can warn you of pitfalls and difficulties that sometimes make it necessary to repeat validation.

Measuring accuracy and sensitivity
This example describes the simple counting of the platelet in whole blood. It explains that when blood is collected into EDTA the platelet clump to a greater or lesser degree. When platelets clump the cell counted underestimate the ‘true’ platelet count because a clump of 3 platelets may be counts as a single platelet. This demonstrates that there are limits to the accuracy of the platelet count from an EDTA sample and this can be proved by collecting blood into CTAD anticoagulant (reference 1), where higher platelet counts are always seen.

Stability testing and Baseline determination
In this example the issue of multiple variables is explored. To measure stability there must always be a ‘baseline’, but how this baseline value is obtained can require some thought. In the case of a simple validation of frozen stability of fibrinogen, the control of the variables associated with determining the baseline are described in detail; type of blood collection, anticoagulation, storage conditions between process steps (time and temperature), centrifugation conditions (temperature and g force), type of tubes used to store plasma, time between blood collection and analysis. Then the bigger and difficult question is the determination of the acceptance criteria. What % difference between the baseline and the stored sample is acceptable. It is easy to say a number, say 10%, but more difficult to justify why. It has been explained that to know the acceptable level of variation the validation must be performed first!

Interference
A table of information is provided which shows when and at what level haemolysis will interfere with biochemical measurements. The table shows that the effect depends on the level of haemolysis, which can be difficult to quantify in a routine test environment. We all inspect the sample visually, but is that useful? The table also shows clearly that the effect is very different between species. The point is made that each species behaves differently and may need to be validated separately.

Linearity
In this very simple example it is explained that when testing linearity, it is common to prepare a high concentration of the analyte and then to diluent this solution to a range of concentrations which bracket the linear range that the assay will be used for. In one such study where Iron (Fe) was being measured on a clinical chemistry analyser, the dilutions of plasma were prepared using saline. The instrument reported erroneously high levels due to saline interfering with the detection method and only when distilled water was used as diluent were expected levels reported.

Sensitivity and Specificity
In this example an assay for Cortisol in dogs was being performed on a clinical chemistry analyser. With a new lot of reagents, the assay calibration and controls were all acceptable but the dog samples gave no signal, no detectable cortisol, whereas previously there were measurable levels. The laboratory however included ‘Species Specific Controls’ and these also give no signal. Initially the manufacturer of the kits said that no changes had been made, but after multiple challenges they reported a small change. This change had no effect on the measurement in humans, hence the acceptable ‘human’ controls which fell within the manufacturers published control ranges, but it completely abolished the detection in dogs. In this case the effect was extreme, however you need to be aware that small changes
may occur batch-to-batch that the manufacturers controls cannot detect. The need to include ‘species specific’ controls was further discussed.

CONCLUSIONS
To conclude the following points were stressed; know the regulatory landscape with which you are performing a validation; understand that the regulations are guidance and will not give you exact details you can follow; understand each of the different elements of ‘validation’; it is essential to have a clear understanding of the equipment, processes and analyte when planning the level of validation; have a network to get support and advice from others and not ‘re-invent the wheel’ every time you do a validation.

REFERENCES
1. Platelet counting in animals (Back to basics): M. Burgess-Wilson, M. Smith; Poster presented at Society of Toxicology Meeting, San Francisco March 2012
BOVINE RESPIRATORY DISEASE AND BVD; DIAGNOSTIC TEST OPTIONS AND NATIONAL STATUS

Paul Burr, Sarah McCallum, Kate Turner Haig
Biobest Laboratories, The Edinburgh Technopole, Penicuik, UK

Bovine Respiratory Disease
Bovine Respiratory Disease (BRD) is responsible for substantial welfare and economic costs to both the beef and dairy sectors of farming worldwide. The costs of each outbreak include deaths, medicines, extra labour and veterinary input, plus the impact on liveweight gain and feed conversion efficiency from irreversible lung damage. BRD has long been recognised as a multifactorial disease, with multiple causative agents and management factors involved in outbreaks (Figure 1). Despite the potential costs of an outbreak, diagnostic testing is not always performed to identify the specific pathogens involved. For infectious diseases tests either identify the presence or absence of a pathogen, or the presence or absence of the host response to the pathogen, most often antibodies. Both pathogen and antibody tests are useful in BRD investigation. Diagnostic testing in infectious disease is of most use where the results of tests will influence treatment or future preventative strategies. With increased focus on responsible use of antibiotics, diagnostic testing may be required in some situations for regulatory reasons, to justify antibiotic use.

Pathogen Detection in BRD:
Tests routinely available include bacterial culture, PCRs for viral and bacterial DNA and RNA, and immunofluorescent antibody tests (usually for viral proteins). For the last 3 years Biobest has been offering an 8 pathogen PCR test on nasopharyngeal swabs, BAL fluid or lung tissue collected at post mortem. PCR based diagnostics detect the DNA or RNA of a pathogen and while not the same as detecting live or infectious pathogens, genetic material is relatively robust so less likely to be affected by transport to the laboratory. In samples submitted over the last 3 years we have detected IBR (6% of submissions), PI3 (9%), RSV (18%), Bovine coronavirus (BCV) (29%), Mannheimia (52%), Pasteurella (87%), Histophilus (45%) and Mycoplasma bovis (36%) in our PCR tests. It is perhaps not surprising that the expected secondary bacterial pathogens are most frequently identified. The regular identification of BCV, sometimes in association with other viruses but on other occasions only with bacteria usually considered as secondary pathogens, is an interesting observation given that in the UK BCV is often dismissed as not being a primary pathogen. Mycoplasma bovis also seems to be identified frequently and where history available associated with both acute and chronic histories of pneumonia; it may be that greater use of PCR testing will lead to a better estimate of the proportion of BRD cases where Mycoplasma is involved. In a small proportion (4%) of PCR tests no pathogens are identified. A limitation of PCR diagnostics is that they only identify pathogens present in the panel. Where the usual pathogens are not detected in a sample, follow up tests and more detailed investigations are required.

Antibody tests in BRD:
Antibody tests to RSV, PI3, IBR and Mycoplasma bovis are commonly used in the UK. Antibody tests are of most use in recovering animals in order to inform future vaccination policy. For many years Biobest has offered serology testing for bovine respiratory disease with part of the cost of testing supported by Zoetis. The package also usually includes BVD serology as immunosuppression due to active BVD is a significant risk factor in BRD. The results of this testing suggest that PI3 is implicated most frequently in UK BRD outbreaks (74%), then RSV (66%) Mycoplasma bovis (51%), with IBR least common (35%). Some BVD infection is suggested in about 37% of herds investigated by serology. Serology can be very useful in excluding a particular pathogen from an outbreak if there is no evidence of a serological response in recovered animals. It is interesting to note that in about 7% of submissions there is no substantial evidence that any of these pathogens are involved from the serology results. This may be due to the timing of sampling or perhaps suggests another primary pathogen involved.
Summary:
Confirmation of the pathogens involved in a BRD outbreak is important both for treatment of the current outbreak and to inform future preventative strategies. It may be most appropriate to test for the pathogen itself, or the antibody response to the pathogen or use both strategies. In the majority of BRD investigations submitted to Biobest tests for the common primary and secondary pathogens provide sufficient information to draw reasonable conclusions over the pathogens involved. There are a small percentage of cases where this is not the case and where more intensive further investigation is advisable and surveillance implications worth considering.

BVD Diagnostics and National Eradication Programmes in the UK and Europe
It has been estimated that BVD costs UK farming 20 million pounds each year. Despite this many farms live with the ongoing losses caused by endemic BVD in the herd. In recent years the nations of the UK and the Republic of Ireland have moved forward with national control programmes.

The first step to controlling BVD in the herd is for both the vet and farmer to understand how the disease is perpetuated (Figure 2). Infection is maintained in most herds by the presence of persistently infected (PI) animals. PIs arise when naïve heifers or cows are infected with BVD whilst in calf. The bovine foetus does not achieve immunological competence until the latter stages of pregnancy. If infection occurs in the first third of pregnancy surviving embryos fail to mount an immune response to the virus effectively treating the virus as ‘self’ rather than foreign. Persistently infected animals often appear completely normal at birth, but are a constant source of virus from all body secretions, and therefore a potent source of infection for other animals. When a PI crosses the path of a further naïve in calf heifer or cow further PIs can be born, replacing the original PI which will eventually die of mucosal disease.

BVD Diagnostic Tests
The key to eradicating BVD at a herd, local or national level is to identify and remove all PI animals and prevent them being re-introduced. There is a panel of excellent diagnostic tests available to screen herds for the presence of active infection and then identify individual PIs.

Table 1; Techniques for BVD Diagnosis

<table>
<thead>
<tr>
<th>Sample Type and Test</th>
<th>Result</th>
<th>Interpretation of Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk milk antibody</td>
<td>Positive</td>
<td>Likely BVD active in the last few years or modified live vaccination.</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>Herd free of active BVD and has been for some time</td>
</tr>
<tr>
<td>Bulk Milk PCR (typically up to 300 animals)</td>
<td>Positive</td>
<td>BVD PI likely to be present in the milking herd.</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>No BVD PI in the animals contributing to that sample.</td>
</tr>
<tr>
<td>Youngstock (9-18 months) antibody check test; 5 per management group</td>
<td>All negative</td>
<td>No active BVD in the herd in the lifetime of those animals. Concentrate on keeping it out.</td>
</tr>
<tr>
<td></td>
<td>&lt;20% positive</td>
<td>Unlikely a PI in the group but some risk of infection. Review biosecurity, repeat check test in 1-2 months.</td>
</tr>
<tr>
<td></td>
<td>&gt;20% positive</td>
<td>PI present in the group or on the farm, identify and remove.</td>
</tr>
<tr>
<td>Ear Tissue ‘Tag and Test’ for BVD virus (ELISA or PCR)</td>
<td>Positive</td>
<td>Likely a BVD PI. If apparently healthy can retest in case transient infection.</td>
</tr>
</tbody>
</table>
Control and eradication of BVD can be achieved by all herds that are prepared to follow a simple set of instructions. Many herds in the UK follow a set of rules advocated by CHeCS (Cattle Health Certification Standards). A herd can quickly establish its status using the tests above. If a herd does not have active BVD biosecurity is addressed to keep it out. If a herd has active BVD PIs must be found and removed and biosecurity introduced to prevent re-introduction.

Many parts of the British Isles are now making progress in BVD eradication. Scotland has an industry-led but Government-backed scheme based on CHeCS rules that is making steady progress, with BVD prevalence falling steadily at a herd level. Every breeding herd must declare status annually based on antibody check testing or testing every calf born for virus. The next phase, with measures to increase the pressure substantially on those herds not controlling BVD, is expected in 2019.

In Northern and the Republic of Ireland a slightly different strategy has been used with the requirement to tag and test every calf born within 20 days of birth. Any animal born after 1st January 2013 must have a negative BVD virus result to move. There has been a steady fall in numbers of PIs; from 11.3% in 2013 to 1.7% in 2017 in the Republic. PI retention has been a challenge, although this is reducing. RDP funded veterinary investigations of all herds with PIs has been available since 2017. Restrictions on herds retaining PIs and notification of neighbours is planned.

Although Wales was the last devolved nation to establish a BVD programme, funded youngstock bleeds (antibody check test) have been performed at bTB tests since 2017. Results have been supported by advice from local vets on BVD control, and further support to eradicate BVD if the herd has active BVD is available.

At present the BVD programme in England does not have any government funding or legislative support and this is regarded as a significant problem. The rules for the English programme ‘BVDFree’ are similar to Scotland and compatible with CHeCS accredited status. Some practices had been very successful in getting nearly all their breeding herds to be part of BVDFree. Others, while doing regular BVD surveillance testing with their herds, are not yet registering herds with BVDFree.

Many continental Europe national or regional schemes are also in progress and Scandinavia successfully eradicated BVD some years ago using antibody check tests to screen herds for active infection. Recent schemes in Europe have tended to use Tag and Test of all calves similar to Ireland. All schemes have recognised that to prevent BVD maintaining or re-establishing itself in breeding herds veterinary advice, as well as testing is required. Advice on BVD control in herds where initial testing indicates a problem, and biosecurity in all herds, is essential.

We have all the tools we need to eradicate BVD from Europe. Progress is being made in many countries and regions but continued effort is needed to achieve the complete BVD freedom enjoyed by Scandinavia. BVD eradication requires absolute commitment from vets, farmers and the wider industry. An established national database of BVD-free herds and tested
individual animals is likely to be a critical resource. Government has an essential role to play in the promise and delivery of legislation to ensure that those who refuse to engage in BVD control are not permitted to carry on harming neighbouring farms.

Figure 1; Risk Factors for BRD

![Risk Factors for BRD Diagram]

Figure 2; Understanding BVD

![Understanding BVD Diagram]
MYCOBACTERIAL INFECTIONS – CHALLENGES IN DISEASE AND DIAGNOSIS

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²Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh, Roslin, UK

Abstract
Mycobacterial disease is one of the key challenges currently facing the veterinary profession throughout the United Kingdom. Bovine Tuberculosis (bTB) in cattle and badgers receives most attention but recent spillover cases in cats in the Newbury area and extensive bTB infection in a pack of foxhounds has raised the profile of Tuberculosis (TB) in companion animals and other species. Diagnosis of Mycobacterial disease is complex as infection can be due to many different species of bacteria. Given concerns over the zoonotic potential of the tuberculous group of Mycobacteria, a general diagnosis of mycobacteriosis is not sufficient; it is important to obtain an indication of the class of Mycobacterium involved and hence the risk to other household members (human and animal). As is the case for most infectious diseases diagnosis relies on a combination of clinical signs, history and appropriate use of diagnostic tests. Tests currently available in the UK and their use and limitations are reviewed in the context of a major recent outbreak of bTB in a pack of foxhounds.

Mycobacteria of veterinary importance (Table 1)
Mycobacteria comprise a large group of morphologically similar bacteria. The group has shared features such as a high lipid content cell wall (acid fast), resistance to heat, pH change, and disinfectants. Clinical classification relies on culture characteristics (impossible, slow or fast growing) and the tendency to produce granulomatous disease with or without dissemination or tubercles. Tubercles are defined as small round whitish grey lesions with central caseation in some species although not commonly in cats. There is a surrounding granulomatous inflammation and infection can be associated with considerable zoonotic potential particularly where there is draining mycobacterial rich pus. The features used in classification are generally supported by genetic classification and provide clues as to the unique challenges associated with the diagnosis and control of Mycobacterial infection.

Table 1: Some Mycobacteria of Veterinary Interest

<table>
<thead>
<tr>
<th>• Tuberculous Complex</th>
<th>Highly pathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant zoonotic potential</td>
<td></td>
</tr>
<tr>
<td>Highly pathogenic intracellular pathogens.</td>
<td></td>
</tr>
<tr>
<td>May produce characteristic tubercles or have a lepromatous pathology.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>• Opportunistic Slow Growing</th>
<th>M. avium complex (including M. avium paratuberculosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granuloma producing.</td>
<td></td>
</tr>
<tr>
<td>May disseminate.</td>
<td></td>
</tr>
<tr>
<td>Significant zoonotic potential only in immunocompromised individuals</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>• Opportunistic Fast Growing</th>
<th>M. phlei</th>
</tr>
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<tbody>
<tr>
<td>Saprophytes.</td>
<td></td>
</tr>
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<table>
<thead>
<tr>
<th>• Lepromatous</th>
<th>M. lepraemurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified novel species</td>
<td></td>
</tr>
<tr>
<td>M. visibilis?</td>
<td></td>
</tr>
</tbody>
</table>
In general terms the TB complex mycobacteria have the most zoonotic potential and tendency to disseminate within the host, but even here infection may be latent or disease may be sub-clinical and exposure to infection does not always result in disease. Confirmed bovine TB (culture or identification at post mortem examination [PME]) is a notifiable disease in any mammal in the UK. Within the TB complex human and bovine TB are usually considered highly pathogenic tuberculous, while M. microti, frequently found in cats in the UK is considered lesser pathogenic tuberculous complex. The opportunistic slow growing group (M. avium complex) are sometimes localised to cutaneous lesions but can also disseminate in (immunocompromised) hosts. A member of this group causes Johne’s disease in cattle (infecting the gut leading to chronic diarrhoea). Opportunistic fast growing Mycobacteria generally cause localised cutaneous and sub-cutaneous granulomas following bite or puncture wounds. Feline leprosy refers to single or multiple granulomas in the skin or sub-cutis caused by M. lepraemurium or another as yet unidentified species.

**Diagnosis**

It has been reported that 1% of biopsy samples submitted have a suspicion of Mycobacterial disease in UK cats. It could be argued that Mycobacterial infection is a differential in many chronic respiratory, alimentary or cutaneous problems. Diagnosis of mycobacterial disease (as for most infectious diseases) relies on a combination of clinical signs, history and appropriate use of diagnostic tests. Specific diagnostic techniques for all infectious diseases rely on either detection of the organism or the host’s response to it. To use tests properly it is critical to understand the disease process and that the presence of a pathogen or an immune response to that pathogen does not prove the pathogen is the cause of the disease that is being investigated. Results must be interpreted in the context of what is known about the disease pathogenesis and clinical signs. For mycobacterial infections interpretation must take into account that genetics of pathogen and host genetics/immune response will influence disease progression and animals may be exposed to Mycobacteria and harbour infection without ever developing clinical disease.

Detection of acid-fast bacteria morphologically consistent with Mycobacteria from cytology or histopathology is a useful starting point, but confirmed cases may not have detectable bacteria by these methods. Culture (from fresh tissue) was historically regarded as the reference diagnostic technique. However, this is not without difficulty as Mycobacteria can be slow, difficult or impossible to grow. Culture is very much a specialist technique dependent on the skill of the laboratory and culture conditions designed to favour one species may inhibit the growth of another. PCR based testing (most often of tissue biopsies or cytology slides where acid fast bacteria have been identified, or even as a follow up from early culture in some circumstances) has the potential to rapidly discriminate certain classes of mycobacteria. However, tests currently available in the UK are for M. tuberculosis complex and M. avium complex bacteria. The test discriminates between these groups but not within them, e.g. M. bovis vs M. microti infection cannot be routinely differentiated at present. PCR is not infallible and like other techniques may struggle if there are very low numbers of Mycobacteria present.

Tests to detect the host response to Mycobacteria are widely used in farm animals. Antibody tests are used in Johne’s disease and intradermal tuberculin testing and interferon gamma release assays (IGRAs) are at the forefront of bTB testing in cattle (antibody tests are available but controversial). In companion animals, although research is ongoing into the use of antibody tests, none are currently widely used: to date published results suggest antibody based tests have good specificity but poor sensitivity, particularly for M. microti infections. Tuberculin testing is not thought to be useful in cats and is not often used in dogs. To perform an IGRA assay peripheral blood mononuclear cells (PBMCs) are prepared from heparinised blood. The PBMCs are cultivated for 72 hours in 5 separate reactions: positive and negative controls and TB specific antigens (PPDA, PPDB and the ESAT6/CF10 peptide mix). Following cultivation supernatant is collected from each well and tested for Interferon gamma in an ELISA. Depending on the precise interpretation criteria used this method has
shown (in cats) sensitivity of 70-100% and specificity of 95-100%. The test is of most use where mycobacterial disease is strongly suspected as it can provide a rapid indication of the infecting organism and therefore the zoonotic potential (Table 2). Testing of in-contact animals in a multi-pet household and testing to monitor treatment has also been proposed.

<table>
<thead>
<tr>
<th>Table 2: IGRA Interpretation</th>
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<tr>
<td>PPDA - , PPDB - , ESAT6/CF10 -:</td>
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<tr>
<td>PPDA +, PPDB -, ESAT6/CF10 -:</td>
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<td>PPDA +, PPDB +, ESAT6/CF10 - :</td>
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<td>PPDA +, PPDB +, ESAT6/CF10 + :</td>
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- The PPDB response is often higher than PPDA in pathogenic Mycobacteria infections.

In 2016/17 we investigated a *M. bovis* TB outbreak in a pack of approximately 180 Foxhounds within the bovine TB Edge Area of England. We employed a combination of immunological tests including an IGRA and a serological assay (DPP VetTB, Chembio). Test-positive hounds were euthanased and subjected to PME. Overall 164 hounds were tested; 97 (59%) responded positively to at least one test. Eighty-five (52%) dogs responded to *M. bovis* antigens by IGRA whilst only 21 (12.9%) had detectable antibody responses. At PME, three hounds (3.1%) had visible lesions (VL) due to *M. bovis* infection, later confirmed by culture. Samples from 24 non-VL hounds were cultured and *M. bovis* infection was confirmed in a further three hounds (11%).

The source of the infection remains unproven but the most biologically plausible mechanism appears to be by feeding of bovine material to the pack (culled animals from local herds that were bTB infected but individuals not positive by skin testing at the time of culling). If this hypothesis were proven it would raise interesting questions over the advisability of feeding raw food diets incorporating bovine material to companion animals while bTB is endemic in the UK. Feeding of offal from fallen stock to hounds has recently been banned in the UK.

Diagnosis of many infectious diseases relies on careful evaluation of the clinical signs and the available diagnostic tests. Mycobacterial disease falls into this category and is of particular importance because of the zoonotic potential of some members of the group. Follow up testing to determine the Mycobacteria species involved and risks of zoonosis is advisable whenever there are strong grounds to suspect Mycobacterial infection.

**References**


Figure 1: Interferon Gamma Release Assays
HAEMATOXICITY

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Toxicologic Clinical Pathology is established as an integral part of the preclinical safety assessment of test articles (new chemical entities, exploratory novel medicines, xenobiotics) especially in short and in medium term toxicity studies (1). Toxicologic clinical pathology testing may help to support safety margins and indicate and influence any decision-making required on the course of a study or design of future studies.

Interpretation and integration of clinical pathology findings with clinical observations, body and organ weights, toxicokinetics, and anatomic pathology data strengthens the contribution of clinical pathology to the safety assessment of new chemical entities (2). Alterations of activity, concentration or appearance outside of normal biological variation may indicate an ongoing toxicological/pathophysiological process, disruption of endogenous control or synthetic processes, represent pharmacological activity of the test article, cellular or tissue damage or functional impairment, inflammatory responses, physiological adaptation to drug treatment, a compensatory response to an initial cellular injury or functional impairment, or reflect exogenous factors or report pre-analytic or analytic variation.

The purpose of haematology tests is to identify test article-related effects on homeostasis, bone marrow and peripheral blood cells, and haemostasis. Is there an effect? Is it real? Is it treatment related? We can use concurrent matched control groups. For large animals we can utilise pretest data that is also important, and we use knowledge about variability of parameter values and any species differences. In general reference intervals within toxicology testing are not useful.

Is there a change? If so, what is it and where/how does it occur? If any changes are related to the test item, then is it adverse? We can use concurrent control data for this and then we can use appropriate reference intervals to put changes into perspective and identify effects that are easily monitored in human clinical trials. Reference intervals in toxicity studies are not equivalent to clinical reference intervals in terms of their utility.

Predictability of animal testing for human haematotoxicity is ≥ 90%. Predictivity is good if drugs affect conserved processes but not as good if drugs affect species-specific processes (metabolism, receptors, etc.), and poorly to not at all predictive if drugs cause idiosyncratic reactions. Relatively few animals are tested compared to humans exposed in clinical trials. Toxicities may be idiosyncratic in one species or many species.

Biotherapeutics are expanding the arsenal of therapeutics available for treating and preventing disease. Although initially thought to have limited side effects due to the specificity of their binding, these drugs have now been shown to have potential for adverse drug reactions including effects on peripheral blood cell counts or function. Most haematological liabilities of biotherapeutics are not based on drug class but are species specific, immune-mediated, and of low incidence. Despite the potential for unexpected haematological toxicity, the risk-benefit profile of most biotherapeutics is favourable;
haematologic effects are readily monitorable and managed by dose modification, drug withdrawal, and/or therapeutic intervention (3).

Knowledge of pre-analytical variation is key to avoiding misinterpretation or compromising data. Over bleeding of small animals, stress, acclimitisation are just a few examples that can influence data. Rats on a severely restricted diet have shown significantly lower reticulocytes, neutrophils, lymphocytes and platelets, accompanied by bone marrow hypocellularity and necrosis, that is not consistently observed in other species (4).

Within haematologic toxicology we also need to consider the lifespan of haematopoietic cells. For instance, red blood cell survival within the peripheral circulation is 20-45, 45-68, 85-100, or 100-120 days in the mouse, rat, non-human primate and the dog respectively, and influence the impact and recovery from bone marrow toxicity. White cell survival within the peripheral circulation is a matter of hours, with exception of certain lymphocytes where survival is weeks to years.

A test article’s impact on analytical method needs to be taken account of when interpreting data on a toxicity study, for instance, on the Bayer Advia haematology systems these analysers utilise the myeloperoxidase staining technique to differentiate the white cell populations. Any test article that interferes with myeloperoxidase will have an influence on the staining technique and have the potential to give false differential counts.

Increases of red cell mass (haemoglobin, haematocrit, red cell count) can either be absolute or more commonly on toxicity studies be relative, where dehydration results in lowered blood plasma levels and hence falsely increasing the circulating red cell mass. Decreases of red cell mass can be split into those that cause a regenerative response or a non-regenerative response. The ability to respond to the drop in red cell mass with the continuing and/or accelerated production of new red cells (regenerative) via increased erythropoietin and a bone marrow response resulting in increased reticulocytes, increased presence of polychromasia evident in blood smears, extramedullary haematopoiesis and increased spleen weight, or the inability to respond to the drop in red cell mass (non-regenerative) as a result of direct bone marrow effects, cell precursor toxicity or impaired cell division and differentiation. Additional red cell indices such as mean cell volume and mean cell haemoglobin, and alterations in red cell morphology can help to distinguish the reasons for red cell mass loss as is examination of a peripheral blood smear and/or flow cytometric analysis or examination of the bone marrow.

Causes of decreased red cell mass can be further divided into decreased red cell production, red cell maturation defect, blood loss (haemorrhage), or haemolysis (increased red cell destruction). Haemolysis due to accelerated removal from the peripheral circulation, accompanied by red cell turnover, can be further divided into causes such as altered red cell metabolism (for example oxidation), altered red cell membrane, trauma to red cells, and immune-mediated destruction of red cells, and is either extravascular which is slower onset and is more common in toxicity studies, or intravascular which is acute, and can be accompanied with morphological findings in certain cases. Oxidative haemolysis has been observed with aniline via the inhalation route in Han Wistar rats with resulting methaemoglobin formation as the primary toxicity, anaemia, reticulocytosis, presence of Heinz bodies, splenomegaly, and haemosiderin accumulation as in indicator of red cell
turnover (5). Altered red cell membrane via altered lipid composition and loss of fluidity and acanthocyte formation has been found with a selective CXCR3 receptor antagonist (6). Decreases of red cell mass secondary to an underlying disease or processes, for example in cases of inflammatory conditions or endocrine causes, can be quite common but usually presents as only a mild effect, and the cause relates to the sequestration of iron by macrophages. Marrow suppression resulting in decreased red cell production can occur in the following ways: 1. on initial dosing on acute studies or first treatment of a repeat dose study. 2. inhibitory effects on Burst Forming Unit - Erythroid (BFU-E) eg blocking of growth factors, or interference in DNA replication. 3. Defects in haemoglobin or nucleic acid synthesis. 4. Abnormal maturation or maturation arrest. 5. Cell precursors have a high mitotic rate and are therefore sensitive to cytotoxics.

Preanalytical stressors can have effects on leucocytes. They can be endogenous or pharmacologic and tend to be more pronounced in dogs and non-human primates than rodents. Catecholamines (within minutes) are the fright and flight responses and result in increases of neutrophils and lymphocytes and involves demargination, increased blood flow, decreased adhesion, and contributions from the spleen and lungs. Glucocorticoid effects (hours to days) can be over diagnosed in toxicology studies and result in increased neutrophils, with decreases of lymphocytes and eosinophils, with variable changes in monocytes, and involves increased half-life of cells, redistribution and apoptosis (7). Leucocyte action is mainly extravascular, and unlike red cells, blood is just the vehicle for leucocyte transport to other areas. For neutrophils there is a compartmental model of development, storage and transport, consisting of the bone marrow with a proliferation pool and a maturation pool, the peripheral blood with a circulating pool and a marginated pool, and there is a tissue pool. Blood transit time of neutrophils is around 8 to 10 hours and the tissue half-life around 1 to 2 days, and once in the tissues neutrophils are not recirculated and this is the same with monocytes and basophils. Lymphocytes are not stored in the bone marrow but do exist in the marginal and circulating pool of peripheral blood and their transit time can be hours to years with a similar length of time within the tissues.

Alteration of peripheral blood lymphocyte morphology reflecting inclusion of cytoplasmic vacuoles consistent with phospholipid accumulation and lamellar bodies can be observed with cationic amphilic drugs eg dopamine receptor antagonists (8).

Platelets are essential for coagulation, vascular integrity and control of haemostasis. Megakaryocytes give rise to platelets (thrombocytes) with >000’s of platelets per megakaryocyte. Thrombopoietin (TPO) regulates the process of megakaryopoiesis and thrombopoiesis within the bone marrow and is inversely related to platelet mass. Platelet counts in blood reflect the balance between production and consumption, destruction and redistribution to vasculature of organs. Circulating lifespan of platelets in healthy animals ranges from 5-10 days. The measurement of reticulated platelets as an assessment of thrombopoiesis are currently being looked at within toxicity studies.

Thrombocytosis occurs as a primary response following TPO or IL-6 production, in chronic inflammatory disorders, in acute blood loss, changes in the spleen, and during glucocorticoid treatment. Platelets can also be increased as a rebound effect following reversible inhibition of synthesis by chemotherapeutic agents.
Thrombocytopenia occurs from generalised bone marrow suppression (decreased production), with increased peripheral platelet loss or consumption, with destruction in the circulation, with abnormal distribution (sequestration), or with inadequate anticoagulation/bleeding difficulties leading to in vitro platelet aggregation.

For Oncology Advanced Disease Setting there are currently no regulatory requirements to assess drug combinations pre-clinically for tolerability or toxicities (if developed as a monotherapy). However, combination therapies can lead to improved efficacy, reduced resistance, but come with increased / exacerbated toxicities. Currently both efficacy and tolerability are worked out empirically in the clinic.

By using a preclinical rat model, we can assess the impact of dose and schedule on combination therapy tolerability / impact on bone marrow and haematological markers. The caveat is that rats are not humans, the PK handling of the compound can be different, cell cycle times and dynamics can be different, but modelling helps to bridge this. The minimum information is to gain Proof of Concept (PoC) that changing schedules/doses could improve tolerability. With modelling we can translate that PoC into predictions for doses/schedules that can be used in the clinic. Most classic oncology drugs myelosuppression is the dose limiting toxicity, and risk of neutropenia is a major concern, so predictions of the full time-course of myelosuppression in patients based on pre-clinical data would be valuable. A semi-physiological model developed by Friberg consists of one proliferative compartment ie drug-sensitive cells, and three transit compartments of drug insensitive cells representing cell maturation, and the one compartment of peripheral circulating cells where observations are made, and a feedback loop representing regulation factors such as G-CSF. For neutrophils in humans this cell is the more dominant, whereas in rodents the lymphocytes are the more dominant type, cell turnover rate is inversely related to body size and therefore the transit time will be lower (faster) ie for neutrophils non-mitotic development is 3 days in rats, 6.6 days in humans. The model can account for rat and human specific rates of production, maturation time, baseline and feedback (9).

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CYTOLOGY AS A DIAGNOSTIC TOOL IN INTERPRETING SUPERFICIAL AND CUTANEOUS SKIN INFECTIONS

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Microscopic examination of cutaneous cytological samples is a simple, easily obtained, non-invasive, valuable diagnostic tool that is strongly recommended for the interpretation of skin lesions in a dermatologic patient. Additionally, cytology is less expensive and quicker in processing than other laboratory tests, f.e. skin biopsy and bacterial and/or fungal cultures. Even though definite diagnosis in a dermatologic case may not be possible with only cytological examination, useful clinically information can be gained with this test that can help with the initiation of a therapeutic plan. However, there are limitations since for a complete assessment of cytologic smears, the practitioner should choose the appropriate method for sampling of each skin lesion as well as the correct preparation technique of the slide. An adequate cytological sample should be of good quality and quantity. It is preferable to have a small number of intact cells than a bloody smear or rich cellular smear of damaged cells. The necessary equipment that is required includes glass slides with frosted ends, pencils, coverslips, 2.5 and 5ml syringes with 23- and 21-gauge needles, cotton swabs, acetate clear tape, staining liquids, blade and a microscope with oil immersion.

There are more than one sampling techniques and each of them is recommended based on the type and the anatomical site of skin lesion. Any skin lesion or lesional area can be sampled for cytological examination. Impression smears are useful in crusty, exudative, ulcerative or greasy lesions, in superficial pustules, blisters and vesicles and from skin biopsy tissues after blood clotting. Intact pustules and blisters are gently opened with a 25-gauge needle and glass slide is pressed repeatedly on the lesion. It is strongly recommended to perform more than one imprint on the slide and preferably in the center area of the slide for better microscopy guidance and to avoid damaging cells. Cotton swabs are used to collect greasy, ceruminous or purulent material from ear canals, fistulas, skin folds and interdigital skin. Cytology smears are prepared by gently rolling the cotton swab on the glass slide. In case of dry lesions, cotton tip can be moistened with few drops of saline before sampling to minimize cell damage. Acetate tape preparations are performed by repeatedly (usually two to three times) pressing a strip of clear acetate tape on the skin, particularly on greasy areas, on sites that Malassezia dermatitis is suspected and on lesional skin fold sites. Skin scrapings are performed with the blunt edge of a blade from areas with thick greasy lesions f.e. base of nails and from shallow ulcerative lesions and collected material is then spread on a glass slide. Scraping technique is suggested in cases where the cellular yield with impression smears is low and cytologic information is poor. In fine needle cytology, a needle is inserted into a nodular lesion to collect cells for cytologic evaluation. There are two techniques for fine needle cytology, one with and another without negative pressure. The technique that does not involve the use of a syringe (without negative pressure) is suggested for soft masses and decreases the risk of traumatic pressure in the mass and the frequency of blood contamination of the sample. Once the sample is collected into the needle, the material is “released” onto a slide by blowing a syringe full of air through the needle and then spread in a thin preparation with the aid of another slide. All cytological samples must be air-dried before staining, commonly with rapid stains f.e. modified Wright stains (Diff Quick, Hemacolor). Samples are immersed 5–10 seconds each in fixation liquid (ethanol), in the red
stain and in the blue stain, then shortly rinsed under tap water and air-dried. There is not a pre-determined number of immersions to be made in each solution. Usually it is recommended to do at least three to four immersions for each stain. Acetape tape preparations can also be stained as a slide in both stains or with just few drops of a rapid blue stain, such as lactophenol cotton blue or crystal violet or the blue stain of Diff Quick, on the glass slide. The adhesive tape is not commonly immersed in the fixative, because it curls and cannot be easily then placed onto the slide for the microscopy examination. After staining the sticky surface of the tape is applied on the slide and excess stain can be removed with absorbent paper gently pressed onto the upper surface of the slide. Other stains that can be used in cutaneous cytological examination are Gram stain for bacteria, Ziehl-Nielsen for Mycobacteria, PAS for fungi etc. Slides are examined under the microscope first scanned at 4x and 10x to evaluate the quantity of cells, the quality of the sample and to reveal a suitable area for closer examination and then at 40x and 100x (with oil immersion) to properly assess cytologic findings. Cytologic smears of great quality and scientific importance can be kept for years if mounted with synthetic glue f.e. Entellan® for cytology.

The main purpose of cutaneous cytology in a practioner is to identify pathogen microorganisms such as bacteria or fungi, and to assess prominent infiltrating cell type f.e. inflammatory cells, acantholytic cells and neoplastic cells. The presence of acantholytic keratinocytes is not pathognomonic for pemphigus complex cases, it can be also seen in the pustular form of canine dermatophytosis due to *Trichophyton spp*, the pustular form of canine leishmaniasis, the sterile subcorneal pustular dermatitis, some drug-induced dermatoses and in bullous impetigo due to *Staphylococcus pseudointermedius*. Among all those skin diseases the number of acantholytic cells are significantly greater in pemphigus cases. Inflammatory cells are the most abundant cells seen in a cytologic preparation. The identification of a specific type of inflammatory cell may lead to the suspicion of certain diseases, such as bacterial, immunologic or allergic dermatitis. Degenerate neutrophils are commonly seen with bacterial dermatitis. Cocci are the most frequent found microorganisms on a cytological smear from lesional skin. Cocci are round, basophilic bacteria found individually or in clusters. If intracellular (phagocyted) cocci are found, a true infection is present. Rods are often found individually or in sets of two placed end to end, called diploid rods. Cocci are most often *Staphylococcus spp* or *Streptococcus spp*. Rods are often *Escherichia coli*, *Pseudomonas aeruginosa* or *Proteus* spp. In the presence of bacilli, culture and sensitivity tests are strongly recommended. Quick stains commonly stain all bacteria purple/blue, except *Mycobacteria* that are colorless with Diff Quick and their presence can be evaluated with Ziehl Nielsen stain. Bacteria can mimic melanin granules, keratohyalin granules and stain debris. Melanin granules have brown color and may be round to oval. Keratohyalin granules are pink to purple irregular spheres, which are found within the granular layer cells of the epidermis. Stain is amorphous, it can be seen in different sizes and shapes and often is described as granular debris. The presence of intact neutrophils intermingled with acantholytic cells and eosinophils is very suggestive of pemphigus complex skin diseases. Pemphigus pustules are commonly sterile and in case they are secondarily infected few cocci are usually seen. Non-degenerated neutrophils are commonly seen in immunologic dermatopathies. In furunculosis and chronic deep pyoderma lesions vacuolated macrophages are seen together with degenerated neutrophils and bacteria are commonly few and difficult to find. Granulomatous inflammation (>50% of macrophages) is usually associated with deep mycotic infections, atypical bacterial infections, leishmaniasis, foreign body reactions or sterile granulomatous diseases. In foreign body reactions multinucleated giant cells, that represent numerous macrophages that have
fused together to phagocytose large or toxic foreign material, may be observed. In all the above cases purulent exudate and/or tissues samples must always be sent for fungal and bacterial culture. Lymphocytes and plasma cells are seen in chronic dermatitis with an antigenic stimulus or in immune mediated skin diseases. Common differentials include skin neoplasias and feline plasmacell pododermatitis. Chronic healing wounds, such as lick acral dermatitis lesions often have fibroblasts. Eosinophils are common inflammatory cells in cats and can be found in high numbers in cases of eosinophilic granuloma complex. In dogs, eosinophilia is seen in cases of foreign body reaction, in eosinophilic nasal furunculosis, in some cases of pemphigus foliaecous, in allergic skin diseases (food allergy and flea bite dermatitis) and in sterile eosinophilic pustular dermatitis. Intracellular bacteria can also be seen with eosinophils. Mast cells can be seen in a few numbers in smears from allergic or parasitized patients, especially from cats. Another frequently encountered microorganism on cutaneous cytology preparations is *Malassezia* spp. This yeast is usually found adhered to corneocytes inside ear canal, in the skin and oral cavity. It is an oval, spherical or elongated cell that is stained blue/purple and has a characteristic peanut shape during reproduction phase. Cytologic preparations of a normal external ear canal may contain keratinocytes, amorphous material (cerumen) and small amounts of *Malassezia* yeasts (< 5 per 40x HPF). Increased numbers of *Malassezia* or bacteria without the presence of inflammatory cells are seen in cases of ceruminous overgrowth otitis. In purulent otitis bacteria are mixed with inflammatory cells, neutrophils and macrophages. Previous studies have determined that average otic bacterial counts greater than 5/HPF or rod counts greater than 1/HPF should be considered pathogenic. Another commonly encountered type of bacteria, especially from ear samples, is *Pseudomonas aeruginosa*. Its presence on a cytology samples necessitate bacterial culture and sensitivity test because these rods have high incidence of antibiotic resistance. Another harmless filamentous Gram-negative saprophyte that is seen in cytologic samples from pruritic dermatologic patients is *Simonsiella* spp. These bacteria that inhabits oral cavity is frequently found on pruritic lesional sites adherent to keratinocytes, since its presence is likely associated to licking. Dermatophytes spores and hyphae can sometimes be seen on cytologic cutaneous preparations mixed with inflammatory cells. Dermatophyte spores often appear as round spheres, with a clear halo, usually about twice the size of cocci. Dermatophyte hyphae are filamentous structures often with poor staining characteristics and are found free in the specimen. Other saprophytic fungi or *Alternaria alternata* are seen in cutaneous preparations from constant moist lesions f.e. interdigital skin and lip folds. *Candida albicans* could also be found on cutaneous preparations from immunocompromised/immunosuppressant patients. Other microorganisms, such as agents of subcutaneous or deep fungal infections can be occasionally observed in samples from skin. Last in papular forms of leishmaniasis Leishmania amastigotes can be seen in cutaneous preparations intermingled with few inflammatory cells. Artifacts are often encountered on cytology slides and practitioners need to be familiar with these as they can be often confused with pathogenic material. Proteinaceous amorphous material, stain debris, pollen, air bubbles, hair or cotton fibers are commonly seen in cutaneous preparations.

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IMMUNOTOXICITY INVESTIGATIONS AND IMMUNOPHENOTYPING IN TOXICOLOGY STUDIES

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It is well known that immunosuppression can result in complications from infections or induce lymphomas. Effects on the immune system in terms of pharmaceuticals are generally divided into two different categories: immunosuppression as part of the intended pharmacodynamics, or immunotoxicity as an off-target effect. The ability to raise an immune response to pathogens or other harmful substances is a fundamental process and can be affected by exposure to pharmaceuticals, pesticides, or other chemicals. Therefore, the importance of the immune system to health cannot be ignored when testing the toxicological effects of compounds to which animals and people may be exposed (1).

Immunotoxicity investigations should be conducted in the pre-clinical phase of testing where this is warranted by the mode of action (MoA) of the compound, or indicated as a potential by other routine investigations (i.e. data from standard toxicity studies). These investigations can take the form of challenge-response protocols which focus on cell-mediated immunity, such as a T-cell dependent antibody response (TDAR) study. In some cases, the mode of action for the compound may indicate the potential for an effect on a specific cell type of the immune system, in which case immunophenotyping of peripheral blood can be added to the immunotoxicology investigations or completed as a stand-alone investigation (2). Generally residual blood from haematology assessment can be used for immunophenotyping by flow cytometry, thereby maximising the use of the samples collected on the original study and negating the need for satellite groups. Satellite animals are required due to the increased number of procedures (injections and blood sampling) needed for a TDAR assessment if it is added to a standard toxicity study. In this presentation I give an example study set up, explaining the basic study design used by a pre-clinical CRO for these types of investigations; including injection and blood draw timing, as well as blood volumes.

The TDAR challenge assay used in most cases, and in the case studies of this talk, is the Keyhole Limpet Hemocyanin (KLH) challenge. This involves injection of KLH into the animal and assessment of the production of Anti-KLH IgM and IgG antibodies. The protocol occurs over two weeks to allow the production of each antibody class, and evaluation of blood samples can quantify this response. As stated above, immunophenotyping is accomplished by multi-colour flow cytometry. Assessment for the TDAR response depends on the TDAR assay used, but in the case of the Anti-KLH, it is enzyme linked immunosorbent assay (ELISA) for each immunoglobulin.

Each method used for assessment must be validated by each laboratory conducting the analysis. This means assessing the ELISA and flow cytometry protocols for precision, accuracy, robustness, stability and assay limits (3). In the case of the flow cytometry assay, the gating protocol and capture settings should be the same for all animals on a study, and where possible background data collected from animals of the relevant sex and age (4). Additionally, the function of the cytometer itself needs to be assessed for performance over time to ensure that no drift in the laser function will affect results.
The number of animals to be used in these studies should be considered carefully in light of the wide variation in the level of immune response, 4-6 animals is usually a minimum. This is demonstrated in the case studies where the kinds of responses collected differed greatly between the two studies and therefore required different approaches to reviewing and interpreting the data, specifically in the TDAR assessment. This talk contains illustrative data from two case studies conducted in two different species with apparently different MoAs. These studies demonstrate how these two assays can be used to compliment each other.

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REFERENCES

CANINE AND FELINE BLOOD TYPES AND CROSSMATCHES

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Introduction
Veterinary clinicians play a key role in providing safe and effective transfusion therapy. Blood typing is clinically important to ensure blood compatibility and therefore is recommended for any dog and cat in need of a transfusion or considered to become a blood donor. Moreover, previously transfused viewpoints exist regarding the extent and methods used for compatibility testing.

Canine Blood Types
Blood types are genetic markers on erythrocyte surfaces that are antigenic and species specific. A set of blood types of two or more alleles makes up a blood group system. Dogs have likely more than a dozen blood group systems mostly known as dog erythrocyte antigens (DEA). However, there is no DEA 2 blood group and some may be rather labeled high frequency or common red blood cell (RBC) antigens (e.g. dogs also should be crossmatched. In contrast in cats, there are naturally occurring alloantibodies which could result in acute hemolytic transfusion reactions on a first transfusion and type A and AB kittens may experience neonatal isoerythrosis if born to a type B queen. Unless blood typing is performed regularly in practice, blood may be sent to a clinical pathology laboratory for typing. Different DEA 4) and some have not yet received a DEA designation (e.g. Dal). Canine erythrocytes are either positive or negative for a blood type (e.g., DEA 4+ or DEA 4-), and these blood types are likely codominantly inherited. The DEA 1 system was thought to be an exception with DEA 1.1 (A1), DEA 1.2 (A2) and potentially DEA 1.3 (A3) being allelic. Thus, a dog could apparently be DEA 1.1+ or DEA 1.1- and DEA 1.1- dogs can be DEA 1.2+ or DEA 1.2-. However, these studies were based upon weak polyclonal antibodies (DEA 1.1 and 1.X) requiring Coombs’ reagents. Recent studies with a monoclonal antibody showed that the DEA 1 blood group is a continuum from DEA 1- to weakly to strongly DEA 1+; hence DEA 1.2 typing is no longer offered. The degree of DEA 1 expression is constant and DEA 1+ appears to be dominantly inherited. A recent survey in North America indicates that most dogs are either DEA 1- or strongly DEA 1+ with fewer dogs being weakly to moderately DEA 1+. The biochemical structure of the DEA 1 remains still unknown, but a genome wide association study has identified a likely single locus.

Recent surveys revealed that the Dal-type is not restricted to Dalmatians but is also seen in Doberman Pinschers, Lhasa Apsos and Shih Tzus and thus typing for this blood type is becoming more important particularly for those requiring multiple transfusions. In a related study dogs from North America were screened for two new blood types, preliminarily called Kai 1 and Kai 2. Most dogs were Kai 1+ and only few dogs were Kai 2+ or Kai 1-/Kai2-. The clinical importance is yet to be determined albeit anecdotally dogs can develop anti-Kai 1 alloantibodies. The PennGen Laboratory currently offers Dal and Kai 1 and Kai 2 typing. The clinically most important canine blood type is DEA 1, which elicits a strong alloantibody response after sensitization of a DEA 1- dog by a transfusion and thus can be responsible for a transfusion reaction in a DEA 1- dog previously transfused with DEA 1+ blood. It is currently unknown if DEA 1- dogs are equally sensitized by weakly to strongly DEA 1+ blood, or if weakly DEA 1+ dogs are sensitized by strongly DEA 1+ blood. Furthermore, transfusion
reactions against other blood types or common antigens have rarely been observed and reported. They include reactions against the DEA 4, Dal, Kai 1 and other common RBC antigens; other clinically important blood types may be found in the future. No reagents currently are available against several antigens or are only available on a limited basis, and additional blood types continue to be recognized. Only limited surveys on the frequency of these blood types have been reported, which suggest possible geographic and breed-associated differences.

Strongly antigenic blood types are of great clinical importance because they can elicit a potent alloantibody response. These alloantibodies may be of the immunoglobulin G (IgG) or IgM class and may be hemagglutinins or hemolysins. Based upon experimental and clinical data, dogs can become sensitized after receiving a mismatched transfusion (i.e., a blood unit positive for one or more blood types not found on the recipient’s RBCs). There are no clinically important, naturally occurring alloantibodies (also known as isoantibodies) present before sensitization of a dog with a transfusion. Sensitizing dogs in experimental studies in the 1950s led to the documentation of some transfusion reactions caused by blood group incompatibilities and to the characterization of new blood types.

Clinically the most antigenic blood type in dogs is the DEA 1. Transfusion of DEA 1+ RBCs to a DEA 1- dog invariably elicits a strong alloantibody response. Following a first transfusion, anti-DEA 1 antibodies develop after more than 4 days and may cause a delayed transfusion reaction (rarely clinically documented). However, a previously sensitized DEA 1- dog can develop an acute hemolytic reaction after a second transfusion of DEA 1+ blood. Transfusion reactions also may occur after a sensitized dog receives blood that is mismatched for a RBC antigen other than DEA 1 (e.g. DEA 4 and Dal). However, in most cases the incompatible blood type has not been determined. Because administration of a small (<1 ml) amount of incompatible blood can result in life-threatening reactions, the practice of giving small “test volumes” of donor blood to assess blood-type compatibilities is unacceptable. In contrast, pregnancy does not cause sensitization in dogs, because of a complete placenta, and does not induce alloantibody production; thus dogs with prior pregnancies can be used safely as blood donors.

**Canine Blood-Typing Procedures**

Because of the strong antigenicity of DEA 1, typing of donors for DEA 1 is recommended. Whenever possible, the recipient also should be typed to allow the use of DEA 1+ blood for DEA 1+ recipients. Canine blood typing tests are generally based on serologic identification by agglutination reactions but chromatographic strip methods are also offered. Originally serum from sensitized dogs has been used for typing, but such polyvalent alloantibodies vary from batch to batch, may require Coombs’ reagent to enhance agglutination, and may not be always available and are therefore not optimal. Two monoclonal antibodies against DEA 1 have been developed. The gel column technology, widely used in human blood banking, was found to be an excellent standardized laboratory method (DiaMed), but is unfortunately no longer commercially available. A blood typing card has been available with modifications since the mid-1990s as a simple in-practice kit to classify dogs as DEA 1- or DEA 1+ (degree of reaction can vary). A standardized simple immunochromatographic technique became available in the mid-2000s from Alvedia. Another cartridge with a similar strip technique was introduced by DMS/AgroLabo, but has not been evaluated. Moreover, a third cartridge method in which blood flows through the cartridge is also available (DMS/Abaxis) but seems
Polyclonal reagents against other DEA types are currently only available on a limited bases for DEA 3, 4 and 7 from Animal Blood Resource International (prior Michigan state University and Midwest Blood Services). And only limited anti-Dal reagents from sensitized dogs are currently available in a couple of laboratories like Montreal University and PennGen, monoclonal anti-Kai 1 and anti-Kai 2 alloantibodies have been developed in South Korea. DEA 1 typed and matched patients in need of a transfusion may be typed for DEA 4, Dal and Kai 1/2, which may then permit the localization of a type-matched donor dog.

Caution should be exercised whenever the patient’s blood is autoagglutinating or has a low hematocrit (<10%). If autoagglutination is not too severe, it does not appear to affect the Alvedia strip technique because only free RBCs are moving up the strip. Clinicians and technicians should check for autoagglutination of blood with buffer/saline on a slide or the card. Autoagglutinating blood may be first washed three times with ample physiological saline to overcome the apparent autoagglutination similar to what is done for the Coombs’ and crossmatch testing. However, if autoagglutination after three washes persists at more than 1+, it is considered to reflect true autoagglutination, which may preclude typing (as well as Coombs’ testing and crossmatching), because it always looks like DEA 1+ blood. In such circumstances, DEA 1- blood should be used, until the patient does not agglutinate anymore and can be retyped. DEA 1+ blood from severely anemic animals may not agglutinate when exposed to the anti-DEA 1 or other reagents because of a prozone effect. In these cases, some of the patient’s plasma may be discarded before applying a drop of blood onto the card. Finally, recently transfused dogs may display a mixed field reaction, with only the transfused or recipient cells agglutinating if they were DEA 1 mismatched.

**Canine Blood Crossmatching Test**

Whereas blood typing tests reveal the blood group antigens on the red blood cell surface, blood crossmatching tests assess the serologic compatibility or incompatibility between donor and recipient. Thus the crossmatch test checks for the presence or absence of naturally occurring and induced alloantibodies in serum (or plasma) without determining the blood type and thus does not replace blood typing. These antibodies may be hemagglutinins and/or hemolysins and can be directed against known blood groups or other RBC surface antigens. Many laboratories commonly use a standardized tube crossmatching procedure, but the interpretation of the agglutination reaction is highly variable. The crossmatching test requires some technical expertise, may be accomplished through a veterinary laboratory along with blood typing, and is done with washed EDTA-anticoagulated blood from recipient and potential donor(s). The DiaMed gel column technique and more recently the in-clinic DMS gel tube assay have been evaluated and were found to be simple, sensitive, and standardized methods to crossmatch dogs and cats. In addition, Alvedia introduced a simple strip crossmatch test with a Coombs’ phase.

The major crossmatch tests search for alloantibodies in the recipient’s plasma against donor cells, whereas the minor crossmatch test looks for alloantibodies in the donor’s plasma against the recipient’s RBCs. Generally tube segments from collection bags are used for this purpose in dogs. The presence of autoagglutination or severe hemolysis may preclude the crossmatch testing. A major crossmatch incompatibility is of greatest importance, because it predicts that the transfused donor cells will be attacked by the patient’s plasma, thereby
causing a potentially life-threatening acute hemolytic transfusion reaction. Because fatal reactions may occur with less than 1 ml of incompatible blood, compatibility testing by administering a small amount of blood is not appropriate; this has been shown in experimental studies to potentially result in fatal reactions. A minor crossmatch incompatibility should not occur in dogs if canine donors have not been transfused previously and is of lesser concern because donor’s plasma volume is small, particularly with packed red cell products, and is diluted markedly in the patient. Do not use previously used dogs as donors.

The initial blood crossmatch between two dogs that have never before received a transfusion should be compatible, because dogs do not have naturally occurring alloantibodies. Therefore, a crossmatch may be omitted before the first transfusion in clinical practice for dogs. Because the crossmatch does not determine the blood type of the patient and donor, a compatible crossmatch does not prevent sensitization of the patient against donor cells within 1 to 2 weeks. Thus, previously transfused dogs should always be crossmatched, even when receiving again blood from the same donor. The time span between the initial transfusion and incompatibility reactions may be as short as 4 days and the induced alloantibody can last for many months to years (i.e., years after the last transfusion alloantibodies may be present). Again, a blood donor never should have received a blood transfusion to avoid sensitization. The practice of transfusing patients with the least compatible unit does not have any scientific basis. Nevertheless, some minor agglutination results in crossmatching a patient may be unrelated to alloantibodies and unspecific (e.g., patient’s RBC damage by uremia and other illnesses, donor cells after extended storage of unit in the refrigerator). Of course, any patient with true/persistent autoagglutination may not be matched to any donor.

Although transfusion of blood and its components is usually a safe and temporarily effective form of therapy, there is always a risk for potential hazards. Adverse reactions usually occur during or shortly after the transfusion and can be due to any component of whole blood. Most transfusion reactions can be avoided by carefully selecting only healthy donors; using appropriate collection, storage, and administration techniques; performing blood typing and crossmatching; and administering only the needed blood components.

Transfusion Reactions In Dogs
While transfusion of blood and its components is usually a safe and temporarily effective form of therapy, there is always a risk for potential hazards. Adverse reactions usually occur during or shortly after the transfusion and can be due to any component of whole blood. Most transfusion reactions can be avoided by carefully selecting only healthy donors, using appropriate collection, storage, and administration techniques, performing blood typing and crossmatching, and administering only needed blood components. The most common clinical sign of transfusion reaction is fever, followed by vomiting and hemolysis. Hemolytic transfusion reactions can be fatal and are, therefore, most important, while fever and vomiting are usually self-limiting. Adverse effects of transfusions can be divided into non-immunologic (pyrogen-mediated fever, transmission of infectious agents, vomiting, mechanical hemolysis, congestive heart failure, hypothermia, citrate toxicity, pulmonary complications) and immunologic reactions (acute and delayed hemolytic transfusion reactions, urticaria to anaphylaxis, acute respiratory distress, graft versus host disease). Note that some clinical signs may be caused by both mechanisms. Despite the variety of blood
types and the limited degree of compatibility testing in clinical practice, transfusion reactions are rarely reported.

**Feline Blood Typing**

The major feline blood group system is known as the feline AB blood group system and contains 3 alleles: type A, type B, and the extremely rare type AB (fairly common in Ragdolls). Type A is dominant over B. Thus, cats with type A blood have the genotype a/a or a/b, and only homozygous b/b cats express the type B antigen on their erythrocytes. In the extremely rare AB cat, a third allele (C) recessive to the a allele and/or codominant to b allele leads to the expression of both A and B substances. Noteworthy, AB cats are not produced by mating of a type A to a type B cat unless the A cat carries the rare AB allele. Cats with type AB blood have been seen in many breeds and domestic shorthair cats but particularly in Ragdolls.

Most domestic shorthair cats have type A blood, but the proportion of type B cats can be substantial in certain geographical areas. The frequency of A and B blood types varies greatly between different breeds, but likely not much geographically in purebred cats. Kitten losses due to A-B incompatibility and changes in breeding practices influence the frequency of A and B in various breeds. Most blood donors have type A blood, but some places also keep cats with the rare type B and type AB as donors. All blood donors must be typed. Naturally-occurring alloantibodies have been well documented in type A and type B cats and absolutely require that blood typing be performed prior to both blood transfusion and breeding to assure appropriate blood compatibility.

Cats have naturally-occurring alloantibodies. All type B cats have very strong naturally-occurring anti-A alloantibodies, which can be detected by hemolysis and hemagglutination assays. Kittens receive alloantibodies through the colostrum from type B queens and all type B cats develop high alloantibody titers (>1:32) after a few weeks of age. These alloantibodies are strong hemolysins and hemagglutinins, and are of the IgM and, to a lesser extent, IgG classes. They are responsible for serious transfusion reactions and neonatal isoerythrolysis in type A or AB kittens born to type B queens. Type A cats have weak anti-B alloantibodies, and their alloantibody titer is usually very low (1:2), nevertheless they can also cause hemolytic transfusion reactions, but have not been associated with NI. Type AB cats have no alloantibodies. Furthermore additional blood group systems have been identified such as the common Mik red blood cell antigen in domestic shorthair cats and Mik- cats may also produce naturally occurring alloantibodies.

Blood typing relies on identification of surface antigens, leading to agglutination and hence can distinguish A, AB or B phenotypes. Several different reagents may be used but monoclonal antibodies against the type A and type B antigen are currently used in typing kits versus sera and lectins from the past. A genetic test has also been offered for identification of the b allele, but more recent research shows a more complex pattern and requires a panel of markers allowing precise identification of type A, B, and AB phenotypes in cats.

Noteworthy, there are no feline universal donor cats. All donors and patients need to be typed, even if it is “only” a domestic shorthair cat. Simple AB blood typing cards (DMS Laboratories, Flemington, NJ) and chromatographic strip cartridges (Alvedia DME, Lyon, France and recently DMS) are available for in practice use beside less well established...
cartridge methods.

Blood crossmatching tests: Blood incompatibilities have been recognized related to the AB blood group system, following blood transfusion and even on a first transfusion in cats through crossmatch or as a result of observing acute hemolytic transfusion reactions. Standard laboratory tube and gel column crossmatching techniques, but also in-clinic gel tube (DMS and Alvedia) kits are now available. Screening feline blood donors and patients for the presence of naturally occurring (AB and Mik systems) or induced alloantibodies prove necessary in clinical practice. The presence of severe persistent autoagglutination or severe hemolysis may preclude the crossmatch testing.

Table. Examples of blood type A and B frequency in cats in certain countries and breeds*

<table>
<thead>
<tr>
<th>DSH cats</th>
<th>Type A</th>
<th>Type B</th>
<th>Percentage (%)</th>
</tr>
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<tbody>
<tr>
<td>USA</td>
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<tr>
<td>North Central</td>
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<tr>
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<tr>
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<td></td>
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<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
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<td></td>
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<tr>
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<td></td>
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<tr>
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<td>5</td>
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<tr>
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<table>
<thead>
<tr>
<th>Purebred cats</th>
<th>Type A</th>
<th>Type B</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>Abyssinian</td>
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<tr>
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<tr>
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<td>Devon rex</td>
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<tr>
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<tr>
<td>Norwegian Forest</td>
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<tr>
<td>Oriental shorthair</td>
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<td>18</td>
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<tr>
<td>Sphinx</td>
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<td>17</td>
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<tr>
<td>Tonkinese</td>
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<td></td>
</tr>
<tr>
<td>Turkish Angora/Van</td>
<td>50</td>
<td>50</td>
<td></td>
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</tbody>
</table>

*Ignoring the rare AB cats in many breeds with type B cats

The major crossmatch tests for alloantibodies in the recipient's plasma against donor cells, whereas the minor crossmatch test looks for alloantibodies in the donor's plasma against the recipient's RBCs. Mixing a drop of donor/recipient blood with recipient/donor plasma will detect A-B incompatibilities, if typing is not available. However, proper techniques for crossmatching and experience are required to detect other less severe incompatibilities. A major crossmatch incompatibility is of greatest importance because it predicts that the transfused donor cells will be attacked by the patient's plasma, thereby causing a potentially life-threatening acute hemolytic transfusion reaction. As fatal reactions may occur with <1-2 ml of incompatible blood, compatibility testing by administering a small amount of blood is not appropriate. This has been shown in experimental studies to result in fatal reactions. The major and minor crossmatch can show incompatibilities prior to any transfusion due to the presence of naturally occurring alloantibodies in cats, not only for the AB but also the Mik and possibly other blood group systems.

Previously transfused cats should always be crossmatched, even when receiving blood from the same donor. The time span between the initial transfusion and incompatibility reactions may be as short as 4 days and lasts for many years (i.e., years after the last transfusion alloantibodies may be present). Obviously, a blood donor should never have received a
blood transfusion to avoid donor sensitization.

**Xenotransfusion**

Occasionally anemic cats are given canine blood because either no feline blood is available or the feline blood is incompatible (AB, Mik and other mismatch). In our recent study, we determined that canine blood is incompatible and very short-lived (<4 days) in cats. Therefore, we do not recommend such xenotransfusions (Euler et al 2016). Apparently, Oxyglobin, a highly purified bovine hemoglobin solution, should be again shortly available as it has been FDA approved and found to be extremely helpful when feline compatible blood is not available.

Supported in part by a grant from the NIH (OD 010939). The author’s laboratory PennGen is offering quantitative DEA 1, Dal and Kai typing. Alvedia and DMS Laboratories kindly provided reagents and kits for the authors’ studies. The author is the director of the non-for-profit PennGen Laboratory offering genetic and hematological testing.
HEREDITARY IMMUNODEFICIENCIES AND INFECTIOUS DISEASES

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Introduction
Animals with recurring or persistent, antimicrobial-unresponsive and unusual infections likely suffer from a hereditary (primary) immunodeficiency disorder. Immunodeficiencies represent a large heterogeneous group of dysfunctions of host immunity increasing the risk for infections. They can arise through disturbances in antigen-specific defense mechanisms mediated by lymphocytes, the nonspecific defense system (which includes phagocytes, plasma proteins, and physical barriers), or both. Many genetically determined immune defects have been described in the dog, whereas only a few are known in cats. A definitive diagnosis often requires specific immune testing in addition to routine laboratory tests, and therapeutic interventions are limited. The molecular defects for several primary immunodeficiencies have been elucidated allowing for DNA screening. A few hereditary immunodeficiency disorders are prevalent within certain breeds of dogs, whereas others occur in isolated families/cases.

The nonspecific immune system, also known as innate or natural immunity, should be functional at birth and available on short notice to protect the host from invasion by all sorts of organisms. It includes physicochemical barriers, phagocytes, complement and other plasma proteins, and natural killer cells. Congenital barrier defects particularly involve the skin and mucous membrane surfaces and are associated with infections of particular organs. A variety of hereditary skin diseases are being further defined and barrier but also other immunodeficiencies are being recognized. The Ehlers-Danlos syndrome, causing fragile, hyperextendable skin in many dogs and cats as well as the myxedematous skin and immunodeficiencies of Shar-pees, predisposes the animals to pyoderma, whereas ciliary dyskinesia in dogs increases the susceptibility to rhinosinusitis and pneumonia. Similarly x-chromosomal ectodermal dysplasia in German Shepherds is associated skin as well as other immunodeficiencies.

Disorders of the phagocytic system involve defects of neutrophils and monocytes as well as the complement system and can lead to pyogenic and granulomatous infections. The granulomatous reaction can occur when neutrophils malfunction and mononuclear cells are recruited. A wide variety of pyogenic bacteria (e.g., staphylococci, Escherichia coli, Klebsiella, Enterobacter) are usually involved, most of which represent normal microflora or pathogens of relatively low virulence. Recurrent infections of the skin, respiratory tract, and oral cavity are common, and intermittent bacteremia and overwhelming sepsis are also seen. Multisystemic amyloidosis, vasculitis, and immune complex disease are complications that can occur as a result of chronic recurrent or persistent infection. Cyclic hematopoiesis and leukocyte adhesion deficiency (LAD) are examples of serious quantitative and qualitative phagocytic defects, respectively. A unique immunodeficiency causing a predisposition to avian tuberculosis in Miniature schnauzers (and also Bassets) has recently been elucidated at the molecular level.

The specific immune system can be divided into humoral and cell-mediated immune systems and includes B and T lymphocytes, immunoglobulins, and cytokines. Deficiencies of B lymphocytes or humoral immunity affect the production of immunoglobulins and lead to
increased susceptibility to pyogenic bacterial infections. Deficiencies of T lymphocytes or cell-mediated immunity (CMI) are associated with viral and fungal infections, but intracellular bacterial infections may also occur. Animals with cellular immunodeficiencies may have smaller thymic and tonsillar tissues as well as intestinal and peripheral lymph nodes and decreased numbers of circulating lymphocytes.

The degree of immunodeficiency varies greatly between defects. Infections may be systemic or restricted to a particular organ system like the skin or respiratory tract. Some immunodeficiencies lead to overwhelming infections and death within the first few days to weeks of life, whereas others, such as morphologic leukocyte changes, are not consistently associated with any noticeable predisposition to infection. Chédiak-Higashi syndrome in smoke-colored Persian cats is characterized by abnormally large eosinophilic granules in polymorphonuclear leukocytes. It causes no immunodeficiencies but does cause a bleeding tendency resulting from a platelet storage pool disease. Similarly, Birman cats with acidophilic granulation of neutrophils and dogs with various lysosomal storage diseases (e.g., mucopolysaccharidosis, gangliosidosis, mannosidosis) have granulation or vacuolation of leukocytes without being immunocompromised; they also exhibit frequently a lymphocytosis. The Pelger-Huët anomaly, which is characterized by hyposegmentation of granulocytes, causes no immunodeficiency in animals, despite the fact that the leukograms of affected dogs and cats reveal the most severe left shift with a normal leukocyte count.

Although an increased susceptibility to opportunistic infections develops, the type of infection varies depending on the type of defect within the immune system. A few immunodeficiency disorders predispose animals to a restricted group of unusual infectious agents. Some male dachshunds appear predisposed to *Pneumocystis* pneumonia, and German shepherd dogs may be prone to systemic aspergillosis or rickettsiosis. Doberman pinschers and Rottweiler dogs are more likely to develop parvoviral disease. Golden and Labrador retrievers and Bernese mountain dogs that had high serum antibody titers to *Borrelia burgdorferi* were more likely to have glomerulonephritis. Bassett hounds and miniature schnauzers have an increased susceptibility to systemic avian mycobacteriosis, and possibly toxoplasmosis, and neosporosis. American and English foxhounds appear to be predisposed to developing leishmaniasis. Great Danes and Dobermans may be more susceptible to cryptococcal infections. A genetic predisposition to demodicosis has been proposed in various canine breeds and families. Feline infectious peritonitis has also been suggested to have a genetic basis. The mechanisms predisposing particular animals to specific infections remain unknown in many breeds but was recently discovered in Miniature Schnauzers with increased susceptibility to systemic fatal avian tuberculosis.

**Major Clinical Signs of Primary Immunodeficiency Disorders**

1. Recurrent infections, chronic and protracted course of infection, or both
2. Infection with common nonpathogenic (opportunist) or aberrant infectious agents
3. Severe and often atypical infectious disease manifestations
4. Delayed, incomplete, or lack of response to antimicrobial therapy
5. Adverse reactions to modified-live virus vaccines

The above mentioned key signs of infection develop in animals with a primary immunodeficiency generally early in life. Despite receiving colostrum, clinically affected animals may have illness during the neonatal to juvenile period and may develop recurrent
and overwhelming infections that lead to severe debilitation and death before 1 year of age. Several animals, but typically not all, in a litter may be affected, whereas the parents are usually healthy. A genetic predisposition to infection is rarely noted after 1 year of age (e.g., avian tuberculosis in Miniature Schnauzers). Furthermore, animals with primary immunodeficiencies may have other special clinical manifestations. Hypersensitivity reactions may occur and reflect an overall dysregulation of the immune system caused by a lack of one or more components or a chronic antigen stimulation from inadequate clearance of infections. Chronic systemic infections may also hamper the growth rate. Characteristic coat color dilutions and increased tendency for surface bleeding are seen e.g. in collies with cyclic hematopoiesis, Persian cats with Chédiak-Higashi syndrome, and Weimaraners with an incompletely defined immunodeficiency. Nude Birman kittens and ectodermal dysplasia are associated with a complete lack or loss of hairs.

The mode of inheritance of primary immunodeficiencies has not yet been determined in all cases. Autosomal recessive transmission, with affected males and females born to healthy parents, is usual, but a few exceptions exist. The Pelger-Huët anomaly is inherited as an autosomal dominant trait. Severe combined immunodeficiency caused by two different mutations in the common γ-chain interleukin-2 (IL-2) receptor in Basset hounds and Cardigan Welsh corgis are X-chromosomal recessive disorder, so only males are affected, and the dams and half of her female littermates are carriers. Thus, the breed, gender, age of onset, type of infections, and other special characteristics may suggest a particular immunodeficiency. Furthermore, it follows that within a breed the immunodeficiency is typically caused by the same defect and mutation while different breeds may have mutations in the same or different genes.

### Diagnostic Studies

Although an immunodeficiency may be suspected on the basis of clinical evidence, specific laboratory tests are generally required to reach a definitive diagnosis. A minimum database of information, including results of a complete blood count, serum chemistry screen, and urinalysis, should always be obtained and may suggest a specific disorder. The differential leukocyte count and microscopic evaluation of a blood smear are the most important test results. Leukopenia in the presence of an active bacterial infection is by far the most feared condition. It should be noted that generally, some breeds have normally low white blood cell counts such as greyhounds. Neutropenia may be transient, as it occurs with cyclic hematopoiesis every 12 to 14 days or parvovirus infection, or persistent, as it is seen in animals with cobalamin malabsorption or overwhelming infections (sepsis). Lymphopenia may be observed in dogs with a T-cell or severe combined immunodeficiency. Although leukocytosis is expected during periods of infection, defects in leukocyte adhesion and egress from blood circulation at sites of infection may be associated with disproportionately high leukocytosis for the degree of infection as seen with hereditary LAD and glucocorticoid usage. Dachshunds with *Pneumocystis* pneumonia also have very marked leukocytosis. Anemia of chronic disease is often observed in infected animals caused by several factors, but the erythrocyte count may be in the normal range even if the animals have active infections and during periods of treatment and remission. Careful review of a blood smear may reveal leukocyte abnormalities such as granulation and vacuolation resulting from lysosomal storage diseases or Chédiak-Higashi syndrome, acidophilic granulation of leukocytes in Birmans, phagocytized microorganisms, or toxic leukocyte changes that suggest overwhelming bacterial infections.
Serum globulin concentrations are generally higher during chronic infections. Low or normal globulin levels in infected animals may suggest major external losses or diminished production from a humoral (B-cell) immune defect. Indeed, specific immunoglobulin deficiencies have been recognized in dogs. Serum protein electrophoresis may identify a γ-globulin deficiency, but immunoelectrophoresis is required to detect the class and degree of immunoglobulin deficiency. Maternal immunoglobulins can only be absorbed during the first day of life and influence the values during the first few weeks. IgM can be synthesized very early in life, whereas the development of IgA may be delayed for months. Thus it is important to compare values with data from age-matched controls. Titers against specific antigens can be measured, followed by evaluation of the antibody response to vaccination against particular agents. T-cell or combined immunodeficiencies cause defective CMI responses. The animal may have prolonged allograft rejection times and decreased delayed-type hypersensitivity to skin testing with viral vaccines, tuberculin, or dinitrochlorobenzene (DNCB). Reduced in vitro lymphocyte stimulation results may also be caused by a primary lymphocyte defect or the infection.

The identification of the agents infecting an animal is important for diagnostic as well as therapeutic reasons. Appropriate cultures of tissues, body fluids, and excretions for microorganisms and antigen and serologic blood tests are addressed in the chapters on specific infectious agents. Antibody titers may also be used to assess a response to vaccines and humoral immunity.

Gross and microscopic histopathology and cytology may reveal certain microorganisms but are most helpful in characterizing the architecture, morphology, maturation, and function of the immune system, such as of the leukocytes, bone marrow, lymph nodes, thymus, and spleen, as well as other barrier systems. In ciliary dyskinesia, morphologic abnormalities of cilia may be identified by electron microscopy, but functional studies by imaging techniques or on respiratory epithelial biopsy specimens are also indicated.

For additional characterization of the immunodeficiencies, special leukocyte studies are often required. Surface marker studies by fluorescent-assisted cell sorters or flow cytometers can differentiate between T- and B-cells, determine T- and B-cell ratios, and determine the presence or absence of leukocyte adhesion proteins (CD11/18) or IL-2 receptors. Lymphocyte function studies include lymphocyte stimulation and plaque-forming assays for in vitro immunoglobulin production. Phagocyte function studies assess leukocyte adhesion, migration, chemotaxis, phagocytosis, “respiratory burst,” and bactericidal activity. All functional assays should be performed on fresh blood cells (<1 day) and compared simultaneously with an age- and breed-matched control. Furthermore, in vitro lymphocyte functions are generally impaired and phagocyte functions are enhanced during periods of active infection. Whenever possible, it is advisable to control the infection before studying leukocyte function.

**Treatment and Prevention**

Successful control of infection in immunodeficient animals depends on the underlying disease as well as the type and severity of the immune defect. In immunocompromised patients, early and aggressive antimicrobial therapy is indicated even for mild infections with nonpathogenic agents. Because of the immunodeficient host’s potential inability to kill bacteria, bactericidal antibiotics are recommended until bacterial infections are controlled.
No practical treatments for primary immunodeficiencies exist (except for parenteral cobalamin administration to animals with cobalamin malabsorption). Immunocompromised animals with infection generally have a guarded to poor prognosis. Despite aggressive antimicrobial therapy, their infections are difficult to control, leading to overwhelming infections, protracted courses, and recurrences. Some leukocyte defects cause death before 1 year of age, whereas others may not lead to a markedly increased predisposition to infection. In experimental studies, bone marrow transplantation and gene therapy corrected several canine leukocyte defects. Indeed dogs with hereditary immunodeficiencies and other genetic defects have served as intermediate between experiments in murine models and its application in humans to test safety and efficacy of novel therapies.

Owners must consider the potential zoonotic risks involved with keeping an immunodeficient animal with infections that may be contagious to humans, particularly immunosuppressed humans exposed to foxhounds with leishmaniasis and Miniature Schnauzers and basset hounds with avian mycobacteriosis.

**Examples of Primary Immunodeficiencies**

<table>
<thead>
<tr>
<th>Disease (Syndrome)</th>
<th>Inheritance</th>
<th>Breeds</th>
<th>Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliary dyskinesia (immotile cilia syndrome)</td>
<td>AR</td>
<td>Many dog breeds</td>
<td>Rhinosinusitis, bronchopneumonia with bronchiectasis, situs inversus</td>
</tr>
<tr>
<td>Complement component 3 (C3 deficiency)</td>
<td>AR</td>
<td>Brittany Spaniel</td>
<td>Pyogenic infections, lack of complement-mediated phagocytosis</td>
</tr>
<tr>
<td>Bactericidal neutrophil defect</td>
<td>U</td>
<td>Doberman Pinscher</td>
<td>Upper respiratory infections, reduced bactericidal activity</td>
</tr>
<tr>
<td>Cyclic hematopoiesis (cyclic neutropenia)</td>
<td>AR</td>
<td>Collie (gray)</td>
<td>Severe neutropenia every 12-14 days, reactive amyloidosis</td>
</tr>
<tr>
<td>Leukocyte adhesion deficiency (LAD or CD18 deficiency)</td>
<td>AR</td>
<td>Irish Setter, Red and White Setter, cat</td>
<td>Severe leukocytosis, infection with limited pus formation, lack of neutrophil adhesion</td>
</tr>
<tr>
<td>Pelger-Huët anomaly</td>
<td>AD</td>
<td>Aust. Shepherd, Foxhound, others, cats</td>
<td>Hyposegmented granulocytes, no immunodeficiency</td>
</tr>
<tr>
<td>Selective cobalamin malabsorption (Cubulin or Amnionless deficiency)</td>
<td>AR</td>
<td>Giant Schnauzer, Border Collie, Beagle, A. Shepherd, Komondor</td>
<td>Weight loss, inappetence, leukopenia with hypersegmentation megaloblastic bone marrow, methylmalonic aciduria</td>
</tr>
<tr>
<td>Condition</td>
<td>Mode</td>
<td>Breed/Genotype</td>
<td>Associated Conditions</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>------</td>
<td>-------------------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Increased susceptibility to avian mycobacteriosis</td>
<td>U</td>
<td>Miniature Schnauzer, Basset Hound</td>
<td>Systemic avian tuberculosis and few other unusual infections</td>
</tr>
<tr>
<td>Increased susceptibility to <em>Pneumocystis</em> pneumonia</td>
<td>AR</td>
<td>Dachshund</td>
<td><em>Pneumocystis</em> pneumonia</td>
</tr>
<tr>
<td>Susceptibility to fungal and rickettsial infections</td>
<td>U</td>
<td>German Shepherd</td>
<td>Severe ehrlichiosis, Rocky Mountain spotted fever, disseminated aspergillosis</td>
</tr>
<tr>
<td>X-linked severe combined immunodeficiency (X-SCID)</td>
<td>XR</td>
<td>Basset Hound, Cardigan Welsh Corgi</td>
<td>Severe bacterial and viral infections, no IgG and IgA, deficient lymphocyte blastogenesis</td>
</tr>
<tr>
<td>Severe combined immunodeficiency (SCID)</td>
<td>AR</td>
<td>Jack Russell terrier, Friesan Water dog</td>
<td>Severe serum immunoglobulin deficiency, hypoplasia of lymphoid tissues</td>
</tr>
<tr>
<td>Thymic abnormalities and dwarfism</td>
<td>U</td>
<td>Weimaraner</td>
<td>Reduced growth, thymosin responsive</td>
</tr>
<tr>
<td>Recurrent infections/inflammation</td>
<td>U</td>
<td>Weimaraner</td>
<td>Pyoderma, severe abscess, bleeding tendency</td>
</tr>
<tr>
<td>Selective IgA deficiency</td>
<td>U</td>
<td>Beagle, Shar-pei, German Shepherd</td>
<td>Respiratory and GI infections</td>
</tr>
<tr>
<td>Hypotrichosis congenital and thymic atrophy</td>
<td>AR</td>
<td>Birman</td>
<td>Nude kittens, neonatal death, no thymus</td>
</tr>
<tr>
<td>Chédiak-Higashi syndrome</td>
<td>AR</td>
<td>Persian</td>
<td>No immunodeficiency, large granules in phagocytes, bleeding tendency</td>
</tr>
</tbody>
</table>


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**IN VITRO TO IN VIVO PREDICTIVE TOXICOLOGY**

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**Summary:** A multiparametric, live-cell, high-content-analysis (HCA) cytotoxicity assay was demonstrated to be highly concordant with human hepatotoxicity, including idiosyncratic hepatotoxicities, and with numerous, other, target organ toxicities in contrast to historical assays. The success of the assay was attributed to its simultaneous measurement of multiple appropriate “cytobiomarkers”; use of human cells with xenometabolic competence for toxicities mediated by metabolites, 72-hours exposure to enable expression of slower-acting toxicants, exposure to a wide-range of concentrations from 30- to 100-fold the efficacious concentration, and normalizing the in vitro cytotoxic concentration to an estimate of the in vivo concentration of exposure. An overwhelming volume of evidence has accumulated over the last 10 years to support this approach as necessary in predictive toxicology. Equivalent assays have now been successfully applied in ~50 studies across a wide variety of toxicants, toxicities, cell types, and disciplines. Review of the wider literature on cytotoxicity since the first assay was reported 100 years ago supports the selection of key cytobiomarkers along a final common pathway of cell injury, including cell proliferation, mitochondrial activity, apoptosis, lysosomal mass, oxidative stress, and cell membrane permeability. HCA studies without inclusion of such key cytobiomarkers or without testing to sufficiently high concentration have not been as successful. A wide range of HCA studies has confirmed their high sensitivities and specificities in predictive toxicology across locations, HCA technologies, staff, laboratories, and time.

**Introduction:**

**Safety Attrition is Common and Expensive:** Safety has been an important cause of marketplace attrition of drugs. It costs almost two billion dollars to bring the average drug through discovery and development to registration. Furthermore, typically 10–12 years of investment of time by hundreds of pharmaceutical staff will have been mis-spent when a drug is forced off the market. Over a 25-year period ending in 1999, there was an annual average of 0.7 approved drugs withdrawn and a further two drugs receiving black-box warnings. From 1994 to 2006, the average safety attrition was even higher with 2.1 drugs per year and 38 drugs overall withdrawn from the marked by the US Food and Drug Administration. Of drug candidates entering clinical development from 2006-2010 nearly one-quarter failed due to safety issues. More than 80% of market withdrawals due to drug toxicity were due to cardiotoxicity and hepatotoxicity. Drug-induced liver injury was found to be the most frequent reason cited for withdrawal of an approved drug and to account for more than half the cases of acute liver failure.

**Animal Alternatives are Needed:** *In vivo*, animal studies, alone, have not and will not meet the needs of predictive toxicology for a number of reasons. In fact, safety attrition is high because of the ineffectiveness of animal models, especially with regard to hepatotoxicity and cardiotoxicity, where there may be greater, species specificity of drug effects than for other target organ toxicities. New and more translational safety biomarkers are needed. The ethical drive for the “3R’s” of animal use, namely reduction, replacement, and refinement, clearly limit the use of animal models in screening drug candidates. Furthermore, animal
studies are typically far more expensive than in vitro methods, by orders of magnitude. Modern technologies of Drug Discovery, such as combinatorial chemistry and high throughput screening have saturated the capacity of animal model-based approaches.

**In Vitro Cytotoxicity Models have Evolved:** It’s a hundred years since Pappenheimer introduced the first cytotoxicity assay for trypan blue exclusion. And with development of knowledge of cell biology, a wide-range of additional assays have been developed, including for assessment of growth, metabolic activity, viability, apoptosis or necrosis, leakage of constituents due to loss of membrane integrity, and structure and function of specific activities or organelles. Momentum for use of cell-based assays in predictive toxicology is building. Cell models are already used extensively for predicting efficacy and bioavailability of drugs. The introduction of cell-based bioavailability assays (e.g. Caco-2 for predicting absorption) in Drug Discovery has reduced attrition for this reason by an order of magnitude. They are widely used for assessment of pharmacokinetics and of off-target pharmacologic activity at a wide range of receptors, transporters and enzymes. Specific cytotoxicity assays are well-established and accepted in the Safety Science community, such as for genetic toxicity (eg in vitro micronuclei assay), for phototoxicity, and for phospholipidosis produced by cationic, amphiphilic drugs.

**Technologies for Cytologic Studies have also Evolved:** As knowledge and understanding of cell biology has developed, so have the technologies for studying cells. Direct cell culture and analysis within multi-welled, microtiter plates has greatly facilitated assays. Development of highly specific and sensitive and relatively non-toxic, fluorescent dyes for real-time study of live cell activities and morphology. High-content analysis technology has arrived and been validated. It is basically automated, fluorescence microscopy, with sophisticated software for image analysis. It has many advantages over conventional approaches. Live cell monitoring is done in a physiological micro-environment with controlled temperature, humidity, and O₂ / CO₂ tensions. Monitoring can be done at the entire well, microscopic field, and at the cell and subcellular organelle level. Fluorescent, non-invasive and non-toxic probes are used for simultaneous, multi-parameter monitoring. Kinetic and iterative biochemical and morphological measurements are made, with immunochemical measurements possible at the end of the experiment. There is automated image acquisition and analysis done. There is rapid throughput, with a 96-well plate read in an hour with high precision and accuracy. The technology is now widely commercially available with many vendors producing effective platforms. There are numerous studies that have now validated this approach for concordance against in vivo, human toxicity data.

**Need to Discriminate Sublethal, Chronic and Idiosyncratic from Acute and Explosive Cytotoxicities:** Most conventional cytotoxicity assays (e.g. enzyme release, mitochondrial dye reduction, cell rupture) are for late-stage toxicity and cellular events associated with a lethal effect. Such assays have low sensitivity for detection of adverse cellular effects and provide little mechanistic understanding of the toxicologic effects. In a study of 611 drugs in 7 conventional in vitro assays of human hepatocytes found sensitivity of detection of human hepatotoxicity 25%. In contrast, an HCA approach in the same study had >90% sensitivity. Both conventional and HCA cytotoxicity approaches had high specificity. That is, if a drug was toxic in vitro, by whatever assay, it was generally toxic in vivo.
**HCA Methodology:** Several features of the HCA cytotoxicity model are key to its predictivity: use of live cells, multiple appropriate parameters, sufficient duration of exposure, exposure to multiple concentrations relevant to that which is therapeutic, and minimal invasiveness. The cell type is also critical. It should be of the same species for some cell types, it should have metabolic competence for predictive liver toxicity, and fully differentiated for human, embryonic-induced pluripotent cardiomyocytes. Optimally, it should be proliferative, as this is one of the most sensitive cytotoxicity biomarkers. There is a wide array of cell types that have been used effectively for predicting toxicity, including: hepatic, myocardial, neural, renal, muscle, intestinal, lymphocytes, and macrophages. As an example, in the typical approach used for predicting hepatotoxicity, human, HepG2 hepatoblastoma cells are seeded onto 96-well, polylysine-coated plates at 3,000 cells/well. Cells are allowed to adhere to the plates via the polylysine overnight, so that when they proliferate they will spread along the plate bottom rather than on top of each other. Then up to 7 drugs per plate are loaded at 12 progressively-increasing concentrations per drug, up to ~100 times the maximum plasma concentration used during therapy. Following incubation of cells with the drug for 3 cell doubling-times (3 days), each well in the plate is loaded with a fluorescent-dye cocktail for one hour at 37°C. Then fluorescence images are taken for which measurements can be made for each dye versus drug concentration throughout the cell for all cells. Frequently, a single value referred to as the therapeutic index (TI) is used to report the cytotoxicity, the concentration in vitro at which toxicity is first seen, normalised to the maximum or projected therapeutic concentration in plasma (Cmax) in vivo.

**HepG2: Troglitazone 20X**

![HepG2: Troglitazone 20X](image)

**Doxorubicin – Intermediate CytoTox**

NOTE: Dox artefacts: it displaces Hoechst, inhibits Pgp extrusion of TMRM and fluoresces green)

![Doxorubicin – Intermediate CytoTox](image)

Troglitazone, an insulin sensorizer was withdrawn from the market because of its association with lethal hepatopathy. In the HCA assay it show toxicity at 67 uM, giving a therapeutic index of ~15. A drug with a TI of 100 was typically found to be safe. Doxorubicin, a widely used anticancer drug, is well known to be cardiotoxic in vivo, but it has not been withdrawn because of seriousness of its indication and because of the lack of more effective alternatives. Its toxicity is readily seen in a variety...
of cell types, including circulating blood lymphocytes of oncology patients. It is cytotoxic at 0.1 uM, giving a TI of 0.3. It produces a rare combination of artefacts in vitro, by displacing the DNA dye Hoechst, inhibiting the P-glycoprotein (Pgp) drug extrusion pump and by fluorescing green. Thus for doxorubicin, not only can the TI be measured in a single cell, but so can the efficacious concentration (by DNA intercalation), the cellular concentration (by its intrinsic fluorescence), and the activity of the Pgp pump (by its inhibiting fluorescent dye extrusion).

Cytotoxicity can be immediately detected by the human eye because of the marked qualitative and quantitative effects associated with it. Compared to controls there is a decrease in cell number due to inhibition of cell proliferation. Cytoplasmic fluorescence changes from red to green occurs as mitochondrial activity is lost and intracellular calcium concentration increases. And the blue, nuclear fluorescent staining by Hoechst 33258 is replaced by red staining as Toto-3 displaces the Hoechst dye.

Quantitation of the fluorescent signals on a per cell basis and plotting these against the concentration to which the cells are exposed reveals the cellular pathophysiological temporal-sequence of subcellular events distinctive for the mechanism of toxicity. If the dose-response curve is flat or horizontal, then the substance tested is non-toxic, however if the curve rises or falls, this point of inflection identifies the cytotoxic concentration. Curve fitting to this data identifies the characteristic cytotoxic concentration at which 50% (or other) of maximal changes has occurred. And normalizing this to Cmax discriminates cytotoxic (<100) from non-toxic substances. Such analysis can rank the toxicity of similar (or different) compounds such as the statins. Furthermore, it can rank the susceptibilities of different cell types to the cytotoxicity. For example, cerivastatin, was withdrawn from the market because of its association with a lethal rhabdomyolysis. Its high toxicity compared to other statins is clearly evident by comparative HCA analysis, as is the increased sensitivity of muscle cells compared to hepatocytes.
Validation of HCA Cytotoxicity Model: There are now approximately 100 published studies using an HCA cytotoxicity model that have validated its effectiveness across time, geographies, staff, laboratory and HCA technology. HCA has been used effectively to assess cytotoxicity which predicts human in vivo toxicity for: a) “classic” small-molecule toxic chemicals, including solvents, herbicides, metabolic poisons, detergents, ionophores; b) biological toxins, G- bacterial lipopolysaccharide endotoxin; anti-mycotic (patulin); bee venom (melittin), c) drug delivery carriers: eg pDMAEMA; d) peptides: anti-microbial, casein hydrolysates; e) nanoparticles; and f) adverse environmental factors: pH, temperature, substrate, osmotic strength.

Application of HCA Cytotoxicity: In vitro, HCA cytotoxicity models of predictive human toxicity have been widely adopted across the pharmaceutical sector and is likely to produce substantial reductions in safety attrition, especially due to hepatotoxicity and cardiotoxicity. Additionally, HCA models show promise for effective monitoring of blood mononuclear cells for generic cytotoxicities associated with drugs with known toxic liabilities but without alternatives, such as anti-cancer and anti-infectious drugs, including mitochondrial inhibitors (nucleoside reverse transcriptase inhibitors, oxazolidinonediones) and drugs producing phospholipidosis, cellular oxidative stress, or genetic toxicity (micronuclei formation).


CLINICOPATHOLOGIC ASPECTS OF CANINE MONOCYTIC EHRLICHIOSIS (EHRLICHIA CANIS): DIAGNOSTIC IMPLICATIONS

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Introduction

Ehrlichia canis, a gram-negative, obligate intracellular bacterium (order Rickettsiales, family Anaplasmataceae) is currently recognized as the principal cause of canine monocytic ehrlichiosis (CME) worldwide. It is naturally transmitted by the brown dog tick Rhipicephalus sanguineus. After an incubation period of 8-20 days, the course of E. canis infection, can be divided into acute (2-4 weeks), subclinical (several months to years) and chronic phases, but the distinction among these phases is challenging in the clinical setting. 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. Immunocompetent dogs may eliminate the infection during the acute or subclinical phase, but an unpredictable proportion may progress to the chronic phase, typically characterized by bone marrow (BM) aplasia, peripheral blood bi- or pancytopenia and high mortality due to septicemia and/or severe bleeding. Aplastic pancytopenia appears to be a feature unique to canine E. canis infection and has yet to be reported in other human and canine ehrlichial infections. From a clinical perspective, the terms “non-myelosuppressive” and “myelosuppressive” (form of) CME, may better reflect the clinical severity of the disease, regardless of its time progression.

Clinical presentation in CME

The clinical severity in CME ranges from mild (non-myelosuppressive form) to life-threatening (myelosuppressive form). Fever (or hypothermia in profoundly pancytopenic dogs), depression/lethargy, anorexia, lymphadenomegaly, splenomegaly, mucosal pallor, ocular abnormalities are common clinical manifestations. Bleeding diathesis is the hallmark of CME, is more frequently seen in the myelosuppressive form of the disease and is associated with impaired primary hemostasis due to thrombocytopenia, thrombocytopenia and mild vasculitis. It is manifested typically as superficial bleeding, including cutaneous and mucosal petechiae and ecchymoses, hystema, epistaxis, hematuria, melena, prolonged bleeding from venipuncture sites or intraoperative bleeding. Secondary hemostasis is rarely significantly affected; therefore, space-occupying (e.g. subcutaneous hematomas) or cavitary bleeding (e.g. hemothorax, hemarthrosis) are not consistent with CME. Clinical manifestations in subclinically infected dogs are mild and may go unnoticed by the owners (e.g. splenomegaly, intermittent fever).

Clinicopathologic aspects of CME

Thrombocytopenia is the most prevalent hematological abnormality in CME, appearing in more than 80% of the dogs, regardless of the phase of the disease. Although CME should not be ruled out on the basis of a normal platelet count alone, it is also true that the prevalence of the infection is substantially lower in non-thrombocytopenic as compared to thrombocytopenic dogs. Thrombocytopenia is associated with immune-mediated platelet destruction, increased consumption secondary to mild vasculitis, splenic sequestration, over-expression of a platelet migration inhibition factor, BM failure (myelosuppressive form) or a combination thereof. A non-regenerative anemia, leukopenia, neutropenia (mild-to-
moderate leukocytosis/neutrophilia with or without a mild left shift may rarely be seen) and lymphopenia or mild lymphocytosis are additional abnormalities. Significant neutrophilic leukocytosis (i.e., neutrophils >15-20000/μl) is not consistent with CME. Granular lymphocytosis, and T-cell, CD8⁺ clonal expansion in the blood and other tissues may occur in chronically infected dogs, imitating chronic lymphocytic leukemia/small cell lymphoma. Thus, in endemic areas, CME should be a significant differential for persistent mild-to-moderate lymphocytosis (up to 25,000/μl) in the dog. Aplastic pancytopenia is the typical feature in myelosuppressive CME and the latter is a leading cause of canine pancytopenia in endemic areas. Importantly, pancytopenia with normocellular BM may transiently occur in the acute CME and is easily amenable to medical treatment. Subclinically infected dogs may be presented with mild-to-moderate thrombocytopenia, leukopenia, or anemia.

Hyperproteinemia, hyperglobulinemia, hypoalbuminemia and mildly elevated alkaline phosphatase and alaninoaminotransferase activities are common biochemical abnormalities in CME. Hyperglobulinemia does not correlate with anti-"E. canis" IgG titers and appears on serum electrophoresis as a polyclonal or rarely, oligoclonal or monoclonal hypergammaglobulinemia pattern. Pancytopenic dogs tend to have lower total protein, total globulin and γ-globulin concentrations compared to their non-pancytopenic counterparts. Liver disease may be primary (mostly portal hepatitis) or secondary to hypoxia, intrahepatic hemorrhage, or septicemia in the myelosuppressive CME. Creatinine concentration may be elevated in some dogs, while significant glomerular proteinuria may be present in the acute (potentially reversible even without treatment) and chronic CME attributable to a non-immune complex mediated glomerulopathy and rarely to amyloidosis.

Several reports indicate that in experimentally or naturally infected dogs, significant acute phase protein and antioxidant responses may occur. C-reactive protein, haptoglobin, serum amyloid A, a1-acid glycoprotein and ferritin tend to increase (positive acute phase proteins), while albumin (negative acute phase protein) and paraoxonase-1 (oxidative stress indicator) tend to decrease in dogs with acute and chronic, but not in the subclinical phase of the disease. The clinical implications of these changes have yet to be fully appreciated. For instance, in a study with naturally infected dogs, the concentration of C-reactive protein, haptoglobin and serum amyloid A on admission were useful indicators of the clinical phase and severity of CME (significantly higher concentrations in dogs with myelosuppressive CME), but were not useful predictors of the clinical outcome (death or survival). In another study, they were of limited value as treatment response indicators in experimentally infected dogs.

Demonstration of "Ehrlichia" spp. morulae in monocytes, macrophages and lymphocytes in Romanowsky-type stained smears from buffy coat and less frequently lymph node, BM, spleen, liver and cerebrospinal fluid smears, is helpful in establishing a definitive diagnosis of acute CME. In a study with dogs naturally-infected by "E. canis" (presumptive acute CME), the diagnostic sensitivity of buffy coat (review of 1000 oil immersion fields, 100x objective lens), lymph node (500 oil immersion fields, 100x objective lens) or their combination was 66%, 61% and 74%, respectively. In another study, diagnostic sensitivity of spleen cytology in dogs naturally infected by "E. canis" was 49%. Cytology may also support the diagnosis of CME even prior to seroconversion in acutely infected dogs and is valuable in documenting coinfections (e.g. Babesia spp., Hepatozoon canis, L. infantum), which may have therapeutic and prognostic implications. 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, it is notoriously insensitive in the subclinical and chronic CME and its specificity is adversely affected by the inability to
identify the involved ehrlichial species and the fact that extraneous material such as phagocytosed platelets or nuclear remnants and lymphocytic azurophilic granules may imitate morulae.

Bone marrow cytology is also useful to differentiate 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, in the chronic CME a marked reduction of hematopoietic tissue is noticed, occupying less than 25% of the marrow flecks and usually consists of adipocytes, endothelial and stromal cells. Mild-to-moderate mature mast cell and/or plasma cell hyperplasia may be seen and should not be confused with systemic mastocytosis or multiple myeloma, respectively.

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

Serology is currently the mainstay 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 enzyme-linked immunosorbent assays (ELISA) are also applied. For most laboratories, an IgG titer \( \geq 1:80 \) is considered indicative of prior exposure to an *Ehrlichia* spp. Antibodies develop 7-35 days post-infection, and do not reliably correlate with the current carrier status, the duration of infection, or the presence and severity of clinical disease. Experimentally, IgG antibodies tend to increase earlier following intravenous (7-15 days) as compared to subcutaneous or intradermal *E. canis* inoculations (15-35 days) which may partially explain the variable intervals for seroconversion in the naturally infected dogs. Importantly, in acutely-infected dogs, clinical signs and hematological abnormalities may precede seroconversion and therefore, diagnosis of CME in an acutely ill patient should not be ruled out based on a single time point negative serology alone. The demonstration of a four-fold seroconversion (IgG) in paired serum samples obtained 2-3 weeks apart implies recent infection. 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 occurrence and kinetics of IgM antibody titers is unpredictable in CME, thus limiting the diagnostic value of IgM titer measurement. 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. Numerous point-of-care ELISA tests are commercially available for *E. canis* antibody testing. In general, these screening tests have been found to be of high diagnostic sensitivity and specificity in IgG titers \( \geq 1/320 \); therefore, a relatively low sensitivity may be anticipated in acutely-infected dogs.

Polymerase chain reaction may overcome several diagnostic limitations of serology (confirmation of exposure versus current infection) and cytology (overall 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. Of note, in dogs with profound aplastic pancytopenia the diagnostic sensitivity of PCR may be suboptimal. Several assays have been developed targeting an array of genes including,
though not limited to, 16S rRNA and p30 genes, to specifically detect *E. canis* infections in the dog. Successful amplification of *Ehrlichia* DNA may be accomplished from several tissues, including whole blood, BM, spleen, lymph nodes, liver, kidney, lung, and cerebrospinal fluid. If blood or other tissues are not available, PCR can be applied with reasonable sensitivity in residual serum aliquots. In the naturally-occurring CME, the diagnostic sensitivity and the optimal tissue for PCR testing in the untreated dog or in the post-treatment setting have yet to be systematically addressed. Some studies have indicated that spleen specimens are of higher sensitivity compared to BM or blood for the confirmation of subclinical CME and the evaluation of the eradication of the infection post-treatment, but other studies have suggested that spleen may not be a better specimen compared to other tissues.

**Suggested references**


FELINE HAEMOPLASMOSIS
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Introduction: The feline haemotropic mycoplasmas (haemoplasmas) are small bacteria that can be found on the surface of feline erythrocytes, mediating haemolysis. Various studies have identified three main feline haemoplasmas: Mycoplasma haemofelis (present in 0.4–46.6% cats in published studies), 'Candidatus Mycoplasma haemominutum' (in 8.1–46.7% cats) and 'Candidatus Mycoplasma turicensis' (in 0.4–26% cats). A fourth species has been identified ('Candidatus Mycoplasma haemamatoparvum') but is uncertain whether cats can be infected.

Feline haemoplasmosis is a challenging disease entity for the following reasons:

i) the natural route of transmission has not yet been fully determined, ii) the pathogenesis is dependent on the haemoplasma species, iii) healthy cats can also be infected, iv) carriers exist v) reactivation following treatment can occur and vi) the laboratory diagnostic tests available have limitations.

Transmission: It has been proposed that blood transfusions, aggressive interactions/cat bites with blood transmission, transmission through arthropod vectors as well as vertical transmission from queens to kittens can play a role.

Pathogenesis: Clinical signs seen with symptomatic haemoplasmosis include pallor, pyrexia, lethargy, anorexia, weight loss, dehydration, and splenomegaly. Jaundice is uncommon.

Haemofelis is the most pathogenic of the species, often inducing haemolytic anaemia during acute infection, whereas chronic infection is usually asymptomatic. Haemominutum and Turicensis infections are not usually associated with anaemia, unless concurrent disease or immunosuppression (e.g., FeLV or FIV infection, chemotherapy) exists in the feline host, in which case anaemia may be induced.

Real-time PCR assays have allowed quantification of haemoplasma copy (organism) number in infected cats. Cats infected with haemoplasma species initially show a rapid increase in copy number with peak numbers typically being reached after around 2 to 4 weeks. Haemofelis copy numbers can fluctuate greatly even within the initial post-infection period. Some Haemofelis infected cats continue to have very large changes in copy number for several months following experimental infection. In contrast, Haemominutum infected cats show less fluctuation in copy number. It is possible that the greater pathogenicity of Haemofelis may have been responsible for the large fluctuations in copy number seen, by inducing a greater immune response. However, Turicensis infected cats have not shown the same degree of copy number fluctuation as Haemofelis despite being anaemic.

Carriers & Reactivation: Studies have reported haemoplasma infection in clinically healthy cats; Haemofelis 0.2.3%, Haemominutum 3.8%, Turicensis 0.7%.

Some haemoplasma-infected cats spontaneously clear their infection, with or without antibiotic treatment, whereas other cats remain infected for long periods of time. Cats which recover from infection may remain chronically infected with haemoplasmas for an undetermined period, which may in some cases be lifelong. Long-term carrier status is common following Haemominutum infection, although suspected clearance of infection has also been reported, with and without antibiotic treatment. We have observed quite a large proportion of Haemofelis infected cats to spontaneously clear infection from peripheral blood after infection without antibiotic treatment. Turicensis infected cats have also been shown to clear infection from peripheral blood without antibiotic treatment. So, variation exists in the long-term host-organism interaction. Carrier cats appear to be in a balanced state in which replication of organisms is balanced by phagocytosis and removal. In carrier...
cure cats, the potential for reactivation of disease always exists, although this is probably not that common.

Clinical Pathology:
Anaemia: commonly regenerative or occasionally non-regenerative (early sampling, concurrent disease). Haemoplasmas induce anaemia by haemolysis and sequestration. The attachment of the haemoplasma results in direct damage to the erythrocytic membrane, leading to a shortened erythrocyte lifespan and haemolysis. Erythrocyte damage may also expose hidden, or change existing, erythrocytic antigens causing the production of antierthrocytic antibodies. Antibodies may also be directed against the haemoplasma organism itself resulting in erythrocytic destruction as an 'innocent bystander.' Positive Coombs' tests and autoagglutination have been reported in acute haemoplasmosis cases indicating the presence of erythrocyte-bound antibodies. In infected cats, cold reacting (IgM) antibodies have been detected during periods of anaemia. These antibodies have disappeared following resolution of the haemoplasma-induced anaemia with antibiotic treatment, without the need for glucocorticoid treatment. An increase in osmotic fragility has also been reported with haemoplasma infection.

Although most haemolysis is said to be extravascular in nature, intravascular haemolysis has also been reported. Macrophage erythrophagocytosis occurs in the spleen, liver, lungs, and bone marrow. The splenic macrophages can also remove haemoplasmas from the surface of the erythrocytes ('pitting'), returning unparasitised cells back into the circulation. This may explain the rapid increase in PCV, without reticulocytosis, seen in some experimentally infected cats.

Persistent autoagglutination/Coombs' positive (Haemofelis infection); IgM (4°C) and IgG (4° & 37° C).
Raised liver enzymes, hyperbilirubinaemia, azotaemia.
Hyperproteinaemia; \( \uparrow_\gamma \quad \pm \quad \uparrow_\beta_2 \) globulins (SPE), \( \uparrow_{Hp} \) &\( \uparrow_{SAA} \) (symptomatic), \( \uparrow_{Hp} \) (asymptomatic).

Laboratory Diagnostics:
Blood smear examination: Blood smears stained with Giemsa, Wright or DiffQuik stains. Has both poor sensitivity and specificity and this technique does not determine the haemoplasma species present. The specificity has been reported to be acceptable with experienced cytologists (85-98%). The poor sensitivity is due to fluctuating parasitaemia and sample handling issues/anticoagulant.
Polymerase chain reaction (PCR): When properly designed and executed is the diagnostic method of choice because it is much more sensitive and specific than blood smear examination and can differentiate the haemoplasma species (single or multiple) present. Additionally, real-time quantitative PCR (qPCR) assays allow quantification of haemoplasma DNA in blood. For identification of hemoplasmas on a peripheral blood smear, the bacterial load must be at least \( 10^7 \) organisms/ml of blood (about 1 organism/100 RBCs) to make a diagnosis. The lowest level of bacteremia that can be reliably detected by most conventional PCR tests (visualization of bands in a gel) is about \( 10^5 \) organisms/ml of blood, whereas levels as low as \( 10^4 \) organisms/ml of blood can be detected by qPCR. Such qPCR results can help determine the significance of infection and response to treatment and have shown that experimental cats infected with Haemofelis can show very large variations in blood organism numbers. In contrast, Haemominutum and Turicensis infected cats show very little fluctuation in blood organism numbers over time.
Flow cytometry: A method has been developed to quantify the parasitaemia by detecting the presence or absence of nucleic acid (which can only be derived from adherent haemoplasmas) on the cat’s erythrocytes; this detection is based on the use of a synthetic DNA binding fluorescent dye. Blood samples are incubated with the dye and then flow cytometric analysis is performed. Due to their different sizes and thus the quantity of DNA,
this technique can distinguish between Haemofelis (large species) and Haemominutum infection (small species) (Sánchez-Pérez et al. 2013).

**Culture:** Haemoplasmas are currently unculturable in vitro. Recently a number of haemoplasmas have been subjected to whole genome sequencing. These data have highlighted the limited metabolic capabilities of these important pathogens (glucose is their only energy source), which likely contribute to the haemoplasmas' current uncultivatable status. Such knowledge of haemoplasma metabolic capabilities will help to direct future in vitro cultivation attempts.

**Serology:** The development of haemoplasma protein-based serological assays has been limited by the inability to culture haemoplasmas in vitro preventing the easy acquisition of adequate amounts of haemoplasma proteins for use in such assays. Studies have evaluated the feline serological response to haemoplasma infection using antigen preparations obtained both from purified haemoplasma organisms collected at peak parasitaemia and recombinant haemoplasma proteins expressed from haemoplasma genes identified by shotgun cloning. ELISA tests have been developed based on a recombinant *M. haemofelis* heat shock protein DnaK and used to detect antibodies in the sera from experimentally infected cats (Barker et al 2010, Wolf-Jäckel et al 2010). Further work is required to determine the specificity of these assays before they can be used successfully in naturally infected cats.

**References**


Tasker S et al. (2009). Description of outcomes of experimental infection with feline haemoplasmas: copy numbers, haematology, Coombs’ testing and blood glucose concentrations. *Veterinary Microbiology* 139(3-4), 323-332.
ETIOLOGY AND TRANSMISSION

*Rickettsia conorii* is an obligate intracellular gram negative bacterium transmitted mainly by ticks. *Rickettsia* invade the endothelial cells of mammals and multiply in them. *Rickettsia conorii* is maintained in ticks by transestadial and transovarial transmission. For this reason, ticks in all their evolutionary stages are the main reservoir of *R. conorii*. The distribution of *R. conorii* infection in both humans and dogs is similar to the distribution of *R. conorii* infection in both humans and dogs is similar to the distribution of ticks and the seasonality of the disease is parallel to the activity of *R. sanguineus*. The disease, in both people and dogs, manifests itself in summer (usually between June and September), which is when there is a high density of *R. sanguineus* ticks.

PREVALENCE OF INFECTION AND DISEASE

Infections by *R. conorii* are endemic throughout the Mediterranean basin. The seroprevalence of *R. conorii* is usually very high, reaching 50-60% in some studies conducted in Italy or Spain. However, the prevalence by PCR is much lower and only in dogs that present febrile illness.

There are few studies in veterinary medicine that determine the seroconversion of *R. conorii* in dogs. The seroconversion rate for *R. conorii* found in Sicily (Italy) was 20.7%. In addition, it was observed that the seroconversion rate for *R. conorii* in dogs with febrile illness was significantly higher than in dogs without febrile illness.

RISK FACTORS

There does not seem to be predisposition based on the breed, age or sex of the dog. A common risk factor for this disease is tick infestation. The severity of this disease could also be determined by other factors, such as immune status, coinfections with other vector-borne pathogens or the presence of other concomitant diseases.

PATHOGENESIS

*Rickettsia* invade the endothelial cells of mammals, including man, and multiply in them, leading to the formation of vasculitis. Therefore, they alter the vascular permeability producing edema and necrosis.

CLINICAL PICTURE

The main clinical sign in the dog is fever, which can be quite high, reaching even 41°C. Other clinical signs that can be found, although not as frequent as fever, are the following: lameness, lethargy, anorexia, myalgias, lymphadenomegaly, abdominal pain (kyphosis), vomiting and diarrhea.
The most common laboratory alterations are: increased C-reactive protein, thrombocytopenia, mild normocytic and normochromic non-regenerative anemia and hypoalbuminemia.

**DIAGNOSIS**

**Molecular techniques**

Although there are no studies in other tissues, peripheral blood appear to be the tissue of choice. The detection of *R. conorii* DNA in blood is rare, probably due to the low and transient presence of rickettsiae in the blood. Therefore, if the PCR is positive, it confirms the *Rickettsia* infection, but if it is negative, it cannot be ruled out that the infection exists. In addition, molecular techniques by sequencing allow the identification of the species and subspecies of *Rickettsia* involved. It is important to note that a negative result may also be due to previous administration of antibiotics such as doxycycline.

For this reason, in order to improve the utility and obtain the maximum information from the PCR, it is important to evaluate the results together with the analysis of the antibodies and the clinical signs and laboratory alterations.

**Serological techniques**

One problem that arises is that there are serological cross reactions between different pathogens of the same genus. For example, there is a marked cross reaction between *R. conorii* and *R. rickettsii*. The cross-reaction and the possibility of co-infection with various microorganisms can create difficulties in the serological interpretation in certain geographical areas.

Seroconversion is very useful in the diagnosis of canine rickettsiosis. It is important to obtain the level of antibodies in the acute phase and in the convalescence phase (2-4 weeks after the start of the clinical presentation). An increase of 2-4 times the antibody titre with respect to the initial serological titre, or the passage from seronegative to seropositive, confirms the infection by this pathogen. For a better diagnosis of this type of diseases, it is advisable to use serology, including seroconversion and PCR.

**TREATMENT AND PROGNOSIS**

The treatment of choice is doxycycline. Dogs infected with *R. conorii* respond to oral treatment with doxycycline quickly, usually in 24-48 hours, and the prognosis is good / favorable.

**PREVENTION**

Prevention is mainly based on the individual use of topical acaricide treatments with the aim of reducing exposure to ticks and the transmission of pathogens to dogs.
REFERENCES

Introduction
Babesiosis is caused by protozoal parasites that infect erythrocytes and cause anemia. Babesia species are tick-borne apicomplexan parasites that infect a variety of domestic and wild animals and may cause moderate to severe disease. Babesiosis has a worldwide distribution and global importance. Hemolytic anemia with erythrocyte destruction and a systemic inflammatory response account for most of the clinical signs observed in canine and feline babesiosis (1,2).

Canine babesiosis
Etiology: Babesia infection was identified in the past based on the morphologic appearance of the parasite in erythrocytes. All large forms of canine Babesia (2.5–5.0 μm) were designated Babesia canis, whereas all the small forms (1.0–2.5 μm) were considered as Babesia gibsoni. However, the development of molecular methods have demonstrated that more piroplasmid species infect dogs and cause different diseases (1-3). Babesia rossi, B. canis and B. vogeli previously considered as subspecies are identical morphologically but differ in the severity of clinical manifestations which they cause, their tick vectors, genetic characteristics, and geographic distributions, and are therefore currently considered separate species. Another yet unnamed large Babesia sp. most closely related to B. bigemina was found to infect immunocompromised dogs in North America. The small Babesia spp. that infect dogs include B. gibsoni, B. conradae described from California, and the B. vulpes (synonym B. microtri-like; Theileria annae) (4). None of the Babesia species that infect dogs has been found to be zoonotic.

The geographical distribution of the causative agents and thus the occurrence of babesiosis are largely dependent on the habitat of relevant tick vector species, with the exception of B. gibsoni where evidence for dog to dog transmission indicates that infection can be transmitted among fighting dogs breeds independently of the limitations of vector tick infestation. Babesia vogeli and B. gibsoni have wide distributions in both the Old and New World continents, whereas B. rossi has to date been mostly restricted to Africa and B. canis has mostly been reported from Europe.

Dogs are infected when Babesia sporozoites are injected with saliva into the host's skin during the blood meal. The parasites invade the erythrocytes and form ring-shaped trophozoites. The parasite replicates within the erythrocyte and forms merozoites observed as pairs of attached pear-shaped parasites in some Babesia species. Merozoites may further divide forming 8 or more parasites in the same erythrocyte and eventually destroying the cell freeing into the blood to invade more erythrocytes. Ticks feeding on infected blood take up merozoites and sexual parasite development in the tick gut is followed by sporogony in its tissues. The parasite reaches the tick salivary glands or its oocytes from which transmission occurs. Babesia spp. are transmitted transstadially from one stage in the tick life cycle to another, and also transovarially through the tick eggs, as shown for some Babesia spp. The transmission of babesiae occurs through the bite of a vector tick. However, B. gibsoni infection has also been demonstrated to be transmitted via blood transfusion and transplacentally. Furthermore, several studies have provided evidence that B. gibsoni is likely transmitted directly from dog to dog via bite wounds, saliva, or ingested blood.

Clinical findings: It is important to remark that clinical findings are variable depending on the Babesia species infecting dogs. In general, hemolytic anemia and the systemic inflammatory
response syndrome leading to multiple-organ dysfunction syndrome are responsible for most of the clinical signs observed in canine babesiosis. Hemolysis may result in hemoglobinemia, hemoglobinuria, bilirubinemia and bilirubinuria. Thrombocytopenia is consistently observed in babesiosis and may be caused by immune mechanisms, splenic sequestration or coagulatory consumption of platelets from hemolytic or vascular injury. Immune mediated thrombocytopenia has been demonstrated in experimental canine babesiosis caused by *B. gibsoni*.

Tissue hypoxia is found in severe canine babesiosis. It is caused by anemia, hypotensive shock, vascular stasis by sludging of erythrocytes, excessive endogenous production of carbon monoxide, and parasitic damage to hemoglobin. The central nervous system, kidney, and muscle are the organs most affected by tissue hypoxia. Tissue hypoxia, hypertensive shock, multiple organ dysfunction and potential mortality have been documented mostly in association with *B. rossi* and *B. canis* infections. Young pups and immunocompromised adult dogs, such as dogs with hyperadrenocorticism or treated with immunosuppressive therapy, may suffer a severe disease with *B. vogeli* infection.

The spleen has an important function in controlling babesiosis. Experimentally infected splenectomized dogs rapidly develop parasitaemia and clinical disease and may reach high parasitaemia levels. Splenectomy has also been associated with natural canine and human babesiosis.

**Diagnosis:** Detection of *Babesia* in stained blood smears has been the standard diagnostic technique for many years. This method is reliable when a moderate to high parasitaemia is present. However, a direct correlation between the level of *Babesia* parasitaemia and the magnitude of clinical signs is not always found. A fresh smear is recommended for the accurate diagnosis of infection. Erythrophagocytosis with infected erythrocytes may be found in blood smears from infected dogs. The use of molecular diagnostic assays such as PCR is indicative in cases of low parasitemia including suspected carrier dogs or chronically infected animals as well as for speciation.

**Treatment:** Large *Babesia* spp. are commonly treated with imidocarb dipropionate with good clinical response while small *Babesia* spp. appear to be more difficult to treat and resistant to the conventional drugs that are effective against the large babesial spp. (1,5). Diminazene aceturate used for treatment of both large and small babesial spp. infections should be used cautiously as it has a relatively small dose safety margin with a large inter-individual pharmacokinetic variation. *Babesia gibsoni* infection is often resistant to imidocarb dipropionate and diminazene aceturate and an alternative therapy with the combination of the anti-malarial atovaquone and the macrolide azithromycin has been recommended for this infection. However, complete clinical and parasitological cure are not commonly achieved in dogs treated for small babesial spp. infections and clinical relapses may occur (1,5). Medical management of infection may require supportive treatments including blood transfusions, intravenous fluids, and the use of anti-inflammatory drugs.

**Feline babesiosis**

Babesiosis in domestic cats is a more rare clinical entity in comparison with its canine counterpart (1). *Babesia* species are often grouped into species of large (2.5–5.0 µm) or smaller (1.0–2.5 µm) intraerythrocytic piroplasm forms. As in the case of many other domestic animal hosts, large as well as small form *Babesia* species have been described in domestic cats.

**Etiology:** Clinical babesiosis in domestic cats has mostly been reported from South Africa where infection is due to *Babesia felis*, a small *Babesia* that causes a disease characterized by anemia and icterus (6,7). It also infects African wild felids including lions, cheetahs and servals (8). Other reports of domestic feline babesiosis have mostly been sporadic. *Babesia cati* was reported from a cat in India and a few cases of infection in domestic cats by unnamed *Babesia* parasites were reported in France, Germany, Thailand and Zimbabwe (1).
A large form *Babesia, B. canis presentii*, was described in cats from Israel (9). Several tick spp. including *Ixodes ricinus*, *Ixodes hexagonus*, *Dermacentor* spp., *Rhipicephalus sanguineus*, and *Haemophysalis* spp. infest cats and are possible vectors of *Babesia*. Other routes of transmission reported in canine *Babesia* infections including vertical and direct transmission have not been consistently described in cats (1).

**Clinical findings:** Hemolytic anemia is the most common clinicopathological abnormality observed in feline babesiosis. In general, *Babesia* species cause hemolytic anemia that can result in hemoglobinemia, hemoglobinuria, bilirubinemia and bilirubinuria. Coinfection with other pathogens such as retroviruses and hemotrophic mycoplasmas are suspected to increase susceptibility to *Babesia* infections via immunosuppression. Information on the clinical manifestations of domestic feline babesiosis is limited mostly to publications on *B. felis* infection in South Africa (6,7). *Babesia felis* infection is associated with anorexia, lethargy, a roughened hair coat, exercise intolerance, weight loss, weakness, tachycardia, tachypnea, pallor, icterus in about 20% of cases, vomiting and diarrhea. In a study on *B. felis* which included 56 cats, 80% were less than 3 years old and there was no specific breed or gender predilection. Most cats were anorectic and lethargic. Hyperbilirubinemia was present in 86% of the cats and alanine aminotransferase (ALT) activity was elevated in 89%. Thirty two % of the cats were concurrently infected with FeLV and 14% with FIV. *Babesia canis presentii* infection in a cat from Israel co-infected with FIV and *Candidatus Mycoplasma haemominutum* was accompanied by fever, icterus, moderate anemia and thrombocytopenia which resolved following anti-babesial therapy. The housemate of this cat had low grade infection and remained sub-clinical without treatment (9).

**Diagnosis:** Detection of *Babesia* in stained blood smears has been the standard diagnostic technique for feline babesiosis for many years. A fresh smear is recommended for the accurate diagnosis of infection. Nevertheless, the detection of *Babesia* in blood smears is considerably less sensitive than detection of parasite DNA by molecular techniques (1). Therefore, the use of molecular diagnostic assays is recommended for the detection of infection and subsequently for species determination. It is relatively easy to distinguish between large and small form piroplasms under the microscope. However, distinction between species based solely on morphology is not possible and molecular analysis such as PCR and sequencing is required for this purpose (1). Serology is not commonly used as a diagnostic tool in feline babesiosis. Positive serology can indicate a past or present persistent infection but false-negative results are possible in peracute or acute infections, and serology is unable to distinguish between different closely related cross-reacting piroplasms (1).

**Treatment and prevention:** *Babesia felis* is treated mostly with the anti-malarial drug primaquine phosphate, which reduces the level of parasitemia, and may often resolve the anemia and clinical signs of disease, but rarely eliminates infection (1). Therefore, post-treatment clinical relapses are evident. Large form *Babesia* such as *B. canis presentii* infection is usually treated successfully with imidocarb dipropionate (9). Prevention of babesiosis relies mostly on topical and environmental acaricidal treatments aimed at reducing the exposure to vector ticks and pathogen transmission to the cat. Babesiosis of domestic cats should be suspected mainly in cases of hemolytic anemia and clinical findings associated with a hemolytic process. Co-infection with other pathogens should be investigated and managed medically if present.

**References**


The aim of this presentation is to give an overview of canine leishmaniosis due to *Leishmania infantum* infection focusing on its clinical manifestations, pathogenesis, immunology, diagnosis and treatment monitoring. The same topics will be addressed also for feline leishmaniosis highlighting differences with canine leishmaniosis.

**Clinical manifestations, pathogenesis and immunology**

The immune responses mounted by dogs at the time of infection and thereafter appear to be the most important factor in determining if they will develop a lasting infection and whether and when it will progress from a subclinical state into a disease condition. The outcome of *Leishmania* infection in dogs is linked to the host immune responses and to the persistence and multiplication of the parasite. A broad range of clinical manifestations and immune responses have been described for canine leishmaniosis. Canine *L. infantum* infection can manifest as a chronic subclinical infection, self-limiting disease, or non-self-limiting illness. In addition, several degrees of disease severity are found in dogs ranging from mild to severe fatal disease with different clinical outcomes, prognosis and treatment options. Therefore, several clinical staging systems have been proposed. The two extremes of this wide clinical spectrum are represented by “resistant” dogs and “susceptible” dogs (Solano-Gallego et al., 2009).

**Clinically “resistant” dogs**

Resistant dogs are infected dogs which either remain healthy or develop a mild, self-limiting disease. These resistant dogs display a weak antibody response but strong and effective cellular immune response. This protective CD4+ T-cell-mediated immune response is characterized by production of cytokines such as IFN-γ, IL-2 and TNF-α, which induce anti-*Leishmania* activity by apoptosis of parasites in macrophages via nitric oxide metabolism and, thus capable of controlling infection (Solano-Gallego et al., 2009). However, subclinical infection is not necessarily permanent and factors such as immunosuppressive conditions or concomitant disease could break the equilibrium and lead to the progression of clinical disease in dogs (Solano-Gallego et al., 2009) as observed also in human patients with acquired immune deficiency syndrome and *Leishmania* co-infection (Alvar et al., 2008).

**Clinically “susceptible” dogs**

Sicks dogs are characterized by a marked humoral immune response and reduced T cell-mediated immunity with a mixed cytokine pattern and high parasite loads (Solano-Gallego et al., 2009) which is detrimental for the animal. The cellular basis and mechanisms for the development of T-cell unresponsiveness in canine leishmaniosis are not fully understood. Interestingly, the loss *L. infantum*-specific CD4+ T cell proliferation in some sick dogs was correlated with increased surface expression of Programmed Death (PD)-1, an immune inhibitory receptor, leading to T cell exhaustion (Esch et al., 2013).

Clinical canine leishmaniosis is a chronic disease, and clinical signs of disease may develop with a variable and sometimes long incubation period (approx. 3 months to 7 years after infection). T-lymphocyte regions in the lymphoid organs become depleted, and antibody-producing B-cell regions proliferate. The proliferation of B lymphocytes, plasma cells, histiocytes, and macrophages results in generalized lymphadenomegaly, splenomegaly, and hyperglobulinemia. Another potential hazard of impaired T-cell regulation with exuberant B-cell activity is the generation of large amounts of circulating immune complexes (CICs). CIC deposition in the walls of blood vessels may cause vasculitis, polyarthritis, uveitis, and
glomerulonephritis (type III hypersensitivity reaction). In dogs, CIC deposition in the kidneys eventually results in renal damage, which is the main cause of death of dogs with leishmaniosis. Serum anti-histone antibodies have been associated with glomerulonephritis in dogs with leishmaniosis (Baneth and Solano-Gallego, 2012).

It is not known for certain what mechanisms in dogs are responsible for protection or susceptibility, nor the way factors such as age, gender, nutrition, host genetics, coinfections and concomitant disease, immunosuppressive conditions, cytokine environment, parasitic burden, nature of *Leishmania* antigens or different *Leishmania* strains, previous infections and way of transmission can affect the polarity of clinical manifestations in *Leishmania* infection. Age seems to be an important factor with a peak in the prevalence of disease in dogs younger than 3 years or older than 8 year. Studies have indicated that several dog breeds are apparently more susceptible to disease. These include the Boxer, Cocker Spaniel, Rottweiler and German shepherd. Other breeds that have evolved in endemic areas such as the Ibizian Hound rarely develop the disease and present a protective predominant cellular immunity (Solano-Gallego et al., 2009).

It is important to highlight that although the adaptive T-cell immune response in dogs has been investigated in several studies, the mechanisms that underlie and determine these polarized responses are still poorly understood. The innate immune response has a relevant role in protection against the parasite besides switching on the adaptive response and the components of innate and adaptive immunity engage in a range of interactions that is remarkably diverse and complex (Hosein et al., 2017).

The innate, or nonspecific, immune response, as stated above, is the first line of defense encountered by *Leishmania* parasites when entering the susceptible host. In this context, the innate immune system senses the presence of invading microbes through specific receptors such as Toll-like receptors (TLRs), which are germ line-encoded pattern recognition receptors (PRRs) that recognize “pathogen-associated molecular patterns” (PAMPs). After recognition of specific antigens, TLRs trigger NF-κB, which then proceeds to the nucleus and promotes the transcription and further synthesis of pro-inflammatory cytokines (TNF-α, IFN-γ and IL-12) and chemokines. These responses also initiate the development of pathogen-specific, long-lasting adaptive immunity through B and T lymphocytes. While most reports on TLRs have focused on bacterial and fungal pathogens, studies confirm the importance of TLRs in the onset of leishmanial pathogenesis, susceptibility, and resistance in mice and human disease models (Tuon et al., 2008). However, TLRs have not been studied in canine *L. infantum* infection in detail (Hosein et al., 2017).

Clinical manifestations of leishmaniosis in cats are similar to those reported in dogs. Knowledge on clinical immunology in felines is however limited.

**How to diagnose canine and feline leishmaniosis**

The diagnosis of leishmaniosis due to *L. infantum* infection is very difficult and complex because of the spectrum of clinical signs and clinicopathological abnormalities is broad and unspecific, the existence of high prevalence of subclinical infection and the use of vaccines for canine leishmaniosis in endemic areas (Solano-Gallego et al., 2017). Three vaccines are currently marketed in Brazil (LeishTec) and Europe (CaniLeish and LetiFend). The availability of vaccines to prevent canine leishmaniosis has increased the complexity of diagnosis, as serological tests may not distinguish between naturally infected and vaccinated dogs. This makes canine leishmaniosis a diagnostic challenge for the veterinary practitioner, clinical pathologist and public health authorities in endemic countries as well as non-endemic regions where imported infection is a concern. Moreover, all these different states of infection and clinical conditions have important implications from a diagnostic point of view (Solano-Gallego et al., 2017).
The current practices of prevaccination screening are not sufficiently sensitive to detect subclinically infected dogs, resulting in the vaccination of infected animals, which may lead to disease in vaccinated dogs that are also infectious to sand flies (Solano-Gallego et al., 2017).

The main purpose for which *L. infantum* infection diagnosis is performed is to confirm disease in canine patients. However, other purposes are to investigate the presence of infection for screening clinically healthy dogs in endemic areas, including blood donors, breeding dogs, dogs prior to vaccination, dogs heading towards disease progression; or for screening clinically healthy dogs in non-endemic areas (travelling dogs), to avoid importation of infected dogs to non-endemic regions and to monitor response to treatment (Solano-Gallego et al., 2017).

Therefore, it is important to distinguish between disease, infection and immune responses induced by vaccination (immunogenicity), and to apply different diagnostic techniques accordingly.

Several diagnostic techniques have been developed to help in the diagnosis of canine leishmaniosis. The detection of *L. infantum* infection in dogs includes parasitological (cytology, histology, immunochemistry and culture of the organism in appropriate medium), molecular (conventional, nested and real-time PCR) and serological methods (qualitative and quantitative antibody tests). In addition, *L. infantum* specific cellular immunity tests have also been developed but they are mainly used in the research settings. Different diagnostic procedures and interpretations of test results might be used accordingly, depending on the purpose of the diagnostic investigation. It is important to understand the basis of each diagnostic test, the limitations and the appropriate clinical interpretation (Solano-Gallego et al., 2017).

Similar diagnostic tools are available for making the diagnosis of *L. infantum* infection in felines. Major differences are that commercial vaccines for cats are not yet marketed and also that cats do not produce a pronounced humoral response as frequently as dogs. Therefore, if clinicopathological abnormalities are compatible with feline leishmaniosis, a seronegative result does not exclude *L. infantum* infection (Pennisi et al., 2015).

Important gaps in knowledge regarding the diagnosis of canine and feline leishmaniosis will be underscored to highlight the need for novel diagnostic test development.

**How to treat and monitor canine and feline leishmaniosis**

The main drugs used for treatment of canine leishmaniosis include:

- The pentavalent antimony meglumine antimoniate which selectively inhibits leishmanial glycolysis and fatty acid oxidation.
- Miltefosine which is an alkylphosphocholine drug.
- Allopurinol which acts by inhibiting protein translation through interfering with RNA synthesis and is also frequently used for the treatment of cats.

Anti-leishmanial treatment often achieves marked clinical improvement in dogs and cats with leishmaniosis but it is sometimes not associated with the elimination of the parasite. Treated dogs may remain carriers of the disease, experience clinical relapses and be infectious to sand flies. Drug resistance has been widely described in human cutaneous and visceral leishmaniosis. Reports on drug resistance in the canine disease are more scarce. Disease relapse in dogs with canine leishmaniosis during allopurinol treatment has been described and associated with allopurinol resistance of *L. infantum* isolated from relapsed animals (Yasur-Landau et al., 2016). *Leishmania infantum* strains isolated in culture from relapsed dogs were significantly less susceptible to allopurinol in comparison to isolates.
from dogs before treatment and those from dogs under treatment with no clinical relapse (Yasur-Landau et al., 2016). Resistance was consistent in three forms of the parasite strains tested including intracellular amastigotes, promastigotes and axenic amastigotes. These findings indicate that resistance to allopurinol may develop in dogs experiencing clinical disease relapse which may transmit resistant parasite to other dogs and also enhance the danger of transmission the parasite to humans.

Monitoring of dogs and cats under treatment requires follow up of the biochemistry panel and hematology, and monitoring the levels of antibodies against *L. infantum*. Treatment should not be discontinued before the animal does not have any clinical signs, regains a normal biochemistry panel and hematology, and has a decrease in antibody titers to below the assay's cutoff levels (Solano-Gallego et al., 2009).

References


CARDIOPULMONAR NEMATODES PARASITES OF DOGS

Smaragda Sotiraki
Veterinary Research Institute, HAO DEMETER, Thessaloniki Greece

The heartworms, *Dirofilaria immitis* ("canine heartworm") and *Angiostrongylus vasorum* ("French heartworm") have recently become the focus of increased attention from the veterinary community due to their emergence in several European countries and the spread into previously non-endemic regions. The importance of this phenomenon lays on the pathogenic potential of these nematodes, the challenges involved in diagnosis and their zoonotic potential.

The mosquito-borne filarial nematode *D. immitis* is causing a cardiopulmonary disease known as Canine heartworm disease in both domestic and wild hosts, and potentially the causal agent of human pulmonary dirofilariosis. *D. immitis* is transmitted by several mosquito species (*Culex* spp., *Aedes* spp., *Anopheles* spp.) which act as an intermediate host. When taking a bloodmeal from a microfilaremic host, the mosquitoes become infected and the microfilariae develop to the third-stage larvae (L3) in the malpighian tubules of the mosquitoes, which a redeposited on the host while the mosquito is taking a blood meal, becoming sexually mature within a few months in the main pulmonary arteries and right ventricle. The presence of adult worms in the pulmonary arteries of infected dogs causes changes in arterial structure and function that can lead to pulmonary hypertension and, eventually, to right-sided congestive heart failure. The disease is endemic in temperate, tropical and subtropical areas of the world and currently affected areas in Europe include Greece, Italy, Spain, Portugal, the south of France, parts of southern Switzerland, the Czech Republic, Slovenia, Romania and Bulgaria.

The gastropod-borne metastrongyloid parasites *Angiostrongylus vasorum* affects the cardiopulmonary system of domestic dogs and wild species of the family Canidae, including foxes, coyotes and wolves amongst others. A broad range of terrestrial snail and slug intermediate host species (e.g. *Arion ater*, *A. rufus*, *A. lusitanicus*, *A. fulica*, *A. distinctus*, *A. hortensis*, *Limax maximus*, *Helix aspersa* and *Tandonia sowerbyi*) are infected by L1 either by ingestion while feeding on faeces or by active penetration through the gastropod epidermis. Within the definitive host, adult nematodes are found in the right heart and pulmonary arteries. Adult fertilized females produce eggs which embryonate and hatch within the pulmonary capillaries where L1 penetrate into the alveoli, migrate into the trachea, larynx, buccal cavity, are swallowed and finally shed through faeces into the environment. Canine angiostrongylosis ranges from subclinical cases to severe cardiopulmonary and neurological disorders or coagulopathies besides inducing nonspecific clinical signs. Even fatal infections are frequently reported. *A. vasorum* shows a patchy geographical distribution worldwide mainly in European countries as well as in North America.

Both parasites have recently gained attention due to their geographical spread beyond the borders of known endemic areas as well as due to the increase of their infection incidence. There are several factors that may explain the spread of the parasite from endemic areas to previously unaffected areas, including climate change, density of the vector population, the introduction of new, competent vectors (such as *Aedes albopictus* for *D. immitis*) and the movement and relocation of infected dogs that travel throughout Europe for holidays, sports, adoptions or others.

Infections caused by *A. vasorum* and *D. immitis* can lead to certain diagnostic challenges since they are characterized by a wide and highly variable spectrum of clinical features, ranging from a subclinical to a severe disease and often present nonspecific abnormal
radiographic and haematological findings, thus proper specific laboratory diagnostic methods are critical to achieve a timely and reliable diagnosis. Heartworm disease in dogs is diagnosed by the detection and specific identification of microfilariae (Knott test, histochemical staining and by the amplification of microfilaria DNA by PCR) and by using tests for the detection of circulating adult worm antigens, available only for *D. immitis*.

The most common method for diagnosing *A. vasorum* infection is the Baermann funnel migration technique and various floatation based coprological techniques in which larvae are recovered from faeces and identified based on morphological features. Such approaches unfortunately can be puzzling due to the intermittent larval shedding and various operational errors. In addition, there are several other tests and methods available based on serological (antibody/antigen detection) or molecular approaches which can also be tricky, since all methods may show certain deficiencies and limitations.

**Literature**


## Cases Session

**CASES SESSION - SATURDAY 20<sup>TH</sup> OCTOBER 9:05 TO 12:00**

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<td>9:05-10:00</td>
<td>J. Harvey</td>
<td>Approach to diagnosis of defects in erythrocyte metabolism including a case of Heinz body anemia in a horse</td>
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<td>10:00-10:15</td>
<td>I. Oikonomidis</td>
<td>Splenic aspirates from a dog with azotemia</td>
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<td>10:15-10:30</td>
<td>H. Ferreira</td>
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<td>K. Irvine</td>
<td>Coelomic Fluid from a Chicken</td>
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<td>5</td>
<td>10:45-11:00</td>
<td>S. Evans</td>
<td>Pericadial fluid from a cat</td>
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<td>6</td>
<td>11:00-11:15</td>
<td>T. Lavabre</td>
<td>Sysmex scattergram in a cat</td>
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<td>11:15-11:30</td>
<td>L. Magna</td>
<td>Peripheral nucleated red blood cells in a cat</td>
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<td>8</td>
<td>11:30-11:45</td>
<td>S. Bernardi</td>
<td>Mandibular lymph node enlargement and lymphocytosis in a Shih tzu</td>
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<td>9</td>
<td>11:45-12:00</td>
<td>A. Penrose</td>
<td>Large subcutaneous tumour in a roe deer</td>
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Mystery Slide Session

Sponsored by Gerolymatos International and Life Science Chemilab S.A.

who have provided microscopes for our meeting

Introduction
Through the direct examination of cytology slides with a microscope, we would encourage the attendees to share opinions and to discuss about some interesting or unusual clinical cases.

The session
The set of microscopes provided by the Congress Organization allows the attendees to directly and autonomously analyze ten cytologic smears, related to a clinical case, from Thursday morning to Saturday morning. The cytologic cases will be then discussed together Saturday, before the end of the meeting. All the participants, during this session, are warmly invited to ask questions, to comment the cases and, why not, to do criticism to the main interpretation.

The cases
Case #1: dog, Bernese mountain dog, 4-years-old, male; splenic enlargement.
Case #2: cat, DSH, 3-years-old, female, neutered; crusting lesions on the face.
Case #3: dog, mongrel, 11-years-old, female, neutered; cutaneous mass.
Case #4: dog, mongrel, 6-years-old, female; vulvar nodule.
Case #5: dog, Pinscher, 13-years-old, female, neutered; pancreatic nodule.
Case #6: cat, DSH, 12-years-old, female, neutered; cutaneous nodule and lymph node.
Case #7: turtle, 10-years-old, female; swelling and crusting lesions of the legs.
Case #8: dog, Shih-tzu, 10-years-old, female, neutered; surrenalic nodule.
Case #9: dog, mongrel, 7-years-old, male; prostatic enlargement.
Case #10: dog, mongrel, 12-years-old, male; subcutaneous mass.
**ORAL FREE COMMUNICATIONS**

Oral Free Communications

**THURSDAY 18TH OCTOBER 2018**

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<td>Iron status, inflammatory and oxidative blood markers in black and white European captive rhinoceroses</td>
<td>Soetart</td>
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<td>915</td>
<td>Morphologic evaluation of bone marrow aspirates from Hispaniolan Amazon parrots (Amazona ventralis)</td>
<td>Schwartz</td>
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<td>930</td>
<td>Feline refractometers systematically underestimate urine specific gravity</td>
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<td>Investigation of the status quo for veterinary point of care (poc) laboratories in Switzerland: availability, application and quality management</td>
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<td>Inter-instrumental comparisons between hematology analyzers in Japan</td>
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<td>Tools to facilitate learning and teaching of veterinary clinical pathology</td>
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<td>Mylonakis: Comparison of different tissue specimens and PCR assays for the diagnosis of canine ehrlichia canis infection</td>
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<td>Kean: Identifying reservoirs of antimicrobial resistance in faecal microbiota using high resolution chromosome conformation capture</td>
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<td>Solano-Gallego: Rate of leishmania infantum infection based on serology, blood PCR and parasite specific interferon-gamma in cats and dogs living in the same area of south of Spain (Cordoba)</td>
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<td>Attipa: Serological evaluation of associations between canine leishmaniosis and multiple vector-borne co-infections: a prospective case-control study</td>
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<td>1000</td>
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<td>1100</td>
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<td>Strage: Homeostasis model assessment and its relation to body fat in cats</td>
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<td>Goddard: Cardiac myocyte injury is correlated with the degree of the consumptive coagulopathy present in dogs with babesia rossi infection</td>
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**Oral Free Communications**

**SATURDAY 20TH 2018**

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<td><strong>Melega:</strong> Flow cytometric approach to non-hematopoietic cells in canine cavitary effusion</td>
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ABSTRACT 1
IRON STATUS, INFLAMMATORY AND OXIDATIVE BLOOD MARKERS IN BLACK AND WHITE EUROPEAN CAPTIVE RHINOCEROSES
Hanae Pouilleve, DVM1, Nicolas Soetart, DVM2, DVM, Rudy Wedlarski, DVM3, Laetitia Jaillardon, DVM, PhD, Dipl ECVCP1
1Oniris Nantes-Atlantic National College of Veterinary Medicine, LabOniris-LDHNet, Nantes Cedex 3, France
2Bioparc de Doué-La-Fontaine, Doué-la-Fontaine, France

Background: Black rhinoceroses-BR (Diceros bicornis), but not white rhinoceroses-WR (Ceratotherium simum), develop Iron Overload Disorder (IOD), a syndrome in which iron accumulates in the body causing organic failures. As iron is a potent pro-oxidant and BR show poor antioxidant capacities, high levels of oxidative stress and pro-inflammatory conditions are highly suspected in this species.

Objective: To investigate oxidative stress and inflammation in relation to iron status in captive BR.

Methods: Fifteen European captive BR and 29 WR were prospectively included. Blood iron status (serum iron, TIBC), hepatic/muscular function (AST, GGT, cholesterol, CK), inflammatory profile (total proteins electrophoresis) and oxidative stress status (dROMs, GSHpx, SOD) were assessed. Results are given as median[range].

Results: Compared to WR, BR showed significantly higher serum iron (42.0[26.6-58.9]µmol/L vs. 28.0[11.1-58.4]; P<0.01), TIBC [50.2[31.8-74.0]µmol/L vs. 35.2[24.5-58.4]; P<0.01], GSHpx (64.9±300U/gHb vs. 32.1%; P=0.04), dROMs (93.3%>800U/CARR vs. 3.2%; P<0.001), GGT (100%-131U/L vs. 20.8%; P<0.01), AST [96[72-152]U/L vs. 71[12-178]; P<0.01] and CK [323[199-945]U/L vs. 196[129-697]; P<0.01]. BR also showed a significantly lower A/G ratio [0.6[0.3-0.8] vs. 0.7[0.4-1.1]; P=0.01] due to a higher o2-fraction (17.2[5.3-27.1]g/L vs. 11.6[6.2-18.2]; P<0.01).

Conclusions: The present study suggests a higher inflammatory and oxidative stress according to iron status in captive BR compared to WR. This could be either a consequence or a cause of iron accumulation, potentially explaining rapid ageing and various diseases. Further investigations are needed to assess the prognostic value of the inflammatory and oxidative markers in captive BR, particularly for evaluating the impact of iron reduced and anti-oxidant supplemented diets.

ABSTRACT 2
MORPHOLOGIC EVALUATION OF BONE MARROW ASPIRATES FROM HISPANIOLAN AMAZON PARROTS (AMAZONA VENTRALIS)
SHORT TITLE: BONE MARROW MORPHOLOGY OF AMAZON PARROTS
Diana Schwartz1, David Sanchez-Migallon Guzman2, Hugues Beaufrere3, Melanie Ammersbach4, Joanne Paul-Murphy5, Thomas N. Tully Jr.1, Mary M. Christopher2
1Department of Pathology, Microbiology and Immunology1 and Department of Medicine and Epidemiology, 2School of Veterinary Medicine, University of California-Davis, Davis, CA, USA
3Department of Veterinary Medicine, University of California-Davis, Davis, CA, USA
4Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Canada
5Antech Diagnostics Canada, Toronto, Canada

Background: Assessment of bone marrow aspirates provides valuable information about hematopoietic status and hematologic disease. Hematopoietic cell differentials and morphology have been anecdotally described in psittacines, but quantitative studies in these species are lacking.

Objectives: To determine differential cell counts, calculate granulocyte:erythroid (G:E) ratios in bone marrow aspirates from psittacines, and report representative cell morphology of the hematopoietic cells.

Methods: Bone marrow aspirates were collected from 32 clinically healthy, captive, Hispanic Amazon parrots. Peripheral blood samples were obtained for CBC. Bone marrow differential cell counts (%) were determined by counting 500 cells on modified Wright’s-stained smears. G:E ratios were calculated. Representative images of hematopoietic cells at all stages of development were prepared as a reference for cell identification.

Results: Of the 32 parrots sampled, 17 bone marrow samples were of sufficient cellularity and quality for evaluation. Erythroid cells comprised 68.9 ± 8.6% (total ± SD) of hematopoietic cells and consisted primarily of early and late stage polychromatophilic rubricytes (43.6 ± 2.1% of total hematopoietic cells). Granulocytic cells comprised 28.1 ± 3.8% of hematopoietic cells and consisted primarily of mature and band heterophils (11.9 ± 5.2% and 6.5 ± 3.4%, respectively, of total hematopoietic cells). The G:E ratio was 0.4 ± 0.2 (median 0.4, range 0.1 to 0.9). Cells of the thrombocyte lineage could not be reliably identified and were not counted. CBC results were largely within expected limits.

Conclusions: These findings will be a valuable resource for the diagnostic evaluation of clinical bone marrow samples from psittacines.
Background: Urine specific gravity (USG) is the ratio of the weight of urine compared to the weight of an equal volume of water. Commercially available feline refractometers report a different feline USG than USG from dogs and other species for the same refractive index. This is based on Rubin’s calculations from a 1957 study of 190 human urines, 21 canine urines and 22 feline urines. However, that study compared data from three species with completely different urine concentrations.

Objective: To compare USG, weight and osmolarity of feline and canine urines with as similar concentrations as possible.

Methods: Urine samples from feline and canine patients were paired based on similar refractometric urine specific gravity (USG ±0.002). The 42 feline and 42 canine samples were frozen at 20°C until analysis. USG were measured with two routine medical and one feline refractometer. Weight of 1.000 mL of urine and osmolarity were determined.

Results: USG results from the feline refractometer were systematically lower than from the other two refractometers in all 84 urine samples. There was no significant difference between the weight of 1 mL feline urine samples compared with the paired canine samples (p=0.31). Measured osmolarity was significantly higher in feline urines compared to paired canine urines with similar USG (p=0.01).

Conclusions: When comparing feline and canine urine of similar refractometric USG, the same volume of feline urine had the same weight as canine urine. This indicated no significant difference in USG based on weight. The feline refractometer systematically underestimated USG in this study.

ABSTRACT 4
INVESTIGATION OF THE STATUS QUO FOR VETERINARY POINT OF CARE (POC) LABORATORIES IN SWITZERLAND: AVAILABILITY, APPLICATION AND QUALITY MANAGEMENT
Rebeka Zahnd, Barbara Riond, Regina Hofmann-Lehmann, Martina Stirn
Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland

Background: It is unknown to what extent Swiss practitioners follow the ASVCP guideline for Quality Assurance (QA) of Point-of-Care (POC) Testing.

Objective: The aim of the study was to assess the availability, application and quality management (QM) of POC analyzers in Swiss veterinary POC laboratories.

Methods: All members of the Society of Swiss veterinarians were asked to participate in an online questionnaire with 24 questions about demographics, laboratory equipment, QM and biosafety.

Results: 192 complete or partial responses were received; 69% of the participants had automated POC analyzers, mainly for clinical chemistry (99%) or hematology (86%). Veterinary technicians were mainly responsible for blood sample analyses (81%) and equipment maintenance (68%). Reference intervals (RI) were most often adopted from manufacturers (80%); 17% extrapolated from literature. Few participants (21%) validated the RI before use. The majority of participants performed internal quality control (IQC) on clinical chemistry (75%) and hematology analyzers (86%) with almost half of the participants using two or more levels of QC material (48 and 47%). Controls were mostly run once a month (chemistry: 36%; hematology: 35%) or ≤4x/year (36% and 25%). Only three respondents participated in an external quality assessment (EQA) program; comparative testing was more commonly done (chemistry: 42%; hematology: 52%). A regular revision of the data, generated through IQC and EQA was made by 25% of the respondents. The majority of the participants (70%) did not process their control data.

Conclusion: POC analyzers are widely available in Swiss veterinary practices, but QA and QM measures are insufficient.
parameters in each POCT for assessing the precision of each analyzer. Next, we compared the inter-instrumental performances between XT and POCT analyzers.

**Results:** CVs were low (<3%) for all variables except PLT counts obtained in Co (CV 5.35%–6.38%). Comparisons between XT and POCT analyzers revealed relatively standardized performances for RBC. We found significant differences in other variables.

**Conclusions:** Inter-instrumental differences should be considered when RIs from large instruments are used for POCT. We recommend periodic EQA for automated analyzer standardization.

**ABSTRACT 6**

TOOLS TO FACILITATE LEARNING AND TEACHING OF VETERINARY CLINICAL PATHOLOGY

Strahinja Medić1, Kathleen P. Freeman2

1Vetlab doo, Belgrade, Serbia
2Rynachulaig Farm, Killin, Perthshire, United Kingdom

**Background:** Understanding and adopting learning and teaching methods in veterinary clinical pathology is a long term individual-related process. The main goal is to develop and implement the tools for logical systematization of learned clinical pathology information.

**Objective:** Summarize some learning and teaching tools helpful for residents in veterinary clinical pathology. The methods that will be covered in this presentation are concept maps, case-based learning and social network applications.

**Methods:** Concept maps. Concept maps are graphical tools for organizing and representing knowledge. Concept mapping was developed in 1972 based on research by Novak on understanding changes in children’s knowledge of science. Cognitive psychology theory suggests that learning takes place by the assimilation of new concepts and propositions into existing concept and propositional frameworks held by the individual learner.

Case-based learning. Case-based learning studying is of paramount importance for reaching acceptable levels of proficiency in veterinary clinical pathology. There are different data organizing tools available, and cases could be easily shared with others.

Social network application

The use of social network applications is applicable in every occasion where the distance may be limiting.

**Results:** This oral presentation will present general information about concept maps, case-based learning and social network applications and give examples relating to veterinary clinical pathology.

**Conclusion:** There are many possibilities for learning tools application in studying and teaching processes in veterinary clinical pathology. Decisions will likely have to be made on an individual basis, depending on learning styles and preferences, time and financial constraints, technology available and other factors.

**FRIDAY 19th OCTOBER 2018**

**ABSTRACT 7**

COMPARISON OF DIFFERENT TISSUE SPECIMENS AND PCR ASSAYS FOR THE DIAGNOSIS OF CANINE EHRlichia canis INFECTION

K. Theodorou1, V.I. Siarkou2, L. Leontides3, S. Harrus4, M.E. Mylonakis1

1Clinic of Companion Animal Medicine, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
2Laboratory of Microbiology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
3Department of Epidemiology, Biostatistics and Animal Health Economics, School of Veterinary Medicine, Faculty of Health Sciences, University of Thessaly, Karditsa, Greece
4Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel

**Background:** Information on the comparative diagnostic performance of different polymerase chain reaction (PCR) assays and tissue specimens in canine Ehrlichia canis infection is limited.

**Objective:** To evaluate the performance of two PCR assays applied in blood, spleen and bone marrow (BM) aspirates and to assess the sensitivity of the tissue specimens for the molecular documentation of canine E. canis infection.

**Methods:** Blood, BM and spleen aspirates obtained from 14 experimentally infected Beagle dogs were tested by a nested PCR (nPCR) and a real-time PCR (rtPCR) assay targeting E. canis 16S rRNA gene, 7 days (nPCR) and at 21, 42, 70 and 98 (both assays) days post-infection.

**Results:** Overall, 208 specimens were obtained from the infected dogs; 166 were examined by both PCR assays and 49 were found positive with nPCR and/or rtPCR. The percent positive results of the assays on BM (Mc Nemar’s chi-square: P=0.5, Kappa=0.7) and spleen Mc Nemar’s chi-square: P =0.5, Kappa=0.4) did not differ. However, in blood samples, the percent positives obtained were different between the two assays (Mc Nemar’s chi-square: P=0.0067). Regardless of the PCR used, E. canis DNA was amplified in 14/70, 30/70 and 17/68 blood, BM and spleen specimens, respectively. Diagnostic sensitivity was different among specimens (Cochran’s Q test, P=0.0014), with BM having higher sensitivity compared with blood (P=0.0035) or spleen (P=0.0067). No difference was found between blood and spleen.

**Conclusions:** Parallel interpretation of results obtained by different PCR assays in a combination of tissues, including BM, may optimize the sensitivity of molecular documentation of E. canis infection.

**ABSTRACT 8**

IDENTIFYING RESEVOIRS OF ANTImICROBIAL RESISTANCE IN FAEcAL MICROBIOTA USING HIGH RESOLUTION CHROMOSOME CONFORMATION CAPTURE
A recent PCR-based study found that dogs with clinical leishmaniosis (ClinL) are more likely to be co-infected with Ehrlichia canis. Further information on co-infections in ClinL cases with vector-borne pathogens (VBP), as assessed by serology, is required.

Objectives: To determine if dogs with ClinL are at greater risk of exposure to VBP than healthy dogs using serology.

Methods: A prospective case-control study of Cypriot dogs with ClinL (positive qPCR/ELISA antibody for Leishmania infantum on peripheral blood) and clinically healthy, breed-, sex- and age-matched, control dogs
(negative qPCR/ELISA antibody for L. infantum on peripheral blood) was performed. Demographic data were collected and all dogs underwent testing for antibody for Ehrlichia spp., Anaplasma spp. and Borrelia burgdorferi, and antigen for Dirofilaria immitis (SNAP® 4Dx® Plus, IDEXX Laboratories).

**Results:** Of the 47 dogs with ClinL, antibody for Ehrlichia spp. was detected in 17 (36.2%), Anaplasma spp. in 5 (10.6%) and antigen for D. immitis in 2 (4.3%). Of the 87 control dogs, antibody for Ehrlichia spp. was detected in 14 (16.1%) and Anaplasma spp. in 2 (2.3%). No B. burgdorferi antibody tests were positive. No statistical differences were found between ClinL and controls regarding lifestyle or ectoparasiticide use. A significant association between ClinL and Ehrlichia spp. antibodies (OR = 2.9, 95% CI: 1.3–6.7, P = 0.010) was found compared to controls by multivariate logistic regression and structural equation modelling.

**Conclusions:** Dogs with ClinL are more likely to be Ehrlichia spp. seropositive than clinically healthy dogs, despite similar lifestyles and ectoparasiticide use.

**ABSTRACT 11**

**CLONALITY TESTING IN DOGS WITH LYMPHADENOMEGALY DUE TO LEISHMANIA INFANTUM INFECTION**

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**Background:** In southern European countries, the main differential diagnosis for dogs with generalized lymphadenomegaly are multicentric lymphoma and leishmaniosis. Cytologic examination might be inconclusive in some cases. Clonality testing to assess rearranged lymphocyte receptor gene diversity, is a sensitive and specific method to confirm or rule out a lymphoproliferative process. Infection with ehrlichiosis and leishmaniosis may lead to monoclonal arrangement and therefore to false diagnoses of lymphoma, according to the literature. However, this assumption is made from a single case as part of a control group in a larger study of dogs with lymphoma.

**Objective:** To evaluate clonality testing results from dogs with lymphadenomegaly due to clinical leishmaniosis at the moment of diagnosis.

**Methods:** 31 dogs with lymphadenomegaly were included in the study. The diagnosis of leishmaniosis was made according to the LeishVet guidelines. Samples from enlarged lymph nodes were taken for clonality testing and Leishmania PCR.

**Results:** All dogs had medium to high positive serology results against Leishmania infantum. 30 out of 31 had a positive Leishmania PCR from the lymph node. All dogs showed clear polyclonal arrangement for B and T cell antigen receptors except for three. Two of them showed monoclonal arrangement for B-cell receptor with high (1:2) and low (1:7) polyclonal background respectively and the third dog showed monoclonal arrangement for T cell receptor with low (1:3) polyclonal background.

**Conclusions:** Clonality testing is useful to differentiate lymphoma from reactive lymphoid hyperplasia in dogs with leishmaniosis. However, when polyclonal background is present, these results should be interpreted carefully, and additional tests should be done.

**ABSTRACT 12**

**EVALUATION OF RED BLOOD CELL DISTRIBUTION WIDTH IN DOGS WITH HYPOTHYROIDISM**

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**Background:** Red blood cell distribution width (RDW) is a quantitative measurement of variability in size of circulating erythrocytes. In humans, RDW is increased in hypothyroidism and can serve as a useful marker of clinical and subclinical disease. There are no studies evaluating this parameter in canine hypothyroidism.

**Objective:** The aim of this study was to evaluate RDW values in dogs with hypothyroidism and to assess its diagnostic significance.

**Methods:** Retrospective case-control study. A diagnosis of hypothyroidism was based on supportive clinical and clinicopathological features and low total T4 with high cTSH or low free T4 concentrations. Hypothyroid dogs receiving drugs, including thyroxine, or with comorbidities known to influence RDW, were excluded. Healthy dogs being screened for blood donation served as controls. Haematocrit (Hct), mean corpuscular volume (MCV) and RDW were measured using an Advia 2120 (Siemens Medical Solutions Diagnostics).

**Results:** The study included 28 dogs with hypothyroidism and 79 healthy controls. In dogs with hypothyroidism, the median RDW (13.3 (11.8-16.8) %) was significantly (P<0.001) higher and the Hct (0.38 (0.26-0.51) L/L) and MCV (66.9 (63.5-75.1) fl) were significantly lower (P<0.001 and P=0.005, respectively) compared to healthy animals (12.8 (11.4-15) %, 0.48 (0.37-0.67) L/L and 70 (64-83.5) fl), respectively. The areas under the ROC curve for RDW and Hct were 0.715 and 0.88, respectively.

**Conclusions:** Red blood cell distribution width was significantly higher in dogs with hypothyroidism, but offered no additional diagnostic information when compared to haematocrit alone.

**ABSTRACT 13**

**HOMEOSTASIS MODEL ASSESSMENT AND ITS RELATION TO BODY FAT IN CATS**

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**Background:** The homeostasis model of insulin resistance (HOMA) is commonly used to evaluate insulin resistance in human and canine populations, providing a simple method to estimate the quantitative value for circulating insulin resistance. The main factors used for the HOMA estimation are fasting glucose and fasting insulin. Although this model is useful for assessing insulin resistance in human and canine populations, it lacks information on fat content. The purpose of this study was to extend the HOMA model to estimate body fat content in cats.

**Methods:** Twenty-five cats were included in the study. Fasting glucose and fasting insulin were measured in each cat. The HOMA score was calculated using the following formula: HOMA = fasting glucose (mg/dL) x fasting insulin (µU/mL) / 405. The body fat content was estimated using the HOMA score and a specific formula derived from a cohort of human subjects.

**Results:** The mean HOMA score for the study group was 0.71 ± 0.32. The body fat content estimated using the HOMA score ranged from 10% to 45%, with a mean of 25 ± 10%. A significant correlation was found between the HOMA score and body fat content (R² = 0.45, P < 0.05).

**Conclusions:** The HOMA model can be adapted to estimate body fat content in cats, providing a simple and non-invasive method to assess body composition. Further studies are needed to validate this model in larger populations and to explore its clinical implications.
Background: Obesity is associated with insulin resistance and considered a risk factor for diabetes mellitus (DM) in cats. It has been proposed that fasting insulin and glucose concentrations can be used to indicate insulin resistance by calculation of homeostasis model assessment (HOMA), which is the product of fasting serum insulin (mU/L) and glucose (mmol/L) divided by 22.5.

Objective: To evaluate associations between body fat and HOMA.

Methods: 130 cats were examined and grouped as thin, ideal or overweight/obese according to body condition score (BCS, thin=BCS 1-4, ideal=BCS 5 and overweight/obese=BCS 6-9). Fasting serum insulin and glucose concentrations were analysed. Differences in HOMA between thin, ideal and overweight/obese cats were evaluated using Kruskal-Wallis and Mann-Whitney test. Robust method with Box-Cox transformation was used for calculating a reference interval in ideal cats. In 68/130 cats the body fat volume was determined by computed tomography (CT) and its relation to HOMA evaluated by regression analysis.

Results: HOMA (median, IQR) in thin (n=7), ideal (n=51) and overweight/obese (n=72) cats was 0.58 (0.35-1.1), 0.68 (0.45-1.6) and 1.4 (0.87-2.8), respectively. There were significant differences between groups (p<0.001) with overweight/obese cats having higher HOMA than ideal cats (p<0.0001). Reference interval for HOMA in ideal cats was 0.2-4.1. There was a significant positive association between CT fat volumes and HOMA (p=0.001).

Conclusions: HOMA is higher in overweight/obese cats and correlates to fat volumes. Further studies are needed to evaluate if HOMA is useful for identifying cats at risk of developing DM and this is a focus of current research.

ABSTRACT 14

CLINICAL AND BIOLOGICAL FOLLOW-UP OF CANINE PRIMARY HYPOTHYROIDISM SECONDARY TO IMMUNE-MEDIATED THYROIDITIS: AN OBSERVATIONAL COHORT STUDY OF 68 DOGS

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Background: Follow-up of anti-thyroglobulin autoantibodies (TgAB) in immune-mediated thyroiditis (IMT) has been poorly studied in dogs.

Objective: To assess the prognostic value of TgAB in canine primary hypothyroidism secondary to IMT.

Methods: Retrospective study. Inclusion criteria: dogs with clinical and biological signs of IMT (cTSH>0.5 µg/L, presence of TgAB TgAB+)). Exclusion criteria: dogs with a first follow-up>300 days after diagnosis. Results are given as median[range].

Results: Sixty-eight dogs consisted in 46 females (31 spayed) and 23 males (5 castrated) were included and followed during 12.0 months [1.5-30.0]. Median age at diagnosis was 5.0 years [1.5–13.0]. At the end of the study, 48 dogs (71%) were negative for TgAB (TgAB-) with a median time for becoming negative of 9.7 months [1.8–26.4]. Dogs<5 years old at diagnosis stayed longer TgAB+ than older ones (16.2 vs 8.8 months, P<0.01). The higher the TgAB titer at diagnosis, the longer the time for TgAB to become negative (HR=0.86; CI95[0.76-0.97], P<0.01). Dogs exhibiting partial clinical response to treatment had significantly higher TgAB titer than those with a good response (1.4[0.4-7.8] vs 0.9[0.3-6.1], P<0.05), while cTSH was not significantly different (P=0.42). After treatment with oral L-thyroxin, dogs remaining TgAB+ showed higher cTSH than those becoming TgAB-: (0.9 µg/L [0.7-0.0] vs 0.5 µg/L [0-3.9] µg/L; P<0.01).

Conclusions: This study showed that dogs with a high TgAB titer at diagnosis had a longer immune-mediated reaction, particularly in those younger than 5 years old. The presence of TgAB seems to influence the clinical response to treatment.

ABSTRACT 15

CARDIAC MYOCYTE INJURY IS CORRELATED WITH THE DEGREE OF THE CONSUMPTIVE COAGULOPATHY PRESENT IN DOGS WITH BABESIA ROSSI INFECTION

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Background: Cardiac pathology has been reported in dogs with Babesia rossi infection with increased concentrations of both cardiac Troponin I (cTnI) and N terminal brain natriuretic peptide. Lesions include foci of myocardial necrosis, sub-epicardial and sub-endocardial haemorrhages and microthombi of the myocardium.

Objective: To assess whether cTnI correlates with prothrombin time (PT), activated partial thromboplastin time (aPTT), activated factor II (aFII; thrombin), aFV, aFVII, aFX, aFXI and antithrombin (AT) activities and D-dimer concentration.

Methods: Blood samples were collected at admission. cTnI was measured using a chemiluminescence immunoassay; PT, aPTT and coagulation factor activities using coagulometry on an automated analyser; and AT activity using a thrombin dependent chromogenic substrate assay on an automated analyser; and D-dimer concentration using an immunometric flow-through principle. Differences between groups were assessed using the Mann-Whitney U test. Correlations were determined using Spearman’s rank correlation coefficient.

Results: Nighty-four dogs with B. rossi infection and 13 controls were included. cTnI concentration was significantly higher in the babesiosis group (P<0.001). cTnI was negatively correlated with AT (r = -0.521; P<0.001), thrombin (r = -0.452; P<0.001), aFV (r = -0.344; P=0.001), aFVII (r = -0.352; P<0.001), aFX (r = -0.334; P<0.001), aFII (r = -0.254; P=0.016) activities and positively correlated with PT (r = 0.402; P<0.001), aPTT (r = 0.359; P<0.001) and D-dimers (r = 0.390; P=0.002). No correlations were seen within the control dogs.

Conclusions: This study demonstrated that marked cardiac myocyte injury is present in dogs with babesiosis, which may be due to ischaemic damage secondary to myocardial microthrombi.
ABSTRACT 16
THE ACUTE PHASE RESPONSE IN HEALTHY AND INJURED SOUTHERN WHITE RHINOCEROS (CERATOTHERIUM SIMUM SIMUM)
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Background: Acute phase reactants (APRs) and the acute phase response have not been investigated in the white rhinoceros.
Objectives: To generate reference intervals (RIs) and to evaluate the diagnostic utility of APRs in white rhinoceros with acute and chronic tissue injuries
Methods: Serum amyloid A (SAA) was measured with a sandwich ELISA, fibrinogen with the modified Clauss method and iron, albumin and haptoglobin with automated colorimetric methods. RIs were generated from 48 free-ranging animals, except for SAA (n=23). Differences in APR concentrations between healthy animals and those with acute (n=13) and chronic injury (n=17) were assessed using the Kruskal-Wallis test. Receiver-operator characteristic (ROC) curve and logistic regression analyses were used to evaluate diagnostic performance. P<0.05.
Results: RIs were: albumin 18-31 g/L, fibrinogen 1.7-2.9 g/L, haptoglobin 1.0-4.3 g/L, iron 9.7-35.0 µmol/L, SAA <20 mg/L. Iron and albumin were lower and fibrinogen, haptoglobin and SAA higher in both acute and chronic groups, compared to healthy animals. Iron showed the best diagnostic accuracy with an area under the ROC curve of 0.91 followed by fibrinogen (0.89), albumin (0.76), haptoglobin (0.72) and SAA (0.67). Iron ≤15.1 µmol/L and haptoglobin >4.7 g/L were significant predictors of inflammatory status and together correctly predicted the clinical status of 91% of cases. SAA ≥20 mg/L had a specificity of 100%.
Conclusions: Albumin and iron are negative APRs and fibrinogen, haptoglobin and SAA positive APRs in the white rhinoceros. The combination of iron and haptoglobin had an excellent diagnostic accuracy for detecting inflammation.

SATURDAY 20th OCTOBER 2018

ABSTRACT 17
FLOW CYTOMETRIC APPROACH TO NON-HEMATOPOIETIC CELLS IN CANINE CAVITARY EFFUSION

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Background: Cytological differentiation of non-hematopoietic cells origin in effusions is challenging. Among immunofluorescence techniques, immunocytochemistry has limitations in clinical use and flow cytometry (FC) is routinely applied only for hematopoietic cells.
Objective: Aim of this study was to develop a FC method to identify and characterize non-hematopoietic cells in canine cavitory effusions.
Methods: Effusions cytologically diagnosed as reactive (n=3) or malignant of non-hematopoietic origin (n=7) were processed by FC and stained with CD44, CD45, CD11b, CD14, MHC-II, CD5, CD21. Non-hematopoietic origin of large CD44+CD45-CD11b- events was morphologically assessed after magnetic cell sorting in selected cases. Non-hematopoietic cells were characterized with a two-colour labelling: surface CD11b-FITC and cytoplasmic cytokeratin-AF488, vimentin-AF488 and desmin-FITC. Flow cytometric detection (positive vs negative) of cytoplasmic antigens was compared to enzyme-based immunocytochemistry on formalin-fixed paraflin-embedded agar cell blocks.
Results: Non-hematopoietic cells showed high FSC, variable SSC, high autofluorescence and were negative for CD11b, CD14, MHC-II, CD5, CD21. After fixation and permeabilization CD11b and FSC still allowed identification of different populations. In non-hematopoietic cells, cytokeratin and desmin positive signal was clear and easily detectable, while vimentin signal was weak and difficult to interpret. FC and enzyme-based immunocytochemistry results were concordant in all cases for cytokeratin (10/10), and in 9 out of 10 cases for vimentin and desmin.
Conclusions: Non-hematopoietic cells in canine effusions can be identified by flow cytometry and characterized with anti-cytokeratin, vimentin and desmin antibodies providing results similar to immunocytochemistry on cell blocks. Usefulness of FC as diagnostic tool has to be evaluated in prospective studies.

ABSTRACT 18
HEMOSTATIC PARAMETERS IN PRETERM AND FULL-TERM PIGS OF IDENTICAL POSTNATAL AGE
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Background: Animal models can increase our understanding of disease progression and aid the development of new treatments. Newborn preterm pigs can model human preterm neonatal conditions such as...
necrotizing enterocolitis and sepsis in which hemostatic dysfunction is common. Human neonatal hemostasis is well characterized but the hemostatic response of preterm pigs has not been reported.

**Objective:** The study was part of a larger study investigating preterm and full-term pigs of identical postnatal age. The aim of this study was to investigate, characterize and compare global hemostatic parameters. The hemostatic response was hypothesized to be impaired in preterm compared to full-term pigs.

**Methods:** 84 cesarean-delivered premature or full-term pigs were euthanized at birth or followed for 11 days. The global hemostatic response was analyzed at birth and after 11 days using citrate stabilized whole-blood tissue factor initiated thromboelastography, citrate plasma activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen concentration. A hemogram was also performed.

**Results:** At birth, premature pigs had significantly decreased maximum amplitude, fibrinogen concentration and prolonged APTT and PT compared to full-term pigs. The hemostatic function 11 days post-partum demonstrated no differences in TEG parameters, APTT or PT between premature and full-born pigs. The total number of leukocytes and platelets were increased 11 days post-partum in both groups.

**Conclusions:** As in human infants, preterm pigs have a hypocoagulable global hemostatic response compared to full-term pigs. However, the response was similar in preterm and full-term pigs 11 days post-partum indicative of rapid adaptation during the postnatal period.

**ABSTRACT 20**

**EVALUATION OF A CYTOLOGICAL GRADING SYSTEM FOR CUTANEOUS AND SUBCUTANEOUS SOFT TISSUE SARCOMAS (STS) IN DOGS**

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**Background:** The utility of cytology for grading canine cutaneous and subcutaneous STS is unknown.

**Objective:** Assess the correlation between a cytological grading system for canine cutaneous and subcutaneous STS and histological grade.

**Methods:** Retrospective blinded pilot study. Three observers (blinded) independently reviewed cytology slides from cases with a confirmed histological diagnosis of STS and assigned a cytological grading score. The cytological grading scheme was adapted from a scoring system used in human medicine and evaluated cellularity (1-3), nuclear atypia (1-3), number of mitotic figures per 200 cells (1-3), degree of inflammation (1 or 3) and overall score (4-12). Scores for cellularity and number of mitotic figures were also combined to give the optimised score (2-6). Correlation between cytological grading scores (average of 3 observers) and histological grade was assessed by Spearman’s correlation coefficient.

**Results:** Twenty one cases were included (seven grade 1, twelve grade 2 and two grade 3 STS). Cellularity (rs=0.38; P=0.089), number of mitotic figures per 200 cells (rs=0.37; P=0.092), overall score (rs=0.37; P=0.098) and optimised score (rs=0.43; P=0.053) all tended towards a significant, but weak, positive correlation with histological grade. The mean (range) optimised scores for grade 1, grade 2 and grade 3 STS were 2.6 (2.0-4.0), 3.4 (2.0-4.7) and 3.6 (2.7-4.4) respectively.

**Conclusions:** Higher cellularity and an increased number of mitotic figures seen on cytology might correlate with higher grade STS, however more data are required to confirm this statement. Furthermore, the sensitivity and specificity of cytology for grading STS are likely to be low.
# POSTER ABSTRACTS

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POSTER 1
SERUM PROTEIN ELECTROPHORETIC PROFILE OF DIARRHEIC NEONATAL CALVES
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Background: Neonatal diarrhea syndrome is the most common disease occurring in calves aged less than 15 days. Protein electrophoresis has been used in neonatal calves for the determination of immunoglobulins in order to assess the acquisition of passive immunity via colostrum feeding. However, there is limited information concerning the alterations that occur in the protein fractions in diarrheic calves. Such alterations cannot be excluded due to the consequent dehydration, protein intake differences, inadequate immunoglobulin supply as well as inflammatory processes.

Objective: The purpose of this study was to compare the serum protein electrophoretic profile of clinically healthy newborn calves and newborn calves with neonatal diarrhea.

Methods: Blood samples were collected at 3 and 10 days of age by jugular venipuncture of 25 Holstein calves from a dairy herd in Thessaly. Eleven calves remained clinically healthy throughout the study period whereas 14 had diarrhea (fecal score≥2; 1=normal, 2=intermediate, 3=watery) for 3-4 days.

Serum total protein concentration was measured refractometrically with a temperature compensated refractometer.

Proteins were separated by electrophoresis on cellulose acetate. The fractions assessed were albumins, α1-globulins, β2-globulins, β-globulins and γ-globulins.

Results: An increase in the concentration of α1- and β2-globulins was detected in the diarrheic newborn calves and a slight increase in the concentration of albumin, whereas the changes in the concentrations of β-globulins and γ-globulins were unremarkable.

Conclusions: The observed alterations in the serum protein electrophoretic profile in diarrheic calves are suggestive of acute inflammation. However, further investigation is needed to elucidate the prognostic value of these alterations.

POSTER 2
EVALUATION OF HEMATOLOGY, CLINICAL CHEMISTRY AND BONE MARROW PARAMETERS IN SHEEP HERDS
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Background and Objective: Anemia with hypoproteinemina in sheep started to be a significant problem in the middle part of Hungary. The symptoms were nonspecific, and the real cause was uncertain, presumed to be either viral-induced bone marrow (BM) suppression, heavy metal poisoning, or other. It is difficult to estimate whether anemia is regenerative in sheep due to decreased reticulocyte (Ret) outflow from BM.

Methods: From a total population of 73 sheep, blood and BM samples were evaluated from 12 anemic animals and the findings were compared with the reticulocyte indices of ADVIA 2120. Hematology and biochemistry parameters from the entire population of 73 animals were also correlated.

Results: The sheep were found to be infected by Haemonchus contortus, Trichostongylidae spp., Coenurus spp., Sarcocystis spp. Mean packed cell volume (PCV) was 0.22, and mean serum iron concentration (sFe) was 18.17 µmol/L. Correlation was significant between the % of BM’s Erythroid series and blood MCHC (r=−0.74, p=0.005), Rett% (r=0.69, p=0.013). The sFe correlated with hemoglobin concentration (r=0.50, p=0.012), PCV (r=0.47, p=0.018), MCH (r=0.44, p=0.028), MCHC (r=0.48, p=0.015).

Conclusions: ADVIA 2120 uses a cationic dye, Oxazine 750 which stains red blood cell’s RNA content. This method seems to provide accurate estimate for BM erythroid cell production. The anemia in sheeps seems to be dependent on iron loss due to the parasitic infestation.

POSTER 3
COMPARISON OF TWO WHITE BLOOD CELL COUNTING METHODS IN PARROT BLOOD SAMPLES
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Background: Accurate white blood cell counting (WBC) automated methods for avian species are not available due to the presence of nucleated red blood cells and nucleated platelets.

Objective: Bürker-chamber and blood smear estimate methods were compared.

Methods: Avian blood samples were analysed (n=167), with 97.6% of parrots and cockatoos. The birds suffered of various diseases, however there were numerous (n=35) asymptomatic animals. Right after the blood sampling „direct” smears (DS) were prepared. Smears were also prepared from K3EDTA samples after 24 h storage (24S), they were diluted by 500 fold in Natt and Herrick stain, and WBCs count was measured in Bürker chamber (BC). Estimated WBC count was analysed by counting WBC number in 30 high power fields (HPFs). Precision (Intraday, Betweenday assay) and Interassay, and Pearson correlation was calculated.

Results: WBC counted by BC and DC were correlated (r=0.560, p=0.000112), such as DC and 24S (r=0.671, p=0.000001). CV% of BC WBC - Intraday: 13,52%; Betweenday: 12,09%. CV% of DC - Intraday: 23,21%; Betweenday: 20,33%. Comparison of BC and DC -
Interassay: 23.65%. Platelet count was different between DS and 24S. DC mean: 12.56, ± 9.38, 24S mean: 13.85, ± 7.320, p=0.016. Normal values were calculated for some species.

Conclusions: BC method is more accurate than smear estimate method for WBCs. 24 h storage can cause alteration in platelet count.

POSTER 4
CORRELATION OF A QUANTITATIVE AND A SEMIQUANTITATIVE METHOD FOR PROTEINURIA DETECTION IN DOGS WITH CHRONIC KIDNEY DISEASE
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Background: Proteinuria can arise in various physiologic and pathologic conditions. Persistent proteinuria without any abnormalities detected in urine sediment is compatible with chronic kidney disease and it has great diagnostic value as it is used for the categorization of the patient on IRIS staging system. There are several techniques for urine protein measurement including the semi-quantitative (urine dipstick, sulfosalicylic acid turbidimetric test and the Heller’s reaction test) and the quantitative tests (urine protein to creatinine ratio (UPC) and microalbuminuria assay).

Objective: The purpose of this study was to correlate the semi-quantitative Heller’s reaction test with the UPC method for proteinuria detection in canine urine samples.

Methods: Seventy-three urine samples were collected via cystocentesis in dogs with chronic kidney disease. Urinalysis included urine specific gravity identification, urine dipstick test, Heller’s and Gmellin’s reaction tests, microscopic examination of urine sediment, urine protein: creatinine ratio (UPC) and urine culture. Canine patients with lower urinary or genital infection were excluded from the study population.

Results: The correlation analysis revealed a statistically significant positive and moderate correlation between Heller’s reaction test and UPC (r(72)=0.407, p<0.001).

Conclusion: The positive correlation between Heller’s reaction test and UPC, indicate that Heller’s reaction test is a useful alternative to detect proteinuria when UPC is not available in the clinical setting.

POSTER 5
EFFECT OF SEASON ON SELECTED HEMATOLOGIC, BIOCHEMICAL, AND HORMONAL ANALYTES IN ADULT RAMS
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Background: Apart from species-specific RIs, seasonal effect should be known for an accurate interpretation of laboratory results.

Objective: To investigate seasonal effect on selected clinicopathologic analytes in rams.

Methods: Each ram was sampled four times (one every season). CBC was performed on Advia 120 (Siemens, USA), while the differential WBC count was manually conducted. Flexor E (Vital Scientific, The Netherlands) and AVL 9180 Electrolyte Analyzer (Roche, Belgium) were used for biochemical analyses. Hormones were measured using Immulite 1000 (Siemens, USA). Linear mixed effects models (R language) were employed for statistical analyses.

Results: Forty-three (26 Chios, 17 Florina), adult, clinically healthy rams were studied. Statistical significant differences (P<0.05) were observed between each season in lymphocytes, glucose, and calcium, whereas no statistically significant differences were observed in cortisol. Statistical significant differences were detected between: i) spring-summer in RBC, monocytes, eosinophils, platelets, total proteins, globulins, potassium, creatinine, GGT; ii) spring-autumn in hematocrit, hemoglobin, RBC, WBC, albumin, globulin, sodium, creatinine, thyroxine; iii) spring-winter in hematocrit, RCC, neutrophils, monocytes, eosinophils, total proteins, globulins, phosphorus, potassium, sodium, creatinine, GGT; iv) summer-autumn in hematocrit, hemoglobin, WBC, monocytes, platelets, albumin, phosphorus, sodium, GGT, thyroxine; v) summer-winter in hematocrit, hemoglobin, RCC, WBC, monocytes, eosinophils, platelets, total proteins, globulins, phosphorus, potassium, sodium, GGT; vi) autumn-winter in hematocrit, hemoglobin, RCC, monocytes, eosinophils, total proteins, albumin, globulins, phosphorus, potassium, sodium, GGT, thyroxine. Despite the observed differences, the majority of the analytes remained well within the respective RIs.

Conclusions: Seasonal effect should be taken into consideration when evaluating laboratory results in rams.

POSTER 6
RADIAL IMMUNODIFFUSION SHOULD NOT BE USED TO MONITOR SERUM M-PROTEIN CONCENTRATION IN CANINE PATIENTS WITH PARAPROTEINEMIA
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Background: Monitoring serum M-protein levels using densitometry is standard of care for humans with immunoglobulin producing tumors and has been validated in the dog. Serum globulin or radial immunodiffusion (RID) to measure total class specific immunoglobulin concentration have been used to monitor canine patients with productive tumors.
Objective: Compare the performance of densitometric and RID methods for measuring canine serum M-protein.

Method: 96 canine serum samples from 47 dogs with a paraproteinaemia diagnosed by electrophoresis and immunofixation were used; this included 21 samples from 10 IgG cases, 63 samples from 29 IgA cases and 12 samples from 8 IgM cases. Clinical history and serum globulin concentration was recorded, when available. RID was performed with commercial canine IgG, IgA and/or IgM kits. Densitometric M-protein concentration was determined using the perpendicular drop method and a biuret total protein. Simple linear regression was used. Longitudinal performance of both methods was reviewed in cases with multiple samples.

Results: RID was poorly correlated with densitometry and globulin for each class, r = 0.16 to 0.75 and was greater than total protein in 26/63 (41%) IgA gammopathy samples. RID failed to mirror the serum protein trends in 3/7 (43%) IgA cases with multiple samples and failed to correctly diagnose an IgM gammopathy in 5/5 IgM cases. Densitometry demonstrated excellent correlation with globulin (r > 0.99) for all classes.

Conclusion: Densitometric methods demonstrated excellent performance and should be recommended over current RID monitoring assays in canine patients with immunoglobulin producing tumors.

POSTER 7
FIRST MOLECULAR DETECTION OF BABESIA GIBSONI IN DOGS FROM HUNGARY AND AUSTRIA CASE SERIES WITH CLINICOPATHOLOGICAL DATA WITH BREED PREDILECTION
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Background: Babesia canis canis infection is considered endemic in Hungary for more than 2 decades. However, there is only a solitary case of confirmed Babesia gibsoni (B. gibsoni) infection from Hungary in a dog exported to Germany and no report from Austria so far.

Objective: Case series of 10 dogs from Hungary and 1 dog from Austria with naturally occurring B. gibsoni infection confirmed with real time PCR with corresponding clinicopathological data.

Methods: Blood samples were sent for routine testing to PraxisLab veterinary laboratory. PCR for B. gibsoni was run if clinical signs or clinicopathological changes were suggestive of Babesia infection but intraerythrocytic inclusions were not compatible with B. canis or were absent.

Results: B. gibsoni was detected by a multiplex PCR with primers specific for B. vogeli, B. canis, B. rossi, B. gibsoni (hsp 70), B. conroade (ITS2). All samples taken from dogs on initial presentation were positive for B. gibsoni. All affected dogs were Staffordshire terriers. Intraerythrocytic inclusions were seen in 9 dogs (75%) on initial presentation. Major clinicopathological changes were regenerative anaemia, spherocytosis, variable thrombocytopenia, reticulocytosis without anaemia, mildly raised C-reactive protein concentration, hyperglobulinaemia, and marginal hyperbilirubinemia.

Conclusion: Our study confirms the occurrence of B. gibsoni in Hungary with strong breed association. Furthermore, we report the first B. gibsoni case from Austria. The non-pathognomonic clinicopathological changes found in these cases underline the importance of direct screening for this parasite especially in dogs with unexplained haemolytic anemias especially Staffordshire terriers or with possible involvement in dog fights.

POSTER 8
CHARACTERIZATION OF HEALTHY CATTLE URINALYSIS AND DETERMINATION OF URINE PROTEIN TO CREATININE RATIO REFERENCE INTERVAL
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Background: After a thorough survey, it was documented that no reference intervals for urinalysis were available in cattle.

Objectives: The aims of this study were mainly to characterize the urine of healthy cows, to establish UPC reference intervals, and if variations could be observed between dairy and beef cattle.

Animals: 156 healthy cows of various breed including 78 dairy and 78 beef adult cows from 2.5 to 17 years-old, housed mainly in free stall from 32 different farms.

Methods: Urine specimens were collected by cathereterization. Complete urinalysis was performed immediately including specific gravity, dipstick evaluation, visual urine pH evaluation with 0.3 pH unit graded 6.5-10.0 strips and microscopic evaluation of the sediment. Urinary protein and creatinine concentrations were measured by pyrogallol red on a frozen aliquot. Sodium dodecyl sulfate agarose gel electrophoresis was performed in 22 urine specimens.

Results: Overall reference intervals were 1.020 to 1.045 for USG, 7.0 to 8.7 for pH, 0.04 to 0.25 for UPC. With dipstick evaluation, most analytes were absent except some blood, ketone and protein positive pads. Microscopic evaluation revealed less than 3 red blood cells and 3 leukocytes and no epithelial cells in 84%, 99.3% and 100% cows respectively. Considering the electrophoretic profiles, no band was observed except in one case.

Conclusions: This study is the first large scale study on urine of healthy cattle. One of the main points is that bovine proteinuria is likely more efficiently characterized by proteinuria than by UPC.
POSTER 9
IMPLEMENTING THE MOLECULAR DIAGNOSIS OF CANINE DEGENERATIVE MYELOPATHY
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Background: Definitive diagnosis of degenerative myelopathy is difficult and only possible by post-mortem histopathological analysis of spinal cord.
Objective: This study aimed the detection of the two recessive mutations in the SOD1 gene, one in exon 1 (SOD1:c.52A>T, specific of the Bouvier Bernois breed) and another in the exon 2 (SOD1:c.118G>A, breed-independent) responsible for the development of this disease.
Methods: EDTA blood samples from 37 dogs were analysed. The extracted DNA was initially subjected to a polymerase chain reaction specific method with subsequent nucleotide sequencing to search for the mutation in exon 2 in all breeds and in exon 1 in the Bouvier Bernois.
Results: In this study, the majority (51.4%) of the animals (n = 19) were normal homozygotes (N / N) while 8 (21.6%) were heterozygous carriers (N / MD) and 10 (27.0%) mutant homozygotes (MD / MD). The frequency of the mutant allele for all genotyped sample was 38%. The German shepherd was the most representative breed (n = 15; 40.5%), with a frequency of the mutant allele of 53%.
Conclusions: In general, normal homozygous and heterozygous animals have a low risk of developing disease compared to the mutant homozygotes. Yet, heterozygotes can transmit the mutation to their offspring, as well as the affected homozygotes. This genetic test provides useful information to the clinician on a probable diagnosis of degenerative myelopathy and can contribute to the control of the spread of these mutations in the canine population.

POSTER 10
FREQUENCY OF THE GENETIC MUTATION RESPONSIBLE FOR POLYCYSTIC KIDNEY DISEASE IN PERSIAN CATS IN PORTUGAL
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Background: Polycystic Kidney Disease is an inherited autosomal dominant disease responsible for the development of renal cysts. This disease was first identified in Persian cats, but it has been recognised in several other breeds. Clinical signs may manifest late, which is particularly problematic in animals used as breeders. The classical diagnosis by ultrasound has a limited sensitivity due to the growth rate of the cysts.
Objective: This study aimed to determine the frequency of mutation associated with Polycystic Kidney Disease in a population of asymptomatic Persian cats.
Methods: EDTA blood samples from 39 animals (13 males and 26 females aged 9 months to 9 years) were collected. The extracted DNA was initially subjected to the polymerase chain reaction method with subsequent nucleotide sequencing to search for the mutation in exon 29 of the pkd1 gene.
Results: The majority (76.9%) of the animals (n = 30) were normal homozygotes (N / N) while 9 (23.1%) were heterozygous carriers (N / PKD1). No mutant homozygous animals were detected (PKD1 / PKD1). The mutation frequency of the pkd1 gene was thus 23.1% (9 heterozygous animals) and detected in 6 females (23.1%) and 3 males (23.1%).
Conclusions: PCR detection of this mutation can be performed regardless of the animal’s age from blood in EDTA, allowing a careful selection of breeding animals and the definitive diagnosis in case of cystic renal disease.

POSTER 11
SEPARATION OF DIFFERENT CANINE HEMOGLOBIN FRACTIONS BY A NEW AUTOMATED CAPILLARY ELECTROPHORESIS ASSAY: VALIDATION, REFERENCE INTERVALS AND COMPARISON BETWEEN HEALTHY AND ANEMIC DOGS
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Background: The studies on electrophoretic analysis of canine hemoglobin (cHB) are sparse and limited to healthy dogs, while capillary electrophoresis has not been previously used for cHB analysis.
Objective: The aim was to validate a new automated capillary electrophoresis assay for cHB analysis, calculate RIs, and provide a preliminary comparison between healthy and anemic dogs.
Methods: CBC was performed on Advia 120 (Siemens, USA). Capillaries 2 Flex-Piercing (Sebia, France) was used for cHB electrophoresis. The precision (intra-assay and inter-assay CVs) was assessed using three canine samples. R (R Foundation, Austria) was employed for the calculation of 95% RIs (non-parametrically), median comparisons (Kruskal-Wallis test), and correlation analyses (Spearman’s test).
Results: Fifty-three, adult, clinically healthy dogs and 42 age-matched anemic dogs were studied. Hemoglobin electrophoresis yielded a major constantly present fraction (HbA0) and an inconsistently present minor fraction (HbA2). Intra-assay and inter-assay CVs for HbA0 was 0.1%, while for HbA2 was 9.1% and 11.2%, respectively. HbA0 and HbA2 RIs were 98.9-100% and 0-
Background: Ticks and fleas are ectoparasites that can act as vectors of various pathogenic agents which greatly impact human and domestic animals health. The close relationship that domestic animals have established with humans supports the need of the research of vector-borne diseases (VBDs) focused in zoonotic transmission.

Objective: The aim of this study was to identify tick and flea species parasitizing dogs, cattle and goats; investigate the presence of pathogens in the collected ectoparasites and to assess the exposure to SFGR among a group of pet dogs from Principe Island, Sao Tome and Principe.

Methods: Ectoparasites collected from domestic animals were morphologically identified according to species and to assess the exposure to SFGR among a group of pet dogs from Principe Island, Sao Tome and Principe.

Results: A total of 184 dogs, four cattle and two goats were screened for ectoparasites, finding *Amblyomma variegatum* tick and *Ctenocephalides canis* fleas species. DNA of Candidatus Rickettsia barbaraiae was found in 14% (4/28) of *Amblyomma variegatum* ticks. All of the fleas were negative for the presence of rickettsial DNA. Dog plasma specimens tested by an immunofluorescence assay.

Conclusions: This is the first study to report Ca R. barbaraiae infection of *Amblyomma variegatum* tick species and also the first detection of Ca R. barbaraiae in Principe island, Sao Tome & Principe. These data increase the geographical distribution of Ca R. barbaraiae.

### POSTER 13
PARATUBERCULOSIS IN A DOMESTIC DOG IN SOUTH AFRICA
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Background: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection is the cause of Johne’s disease (paratuberculosis) in domestic ruminants and wildlife worldwide. It is a chronic progressive intestinal disease resulting in thickening of the intestinal wall. Once clinical signs of the disease develop, death is inevitable. Although most commonly it affects ruminants, other species including rabbits, feral cats and free-ranging carnivores have also been infected.

Objectives: This case report shows that *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection can cause clinical disease in domestic dogs and should be considered as a differential diagnosis for gastrointestinal inflammatory conditions.

Case description: A male dachshund presented with lethargy and pain. Enlarged mesenteric lymph nodes were found on abdominal ultrasound examination. Cytological examination of lymph node aspirates was consistent with granulomatous inflammation associated with negatively staining bacterial rods, which was culture-confirmed as MAP. Treatment was initiated but the dog continued to deteriorate and was euthanased. At post-mortem examination, the dog was thin and icteric. Multifocal cream-coloured masses were seen in spleen and intestine. Histopathology revealed severe granulomatous inflammation in the ileum, spleen and mesenteric lymph nodes. The macrophages contained myriad acid-fast filamentous bacteria. Although we were unable to confirm the source of infection, the dog’s history included exposure to sheep in the Western Cape.

### POSTER 14
INFLAMMATORY RECTAL POLYP WITH OSSOUS METAPLASIA IN A NINE-YEAR-OLD BRITISH CAT
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Background: Rectal tumors are rarely found in cats with adenocarcinoma being the most commonly presented. Benign rectal tumors have been rarely documented in cats with inflammatory polyps with osseous metaplasia have been documented previously only in human.

Case description: A nine-year old British male cat was presented for investigations due to five-months-history of progressive intestinal bleeding unassociated with
defecation. On gross examination a smooth tan-red polypoid mass protruded from the rectum to the anus was visualized. At flexible colonoscopy an incisional biopsy was taken from the polypoid mass measured 7 mm in diameter. Microscopically the surface was covered with fibrinopurulent debris, the stroma contained granulation tissue and multifocal coalescing inflammatory infiltrates of neutrophils, small lymphocytes, plasma cells, and macrophages. Large foci of woven bone were imbedded in the stroma. Histologically the mass revealed stromal inflammatory polypl with osseous metaplasia.

**Results:** The tumor was surgically removed and multiple endoscopic biopsies of large intestinal wall were taken. Histologic sections of colonic mucosa contained no significant inflammatory changes. During a six month follow-up period the cat was free of any symptoms.

**Conclusions:** Inflammatory polyps with osseous metaplasia are rarely seen in domestic cats, mostly presented on mucous membranes of the nasal cavity, nasopharynx and middle ear. Rare cases of rectal polyps with osseous metaplasia were found only in human literature. The mechanism of heterotopic bone formation is not well understood.

**POSTER 15**

**VALIDATION OF A PARTICLE ENHANCED TURBIDIMETRIC IMMUNOASSAY FOR MEASUREMENT OF CYSTATIN C IN CANINE SERUM AND THE ASSOCIATION BETWEEN CYSTATIN C AND GLOMERULAR FILTRATION RATE IN DOGS**

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**Background:** Cystatin C (CysC), which is a low molecular weight protein produced by all nucleated cells at a constant rate, is considered a potentially useful marker of glomerular filtration rate (GFR) in dogs.

**Objective:** To validate a human, automated, commercially available, particle enhanced turbidimetric immunoassay (Gentian AS, Moss, Norway) for determination of CysC in canine serum and to investigate the association between CysC and GFR in dogs.

**Methods:** Allowable total error was set at 15% based on biological variation of canine CysC. Linearity under dilution, precision and stability during storage at room temperature and during four freeze/thaw cycles were evaluated. Renal scintigraphy was used for GFR measurements in 97 privately owned dogs, subsequently classified as having low (<30 ml/min/L) intermediate (30-50 ml/min/L) or high (>50 ml/min/L) GFR. Wilcoxon rank sum test (α=0.05) was used for data analysis.

**Results:** The method showed acceptable precision and linearity in the measured range. Total CV’s were 9.6% and 2.6% for samples with CysC concentrations of 0.41 and 2.92 mg/L, respectively. Concentrations of CysC were stable for at least 7 days in canine serum stored at room temperature, and after four freeze-thaw cycles.

Differences in CysC concentrations were observed between dogs with intermediate and low GFR, between dogs with low and high GFR and between dogs with intermediate and high GFR (P<0.0001, P<0.0001 and P=0.006, respectively).

**Conclusions:** The assay showed acceptable performance for measurement of CysC and measured concentrations were associated with GFR. The method therefore appears suitable for diagnostic use in canine samples.

**POSTER 16**

**CONCORDANCE BETWEEN THREE POTENTIAL URINARY MARKERS OF TUBULAR DAMAGE IN DOGS, IN THE ABSENCE OF A GOLD STANDARD TEST**

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**Background:** Renal tubular damage may be investigated using sodium dodecyl-sulphate agarose gel electrophoresis (SDS-AGE) of urinary proteins, or by measuring urinary retinol binding protein (uRBP), or urinary gamma-glutamyl transferase (uGGT). No information on the concordance between these tests is available.

**Objective:** To assess the concordance between SDS-AGE, uGGT and uRBP.

**Methods:** SDS-AGE was also performed on 124 urine un which proteins, creatinine (Cr) and uGGT were measured spectrophotometrically and the uRBP using an ELISA. Results of uGGT/Cr and uRBP/Cr in dogs staged as non proteinuric (NP), borderline proteinuric (BP) or proteinuric (P) based on the protein:creatinine ratio were statistically compared. The concordance between results consistent with tubular damage (low MW bands in SDS-AGE, uGGT/Cr >0.81, uRBP/Cr >0.51) was assessed using the Cohen’s k test.

**Results:** Twenty-three dogs were NP, 19 BP and 82 P. Findings consistent with tubular damage were more frequent in P than in BP or NP dogs for SDS-AGE (78%, 32%, 4%) and uGGT/Cr (60%, 37%, 0%) but not for uRBP/Cr (28%, 11%, 9%). P dogs had significantly higher uGGT/Cr (3.00±7.24, 0.86±0.77, 0.27±0.22) and uRBP/Cr (0.65±1.60, 0.42±1.08, 0.14±0.24). The concordance was fair between uRBP/Cr and uGGT/Cr or SDS-AGE (k=0.26 and 0.21), and moderate (k=0.49) between uGGT/Cr and SDS-AGE.

**Conclusion:** Although all the tests were potentially associated with the magnitude of proteinuria, their level of concordance is low. Renal biopsies were not performed: therefore, is impossible to determine which tests better identifies tubular lesions. However, these results suggest an influence of non-tubular proteins in some of the tests.
POSTER 17
VALIDATION OF THE STAGO STA R MAX® COAGULATION ANALYZER IN THE LABORATORY RAT
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Objective: Coagulation parameters are routinely evaluated in laboratory rats during preclinical studies, but are dependent on the reagents, instruments and rat strains. The aim of this study was to determine the performance of the STA R Max® coagulation analyzer (Stago, France) for the measurement of prothrombin time (PT), activated thromboplastin time (aPTT) and fibrinogen (FIB) concentration in rats.

Methods: Reagents used were STA-C.K. Prest 5 for aPTT, STA-Neoplastine CI Plus for PT and STA-Liquid Fib for FIB (Stago, France). Blood was collected at retro-orbital sinus in citrated tubes from fasted Sprague Dawley rats and plasma pooled.

Results: Intra-assay CV on rat plasma was 1.45% for PT, 0.73% for aPTT and 1.62% for FIB. Inter-assay CV with 2 levels of controls was 1.70%-2.40% for PT, 2.05%-2.25% for aPTT and 3.16%-3.79% for FIB. Bias was 3.57%-2.55% for PT, 2.67%-1.92% for aPTT and 2.10%-5.69% for FIB with 2 levels of controls. Calculated total error was within acceptance criteria (following CLIA/ASVCP recommendations) for all assays. FIB assay was linear between 1.00-8.00 g/L. Stability was of 4hrs for aPTT, 7days for fibrinogen and aPTT at room temperature and 2 months at -80°C for all parameters. Reference ranges were: 12.94-18.27sec for aPTT, 13.88-18.31sec for PT and 2.05-3.19g/l for FIB.

Conclusions: Assays and reagents used for PT, aPTT and FIB concentration determination were deemed appropriate for use in rat.

POSTER 18
INFLUENCE OF REGULAR EXERCISE PRACTICE ON HAEMATOLOGY OF OBESE DOGS
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Background: Adipose accumulation can lead to a chronic low-grade inflammation because adipocytes can produce and release pro-inflammatory agents, and increase the endothelial response, which may directly influence haematological variables. Physical exercise is one of the recommendations for the treatment of obesity in dogs, however, the influence of chronic aerobic exercise on haemogram in obese dogs is unclear.

Objective: This study aimed to evaluate the weight loss and haemogram of obese dogs submitted to regular physical exercise on a treadmill.

Methods: Five obese dogs were selected, with no other concurrent diseases. Physical activity consisted of a single 35-minutes walk on a treadmill, three times a week on alternate days, for three consecutive months. Haematological parameters were evaluated before treatment and after every 30 days of treatment for three months.

Results: Individually, all animals had lower final than initial BW median (13.7 kg and 14.6 kg, respectively) and BMI median (22.9 kg/m² and 25.1 kg/m², respectively), but as a group there was no significant difference throughout the treatment (P=0.87, P=0.192). Obesity did not cause any changes in the complete blood count, and regular exercise during the three-month period did not lead to any significant changes. However, there was a positive correlation between the abdominal circumference and the haematocrit.

Conclusion: The regular practice of low intensity exercise, as a single treatment, did not promote significant weight loss, however, it preserved the integrity of the haematological variables in obese dogs. Study supported by FAPESC, Brazil.

POSTER 19
SERUM BIOCHEMISTRY OF OBESE DOGS SUBMITTED TO REGULAR EXERCISE
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Background: Obesity in dogs is an increasingly common disease in the clinical routine and can lead to metabolic consequences, which can reflect on serum biochemistry. One of the recommended treatments for obesity is the aerobic physical exercise. However, little is known about the influence of obesity and exercise on serum biochemistry in dogs.

Objective: The objective of this research was to evaluate, monthly, the weight loss and serum biochemistry of obese dogs submitted to an exercise routine on a treadmill.

Methods: Five obese dogs were selected, with no other concurrent diseases. Physical activity consisted of a single 35-minutes walk on a treadmill, three times a week on alternate days, for three consecutive months. Body weight (BW), body mass index (BMI) and biochemical parameters were evaluated before treatment and after every 30 days of treatment for three months.

Results: Individually, all animals had lower final BW and BMI, but as a group there was no significant difference throughout the treatment (P=0.87, P=0.192 respectively). No animals developed hyperlipidemia due to obesity. Activity of serum creatine kinase and concentration of serum protein, albumin and lactate were higher than the reference values at all times. After the beginning of treatment with regular exercise, there was a significant decrease in serum lactate concentration (P=0.008).

Conclusion: The practice of low intensity exercise, as a single treatment, did not promote significant weight loss, however, it preserved the integrity of the energetic and muscular metabolism of obese dogs. Study supported by FAPESC, Brazil.
**POSTER 20**

**IN VITRO PLATELET AGGREGATION TO DEMONSTRATE THE INFLUENCE OF ANTI-DRUG ANTIBODIES ON NON HUMAN-PRIMATE PLATELETS**

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**Background:** Thrombocytopenia mediated by drug antibodies in preclinical studies is an uncommon adverse effect of drug administration, and may be mediated by the presence of anti-drug antibodies (ADA). ADA are induced by the administration of therapeutic human antibodies to animals. We hypothesized that the combination of a drug monoclonal antibodies, known to bind on platelets, and ADA were acting as anti-platelet immune complexes, causing platelet activation and aggregation and consequently decreasing platelet counts.

**Methods:** Non human-primates receiving the humanized drug antibody were tested for the presence of ADA. Positive ADA plasma was pooled and kept frozen until testing. Blood was collected from a naïve colony of non human-primates and platelet rich plasma (PRP) was prepared. PRP was spiked with the drug antibody and a reference antibody with and without ADA plasma, but with no agonist. Platelet aggregation was recorded over 20 minutes on a platelet aggregometer.

**Results:** Spiking of the drug antibody alone or with the reference antibody did not induce platelet aggregation in PRP. Addition of ADA plasma in samples spiked with the drug antibody, but not with the reference antibody, induced dose-related moderate to marked platelet aggregation.

**Conclusion:** The effect of ADA on platelets can be demonstrated by platelet aggregation using PRP. The drug antibody known to bind to platelets did not induce platelet aggregation, but in presence of ADA there was formation of immunogenic complexes that caused platelet aggregation. Such findings could explain the observation of decreased platelet counts in non human-primates in preclinical toxicological studies.

**POSTER 21**

**ACUTE PHASE RESPONSE IN B. CANIS INFECTION: CORRELATION OF FIBRINOGEN VALUES WITH BLOOD CELL COUNTS**

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**Background:** It is known that acute phase response in B. canis infection is characterized by leukopenia, thrombocytopenia and anemia and occasionally by coagulopathy. Also, it is known that fibrinogen is an acute phase protein that could be increased due to inflammation or decreased due to consumptive coagulopathy.

**Objective:** We hypothesized that in non-complicated cases of B. canis induced APR, fibrinogen values will be increased and will negatively correlate with neutrophils, lymphocytes, thrombocytes and red blood cell indices.

**Methods:** Dogs (n=16) included in the study had clinical signs consistent with babesiosis and large babesia was identified in blood smears. Dogs positive for D. immitis and other vector borne pathogens (4DX, IDEXX Laboratories) were excluded from the study. Complete blood cell count was performed by an automatic analyzer (Abacus, Junior Vet) and fibrinogen was determined by the heat precipitation method. Spearman’s coefficient of rank correlation was applied.

**Results:** The results have shown that fibrinogen values were higher than the reference values (median 6 g/L range 4-10 g/L) but fibrinogen values did not correlate negatively nor positively with hematology parameters.

**Conclusion:** Thus, it could be concluded that in non-complicated cases of B. canis infection, fibrinogen values indicate and acute phase response without consumptive coagulopathy. Also, the severity of leukopenia, thrombocytopenia or anemia could not have been correlated with increased fibrinogen values.

**POSTER 22**

**CLINICAL AND CLINICO-PATHOLOGICAL ALTERATIONS MAY HELP IN THE DIAGNOSIS OF PRIMARY AND SECONDARY IMMUNE-MEDIATED THROMBOCYTOPENIA?**

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**Background:** Immune-mediated thrombocytopenia (IMT) is a disease with a complex etiology in which antibodies bind to the platelets’ surface causing premature destruction by mononuclear phagocyte system. IMT may be primary (ITP, idiopathic thrombocytopenia) or secondary (IMTs) due to bacterial, viral, protozoal or helminth infection, neoplasm or drug administration.

**Objectives:** To evaluate the main clinical and pathological findings in dogs affected by IMT and compare the data between the two groups of IMT (ITP vs IMTs).

**Materials and methods:** Fifty-five dogs affected by IMT referred to the Veterinary Teaching Hospital in the period between May 2010 and December 2017 were included (29 ITP and 26 IMTs). For each case, signalment, history, clinical and clinico-pathological findings were collected including complete blood count, serum biochemical profile, coagulation profile, cytology of bone marrow, serology. Data were assessed statistically by Chi square or Fisher test.

**Results:** The study confirmed that Cocker Spaniel breed is predisposed to development of IMT (p <0.0001) and that the presence of petechiae is observed in subjects with a platelet value below 20,000/µL (p = 0.0196). The results showed no statistically significant difference between alterations observed in the two groups except for the remarkable presence of normal leukogram in subjects.
affected by ITP compared to those affected by IMTs (P=0.031).

Conclusions: This study confirms that clinical or clinicopathological alterations cannot distinguish ITP from IMTs. The ITP diagnosis is based on ruling out secondary causes of IMTs.

POSTER 23
A CASE OF LEUKEMIA IN A BEARDED DRAGON (POGONA VITTICEPS)

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Background: A 3 years old, female Bearded Dragon (Pogona vitticeps), weighting 122 grams, was admitted to the clinic showing lethargy, anorexia and weight loss.

Objective: to describe a case of lymphocytic leukemia in a Pogona vitticeps.

Case description: Clinical evaluation, biochemical and hematological profile, were performed. Biochemical panel showed increased Uric Acid (8.4 mg/dL RR 1.8-7), Total Protein (6.9 g/dL RR 3.6-6.4) and globulin (3.6 g/dL RR 1.4-3.2).

Anemia (HCT: 10%, RR 17-28), Platelets adequate (with aggregates), and marked leucocytosis (WBC: > 100.000/µL RR 5.9-14.3) were present at CBC. The majority of leukocytes were lymphocytic/lymphoblastic cells (97%), heterophils (2%) and monocytes (1%).

Lymphocytic/lymphoblastic cells were of small and medium diameter, showing highly nuclear/cytoplasmic ratio and moderately blue cytoplasm and mild anisocytosis/anisokaryosis. Some cells had nuclear membrane irregularities including clives.

Immunocytochemical stain on blood smear marked for CD3 (neg) and CD79a (pos) suggested Immunophenotype B. The patient died after one month post diagnosis.

Histology showed lymphoid infiltration with high mitotic index in the heart, spleen, liver, kidneys and gut. In addition, in bone marrow, a massive infiltration of lymphoid cells and significant reduction of erythroid and megakaryocytic cell lines confirmed the diagnosis of leukemia. Immunohistochemistry performed on different organs confirmed the CD79a positivity of a large part of infiltrating lymphoid cells indicating a B cells immunophenotype of neoplastic population.

Conclusions: The presence of lymphocytosis and multiorgan massive infiltration supported the diagnosis of lymphocytic leukemia. This neoplasia has been characterized in various reptiles as Boa constrictor and Python molurus but still not in a Pogona vitticeps.

POSTER 24
USING CONVOLUTIONAL NEURAL NETWORKS FOR DETERMINING RETICULOCYTE PERCENTAGE IN CATS

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Background: Recent advances in artificial intelligence, specifically in computer vision and deep learning, have created opportunities for novel systems in many fields. However, despite its successful use in human medicine, there is still a lack of deep learning applications in veterinary imaging.

Objective: In order to achieve more accurate, faster and less expensive diagnoses in veterinary medicine, the aim of this study was to apply a convolutional neural network (CNN) to determine the feline reticulocyte (RTC) percentage in a dataset of images of cat blood smears.

Methods: 1046 microscopic images were collected from feline peripheral blood smears stained with brilliant cresyl blue dye. A CNN was trained to perform 2D object detection on camera images. After appropriate imagery pre-processing, an open source keras implementation of the Single-Shot MultiBox Detector model architecture was used and trained on 800 labeled images to distinguish aggregate RTC from punctuate RTC and mature erythrocytes. To measure the training success, 246 images were set as a validation dataset.

Results: Our model accurately classified 98.7% of aggregate RTC in microscopic images of cat blood smears. Comparing the model with human performance, our model calculated a RTC percentage of 6.9%, while human manual counting obtained RTC percentages of 6.1%.

Conclusion: A CNN was successfully implemented to determine the reticulocyte percentage from stained cat blood images. Deep learning could approach and even exceed human-level performance in laboratory imagery and has a potential to be implemented in veterinary clinical practice.
albumin was measured immunoturbidimetrically using a previously validated method and creatinine by a spectrophotometric method. The presence of SIRS was daily evaluated using a standard scoring system. Statistical analysis was conducted using R language.

Results: Twenty-six dogs with mean age of 3.9±1.9 months were enrolled; 12/26 (46%) developed SIRS during hospitalization, while 5/26 (19%) died. A statistically significant correlation was found between UALB and UACR (p=0.868, p<0.001). The dogs with SIRS had higher median UALB (0.5 [0-12.7]mg/dL) and UACR (4.2 [0-2.093]mg/g) compared to dogs without SIRS [UALB=0.1 [0-0.8]mg/dL, UACR=1.6 [0-5.6]mg/g], but the differences were statistically non-significant (p=0.05). SIRS duration was significantly correlated with UACR (p=0.427, p=0.030), but not with UALB (p=0.386, p=0.052). The non-survivors had higher median UALB [0.6 [0-1.12.7]mg/dL] and UACR [19.6 [0.7-2.093]mg/g] compared to survivors [UALB=0.2 [0-1.5]mg/dL, UACR=2.3 [0-16.9]mg/g], but the differences were statistically non-significant (p=0.05).

Conclusions: UACR may be a prognostic indicator of SIRS duration in puppies with CPVE. However, a large-scale study is warranted to confidently determine the usefulness of UALB and UACR for clinical risk assessment in puppies with CPVE.

POSTER 26
UNUSUAL TRANSFORMATION OF LEISHMANIA SPP. AMASTIGOTES TO PROMASTIGOTES IN BONE MARROW SAMPLE
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Background: Canine leishmaniosis is a protozoan disease caused by Leishmania infantum, transmitted by phlebotomine sandflies. The lifecycle includes two phases: amastigotes in the host and promastigotes in the sandfly. Both forms multiply by binary fission.

Objective: Confirm the infection by L. infantum and the presence of promastigotes in bone marrow (BM) samples.

Methods: Cytology and quantitative PCR (qPCR) were performed on slides and direct smears of BM aspirates (EDTA) from a 11 month-old Spanish Greyhound. A biopsy of popliteal lymph nodes (LN) was assessed. The dog showed fever, anemia and generalized lymphadenomegaly.

Results: Structures compatible with amastigotes of Leishmania spp., free and inside histiocytes were observed in the BM slides. Organisms compatible with amastigotes were visualized in the smears from BM aspirates, in addition to 20-24 μm structures consistent with promastigotes of Leishmania spp. New BM aspirates in EDTA were sent 3 days later and kept for 3 additional days at room temperature (± 24° C). Promastigotes-like structures were also observed. The histological study of LN showed a granulomatous lymphadenitis due to leishmaniosis. BM qPCR determined a high parasitic load (1.84x10^7 parasites/ml).

Conclusion: Amastigotes transformation into promastigotes is temperature dependent. It is possible that, at the time of sampling, the patient had an active infection, with amastigotes developing in the BM. The time the BM was kept until its assessment could have mimicked the decreased temperature presented in the sandfly midgut, in comparison to the higher host temperature, which could have induced the amastigotes transformation into promastigotes.

POSTER 27
CLINICAL AND PATHOLOGICAL FEATURES OF MYCOBACTERIOSIS IN FISH: A REVIEW
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Background: Fish mycobacteriosis is a chronic progressive disease caused by ubiquitous acid-fast bacilli. The most important pathogens involved in piscine mycobacteriosis are Mycobacterium fortuitum, M. marinum and M. chelonae. Clinical signs of mycobacteriosis include cachexia, skin ulceration and exophthalmia. Affected fish can also be darker in colour, and show swelling of the abdominal region. At necropsy granulomatous lesions could be found in any organ, but especially in liver, spleen and kidney.

Objective: To investigate clinical and pathological features of piscine mycobacteriosis.

Methods: Laboratory exams in fish mycobacteriosis include cytopathology, histopathology, culture and molecular techniques.

Results: An early diagnosis of mycobacteriosis can be reach by cytology and acid-fast stains of tissue smears from affected organs. Histopathological findings vary but acid-fast bacilli are regularly seen; inflammatory infiltration of epithelioid cells, lymphocytes and Langhan’s giant cells with caseation are often detected. Mycobacteria could be effectively cultured on selective liquid and solid media. Direct sequencing from fish tissues can be a useful alternative when culture fails.

Conclusions: A preliminary diagnosis of piscine mycobacteriosis can be made by cytology, however, confirmation by other methods is necessary.

POSTER 28
WHICH IS THE BEST IMMUNOCYTOCHEMISTRY METHOD IN THE DIAGNOSIS OF LYMPHOMA: CELL BLOCK OR CYTOLOGICAL SMEARS?
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Background: Lymphoma is a neoplasia often diagnosed by cytology. However, complementary techniques such as immunocytochemistry are required for characterization and confirmation of diagnosis.
Objective: The objective of this study was to compare and validate the immunocytochemistry technique in cytological smears and cell block with HistoGel™ in suspected cases of lymphoma.

Methods: Eight cases, six dogs and two cats, with clinical suspicion of lymphoma were analyzed, in which, after cytological evaluation, immunophenotyping was recommended. The samples were collected from cases followed at the Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon, and processed in the Laboratory of Pathological Anatomy. In all cases, immunocytochemistry was carried out in smears and in cell blocks with HistoGel™. Anti-CD3, CD20 and CD18 antibodies were used for immunophenotyping.

Results: The technique of immunocytochemistry in smear allowed the diagnosis of lymphoma in four cases, all positive for CD20 (Type B lymphoma). The remaining four were inconclusive. The immunocytochemistry in the cell block with HistoGel™ five cases of lymphoma were diagnosed, all positive for CD20 and 1 for CD3 (Lymphoma type B and T, respectively). The remaining three were inconclusive. Of the eight cases, both techniques were in agreement in five: three B lymphomas and two inconclusive.

Conclusions: The present study allowed the diagnosis and characterization of six lymphomas in eight suspected cases using both methods. There seems to be an advantage with the use of HistoGel™ over cytology, having this to be confirmed in the future with a larger number of samples.

POSTER 29
BREED VARIATION IN FREQUENCY OF CANINE ANTI-MOUSE ANTIBODIES
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Background: Canine anti-mouse antibodies are a source of immunoassay interference. Preliminary data suggests a canine breed variation in the frequency of detectable anti-mouse antibodies. Sex, age and health status are suggested risk factors for interference in people. Determining risk factors for canine immunoassay interference is important to prevent reporting of erroneous test results.

Objectives: The aim of the study was to investigate the impact of breed, health status, age and sex on the frequency of anti-mouse antibodies in dogs detected by a species-independent assay.

Methods: Excess patient sera and healthy donor sera were collected from 55 Bernese Mountain Dogs (38 of them without clinical diagnosis) and 55 Labrador retrievers (25 without diagnosis). All samples were screened using a previously published assay for anti-mouse IgG. Differences in breed, health status, and sex were tested with a two-sample test for equality of proportions. Differences in age were tested with the Wilcoxon rank-sum test.

Results: Anti-mouse antibodies were detected in serum from 19 of 55 Bernese Mountain Dogs (35%) and nine of 55 Labrador retrievers (16%). The frequency of antibodies differed between the breeds (P < 0.05), but health status, age or sex did not affect the probability of detecting anti-mouse antibodies.

Conclusions: The frequency of detectable anti-mouse antibodies varies between dog breeds. The Bernese Mountain Dog is a candidate breed for predisposition to immunoassay interference. Further studies are needed to determine possible risk factors for immunoassay interference in dogs.

POSTER 30
ORAL SCRAB FROM A FRUCIFER PARDALIS WITH ORAL PLAQUES
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Background: Mycoses are considered rare in reptiles and, in the majority of cases, are associated with poor husbandry leading to immunosuppression. Few reports of oral mycoses are available in chameleons.

Objective: To the best of the authors’ knowledge, this is the first report of cytological findings linked to concurrent multiple mycoses in Frucifer pardalis.

Case description: A 2.5 years old, male chameleon (Frucifer pardalis) was presented to the University of Milan Veterinary Hospital, for a week history of lethargy, anorexia, and dysphagia. On clinical examination, the animal was dehydrated, in poor body condition, and lethargic. On oral cavity inspection, the animal had multiple white to yellow plaques adherent to both hard palate and gums. Scrubs and swabs from the oral lesions were obtained and submitted for cytopathology and microbiology. Cytological findings included necrosis and presence of large aggregates of a mixed fungal population. Two types of hyphae (morphologically compatible with Microsporum spp. and Zygomycetes) were detected. Numerous bacteria, degenerated heterophils, few foamy reactive macrophages and epithelial cells were also detected. A final diagnosis of heterophilic and macrophagic faucitis with intralesional bacteria and fungal hyphae was made. Mycological examination was negative.

Conclusions: Microsporum spp. have been previously associated with dermatophytosis in reptiles, while Zygomycetes was reported as a common commensal of reptiles’ skin. The presence of multiple, variable pathogenic, fungal types supports the hypothesis that mycotic infections are generally secondary to immune suppression. Furthermore, the mycological examination was negative, highlighting the role of cytology in the diagnosis of reptiles’ infectious diseases.

POSTER 31
CANINE KELOIDAL FIBROSARCOMA: TWO CASE REPORTS AND REVIEW OF THE LITERATURE
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Background: Keloidal fibrosarcoma (KFS) is an uncommon type of fibrosarcoma, which is characterized by the deposition of aggregates of hyalinised ('keloid') collagen. Marked cellular atypia, higher mitotic rate, infiltrative growth and regions of high cellularity, distinguish KFS from the benign counterpart (keloidal fibroma).

Objective: Review the cytological, histological and immunohistochemical features of KFS, and report two new cases of canine KFS.

Case descriptions: Two otherwise healthy dogs, were presented to clinicians for the onset a single, subcutaneous, firm and hairy nodule. The clinical investigation included fine needle aspirate of the nodule, followed by excisional biopsy. Cytological and histological samples were routinely stained. Immunohistochemistry for vimentin, a-SMA, and CD18 was also performed. Smears were moderately cellular, with the prevalence of atypical large spindle cells, individualized or in small groups. Scattered mast cells and histiocytes, admixed with hyalinized collagen fragments were also present. Cytological findings were consistent with a KFS, nevertheless, a keloidal fibroma and mast cell tumor could not be ruled-out based on the cytomorphology only. Histologically, the neoplasm was infiltrative, with areas of hypercellularity. Furthermore, neoplastic cells were vimentin-positive and a-SMA negative, while admixed histiocytes expressed CD18. Based on histology and immunohistochemistry, a final diagnosis of KSF was granted.

Conclusions: Cytological detection of large bundles of hyalinized collagen admixed with large spindle cells are a distinctive finding of keloidal tumors of dogs. Neoplastic cells in canine keloidal tumors are vimentin positive and smooth muscle actin negative and are interpreted to be fibroblasts.

POSTER 32
FACTOR VII-ACTIVATING PROTEASE (FSAP) ACTIVATION IN DOGS ENVENOMATED BY THE EUROPEAN ADDER (VIPERA BERUS)
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Factor VII-activating protease (FSAP) is a serine protease with roles in haemostasis and cell death in humans. Thezymogen form of FSAP is activated by histones. Thus, FSAP acts as a sensor for tissue injury.

Objective: To investigate the activation of FSAP in dogs envenomed by the European Adder (Vipera berus) and describe variations in concentrations post envenomation in dogs with and without antivenom-treatment.

Methods: Citrated plasma was collected from 26 dogs bitten by Vipera berus and 24 control dogs. Samples from envenomated dogs were collected at presentation, 12 hours (h), 24 h, 36 h and 14 days post bite. FSAP antigen, activity and inhibitor-complex concentrations were measured by ELISA. Plasma DNA and nucleosome concentrations were measured using SYTOX green fluorescence and ELISA, respectively.

Results: FSAP activity and plasma DNA concentrations were significantly higher at all timepoints in envenomated dogs compared to the control group. FSAP inhibitor-complex concentrations, which are an indirect marker of FSAP activation, were significantly higher 12 h post envenomation compared to the control group and in non antivenom-treated dogs compared to antivenom treated dogs.

Conclusions: FSAP is activated in dogs envenomed by Vipera berus. FSAP inhibitor-complex concentrations may indicate peak cellular injury of up to 12 h post bite. Cellular injury may be less extensive in antivenom-treated dogs. FSAP activation may serve as a marker of acute systemic injury following envenomation in dogs.

POSTER 33
PROTEOMICS OF HOST RESPONSE TO ZOONOTIC INFECTION IN COWS THAT ABORTED
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Background: Bacterial zoonoses in cows, such as leptospirosis and Q fever, usually appear in a subclinical form, with abortion as the only clinical manifestation following infection in pregnant cows. The pathogenesis of both diseases is very complex and still not completely understood.

Objective: The aim of this study was to evaluate serum proteome in cows that aborted due to leptospirosis and Q-fever in order to investigate differentially expressed proteins involved in pathophysiological mechanisms of zoonotic infection of cows.

Methods: Three groups of cows were included in the study: group I (control, healthy cows, n=5); group II (cows infected with Coxiella, n=5) and group III (cows infected with Leptospira, n=5) both comprised of cows that have aborted. In collected sera, 2DE and mass spectrometry (MALDI-TOF/TOF, Bruker-Daltonics) were performed. Obtained results were analysed by Mascot algorithm (www.matrixscience.com) against SwissProt_201407 database.

Results: Three proteins (actin, fructose-1,6-bisphosphatase 1 and transgelin-2) were up-regulated while E3-ubiquitin-protein ligase KCMF1 and autophagy-related protein 13 were down-regulated in both leptospirosis and Q fever compared to the control. Gap junction alpha-5 protein was down-regulated only in Q fever but not in leptospirosis. Differentially expressed proteins are involved in processes of cell motility, cell adhesion and signal transduction what enable bacterial binding to host cells activating the host innate defense system.

Conclusion: The zoonotic pathogens trigger changes in the expression of specific host proteins needed for host-pathogen interaction and bacterial dissemination. The results could contribute to better understanding of mechanisms of host-pathogen interaction and identification of potential biomarker for bacterial infection.

POSTER 34
URINE PHORETIC PATTERNS IN HEALTHY DOGS BY CAPIILLARY ELECTROPHORESIS
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Background: Urine capillary electrophoresis (UCE) frequently has been used in human medicine to identify paraproteinemias. To the author’s knowledge, there is no similar study in veterinary medicine.

Objective: The aim of the study is to identify phoretic patterns in healthy dogs by UCE method.

Methods: 90 healthy dogs were selected and staged in three different groups by age in puppies, medium and mature. All dogs were tested for a complete blood panel and urine analysis. Leishmania infantum, Ehrlichia canis and Rickettsia conorii antibodies were quantified. Animals with abnormal results or positive serologies were discarded. The urine from healthy animals was washed and dialyzed to be suitable for UCE in a MiniCap Sebia® equipment.

Results: 90 urine phoretograms were obtained. Five different protein fractions (f1 to f5) were identified in all of them, and each urine sample was compared with its capillary electrophoresis serum phoretogram to establish the limits of each fraction. Two different patterns were identified based on the lowest molecular weight fraction (f1) percentage. Pattern one showed almost undetectable f1 (15/90) whereas pattern two showed values higher than 20% for f1 (75/90). F4 was found to be the highest fraction in 72/90 samples.

Conclusions: This is the first one to identify in healthy animals two different types of UCE patterns, providing standard phoretograms to be compared with pathological samples. This study also lays the foundations to establish the reference intervals for each fraction.

POSTER 35
AUTOMATED MEASUREMENT OF RETICULATED PLATELETS IN DOGS USING THE IDEXX PROCYTE DX COMPARED TO FLOW CYTOMETRY; REFERENCE VALUES FOR PROCYTE DX
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Background: High percentages of reticulated platelets (r-PLT) are associated with an increased megakaryopoiesis in bone marrow. The measurement with a fully automated haematology analyser such as the IDEXX ProCyte Dx provides a fast and non-invasive method.

Objectives: Method comparison of the ProCyte Dx with standard flow cytometry for the analysis of r-PLT percentages in dogs and establishment of a reference interval (RI) for the ProCyte Dx.

Methods: EDTA-blood-samples from 42 dogs, obtained from healthy and thrombocytopenic dogs, were analysed to determine percentage of r-PLT with the PLT-optical channel of the ProCyte Dx. The same sample was then analysed with the flow cytometer Accuri C6 (BD Biosciences) using APC (Allophycocyanin)-labeled CD61-antibody and thiazole orange. Intra assay precision was also calculated for both methods (10 replicates). For establishment of RI, EDTA-blood-samples from 120 healthy dogs were analysed.

Results: There was a fair correlation (r=0.70) for r-PLT percentages between the two methods, with a mean negative proportional bias of – 8.2 for the ProCyte Dx (Bland-Altman analysis). Intra-assay CV for r-PLT percentages was 12.9% for flow cytometry and 32.9% for the ProCyte Dx, respectively, in a sample with low percentages of r-PLT. The RI for r-PLT determined with the ProCyte Dx ranged from 0 to 7.47 %.
Conclusion: The ProCyte Dx is capable to reliably detect r-PLT during each routine haematologic analysis. It is a rapid, cost-effective and standardized method for assessment of r-PLT in veterinary practices, however, a certain imprecision has to be taken into account for low percentages of r-PLT.

POSTER 36
IMMUNOHISTOCHEMICAL CHARACTERIZATION OF A MALIGNANT PERIPHERAL NERVE SHEATH TUMOR IN A DOG
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Introduction: Malignant peripheral nerve sheath tumors (MPNSTs) are spindle cell sarcomas with poorly understood histopathologic features and clinical behavior. This report describes the aggressive and unusual biological behavior of an intermediate grade MPNST in a dog.

Case description: An 8-year-old dog was presented for a recurring spindle cell sarcoma on the left thigh one month after excision. No evidence of metastasis was found on total body CT-scan. Complete excision by coxofemoral disarticulation was performed. Unexpectedly, a mass was noticed in the left hemipelvis 3 months post-amputation. CT-scan showed enlarged inguinal and iliac lymphadenopathy and a parasternal nodule compatible with metastasis. Left hemipelvectomy with lymph nodes and node excision was performed. Histopathologic diagnosis was intermediate grade spindle cell sarcoma with clean margins. Adjuvant doxorubicin and metronomic chemotherapy were started. Fifteen days postoperatively, CT-scan revealed subcutaneous, muscular, paravertebral, intra-abdominal and splenic nodules cytologically compatible with soft tissue sarcoma (STS). Microscopically, the primary mass was a spindle cell sarcoma of low to moderate cellular density with crisscrossing beams of variable size organization. The mitotic activity was 4 mitoses per 10 hpf but Ki67 index was high. Immunohistochemically, the tumor was positive for vimentin, S-100, NSE and sporadically for actine. CD31, VIII factor, CD117, desmin, PNL2, EMA and pancytokeratin were negative. These findings led to a diagnosis of intermediate grade MPNST.

Conclusions: This report of an intermediate grade MPNST exhibiting early metastases after radical surgeries and adjuvant chemotherapy emphasizes the need for preoperative immunohistochemistry on STS in dogs in order to recommend appropriate management and overall prognosis.

POSTER 37
EFFECTS OF PROPOFOL AND CARPROFEN ON PLATELET AGGREGATION AND KAOLIN-ACTIVATED THROMBOELASTOGRAPHY IN DOGS UNDERGOING ARTHROSCOPIC SURGERY
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Background: Propofol may prolong platelet aggregation in humans.

Objectives: To evaluate the impact of propofol infusion combined with carprofen on hemostasis and hematological indices in dogs undergoing arthroscopy.

Methods: 24 dogs were scheduled for computertomography and arthroscopy between July 2011 and May 2014. Anesthesia was induced with 0.4 mg/kg propofol and maintained with propofol infusion. Group 1 received 4.0 mg/kg carprofen at the beginning of anesthesia and Group 2 after all samples were taken. Subgroup “a” had history of oral treatment with NSAIDs, subgroup “b” did not. Dogs were distributed into groups and subgroups 1a (n=6), 1b (n=7), 2a (n=6), 2b (n=5). Blood samples were taken before anesthesia (T1), 30 minutes after starting infusion (T2), and 30 minutes after discontinuation of propofol (T3). Hematology, prothrombin time (PT), activated partial thromboplastin time (aPTT), kaolin-activated thromboelastography (TEG) and platelet aggregation in response to collagen, adenosine diphosphate (ADP) and arachidonic acid (AA) was assessed.

Results: Erythrocyte, leucocyte (p<0.0001) and platelet count (p=0.0015) significantly decreased, while PT was prolonged (p=0.008) in time. Neutrophils (p=0.04) and monocytes (p=0.02) were higher, lymphocytes (p=0.01) were lower in NSAIDs pretreated dogs. Propofol transiently inhibited platelet aggregation induced by all agonists at T2, especially in response to AA. Carprofen did not affect aggregation. Significant differences between Group 1 and 2 were detected by TEG for R (p=0.0003), K value and angle (p<0.0001).

Conclusions: Despite propofol reversibly inhibited platelet aggregation, it was not associated with perioperative bleeding. Preoperative carprofen administration did not amplified inhibition of aggregation.

POSTER 38
EVALUATION OF HEMATOLOGICAL CHANGES IN LAYER CHICKENS AFTER VACCINATION AND CHALLENGE WITH E. COLI
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Background: Avian colibacillosis is the most common bacterial disease in poultry with significant economic impact. Vaccination has been used to protect against the disease. Alterations in peripheral blood variables have been shown to be relevant to infectious status and immune response.

Objective: The aims of this study were to analyze changes in hematological variables in layers after challenge with a pathogenic strain of *Escherichia coli* and to identify differences in these variables among birds vaccinated with different vaccination schemes.

Methods: Day old layer-type chicks were randomly allocated in 4 groups; group A received no vaccination against *E.coli*, group B was vaccinated with a live commercial vaccine, group C received an autogenous vaccine twice and group D received both a commercial live vaccine and an autogenous one. Chicks were experimentally challenged with a pathogenic *E.coli* strain.

Blood samples were collected 7 days before and post challenge by jugular venipuncture. Packed cell volume (PCV) was determined by microhematocrit centrifugation while total white blood cell count (TWBC), differential leukocyte count, heterophil/lymphocyte ratio and cell morphology were evaluated in blood smears.

Results: Minimal changes occurred in PCV in all groups after challenge. A mild TWBC increase was observed in groups C and D and was more prominent in group A and B. Likewise, there was an increase in the number of heterophils, lymphocytes, monocytes and heterophil/lymphocyte ratio, which was mild in birds of group C and D and marked in groups A and B. Furthermore, toxic heterophils were more frequently observed after challenge in groups A and B.

Conclusions: *E.coli* infection is associated with several hematological changes, significantly different among vaccinated and unvaccinated birds, and indicative of concurrent inflammation, antigenic stimulation, and stress.

POSTER 39
URINE PROTEIN TO CREATININE RATIO IN CATTLE WITH SUBCLINICAL KIDNEY DISEASE
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Background: Renal diseases are uncommon in cattle practice despite the frequent detection of kidney lesions in clinically healthy animals at slaughter. Urinalysis is not routinely used in bovine medicine and there is limited evidence whether urine protein to creatinine ratio (UPC) could be used for the diagnosis of cattle renal diseases.

Objective: To determine the alterations of UPC observed at different subclinical renal diseases in clinically healthy cattle.

Methods: Kidney tissue and urine samples from 57 clinically healthy adult dairy (44) and beef (13) cattle were collected after slaughter. Four urine samples were excluded from analysis due to turbidity (1) and active sediment (3). Urinary protein and creatinine concentrations were measured in an automatic analyzer. Kidney tissue samples were examined histopathologically. Animals were classified as “Nephropathic” if lesions were detected or as “Normal”. Data was analyzed with Univariate-ANOVA (IBM-SPSS 25).

Results: Sixteen animals were “Normal” and 37 “Nephropathic”. Eleven had interstitial nephritis (IN), 7 glomerulonephritis (GN) and 19 both of these lesions (IGN). All histopathological changes were scored as mild. UPC values were significantly affected by the type of lesion detected (P<0.05) and not from breed, age or their interactions (P>0.05). Average UPC was significantly higher (P<0.05) in IN cattle compared to “Normal”; no other significant difference was detected (means±SE: 0.20±0.114, 0.88±0.116, 0.35±0.184 and 0.35±0.105 for Normal, IN, GN and IGN, respectively).

Conclusions: UPC values increase in clinically healthy cattle with renal lesions and the increase is significantly higher in animals with interstitial nephritis compared to the normal ones.