ECEIM

EUROPEAN COLLEGE OF EQUINE INTERNAL MEDICINE

ECEIM CONGRESS LYON·FRANCE

27-28 OCTOBER 2023

ONLINE PROCEEDINGS BOOK

16th ECEIM CONGRESS 27-28 october 2023

ECEIM Lyon

Contact:
Chair of the ECEIM congress
Dr. Isabelle Desjardins
DVM, DES, Dip.ECEIM, ACVIM-LA, ECVECC-EQ

VETAGRO SUP
Email: isabelle.desjardins@vetagro-sup.fr

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WELCOME WORD

Dear Friends and Colleagues,

On behalf of the organising committee, I'd like to welcome you to the second ECEIM congress in France. The first French town to host the conference was Le Touquet, by the sea, in 2013... This time, you'll be immersed in the land of the Gauls and the Romans! The city is also called the "city of lights". Ever since Lyon was founded, at the beginning of December, the city of Lyon is engulfed in an explosion of colour and light, a very popular tradition.
Lyon is also emblematic for veterinary sciences. Claude Bourgelat, equerry of the king Louis XV, founded the first veterinary school in the world here in 1761.
Like the gastronomic delights on offer in Lyon, the scientific program will be mouth-watering, with carefully selected and renowned speakers. The multi-disciplinary and trans-disciplinary approach is in the spotlight with contributions from physicians, ethologists, specialists in pedagogy, anesthesia and pain management, etc.
Practical pre-congress workshops will be held on the two days preceding the congress (October 25-26) at the Cité Internationale and the Lyon racetrack. Many of you have asked for lectures on intensive care, which will be the central theme of Specialist Day. Topics from other workshops include immunological testing and diagnostics, clinical impact of immune digestive infiltration, practical dermatology, infectious diagnostics and outbreaks management, and medical training.
The main congress will begin with an exciting presentation on antique equine medicine and how concern for the health of farm animals led to the creation of our profession, based on a scientific approach.
The 2-day scientific program of the congress itself will include 2 streams running simultaneously, with a focus on critical care medicine and neurology the first day, and on the second day, immune disorders, as well as recent advances in emerging infectious diseases and their diagnostic approaches, and not to forget, cardiology. A number of "new and hots topics" will also be presented over the two days.
As traditionally, research presentations will be given in the form of lectures and posters. Many equine internal medicine residents will be presenting, and competing for the Luis Monréal Award. We wish them the best of luck !
A special session to prepare the future ECEIM consensus statement on equine kidney disease and diagnosis will also take place. And as a tradition, a training session for the residents will be also provided.
This year, the Annual General Meeting will not take place during the congress but will be held online in November 2023. ECEIM diplomats will nevertheless have the opportunity to exchange views with Board members at the end of the first day of the conference.
On the 25th, the social program will include a welcome cocktail at the sumptuous Palais de la Bourse, in the city's magnificent historic center and close to typical restaurants to extend the evening.
On the second evening, the social dinner will consist of a gala dinner at the Cité Internationale, in a festive and atypical atmosphere, accompanied by an equestrian cabaret and an amazing music group. After the hilarious costumes at last year's gala in Rome, this year's motto is "So Chic!"
For outings and entertainment, the Tete d'Or Park around the Convention Center is a haven of greenery and tranquility, so take the time to stroll around, recharge your batteries and visit the greenhouses and zoo. The Museum of Contemporary Art also welcomes you inside the Cité Internationale. For more details on the social program, please visit the ECEIM website dedicated specifically to its congresses:
https://www.eceim-congress.com/venue

And finally, on behalf of ECEIM, I'd like to extend my sincere thanks to all our sponsors who generously supported the congress. We keep in mind that without them, the congress wouldn't be taking place. The exhibition area will be the heart of the coffee and lunch breaks, allowing you to contact them and find out about their cutting-edge equipment and new diagnostic and therapeutic developments.

Enjoy the French touch, and "au plaisir de vous accueillir à Lyon ! »

Chair of the 2023 ECEIM Congress Organising Committee

ECEIM 2023 CONGRESS ORGANIZING COMMITTEE

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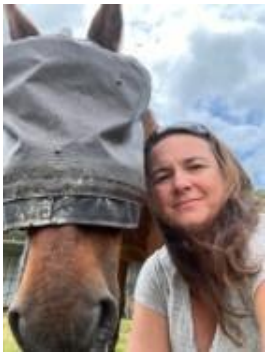
(Chair of Organising Committee)

Marco Duz
(Past chair OC)

Agnès Leblond

Aurélia Leroux

Gwenola Touzot-Jourde



John Keen

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Marco Duz

Anna Kendall

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John Keen (B)

Isabelle Desjardins
(Past Chair)

PROGRAMME FRIDAY 27TH OCTOBER 2023

ALL TIMES ARE BASED ON THE CENTRAL EUROPEAN TIME (GMT+2)

Friday, October 27th					
PASTEUR AUDITORIUM (main)	**TIME**	**LECTURE**	**ROOM RHONE 3 (Second)**	**TIME**	**LECTURE**
	8:10-8:15	Congress opening and welcome			/
	8:15-9:00	OPENING LECTURE: From hippiatry to modern veterinary medicine *C. Degueurce*			
	9:00-10:00	CCARE: An update on Sepsis, septic shock, and biomarkers *D. Wong*		**9:00-10:00**	ECEIM RESIDENT RESEARCH ABSTRACTS (1-4)
	10:00-11:00	CCARE: Transfusion medicine review *B. Dunkel*		**10:00-11:00**	ECEIM RESIDENT RESEARCH ABSTRACTS (5-8)
COFFEE BREAK					
PASTEUR AUDITORIUM	**11:30-12:15**	CCARE: A review of compartment syndromes. *B. Dunkel*	**ROOM RHONE 3**	**11:30-12:15**	ACVIM RESIDENT RESEARCH ABSTRACTS (9-11)
	12:15-13:00	CCARE: Systemic repercussions and MODS / MOF following severe trauma *G. van Galen*		**12:15-13:00**	RESEARCH ABSTRACTS (12-14)
LUNCH					
PASTEUR AUDITORIUM	**14:00-15:00**	RESEARCH ABSTRACTS (15-18)	**ROOM RHONE 3**	**14:00-15:00**	ECEIM Resident's presentation: Acid Base secrets *B. Dunkel*
	15:00-15:45	NEUROLOGY: Integrative therapy for the neurologic patient *G. Touzot-Jourde*		**15:00-15:45**	RESEARCH ABSTRACTS (19-20)
COFFEE BREAK					
PASTEUR AUDITORIUM	**16:00-16:45**	NEUROLOGY : An update on headshaking management *V. Roberts*	**ROOM RHONE 3**	**16:00-16:45**	RESEARCH ABSTRACTS (21-23)
	16:45-17:30	ECEIM Consensus Statement on Equine Kidney Disease *G. van Galen*		**16:45-17:30**	HOT TOPIC: How to manage an obese equid. *R. Morgan*
	17:30-18:30	Q&A session for the Board			/
19.30 – 01.00 GALA DINNER (on site) APERITIF : 19:30-20h EQ. SHOW : 20h-20h30 MEAL : 20h30-22h30 LIVE CONCERT 22h30-24h DJ 24h- 01h					

PROGRAMME SATURDAY 28TH OCTOBER 2023

Saturday, October 28					
PASTEUR AUDITORIUM (main)	**TIME**	**LECTURE**	**ROOM RHONE 3 (Second)**	**TIME**	**LECTURE**
	09.00-09.45	HOT TOPIC: Highlights from ACVIM Forum 2023 *C. Sanchez*		**09.00-09.45**	HOT TOPIC: How to review a paper *C. Marr – G. Hallowell*
	09.45-10.15	IMMUNOLOGY: Immune dysregulation of the human skin. *A. Nosbaum*		**09.45-10.15**	CARDIOLOGY: New insights into Atrial premature depolarizations, atrial tachycardia, atrial fibrillation *G. van Loon*
	10.15-11.00	IMMUNOLOGY: equine immune-mediated skin diseases *D. Pin*		**10.15-11.00**	CARDIOLOGY: Review on equine myocarditis. *G. van Loon*
COFFEE BREAK					
PASTEUR AUDITORIUM	**11.30-12.15**	IMMUNOLOGY: The mechanisms of equine "allergic lung diseases" – focus on equine asthma *S. Sage*	**ROOM RHONE 3**	**11.30-12.15**	INFECTIOLOGY: PCR testing: new tools and rapid testing *A. Waller*
	12.15-13.15	IMMUNOLOGY: Inflammatory bowel disease (IBD) in horses: from the immunological point of view *D. Jean*		**12.15-13.00**	INFECTIOLOGY: Understanding Next Generation Sequencing technology and metagenomics in equine infectiology *A. Waller*
LUNCH					
PASTEUR AUDITORIUM	**14.15-15.00**	IMMUNOLOGY: A review of immune maturation from the newborn foal to the aged horse *J. Felippe*	**ROOM RHONE 3**	**14.15-15.00**	INFECTIOLOGY: Overview of arthropod-borne infectious diseases in horses. *E.J. Cunilleras*
	15.00-15.25	INFECTIOLOGY: Equine piroplamosis: epidemiology of the disease in Europe *L. Malandrin*		**15.00-15.45**	HOT TOPIC: An update on simulation-based learning in equine veterinary medicine *S. Bailie*
	15.25-15.35	INFECTIOLOGY: Presentation of the R.E.S.P.E. *A Couroucé*		**15.45-16.30**	HOT TOPIC: Stem cell and blood product use in equine internal medicine: where are we? *L. Berg*
	15.35-16.00	INFECTIOLOGY: Clinical variability and diagnostic controversies in equine piroplasmosis *A. Leblond*			
COFFEE BREAK					

PASTEUR AUDITORIUM	17.00-17.45	INFECTIOLOGY: Round Table around Equine Piroplasmosis *J. Cavalleri* *A. Couroucé* *E.J. Cunilleras* *A. Leblond* *L. Malandrin*	**ROOM RHONE 3**	**17.00-17.45**	HOT TOPIC: Patient safety and quality of care. *C. Sanchez*
	17.45-18.00	Closure of the Congress			
CLOSURE OF THE CONGRESS					

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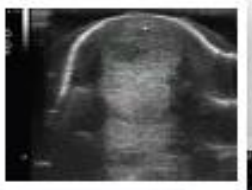

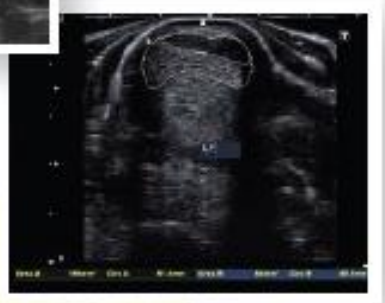

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1. ROBINSON ET AL., VACCINE, 2020
2. FROSTH ET AL., EQUINE VET J, 2021

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SPEAKERS

Sarah Baillie

Sarah worked in clinical practice for many years before undertaking her PhD in computer science. She has developed virtual reality simulators for bovine reproduction and equine colic to train veterinary students. She led a major curriculum review at Bristol Veterinary School, UK and introduced a more outcome-based and integrated curriculum. She was responsible for opening the clinical skills laboratory at Bristol and has created many models (low- and high-fidelity). She is now an Emeritus Professor with the University of Bristol and is fortunate to be able to dedicate more time to educational research projects and collaborations around the world.

Lise Berg

Lise C. Berg, DVM PhD, graduated from University of Copenhagen in 2001. She completed her PhD in 2008 including studies on equine mesenchymal stem cells (MSC), and she has continued her work in this field exploring the mode-of-action and best clinical application of MSC in equine practice. Her main research areas include regenerative medicine, sport horse management, and muscle function. She is currently Associate Professor of Clinical Biomedical Science at the Department of Veterinary Clinical Sciences at University of Copenhagen. She is also certified in Animal Chiropractic and Equine Rehabilitation, founding member of the Sporthorse Welfare Foundation, and involved in equestrian sports at national and international level.

Jessika Cavalleri

Prof. Jessika-M. Cavalleri graduated from Free University Berlin, Germany, in 2000 and has worked at different academic institutions since. In 2006 she finished her doctorate thesis on "Evaluation of muscular relaxation using an electronic pressure measurement under the saddle". In 2010 she became ECEIM Diplomate. In 2016 she finished her habilitation on "Investigation of immunotherapies of the equine malignant melanoma with special reference to the immunological response to therapy and the antitumoral effects of the cytokine gene therapy with equine interleukin 12 and equine interleukin 18 as well as the therapeutic DNA vaccines with human tyrosinase and human glycoprotein 100". Jessika-M. Cavalleri is head of the Clinical unit of Equine Internal Medicine at the University of Veterinary Medicine Vienna. She is Vice-President of the ECEIM. Her major interests are in the field of Equine Internal Medicine, especially infectious diseases and dermato-oncology. Her research focuses 1) on hepatitis associated virus infections like the equine parvovirus hepatitis and the equine hepacivirus, and 2) on treatment of equine melanoma.

Anne Couroucé

Prof. Anne Couroucé graduated from Ecole Vétérinaire de Nantes in 1992 and has worked for a research unit in equine sport medicine "Pégase-Mayenne" up to 2001. She obtained her phD in this field in 1997. In 2001 she entered Nantes Vet School as a lecturer in equine internal medicine. In 2004 she became an ECEIM Diplomate and she is now, since 2016, a professor in equine internal medicine. Her research focusses on equine sport medicine with a special interest in respiratory diseases. Anne Couroucé is the head of the Clinical unit of Equine Internal Medicine at Oniris. She is also past president of the ECEIM, president of the RESPE scientific committee and president of the veterinary committee of the French Equine Federation, and a member of the FEI veterinary committee since 2021.

Christophe Degueurce

Christophe Degueurce is a veterinary surgeon who graduated from the Ecole Nationale Vétérinaire d'Alfort in France in 1990. He has had a career as a professor of anatomy and was appointed curator of the Fragonard Museum in 1993. He has published several books on the museum's collections and numerous articles on the history of veterinary medicine. Since 2017, he has been Dean of the École Vétérinaire d'Alfort (Paris).

Bettina Dunkel

After graduating from Berlin University, Bettina completed a fellowship at Tufts University, an internship at the Marion duPont Equine Medical Center, Leesburg and a large animal internal medicine and emergency and critical care residency at the University of Pennsylvania. Since 2005, she has been a member of the American College of Veterinary Internal Medicine and the American College of Veterinary Emergency and Critical Care. After attained her PhD from the University of London in 2008, Bettina has been working at the Royal Veterinary College where she is now the Head of RVC Equine and Professor in Equine Medicine and Emergency and Critical Care.

Julia Fellipe

Julia Felippe received her veterinary degree in 1989 from UNESP-Campus Botucatu, Brazil, and pursued equine practice in her native country before an internship and residency in equine internal medicine at Kansas State University, United States (1994-1998). She is board certified by the American College of Veterinary Internal Medicine. She has a Master of Science degree from Kansas State University (1997), and a Doctor of Philosophy degree in immunology from Cornell University (2002). She has been a faculty at Cornell University College of Veterinary Medicine since 2002, and runs a research program studying equine developmental immunology and immunodeficiencies as the head of the Equine Immunology Laboratory. Julia also serves as a Cornell Provost's Fellow for Public Engagement.

Gayle Hallowell

Gayle graduated from the University of Cambridge in 2002 and then completed a large animal rotating internship and joint large animal internal medicine and emergency and critical care residency at the Royal Veterinary College, London. She then completed a PhD in equine cardiology. She then stayed on staff at Nottingham for 15 years and held the position of Professor in Veterinary Internal Medicine and Critical Care. In February 2022, she joined IVC Evidensia as Group Director for Veterinary Professional Development. Since 2019, she has been Editor-In-Chief for Veterinary Medicine and Science, a Wiley Journal. Her main clinical interests are cardiology, gastroenterology and imaging (particularly ultrasonography).

Daniel Jean

Daniel Jean has completed his studies in veterinary medicine at the University of Montréal in 1988. After a one-year internship at the University of Montréal Equine Hospital (1988-1989), he completed a residency program in equine internal medicine (1991-1993). He has been certified by the American College of Veterinary Internal Medicine in large-animal internal medicine since 1997. Daniel holds a master's degree from the University of Montréal (1996) and a doctorate from the University of Montréal, Faculty of Medicine. Daniel holds a Master's degree from the Université de Montréal (1996) and a Ph.D. from the Faculté de médecine de Paris (Hôpital Henri Mondor) (France) in 2001. He was Associate Professor at the equine hospital of the École Vétérinaire d'Alfort (France) (1995-2001). He has been Professor of Equine Internal Medicine at the Université de Montréal since 2001. His main clinical and research interests are the clinical use of equine digestive biopsies and renal diseases.

Eduard Jose Cunilleras

Dr. Eduard Jose-Cunilleras LV PhD Dipl. ECEIM, is a Professor of Equine internal medicine at the Universitat Autonoma de Barcelona since 2008.
Eduard was trained at The Ohio State University and has completed a residency in equine internal medicine and a PhD in equine exercise physiology.
He has been a practitioner in the UK as an equine internal medicine specialist (AHT and private practice). Eduard has over 70 peer-reviewed scientific papers and multiple national and international invited conference presentations. His main interests are exercise-related disorders, coagulopathies and infectious diseases.

Agnès Leblond

Agnès is a veterinary surgeon who graduated in Lyon in 1987. She has pursued an academic career and has been a teacher-researcher since 1994, and a full professor at VetAgro sup, Ecole Vétérinaire de Lyon since 2018. Agnès is a European specialist in equine internal medicine.
Since 2000, she has been conducting research into the epidemiology of infectious diseases, mainly vector-borne diseases (including piroplasmosis) and zoonoses, with a focus on setting up surveillance systems for early warning.
Agnès has also been involved in the One Health concept for 20 years, working with ecologists, virologists, entomologists, geographers, statisticians and modellers. After working on the conditions of emergence of the West Nile virus in the northern Mediterranean, then in Morocco and Iran, her focus shifted to the development of decision-making tools for health authorities in the context of global change, and the disciplinary field of her collaborations now includes political scientists, economists, sociologists, urban planners and anthropologists.

Laurence Malandrin

Laurence holds a PhD in microbiology and spent two post-doctoral periods in Sweden and England researching bacteria, before turning her attention to tick-borne pathogens at the veterinary school in Nantes, France. She became fascinated by piroplasmas, their genetic diversity and epidemiology, their mode of transmission by tick vectors and their interactions with red blood cells.

Celia Marr

Celia M Marr graduated from the University of Glasgow in 1985 and she has held positions in the University of Pennsylvania, University of Cambridge and the Royal Veterinary College and worked in racehorse practice in Lambourn. Currently, she is based at Rossdales Equine Hospital and Diagnostic Centre in Newmarket. Celia has published widely on equine cardiac and medical disorders. She is a Diplomate of the European College of Equine Internal Medicine, Fellow of the Royal College of Veterinary Surgeons, Honorary Member of British Equine Veterinary Association, Honorary Professor of the University of Glasgow and Editor-in-Chief of Equine Veterinary Journal.

Ruth Morgan

Ruth Morgan is a veterinary endocrinologist and a lecturer at the SRUC/University of Edinburgh based at the Roslin Institute in Edinburgh. She qualified from Cambridge University and after several years in private equine practice completed her residency in Equine Internal Medicine at Liverpool University. She became a diplomate of the European College of Equine Internal Medicine in 2013. She received her PhD in "Cortisol dysregulation in equine endocrinopathic laminitis" from the University of Edinburgh in 2016. She then undertook her first Wellcome Trust Clinical Career Development Fellowship at the University of Edinburgh and has just been awarded a second fellowship from the Wellcome Trust. Her research focuses comparative glucocorticoid biology and in particular the role of glucocorticoid metabolism in health and disease. Working with experimental models, veterinary clinical cases and human data she investigates the relationships between glucocorticoid metabolism and the morbidities associated with obesity.

Audrey Nosbaum

Audrey Nosbaum, M.D. Ph.D., is an immuno-dermatologist and Professor in Clinical Immunology. Dr Nosbaum sees patients, teaches at the University of Lyon, conducts research and is the Deputy Head of the Department of Allergy and Clinical Immunology, Lyon Sud University Hospital, France. In her role as a researcher, Dr. Nosbaum examines the immunology of skin hypersensitivity reaction and eczema, including atopic dermatitis, focusing on the identification of diagnosis biomarkers and new therapeutic strategies, using both mouse models and patient samples. As a clinician, she treats patients with AD and skin inflammatory conditions. She also serves as coordinator of the Atopic Dermatitis Center of Excellence and Reference in Lyon. She is president of the French Society of Research in Dermatology and president of the French Group on Therapeutic Education in Dermatology.

Didier Pin

Didier Pin has been a professor of veterinary dermatology at Vetagro Sup, Lyon veterinary campus since 2003, and his the head of the Dermatology Department.
He is the author of numerous publications on equine dermatology.
His main research interests are the skin barrier, skin immunology and immunopathology, dermatopathology, and animal models.

Veronica Roberts

Veronica is a European and RCVS Recognised Specialist in Equine Internal Medicine and has experience of working in a number of referral and university hospitals in the UK and Europe. She was awarded a PhD by publication into trigeminal-mediated headshaking and Fellowship of the Royal College of Veterinary Surgeons for Meritorious Contributions to Clinical Practice. She is Senior Lecturer in Equine Medicine at the University of Bristol, as part of which role she sees clinical headshaking cases at B and W Equine Hospital, Breadstone, UK.

Sophie Sage

Sophie graduated from the veterinary school of Lyon (France) in 2015. She pursued an internship at the University of Montreal (2015-2016) and a residency in equine internal medicine at Tufts University, United States (2016-2018). Sophie is a diplomate of the ACVIM (Large Animals) and the ECEIM. She is currently a senior clinician at the University of Bern (Switzerland) and has just completed her phD on the immunologic signature of equine asthma.

Chris Sanchez

Dr. Chris Sanchez is a Professor of Large Animal Internal Medicine and Associate Dean for Clinical Services, Large Animal Operations at the University of Florida's College of Veterinary Medicine. She received her DVM (1995) and PhD (2003) from the University of Florida. She also completed an internship at Equine Medical Associates in Edmond, Oklahoma and a residency in large animal medicine at the University of Florida. She became board certified by the American College of Veterinary Internal Medicine (Large Animal Internal Medicine) in 1999. Dr. Sanchez's research and clinical interests include equine neonatology and gastroenterology, with a focus on pain management. She currently serves as the Specialty President for Large Animal Internal Medicine for the American College of Veterinary Internal Medicine.

Gwenola Touzot-Jourde

Gwenola graduated from the French Vet School of Alfort (ENVA) in 1995 and went in equine private practice after a 2-year internship in equine medicine and surgery with a special focus on equine lameness. I then went onto a north-american journey during which she completed a residency in Anesthesia and Analgesia at the University of Georgia and became an ACVAA diplomate in 2004. Gwenola worked in different university settings (Colorado State University, Ross University, Louisiana State University, University of Montreal) as a clinician and/or an associate professor before coming back to France full time in 2012 at Oniris VetAgroBio, National College of Veterinary Medicine, Food Science and Engineering, where she is currently the head of the anesthesia service. In 2018, she gained her ECVAA diplomate status and completed a phD program studying the

impact of cervical nerve root anesthesia onto equine locomotion (University of Bretagne-Loire, France).
Her research interest focuses mainly on equine anesthesia and pain management including rehabilitation with a special interest in neurologic horses, and animal models to develop and test innovative biotherapies and medical devices. Gwenola has a clinical specific interest in good practice guidelines in bovine anesthesia and pain management and is involved in welfare issues for production and research animals.

Gaby van Galen

Gaby started her career as an equine intern in private practice in the Netherlands immediately after graduation from the University of Ghent, Belgium in 2003. Following this internship she commenced a residency program in equine internal medicine at the University of Liege, Belgium and passed the exam of the European College of Equine Internal Medicine in 2009. In 2011 she left Liege and has since taken up clinical academic positions at the University of Uppsala, Copenhagen and Sydney, and has briefly managed together with her husband a private hospital in Germany. In 2017, she also became Diplomate of the European College of Emergency and Critical care. In January 2022, she returned to Australia and joined the Goulburn Valley Equine Hospital. Gaby's interests and expertise lay in equine internal medicine and emergency and critical care. She has a special clinical interest in critically ill patients such as horses with acute colic or diarrhea, acute neuromuscular disorders and sick neonatal foals, and the use of thoracic and abdominal ultrasonography in those patients. She is author or co-author of over 70 international peer reviewed scientific publications and book chapters, and has given numerous presentations at national and international congresses.

Gunther van Loon

Prof. Gunther van Loon graduated from Ghent University, Belgium, in 1992 and has worked at Ghent University, Department of Large Animal Internal Medicine, ever since. In 2001 he finished his PhD on "Atrial pacing and experimental atrial fibrillation in equines". In 2004 he became ECEIM Diplomate and in 2011 Associate Member of ECVDI. In 2015 he received the British Equine Veterinary Association (BEVA) award for 'Clinical Research' (Liverpool, UK) and in addition the Merial Applied Equine Research Award for outstanding research regarding 'Advances in Equine Cardiology', awarded by the World Equine Veterinary Association (WEVA) (Guadalajara, Mexico). Gunther van Loon is head of the department of Large Animal Internal Medicine at Ghent University and the Equine Cardioteam Ghent. He is Past-President of the Belgian Equine Practitioners Society (BEPS). His major interests are in the field of Equine Internal Medicine, especially cardiovascular diseases, including special focus on arrhythmias, electrophysiology, cardiac pacing, 3D mapping and abla^on of arrhythmias, echocardiography, TDI, 2D ST, biomarkers and interventional cardiology.

Andrew Waller

Andrew Waller has graduated from the University of Bristol and has completed a PhD in Biology at the York University.
Andrew studied the evolution, transmission, and prevention of Streptococcus equi for over 17 years in his role as Head of Bacteriology at the Animal Health Trust. He collaborates with world-leading researchers in over 20 countries around the world and has published over 90 peer reviewed papers on streptococcal infections of animals, utilising these research findings to develop novel diagnostic tests and vaccines with which to improve animal health. Andrew joined Intervacc AB in Stockholm, Sweden, as Chief Scientific Officer in 2020 where he continues to work towards making the lives of animals better.

David Wong

David graduated from Michigan State University and has completed a fellowship at Iowa State University and University of Pennsylvania, followed by a residency program and a MSc at Virginia-Maryland College of Veterinary Medicine.
David is a diplomate of the American College of Veterinary Internal Medicine and the American College of Veterinary Emergency and Critical Care. Since 2020, he is the Department Chair of the Veterinary Clinical Sciences at Iowa State University.
His main research interest are foal medicine and critical care, and equine respiratory diseases.

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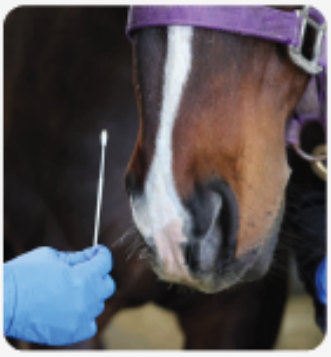


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LECTURES: FRIDAY 27 OCTOBER 2023

STREAM 1 | ROOM: PASTEUR AUDITORIUM

08.15 – 09.00 From hippiatry to modern veterinary medicine - C. Degueurce

Pr. Christophe Degueurce, dean of the Alfort national veterinary college (Paris, France), curator of the Fragonard museum

Hippiatry is the ancient form of veterinary medicine relating to horses, and more generally to knowledge of horse health, equine diseases and their treatments. It generally refers to the ancient and medieval science of horse care, which included rituals, bloodletting, references to the theory of humours and magic formulas. The term was used until the 19th century, when veterinary medicine had already been developing for several decades. But what are the differences between ancient hippiatry and veterinary medicine, and when and how did we move from one to the other?

Veterinary medicine, the modern concept, started with Claude Bourgelat's decision to create a veterinary school in Lyon in 1761. The term *veterinary* was virtually unknown at this time and Bourgelat, choosing the name of the school, hesitated between three ways of naming his school, having the choice between hippiatric school, zooiatric school and veterinary school. In the end, he chose the latter term in reference to the *veterinarius* mentioned by Columella, a first-century Latin agronomist, in his treatise *De re rustica.* Columella used this term to designate the people who treated sick domestic animals; the *veterinarius*, derived from *vetus*, *veteris*, "old". The *veterinarius* was the person who cared for animals that were left to age (*veterina*), those that were not intended for slaughter, i.e. equines and working oxen. In making this choice, Claude Bourgelat was asserting a political will: not to restrict the practice of animal care to horses alone, but to extend it to ruminants and pigs. It was an answer to an order from the former last king of France and his minister, Léonard Bertin, who wanted to decrease the incidence of livestock diseases in order to develop agricultural production, The challenge was to be able to feed a growing population, which was absolutely essential to supply the workers needed to develop the factories. Veterinary medicine thus saw a broadening of practices to include other species that had become of major economic importance, but were poorly understood from the point of view of their health.

As it is often the case, the break with the past represented by the creation of the first veterinary school in Western Europe contributed to an inter-professional conflict that had begun a century earlier, when scholars such as Gervase Markham in England and Jacques de Solleysel in France were writing down the practices of hippiatrists and farriers, who were practitioners of hippiatry but didn’t publish any scholarly literature on their art. Claude Bourgelat, who was a lawyer, did the same between 1750 and 1753 when he published his *Elemens d'hippiatrique*, which was a success and led him to become an encyclopaedist. Hippiatrists were partly dispossessed of their knowledge by educated people capable of writing books. Many hippiatrists took a very dim view of this monopolisation of their knowledge by outsiders. They fought to maintain their advantage, but had few success.

In the 18th century, a French hippiatrist was a professional trained by a guild, in this case that of farriers. They did not follow a standardised school curriculum and apprenticed with a master, before being certified by a jury drawn from this professional body. Hippiatrists were therefore heirs to a routine practice that had remained virtually unchanged for two thousand years. We are very familiar with the methods and medications used by the hippiatrists of late antiquity and, by the eighteenth century, nothing had really changed. Modern hippiatrists practised bloodletting, purging, enemas, treatments using natural products, plucking out soles, cutting off ears, etc., all of which were used to treat the horse.

In creating a veterinary school, Claude Bourgelat broke with the usual rhythm. He was bringing students together in a single place to give them standardised, structured, exhaustive training, ensuring that they would all be professionals with the same skills and

competencies that would be extended to animals other than the horse. In reality, things were less clear-cut than his communication might lead one to believe, and the lack of knowledge about the health of animals, particularly ruminants, limited the training of these new professionals for a time. In reality, an examination of the course books shows that the concepts were mainly aimed at horses and were very similar to those practised by farriers. Everything still had to be worked out, for the horse as for other species, and it took no less than a century for the beginnings of an understanding of ruminant diseases to emerge. The path from hippiatry to veterinary medicine was therefore a long and chaotic one, and it has to be said that the first veterinary surgeons hardly distinguished themselves from their empirical cousins. Worse still, many of them found it difficult to practise their profession because they were competing with locally-established farrier families, who were repeating what their customers expected, i.e. the same gestures that had been practised since antiquity. In the early decades, many vets in France gave up their profession.

So, finally, what were the factors that ensured the switch from the old hippiatry to the new veterinary medicine? Is there a specific date that marks this transition?

At the beginning, the new vets were unable to distinguish themselves from the hippiatrists because they simply did not have the additional knowledge. Worse still, having moved to the city to study, and having left their families and social circles behind, they were suspect in the eyes of their fellow citizens, and most of them had great difficulties making a living from their profession, sometimes even hiding their veterinary training to blend in with the mass of empiricists. One of their few distinctive features was their concern for hygiene, which often ensured them unexpected medical successes at a time when practices left little room for hygiene. By recommending that stables be cleaned, that the sick be kept separate from the healthy, and that ancestral practices such as burying the corpses of animals that had died of plague (i.e. a contagious disease) in the stable dung under the feet of the survivors be abandoned, veterinary surgeons were able to achieve sometimes spectacular improvements in animal health, even though they had no knowledge of the contagious process.

In order to gain recognition, veterinary surgeons set up a process to differentiate themselves from hippiatrists, taking the opposite approach to the practices of hippiatrists. Future veterinarians were recruited via an examination at the two French veterinary schools. In 1777, admission requirements were to be over 16 and under 30 years of age, able to read and write, and of robust build. The veterinary profession sought to raise the level of recruitment of future vets, instituting increasingly difficult entrance exams, and recruiting the children of blacksmiths, particularly from the big cities. The vets joined forces with the doctors, in an attempt to assimilate them. They teamed up with doctors to publish treatises, embraced experimental medicine, shifted from observation to a series of logical experiments, and joined forces with physicists, mechanics and chemists to create instruments and medicines. Hippiatrists, on the other hand, remained outside this process of creating a veterinary science that was closer to human medicine.

In the end, it was these scientific and technical advances that marginalised the empiricists and legitimised the veterinarians. The most important of these revolutions was the development of effective therapy. Hippiatrists used polypharmacy, i.e. mixing a large number of ingredients to form a pharmaceutical panacea that was good for everything and therefore good for nothing. And the first vets did no different. From the 1820s onwards, physiologists in Europe tested simple products, plant extracts and mineral compositions. In this way, the composition of each plant was established and each identified component was tested. In his *Formulaire pour la préparation et l'emploi de plusieurs médicamens*, published in 1821, François Magendie recommended preferring the alkaloid to the natural substance, for example morphine to opium, strychnine to *nux vomica* powder. At the very end of the 19th century, what we now call active ingredients, first natural and then synthetic, were substituted for plant parts. At the same time, physiologists established the quantity of active substance that would produce an effect without generating toxicity, thereby establishing the famous posology. Traditionally, substances were administered orally, and large quantities were needed to produce a visible effect. The active ingredients were expensive and the quantity a horse needed to absorb in proportion to its size was

considerable, making the cost prohibitive; the cost of treatments was fifteen to twenty times the price of a human treatment, at a time when treatment was already a luxury beyond the reach of ordinary humans. Only the richest people could go to the apothecary, and everyone else treated their animals with plants, often toxic, and minerals, all of them gathered from their immediate surroundings. And the oral route had another limitation: when the substance had an unpleasant taste, the veterinary surgeon had to try to hide it or fight with the animal, which they were very good at doing, since restraining animals was an art particularly well mastered by people of the time.

The second revolution was the idea of injecting active ingredients directly into the body of animals. At the end of the 17th century, Professor Erik Viborg of the Copenhagen Veterinary School described the intravenous injections of a powerful purgative, which was white hellebore or white veratrum. The root was infused in brandy, which was then cut with water. The resulting solution, heated to blood temperature, was poured into a small clyster named a syringe. The external jugular vein was opened by phlebotomy. A channel was inserted into the wound to guide a cannula. The syringe was then slowly emptied into the bloodstream and the product took effect in two to three minutes; the horse's breathing became altered, the pulse became rapid and superficial, and the animal evacuated abundant excrement. After confirming his method on twenty-two horses, Viborg varied the dilution of the decoction to fix the optimum dosage, minimizing undesirable disturbances. He also repeated the experiment on six cattle, in which, in addition to the expected reactions, he noticed the ejection of ruminal bowls into the oesophagus. The article concluded that veterinary surgeons would benefit from a route of administration that was as effective as it was economical. The solution to the two problems - the low potency of plants given orally, and the difficulty of getting the plants to swallow - was to come from injecting the product directly into the animal's body which was a major revolution that took over a century to implement. The experiment was repeated in other countries and with other substances from the 1820s onwards, but it was still used on an experimental basis. It was far from being used routinely in clinics, as veterinary surgeons did not yet have the equipment to carry out injections.

The third revolution was the creation of the syringe and the needle. In France, a physician from Lyon, Charles-Gabriel Pravaz, wanted to inject iron perchloride into an aneurysm; in 1841, he designed a silver syringe, 3 cm long and 5 mm in diameter. The plunger advanced by screwing, allowing to control of the quantity of substance injected. To inject the product, he used cannulas and trocars made of gold or platinum. The trocar, made of a tube supported by a very thin pointed pin, pierced the vein; the pin was removed and the mandrel was then screwed onto the syringe delivering the product. Several manipulations were therefore required. The first trial of the device in France was carried out on sheeps at the Lyon Veterinary School in 1852 and it was a success. The next step was to create a hollow needle that was rigid enough to penetrate the skin, which was done ten years later.

The fourth revolution was the development of vaccination, which will not be discussed here.

The fifth one was the development of synthetic chemistry at the very end of the 19th century. One family of medicines marked a decisive stage, that of arsphenamines, which your colleagues in the 1970s still used extensively on horses suffering from febrile syndromes. Veterinary surgeons and farriers in the 19th century made extensive use of arsenical derivatives; Fowler's liqueur in particular was used to treat asthma in horses. In 1907, arsphenamine was first synthesized in Paul Ehrlich's lab by Alfred Bertheim. It was first used to treat the disease syphilis because it is toxic to the spirochete *Treponema pallidum*. Arsphenamine was originally called *606* because it was the sixth in the sixth group of compounds synthesized for testing; it was marketed by Hoechst AG under the trade name *Salvarsan* in 1910. *Salvarsan* is often regarded as the first modern chemotherapeutic agent, and Ehrlich as the creator of the modern concept and term of chemotherapy. In 1911, Rips and von Kirsten in Germany, at Ehrlich's instigation, used arsenobenzene on a large scale to treat contagious equine pleuro-pneumonia - equine influenza or Brustseuche der Pferde - at a dose of one gram of powder dissolved in half a liter of physiological serum. Injection was risky because the product was liable to trigger dreadful phlebitis, which meant that effective needles had to be available and infusion was

preferred to injection. The product was extremely effective, generally curing after eight days. The Russians, Norwegians and Germans began to use it, but the French, still competing with the Germans, didn't use the product. In 1912, Ehrlich's team created neo-arsphenamine, or *914,* or *Neo-Salvarsan*, which had the immense advantage of being very soluble. The use of Neo-Salvarsan thus made the method easier to apply. The German Empire used it extensively during the First World War, which gave it a clear advantage.
Subsequently, organic syntheses multiplied and with them the number of active and injectable products, and the oral administration disappeared. The development of sulphonamides and antibiotics, and the effectiveness of chloral, among others, were to provide veterinary surgeons with formidable tools. This was probably the turning point, the transition from the old hippiatry to veterinary medicine, from the hippiatrist to the veterinary surgeon, hippiatrists being systematically excluded from these advances. As therapeutics developed, non-veterinarians found themselves increasingly excluded from modern practice, including by legislation.
The final question is: when did hippiatrists, or rather non-veterinarians, stop treating horses? Each country had its own approach to the matter. In France, the veterinary profession went through a major crisis after the First World War. The mechanisation of transports and then agriculture led to a decline in the number of horses, although dog and bovine medicines and the development of horse riding had not yet created new outlets for vets. The crisis was having a severe impact on French veterinary schools, which were no longer able to recruit students. A number of measures were taken, including aligning the veterinary and medical curricula, with the creation of a veterinary doctor's diploma in 1923. Finally, negotiations between veterinarians and non-veterinarians led to the adoption of the law of the 22th of June 1938. Only veterinary surgeons were then authorised to practise animal medicine and surgery, with the exception of non-veterinary surgeons practising in 1938 or in training at that date, who were granted an exemption until they retired. The latter ceased their activities at the end of the 1980s. In France, therefore, there have officially been no non-veterinarians practicing this art for only forty years. The disappearance of the descendants of horse doctors is therefore a very recent phenomenon.
Finally, what were the factors that led to the replacement of hippiatrists by vets? During the 19th century, advances in medicine and especially pharmacy were to provide veterinary surgeons with new and effective resources to treat animals. The veterinary surgeons, thanks to the uniform teaching they received in veterinary schools, thanks to the links they forged with doctors, scientific and medical societies, faculties and universities, were the great beneficiaries of this progress. It was undoubtedly through the development of effective drugs and the sanitary control of farm animals that veterinarians found their place in society, almost two centuries after the profession was created.

NOTES

09.00 – 10.00 CCARE: An update on Sepsis, septic shock, and biomarkers - D. Wong

David Wong, DVM, MS, DACVIM, DACVECC Iowa State University, Ames Iowa

Despite an enormous amount of research surrounding sepsis in people, this disease process remains highly prevalent and a target of continued clinical research in efforts to improve early diagnosis and outcome. The definition of sepsis and the systemic inflammatory response syndrome (SIRS) in people has been modified and changed over the past several decades and in the most current definition (Sepsis-3, 2016), the systemic inflammatory response syndrome has actually been removed from the definition of sepsis. Sepsis-3 defines sepsis in people as life-threatening organ dysfunction caused by a dysregulated host response to infection while septic shock is defined as a subset of sepsis with circulatory and cellular/metabolic dysfunction associated with a higher risk of mortality.[1] A consensus definition of equine neonatal sepsis has not been agreed upon, but a few suggestions have been put forward. For example, Sheats suggested that sepsis be defined as a dysregulated host systemic inflammatory response to infection.[2] Alternatively, Wilkins proposed a definition of sepsis as a SIRS patient with suspected or proven infection;[3] a positive blood culture or single site of infection does not define sepsis without evidence of systemic inflammation. In addition, sepsis severity groups have also been used in some equine research projects including normal sepsis (sepsis without organ dysfunction, oliguria, hypotension or hypoperfusion, blood lactate <2 mmol/L), severe sepsis (sepsis plus presence of organ dysfunction, hypotension, blood lactate >2 mmol/L, oliguria or MAP <60 mmHg) and septic shock (sepsis plus persistent hypotension after fluid therapy or patients in need of/in treatment with dobutamine and/or vasopressors).[4]

Sepsis also remains a prevalent disease entity in the neonatal foal. In various equine research publications, the incidence of positive blood cultures from hospitalized foals has typically ranged from 25-35%, although a few studies have reported higher incidences of positive blood culture.[5-11] Interestingly, several studies have documented positive blood cultures from apparently healthy foals in the range of 29-57%.[12-14] This fact, that is healthy foals can be transiently bacteremic, reiterates the proposed definitions of equine sepsis in that a positive blood culture alone does not define sepsis without a systemic inflammation. Moreover, transient bacteremia in newborn/neonatal foals is more common than anticipated.

A variety of diagnostic tests have been used to document sepsis, with blood culture remaining the gold standard. Unfortunately, there are no specific guidelines or standard protocols on performing blood cultures in foals. Variables such as sample volume, sample timing, site of sample collection, and sample frequency can impact the results of blood cultures. For example, studies in people have indicated a positive correlation between larger blood sample volumes (e.g., 5-20 mls) and yield of colony forming units (CFU) cultured.[15,16] Although no specific correlation of timing of blood cultures (e.g., culture at the time of patient febrile episode) and positive blood culture has been documented, other studies indicate that serial/multiple blood draws increased the likelihood of sampling during bacteremia. The clinician must be cognizant of the fact that submission of blood culture(s) has moderate sensitivity in identifying bacteremia, but attempting to collect multiple samples, using larger volumes of blood per sample (10-20 mls) may improve the likelihood of identifying bacteremia in the foal.

Other mechanisms that have been used to help identify septic foals include scoring systems (sepsis score), advanced molecular techniques such as matrix-associated laser desorption/ionization time-of-flight mass spectrophotometry (MALDI-TOF MS) and multiplex PCR. Additionally, the scientific community has repeatedly tried to identify a biomarker that would be highly suggestive of the presence of sepsis in people and foals. Some of these biomarkers will be discussed further (below), but inevitably, sepsis in clinical practice is often relegated to the clinician's intuition, based on physical examination and various clinicopatholgic tests (blood lactate, CBC).

In the 1980s, Koterba *et al.* developed a sepsis scoring system to help identify foals at risk of sepsis based on a variety of weighted criteria including variables found on the CBC (neutrophil count, band neutrophils, toxic morphology), laboratory data (blood glucose, IgG, fibrinogen), physical exam findings (petechia, scleral injection, fever, presence of diarrhea) and historical information (placentitis, vulvar discharge, others).[17] In the original study evaluating 148 foals, the sensitivity was 93% and specificity 86%. However, subsequent evaluation of the sepsis score at different referral hospitals have documented lower sensitivities and specificities.[18] A more recent attempt to enhance the sensitivity of the sepsis score (updated sepsis score) added additional variables such as blood lactate, serum creatinine concentration, and the SIRS criteria; however, despite the addition of these variables, improved sensitivity was not gained.[19]

Over the past decades, researchers have investigated a multitude (hundreds) of different biomarkers in an attempt to provide early identification of a septic patient, facilitate prognostication, and/or serve as a marker to monitor response to treatment.[20] A biomarker can be defined as a measurable substance in an organism whose presence is indicative of some phenomenon such as disease, infection, or environmental exposure. These biomarkers can be broadly categorized under soluble or membrane receptors, damage associated molecular patterns (PAMPs), cytokines/chemokines, and acute phase proteins. Although hundreds of biomarkers have been investigated in people, only a fraction of these markers have been examined in equine neonatal sepsis and will be briefly reviewed below.

A. **C-reactive protein** (CRP): CRP is a protein secreted by the liver in response to inflammatory cytokines with levels of this molecule increasing and decreasing rapidly in response to trauma, inflammation, or infection.[21] CRP is part of the innate immune system and activates complement, binds Fc receptors, and acts as an opsonin for various pathogens. In one multi-institutional study, CRP was measured in healthy (n=39), sick-non septic (40), and septic foals (40).[22] In this study, CRP was not significantly associated with the presence of sepsis in foals. However, plasma CRP concentrations were significantly associated with band neutrophil count and rectal temperature.
B. **Procalcitonin** (PCT): PCT is a precursor peptide for the hormone calcitonin.[23] The expression of PCT is upregulated in many tissues during infection. In a study by Bonelli *et al*., blood was collected from 16 healthy foals and 35 sick foals and PCT was measured via ELISA.[24] The PCT concentration was statistically higher in septic SIRS foals (178.9 ± 76 pg/ml) when compared to control group (30.0 ± 33.1 pg/ml) and a positive linear correlation between PCT concentration and SIRS scale was observed. The authors suggested a PCT cutoff value to determine septic SIRS of 73.04 pg/ml (87.5% sensitivity, 97.1% specificity). Interestingly, in another earlier study by Pusterla *et al*., no significant difference was observed in PCT between healthy and sick/septic foals via evaluation of gene expression.[25]
C. **Serum Amyloid A** (SAA): SAA is an acute phase protein that is induced by a wide array of stimuli including inflammatory cytokines (IL-1, IL-6, IFN-gamma, others). The precise role SAA has is not known but it has been shown to have multiple functions including opsonization, regulation of the inflammatory process, stimulation of monocyte and neutrophil migration, among other functions.[26-29] Several studies evaluating SAA in septic foals. In healthy neonates, mean ± SD (range) of SAA has been reported as <u>Day 0:</u> 60±80 µg/ml (6-222); <u>Day 1:</u> 120±87 µg/ml (11-87); <u>Day 3:</u> 93±68 µg/ml (9-68).[30] In a recent (2022) multi-institutional study involving 586 foals, SAA concentrations were significantly higher in septic foals (1079±1254 mg/L) compared to non-septic foals (312±685 mg/L) and increased with increasing sepsis score.[31] In this study, the optimal SAA cutoff for detection of sepsis was 1050 mg/L (low sensitivity 30%; high specificity 91%). Admission SAA concentrations were lower in surviving foals (435±723 mg/L) compared to non-surviving foals (1063±1449 mg/L). In another recently (2022)

published study, SAA was compared in 397 foals divided into healthy (n=245) sick non-septic (117) and septic foals (35).[32] In this study, the median SAA concentrations were 0, 1.5 and 114 µg/ml, respectively, with septic foals having a significantly higher SAA when compared to healthy foals. Of note, in this study, a commercial point-of-care SAA testing device was used. Older studies have also suggested an association with SAA and sepsis with one study noting a significantly higher SAA (280 mg/L) when compared to healthy (27.1 mg/L) foals.[33] A SAA concentration of > 100 mg/L was suggested as a cutoff value for sepsis in several studies with one study noting a sensitivity of 53% and specificity of 98% when 100 mg/L was evaluated.[32,33]

D. **Neutrophil gelatinase-associated lipocalin** (NGAL): NGAL is a biomarker of renal damage and has also been shown to correspond with severity of sepsis irrespective of the degree of renal dysfunction in human medicine. In a recent study, serum NGAL was evaluated in 91 neonatal foals categorized as septic, sick non-septic, healthy, and uncertain sepsis status.[4] In this study, serum NGAL was significantly higher in septic compared to non-septic foals. Moreover, serum NGAL concentrations were significantly lower in surviving foals than non-surviving foals.
E. **Soluble CD14** (sCD14): CD14 functions as the receptor for LPS and LPS-binding protein and associates with Toll-like receptor 4 (TLR4) on the cell surface. Once LPS/LPS-binding protein complex binds to CD14, it induces signal transduction through TLR4 and triggers synthesis of pro-inflammatory cytokines. In addition to the membrane form, a soluble CD14 (sCD14) is also detectable in serum and is produced by shedding of CD14 from cell surfaces and by exocytosis.[34] In one equine study, sCD14 was compared between healthy (n=15) and septic foals (n=15); in this study, sCD14 was significantly higher in septic foals (median 465, range 40-1592) compared to healthy foals (298, 28-1108).[34] Further evaluation of sCD14 in septic foals have not been published, to the speakers knowledge.
F. **Adrenomedullin** (ADM): ADM is a peptide is synthesized in the adrenal medulla, endothelial cells, and vascular smooth muscle cells, and facilitates tissue perfusion via vasodilation and inotropic effects.[35] In one study, adrenomedullin concentrations (ELISA) were compared between healthy foals (n=61), sick non-septic foals (48), and septic foals (42). In this study, plasma ADM was not significantly different between sick non-septic and septic foals, but the concentration in ill foals (all sick foals) was significantly increased compared to healthy foals.[35] The authors concluded the plasma ADM concentrations was not associated with sepsis or survival in neonatal foals, but a 6-fold increase in the median ADM concentration was observed and may be a marker of health.[35]

Although several of the aforementioned biomarkers show promise in helping identify sepsis in foals (e.g., SAA, NGAL), the clinician must also recall that clinical intuition plays an important role in identifying sepsis suspects. Factors such as physical exam findings (tachycardia, fever, cool limbs/poor peripheral perfusion), point-of-care measurements (hypoglycemia, hyperlactatemia), and clinicopathologic data (leukopenia, left shift, toxic neutrophils, organ dysfunction) serve as important variables that allow the equine veterinarian to make assessments of sepsis in real time clinical cases. As more equine studies are performed, the role of biomarkers in the diagnosis of sepsis may become clearer.

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NOTES

10.00 – 11.00 CCARE: Transfusion medicine review - B. Dunkel

Bettina Dunkel, DVM, PhD, DACVIM, DECEIM, DACVECC, FHEA, MRCVS; The Royal Veterinary College, Hatfield, Herts, UK

The total blood volume of a horse depends on the breed, age, and sex, with Thoroughbreds, light breed horses and draft horses' blood volume being approximately 100 mL/kg, 78 mL/kg, and 61 mL/kg (6%–10% of the body weight), respectively. Blood loss of up to 15% of the blood volume (9–15 mL/kg; 4.5–7.5 L in a 500-kg horse) is tolerated without causing changes in physical parameters but a decrease in central venous pressure can be appreciated when losing 16ml/kg[1]. Up to 25% (15–25 mL/kg; 7.5–12.5 L in a 500-kg horse) might be tolerated without need for a transfusion but most horses with blood loss of > 20 mL/Kg will require fluid therapy to increase the circulating volume and/or a blood transfusion[2]. A whole blood transfusion is indicated when the oxygen-carrying capacity, which is to 98% determined by the hemoglobin concentration, has decreased to a degree that oxygen delivery to the tissues becomes insufficient. Subsequent tissue hypoxia can manifest as colic, acute renal injury, cardiac arrhythmias, and increase in cardiac troponin I (cTnI), presumably caused by tissue and myocardial hypoxia. Arrhythmias have been described in 10% and 73% of horses experiencing significant hemorrhage with ventricular arrhythmias being particularly common[3, 4]. The decision whether or not a transfusion is necessary depends on the amount of blood lost and over what time frame the deficit has occured. In people, a hemoglobin concentration of 7 g/dL has been used as transfusion threshold while many Equine clinicians might use a slightly lower value of 5-6 g/dL corresponding roughly with a PCV of 15-18% in a euvolemic patient. In controlled hemorrhage, the volume deficit is corrected first to quickly reestablish perfusion pressure to all organs. In uncontrolled hemorrhage, for example bleeding into a body cavity, overly aggressive correction might result in a rapid increase in blood pressure and disruption of clot formation and is therefore best avoided[2]. Initial hypotensive resuscitation in form of 20mL/kg boluses of isotonic fluids or 2 to 4 mL/kg of hypertonic saline and maintaining a blood pressure of approximately 60mmHg has been recommended until control of bleeding has been achieved. On the other hand, prolonged hypotension or failure can also be detrimental and achieving a balance is challenging. Concerns about hypocoagulatory effects of synthetic colloids have not been replicated in a more recent study using 6% HES or 4% modified fluid gelatin in healthy horses but cautious use in sick animals might still be warranted[5]. Clinical parameters are strongly influenced by the primary disease process which makes it difficult to base the need for a transfusion solely on these parameters. PCV and plasma protein concentration remain unchanged until lost volume is replaced by interstitial fluid. More objective measures of tissue hypoxia and increased oxygen extraction in the periphery include lactate concentrations, partial pressure of oxygen in venous blood (PvO_2), and oxygen extraction ratio.

Horses have eight known blood groups (A, C, D, K, P, Q, U, T), with each group also having multiple factors giving rise to approximately 400,000 possible blood types. Donkey and mule red blood cells (RBCs) carry an antigen called *donkey factor* that is presumed to contribute to the high risk of neonatal isoerythrolysis in mule foals. A similar antigen is also suspected on donkey platelets, predisposing them to neonatal alloimmune thrombocytopenia. The percentage of horses with alloantibodies without prior exposure to allogenic blood has been estimated at approximately 10-20%. This can vary between populations and more recent studies show alloantibodies in 12-35% of horses but this percentage could be higher if antibodies against currently unidentified antigens are included[6, 7]. Anti-Aa and anti-Qa antibodies are most frequently associated with transfusion reaction or neonatal isoerythrolysis while most antibodies are directed against the Ca antigen[8, 9]. The most antigenic group appears to be Aa, with all Aa-negative horses developing antibodies if transfused with Aa-positive RBCs; those antibodies persisted for over a year.[89,91] Antibodies against other RBC antigens develop infrequently,

can be detected 1 to 154 weeks after transfusion, and might disappear again after 4 weeks[10].

The clinical effects of most alloantibodies appear to be minimal in regards to causing a transfusion reaction and first transfusions are therefore frequently carried out without prior crossmatching. However, use of a matched donor significantly prolongs the life span of transfused RBCs. If blood type is matched and crossmatches indicate compatibility, the mean life span of transfused autologous and allogeneic RBCs is approximately 89 days and 39 days, respectively, with a mean posttransfusion allogeneic RBC half-life of 33.5 days[11, 12]. The half-life was reduced to 11 days and 4.7 days, respectively, if agglutination is noted on the major crossmatch reaction[11]. At least a major agglutination crossmatch is therefore advisable if several blood donors and time for crossmatching are available.

Compatibility of a donor-recipient pair can be assessed based on *in vitro* laboratory methods or, in horses that have been blood-typed and alloantibody screened, on expected compatibility. Unfortunately, neither method corresponds 100% with *in vivo* compatibility which would be the gold standard. Other factors such as leukocytes, platelets, plasma proteins, and poorly documented RBC antigens or unidentified antibodies probably also play a role.

Major (donor's RBCs with recipient's plasma) and minor (donor's plasma with recipient's RBCs) crossmatching ideally includes evaluation for agglutination and hemolysis (requires rabbit complement). Reassuringly, hemolysis in the absence of agglutination appears to be very rare and agglutination alone could be used as a proxy for hemolysis[13]. The most common method used for equine cross matching remains tube agglutination and hemolysis which takes approximately 120-180 minutes. In people and small animals gel column agglutination is performed which can be substantially faster. An Equine Gel Test is commercially available for stall site use which delivers results in 25min according to the manufacturer[13]. The sensitivity of the Equine Gel test compared with expected reactions (based on known blood groups and known presence of ant-RBC antibodies) was 91.4% and specificity was 73.5% but varied widely between blood groups: The test was 100% sensitive for anti-Aa, 70% for anti-Qa and 100% sensitive for anti-Ca reactions. Cohen's κ agreement for the test with expected positive and negative reactions was substantial but only fair compared to standard laboratory methods.

Ideally, blood donors should be Aa and Qa negative. and free of infectious diseases, most notably equine infectious anemia (EIA). Donkeys should not be used as blood donors for horses and only receive horse blood free of anti–donkey factor antibodies. Approximately 20% of the donor's blood volume can be collected (8 L in a 500-kg horse) every 30 days. Volume replacement with isotonic fluids is recommended if greater than 15% of blood volume is collected. The aim is replacing 25% to 50% of the lost blood to ensure adequate oxygen delivery while the patient's RBCs are regenerated. On average, one can expect the PCV to increase by approximately 0.5% to 1.0% per day. Blood should be collected through a large-bore catheter (10–12 gauge) inserted in a rostral direction (against the direction of flow) into the jugular vein into blood bags containing 3.2% sodium citrate at a blood-to-anticoagulant ratio of 9:1. If blood is to be stored, citrate-phosphate-dextrose with adenine (CPDA) should be used and blood kept at 4°C. Autologous equine blood stored for 28 days still has a longer half-life of 29 days compared to compatible allogeneic blood stored for only 24 hours (half-life of 20 days)[12, 14]. Adverse reactions occur in approximately 16% of horses but can be as high as 87.5% in crossmatch-incompatible transfusions[11]. Hemolytic reactions can occur immediately or up to 24 hours and longer after the transfusion. Depending on severity, antihistamines (hydroxyzine 0.5–1.0 mg/kg PO q12h), corticosteroids (dexamethasone 0.1 mg/kg intravenously [IV]), or epinephrine (0.01–0.02 mL/kg of a 1:1000 solution IV; 5–10 mL for a 500-kg horse) may be indicated.

Autologous blood transfusions can be considered if blood loss is anticipated in advance, for example during a planned surgery, or following major body cavity bleeds, most commonly hemoabdomen. The advantage is a longer RBC half-life and minimal risk of a transfusion reaction. The half-life of RBCs obtained from a body cavity has not been investigated in horses. Blood in contact with serosal surfaces for longer than 1 hour becomes defibrinated and it is therefore controversial if it is necessary to add an anti-coagulant if blood is collected from a body cavity. Platelets and coagulation factors are also diminished, making an allogeneic transfusion potentially a better option in cases of hypocoagulation. Recommendations in dogs range from addition of 0.05 to 0.14 mL of anticoagulant per milliliter of blood, whereas in horses a blood-to-anticoagulant ratio of 15:1 (0.07 mL/mL of blood) has been suggested. In horses, blood from the abdomen can be collected using a 30-Fr Foley catheter or 28-Fr chest drain, inserted into the abdomen through a sterile stab incision and connected to a blood collection bag. Blood can then be directly administered through an intravenous blood administration set[15].

Platelet transfusions might be indicated in horses with severe thrombocytopenia or to prepare horses with known functional platelet abnormalities for surgery. The risk of hemorrhage is dependent on platelet numbers, platelet function, and the activation state of the plasma-based coagulation and fibrinolysis system and the endothelium and vascular wall. In horses, a platelet count less than 30,000 to 50,000/µL is likely to be associated with clinical signs such as petechiae or epistaxis. With platelet counts below 10,000/µL, active hemorrhage is likely; this often resolves in otherwise healthy animals once the platelet counts increase above 15,000 to 30,000/µL. In thrombocytopenia secondary to neoplasia and also rarely primary immune-mediated thrombocytopenia (IMT), clinical signs occur over a much wider range from 20,000 to 91,000/µL suggesting that platelet function is compromised. A platelet transfusion is indicated if prolonged bleeding is observed or suspected (e.g., due to a sudden decrease in PCV). Most equine hospitals lack the ability for apheresis or to concentrate platelets but a platelet transfusion can still be prepared. Potential platelet alloantigens similar to people have been identified in equids, but their clinical significance is yet unknown. Citrate-based anticoagulant solutions are recommended; under- or overfill should be avoided as it could affect platelet function. Glass bottles are not suitable for collection as glass surfaces activate platelets. Because of their small size, platelets remain in suspension when RBCs are allowed to settle by gravity and the harvested plasma contains the vast majority of platelets (and leukocytes). If not administered immediately, the platelet-rich plasma should be stored at room temperature (22°C); exposure to temperatures below 15°C induces irreversible changes decreasing platelet function. Platelets can be stored under constant agitation for up to 5 days, but the risk of bacterial contamination is higher at this storage temperature. In dogs, a fresh whole blood transfusion of 10 mL/kg of body weight is expected to increase the recipient's platelet count by a maximum of 10,000/µL. No guidelines are available for horses but similar to slightly lower increases might be expected, depending on the donor's platelet count. Administration of drugs that negatively influence platelet function (acetylsalicylic acid, clopidogrel, heparin) is probably contraindicated. Other nonsteroidal antiinflammatory drugs (NSAIDs) such as flunixin meglumine, phenylbutazone and firocoxib have minimal effects on *in vitro* platelet function.

Plasma transfusions might be indicated in horses with low protein concentrations, to combat systemic inflammation, in foals with failure of passive transfer or in need of *Rhodococcus equi* prophylaxis and in horses with coagulopathies to provide essential coagulation factors. Frozen equine plasma is commercially available or can be prepared fresh by gravity sedimentation from collected blood. Fresh frozen plasma refers to plasma separated from blood within 8 hours of collection and stored at −18°C for up to 1 year. If plasma is separated after 8 hours or stored for longer than 1 year it is referred to as *frozen plasma*. Following preparation of plasma from whole blood by gravity sedimentation over 48 hours at 5°C, clotting factors retain 66% to 95% of their activity, and all activities

remain within the normal reference ranges. Activities for all factors except FX remain within normal ranges after storage at -20°C for 90 days. The amount of plasma needed to increase plasma protein concentrations can be calculated as follows: Volume (L) = ([TP_{Target} − $TP_{Patient}$] × 0.05 × body weight [kg]) ÷ TP_{Donor} but calculations are notoriously unreliable. A 500-kg horse will require approximately 4 to 5 L of plasma to increase the plasma protein concentration by 10g/L. Administration of plasma follows the same guidelines as blood administration, using a filter and beginning slowly, while closely monitoring the patient. Reactions to commercial plasma have a reported incidence of 8.7% in foals and 0% in adults, while the incidence with noncommercial plasma is 10%. In case of a reaction, the plasma infusion should immediately be discontinued and treated as outlined for blood transfusions.

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NOTES

11.30 – 12.15 CCARE: A review of compartment syndromes - B. Dunkel

Bettina Dunkel, DVM, PhD, DACVIM, DECEIM, DACVECC, FHEA, MRCVS; The Royal Veterinary College, Hatfield, Herts, UK

Compartment syndrome describes a pressure increase within a body space with limited compliance that, if sustained, can cause significant pain, decreased perfusion and tissue damage. Compartment syndrome is most commonly described in the musculature of the extremities and within the abdomen; both have been described in horses.

Extremity compartment syndrome has been suspected in horses with a variety of conditions including intramuscular hemorrhage of the caudal antebrachial muscle, periarticular hemorrhage within the fascial planes around the femoropatellar joint, flexor muscle myopathy and a leiomyosarcoma in the crural region. Treatment included multimodal analgesia and in some cases fasciotomy with variable results[1-4].

Normal intraabdominal pressure in people is between 0-5mmHg while intraabdominal hypertension (IAH) is defined as intraabdominal pressures > 12mmHg obtained at two measurements 1-6 hours apart. Compartment syndrome is defined as an acute and sustained increase in intraabdominal pressure > 20mmHg in association with new organ dysfunction[5]. Both conditions are considered under-recognised and under-diagnosed in people and the same is probably true for horses. Risk factors in people include decreased body wall compliance, increased intraabdominal contents such as peritoneal effusion, obesity, cytokine release, reperfusion injuries and IAH after large volume resuscitation resulting in capillary leak syndrome[6]. It is assumed that risk factors in horses are similar[7].

Intraabdominal hypertension and abdominal compartment syndrome are both feared complications as the increased intra-abdominal pressure affects virtually all organ systems negatively, often resulting in organ dysfunction. Complications and organ dysfunctions are largely due to decreased abdominal perfusion pressure (mean arterial pressure minus intraabdominal pressure) and intrinsic or extrinsic compression of organs[6]. With increasing abdominal pressures, the intra-abdominal arterial and venous blood supply is compressed leading to an increased cardiac afterload due to an increased systemic vascular resistance and decreased cardiac preload due to a decreased venous return. This ultimately leads to decreasing cardiac output and mean arterial pressure with subsequent decrease in renal and splanchnic perfusion[8]. In addition, mechanical transfer of pressure from the abdominal to the thoracic cavity results in increased intrapleural pressure which can increase central venous pressure, decreased lung volume and worsen the PaO_2/FiO_2 ratio[8].

In people, direct and indirect measurements of intraabdominal pressure correlate well and one of the most commonly used techniques is indirect measurement of intravesicular pressures in a supine position. In horses, indirectly obtained intravesicular and intragastric pressures unfortunately correlate poorly with direct intraperitoneal measurements and therefore intraperitoneal cannulation in the right or left flank or ventral abdomen are considered the gold standard.[9-12]. Direct measurements in the left and right mid-flank area midway between the height of the tuber ischii and the point of the shoulder and 12cm caudal to the last rib yielded normal values of -5 to -1.0mmHg[9, 11]. Using a ventral position, normal standing horses had an intraabdominal pressure between 20-30mmHg with median 25mmHg[7, 11]. An increase above 32mmHg has been suggested as cut-off point for IAH. IAH has been documented in horses with colic and peritoneal effusion and has been suspected in foals with uroperitoneum[7, 13]. IAH was documented in 30% of horses presenting to a referral hospital for colic. Horses with medical large intestinal lesions were most commonly affected and intraabdominal pressures did not differ between survivors and non-survivors. Transrectal palpation did not affect intraabdominal pressure but a small increase was observed with abdominal bandages[14]. While this increase was clinically insignificant in the examined healthy horses, a more detrimental effect in sick horses cannot be ruled out.

In people, treatment options vary depending on the reasons for the increased intraabdominal pressure but might include gastric decompression, percutaneous drainage of abscesses and surgical decompression[8]. Although the effects on intraabdominal pressure have not been documented, decompression of gas-distended large intestine, either percutaneous in standing horses, or, arguably more effectively during surgery, almost certainly have the same effect. In foals with uroperitoneum undergoing surgical correction hypoxemia during surgery was commonly encountered which frequently improved towards the end of surgery, following peritoneal drainage. This might be associated with decreasing intraabdominal pressures (Dunkel and Wilkins; unpublished data).

In summary, intraabdominal hypertension exists in horses but is probably under-diagnosed. Awareness and rapid resolution of the inciting cause and decompression might help to prevent complications associated with the condition.

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NOTES

12.15 – 13.00 CCARE: Systemic repercussions and MODS / MOF following severe trauma - G. van Galen

Gaby van Galen, DVM, PhD, Dipl ECEIM, Dipl ECVECC
Goulburn Valley Equine Hospital, Australia, Affiliated to University of Sydney, Australia, Affiliated to University of Melbourne, Australia

As vets attending a trauma patient, we tend to focus on the wound, and often systemical effects of trauma are not or only late in the workup considered. A trauma team approach is common in human medicine. On the contrary, in equine medicine often a single vet attends an injured horse. However, in my experience a multi-disciplinary approach would benefit the patient. Horses have different types of injury and often more localised trauma with less systemical impact than human trauma patients, but there is lots to learn from the human trauma experience, especially for the less common multi-trauma equine patient.

Systemic repercussions of severe trauma can follow the direct traumatic impact, for example following thoracic, abdominal or birth trauma events directly damaging or affecting certain organs. However, they can also be caused more indirectly through haemorrhage, infections, systemic inflammatory response syndrome (SIRS) and multi-organ dysfunction (MODS). Each of these problems has been shown in human trauma medicine to have a significant negative impact on the length of hospitalisation and mortality. Currently, there is very little equine data on this topic.

Haemorrhage can lead to significant compromise of the patient due to loss of circulating volume, oxygen carrying capacity and serum proteins. Due to reductions in oxygen delivery to the tissues cellular and organ damage develop, which can further develop into organ dysfunction and failure. One of the organs that can be significantly affected by blood loss is the heart, and myocardial damage with elevated cardiac troponin concentrations and arrhythmias has been described in people and horses with severe haemorrhage. Severely haemorrhaging patients can develop 'the triad of death': a vicious circle with 3 elements (acidosis, hypothermia, coagulopathy) that impact eachother negatively until eventually death occurs.

Local infection or sepsis can also develop. Humans that develop infections after trauma have worse clinical signs on admission, higher mortality and more concomitant complications with organ dysfunctions also noted in organs different than the primary infection.

Following severe trauma, the body often responds with an overwhelming cascade of inflammation, called **SIRS**. Through this pro-inflammatory state SIRS (or SIRS plus sepsis) and the consequences of haemorrhage **multi-organ dysfunction (MODS)** or **multi-organ failure (MOF)** can develop and eventually death. MODS is a fairly new term used in equine medicine, yet, the principle of organ dysfunction has been recognised since long in horses with endotoxemia (colic, colitis, pleuropneumonia,….). However, despite its high incidence in human trauma patients it is often not considered in equine trauma cases. Common organs developing dysfunction or failure in human trauma patients are the respiratory system, the cardiovascular system, the neurological system, coagulation, and liver and kidney.

The **pathophysiology of MODS** is not yet fully understood but tissue damage and bleeding cause a pro-inflammatory state and tissue hypoxia. Critical body parts that are affected by these processes and that seem to be a central driving force in the ongoing vicious circle of MODS are vascular endothelial damage, mitochondrial damage and intestinal barrier injury. There are different **scores** used in human trauma medicine to assess the severity of the trauma or its complications. One of the most commonly used ones is SOFA (Sequential Organ Failure Assessment). SOFA assesses organ dysfunction in the ICU, helps triaging patients, has a good prognostic value, and recognition of MODS through SOFA improves outcomes. A similar score has been developed for horses with

acute surgical GI disease: the MODS SGI score. This score also has shown prognostic value in this subgroup of horses.
The **outcome** is not just determined by the fact whether or not a trauma patient develops MODS, but also how severe it is (higher mortality with more severe SOFA scores) and how long it takes to resolve. Compared to early MODS resolution following trauma, human patients with prolonged MODS resolution have different organ types involved, a much more gradual development and later peak of the maximal organ dysfunction, higher mortality rate, higher infection rate and longer hospitalisation duration.
Human trauma patients have a **trimodal mortality distribution** with immediate deaths, early deaths (hours following the trauma) and late deaths. Such a pattern has not been described in horses with trauma. Uncontrollable haemorrhage is the major cause for the immediate deaths, and MODS is a major cause of the early and especially the late deaths.
Negative prognostic factors in human trauma patients are, as expected, around severity of trauma, severity of MODS and presence of the triad of death. Not much data is available on prognostic parameters in severe equine trauma. One study described a 67% survival rate following traumatic hemoperitoneum, with tachypnoea being a negative prognostic factor. A second study reports a survival rate of 62% in horses that are hospitalised after a traumatic brain injury. Poor prognostic factors identified in this study were high PCV, recumbency for 4 hours or longer after admission and a fracture of the basilar bone. The pathophysiology of development of MODS after traumatic brain injury is however slightly different than in other forms of trauma. Through a surge of catecholamines a **neurogenic shock** with cardiac dysfunction and/or pulmonary oedema can namely develop.
In a small subgroup of human trauma patients that need ICU for more than 14 days **Persistent inflammation, immunosuppression and catabolism syndrome (PICS)** can develop. Clinically this leads to poor wound healing and recurrent infections (often with multidrug resistant bacteria) and weight loss. They often have chronic elevations of inflammatory markers (CRP) and persistent low lymphocyte counts.
Through several equine case studies, the occurrence of these human post-traumatic syndromes is demonstrated in equine trauma patients.

NOTES

15.00 – 15.45 NEUROLOGY: Integrative therapy for the neurologic patient - G. Touzot-Jourde

Gwenola. Touzot-Jourde, DVM, phD, Diplomate ACVAA/ECVAA, Associate Professor in Anesthesia and Analgesia, Oniris VetAgroBio, National College of Veterinary Medicine, Food Science and Engineering, Nantes

What is integrative medicine ?

The term "integrative medicine" emerged in the late 90s in human medicine. It has been defined as a philosophy of care that integrates conventional allopathic medical treatments with complementary and alternative modalities not typically included in conventional care and addresses the physical, emotional, and spiritual needs of the patient.[1] In 2005 the Consortium of Academic Health Centers for Integrative Medicine defined integrative medicine as the practice of medicine that reaffirms the importance of the relationship between practitioner and patient, focuses on the whole individual, is informed by evidence, and makes use of all appropriate therapeutic approaches, health-care professionals and disciplines to achieve optimal health and healing. It has made its way in veterinary medicine and is quite preferred compared to the terminology of "holistic medicine". If the literal translation "entire or whole" of the Greek root "holos" is taken into account, it doesn't look that different from integrative. However, the holistic approach has been often associated to strictly alternative medicine in opposition to conventional "western" medicine.

The main features of veterinary integrative medicine include

- The promotion of the animal well-being / health / welfare (preventive medicine and care) encompassing the physical, mental and social dimensions,
- A real, prolonged/long term partnership between the vet and the owner/caregiver/rider and a long-term follow-up of the horse with well documented medical record and periodic reappraisal of the client objectives
- A team approach to benefit from multidisciplinary competencies (fig. 1)
- A sound clinical reasoning leading to a precise and documented diagnostic
- An array of therapeutic modalities and care measures tailored to the individual horse and owner taking into consideration living and working environment
- The use of evidence-based medicine principles and scientific resources.[2]

This description fits with the everyday "regular" vet tasks but highlights the necessity to take a step back at the end of clinical evaluation/reasoning to review the process and reassess diagnostic and therapeutic choices with a widened angle in the overall horse life/career. This type of veterinary practice may not be applicable to all case presentations and owner complaints. It is easily imagined for sports medicine when the goal is to help the equine athlete have the best prolonged performance possible. In case of a more circumscribed clinical presentations, the emphasis may be to promote disease resolution and restore the animal well-being/ well-feeling with an integrative approach combining conventional and complementary therapeutic modalities. The distinction between complementary and alternative therapeutic intervention is not well defined. It could be interpreted in the context used : complementary as an added modality to a medical treatment and alternative as the replacement. With this interpretation, integrative medicine uses more complementary than alternative treatments, the objectives being to obtain an added effect up to a synergistic result. A non exhaustive list of complementary modalities are presented in table 1.

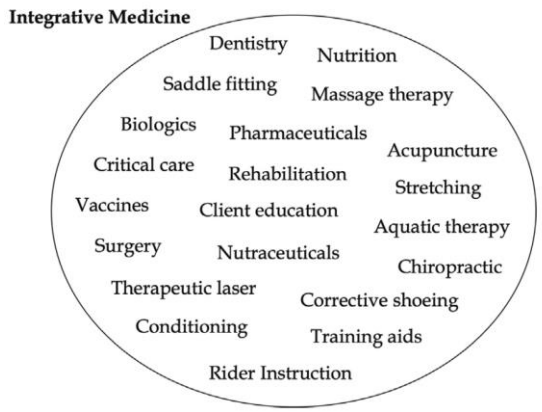

Fig. 1. Illustration of the different tools that might be available to the equine practitioner under the umbrella of integrative medicine (From K Haussler Vet Clin Equine 38 (2022) 445-453)

TABLE 1.
Complementary Therapies Commonly Integrated in Veterinary Medicine

Acupuncture
Nutrition, nutraceuticals, and herbal medicine
Laser therapy
Rehabilitation and sports medicine
Regenerative medicine (ie, stem cell or platelet therapy)
Hyperbaric oxygen
Homeopathy
Therapeutic massage
Veterinary spinal manipulation

Table 1: Integrative medicine services in veterinary hospitals include various combinations of therapies (From J Shmalberg February 17, 2016 Today's Veterinary Practice)

A focus on selected complimentary therapeutic modalities: evidence-based data
As complementary therapies are becoming more readily available et more commonly used, scientific evidence of their effects is starting to be available in horses. Early studies most often lack a control group but scientific quality is improving over time.

Acupuncture has been shown to decrease cervical stiffness and pain in horses as well as back pain. Through pain relief and decreased muscle spasms, acupuncture treatments may allow the horse to properly use his back and neck and strengthen muscles of spinal stabilization.[4] the study didn't investigate the effect of acupuncture on the degree of ataxia but it has been shown in dogs to decrease it by one degree. Acupuncture and electroacupuncture can be integrated in the management of peripheral neuropathies. One study evaluated a positive effect on low grade recurrent laryngeal paralysis especially if the therapy was initiated quickly upon identification of clinical signs in the acute phase of the condition. The use of transcutaneous and percutaneous electrical stimulation has been investigated to treat trigeminal neuralgia with variable results. A retrospective study on suprascapular neuropathy documented the use of acupuncture in the therapeutic interventions.

A systematic literature review on Laser therapy in veterinary medicine has recently been published. [5] More than 2500 abstracts were identified in the electronic databases but only 125 were reviewed leading to 50 studies on the musculoskeletal system, 28 in dogs and 22 in horses. The overall study quality was judged low due to lack of description concerning the laser treatment, other therapies used concomitantly and absence of a control group. Good quality studies in dogs showed the decrease need for pain medication and lameness improvement. Unfortunately, studies in horses focused on tendon and ligament healing and are mostly inconclusive. Pain associated with these lesions was not evaluated. One small study showed no improvement of the lameness evaluated subjectively. Two studies showed positive results for the treatment of back pain. Pain was also diminished in horses suffering from laminitis and analgesia was prolonged by a laser treatment following an epidural anesthesia injection. Only studies in dogs were available for the treatment of neurological disease. A positive effect was found for intervertebral disk disease with a treatment in the postoperative period and also in an experimentally induced sciatic nerve injury. Although still scarce, the evidence of a positive effect of laser therapy is progressing. Better descriptions of the treatment and laser characteristics are still needed.

The neurologic horse

Before illustrating how to use integrative therapeutic modalities, it seems indicated to define the clinical symptomatology of the neurologic horse and if accessible obtain a causal diagnostic. It is then possible to determine the objectives of the therapeutic management. Neurologic signs may be central (brain and spinal cord) or peripheral (peripheral nerve deficit).

The integrative approach is appealing in extreme cases especially encountered with central neurologic disease that often results in trauma and recumbency. Complications due to recumbency can be mitigated by intensive physical care and manual therapy (appropriate nutrition and feeding, padding and skin care, physical therapy through massage and mobilization). Pain from the disease but also secondary pain from trauma and recumbency can also be diminished by an association of analgesic treatments and complementary modalities (laser, acupuncture and electroacupuncture, therapeutic ultrasound, massages). Anxiety and sleep disturbances should be taken into consideration during long hospital stay or immobilization as well as the social environment when mentation is normal. Cervical spinal compression resulting in pain, variable degree of ataxia and locomotion dysfunction is also a good terrain to apply the principle of integrative medicine. A thorough examination and the use of advanced imaging techniques allow to have a good localization of pain and dysfunction. Long term management may include regular intra-articular or periarticular injections of corticosteroids, PRP but rely also on a real rehabilitation program and maintenance of specific exercises to minimize pain while improving flexibility, muscle strength and neuromotor control.[6] A large variety of complementary modalities can be used to decrease pain and improve neck function : acupuncture, electroacupuncture and transcutaneous nerve stimulation (TENS), photobiomodulation (low-level laser therapy), pulsed electromagnetic field therapy, extracorporeal shockwave therapy, manual therapies like massages, mobilization and myofascial release techniques.[7] Proprioceptive stimulation with elastic bands, leg accessories and specific exercises on ground or in water can also used.

Finally, electroacupuncture, TENS and low-level laser therapy (LLLT) can be incorporated in the management of peripheral nerve injury and/or dysfunction. Neuromuscular stimulation can minimize muscle atrophy and promote nerve healing. LLLT promote tissue healing through pain relief, decreased inflammation and edema. Their use has been reported for cranial nerves (facial and trigeminal nerves). They may also be useful for suprascapular, radial and femoral nerve injuries. Case examples of integrative therapeutic management will be presented to illustrate its usefulness in treating the neurologic horse.

Conclusion

Integrative therapy for the neurologic patients encompasses a lot of various modalities that includes instrumental therapy but also manual therapy and human care. The integrative approach is appealing on basis of its paradigm to give the best care by promoting health and preventing disease for the animal well-being. The complementary therapeutic modalities are mostly non-invasive treatments, produce no residues and may allow to reduce drug administration. As sports and integrative veterinary medicine is rapidly developing, more study proving their efficacy and describing treatment regimen for specific disease like the neurologic horse should be available.

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NOTES

16.00 – 16.45 NEUROLOGY : An update on headshaking management - V. Roberts

Veronica Roberts, MA(Oxon) MA VetMB(Cantab) PhD PGCert(HE)DipECEIM FRCVS
European and RCVS Specialist in Equine Internal Medicine, Senior Lecturer in Equine Medicine, University of Bristol (clinical work at B&W Equine Hospital)

Objectives:
We will focus on trigeminal-mediated (TGM) headshaking. We will cover the importance of making a diagnosis. We will consider why might we able to treat TGM headshaking and why it is hard to treat, along with the challenges to the objective assessment of response to treatment. We will cover the published treatment options – those with no proven efficacy, those that might work and try to look into the future!

TGM Headshaking
Just a reminder that this is an acquired condition. The median age of onset is 9 years but with a wide age range. TGM headshakers usually show classic signs of vertical shaking, sharp vertical tics and nasal irritation such as snorting, nose-rubbing and striking at the nose (both sides of the nose). Signs are worse at (any) exercise but may also be present at rest. Signs may be seasonal, there is a complex and not understood, association with environment. There is no gross pathology (Roberts et al, 2017). The infraorbital branch (and the other branches?) are sensitised, firing at too low a threshold (Aleman et al 2014). We think this then results in neuropathic pain (which in people is usually described as varying occurrence and intensity of burning, pins and needles, electric shock like pain).

Importance of diagnosis
There are many reasons why a horse might shake its head more than normal. This affects 4% of the UK equine population with 1% (25% of the 4%) seeing a vet (Ross et al 2018). TGM headshaking is a diagnosis of exclusion, which is imperfect, mostly with a risk of over-diagnosis. History and observation are hugely important for your index of suspicion for TGM headshaking. Of horses that are referred for investigation, 90% receive a diagnosis of TGM headshaking. 8-10% have another diagnosis found on CT. 1-2% have another diagnosis found on endoscopy, oroscopy / oral exam, ophthalmic exam. This may be different in cases with known seasonality.

Why we might be able to treat TGM headshaking
The condition appears to have a 5% spontaneous remission rate and a 25 – 64% seasonal remission (depending whether remission is classed as complete absence of signs or just improvement). The nerve appears to be sensitised, firing at too low a threshold and appears to be a functional, not structural abnormality (so could be reversed?).

Why is it hard to treat?
Neuropathic pain is hard to treat even in people. We know little about TGM headshaking, with an incomplete understanding of trigeminal nerve sensitisation, and incomplete understanding of its role in the aetiopathogenesis of trigeminal-mediated headshaking. There may be more than one cause with the same clinical manifestation.

Other challenge to developing treatment
We need an objective measure of headshaking in order to be able to validate treatments (Roberts et al, presented ECEIM 22) as there is a significant placebo effect – about 30% (Talbot et al, 2013; Pickles et al, 2011). HRE-S scoring system useful (in press, Kloock et al)

Treatments
We will consider published treatments; first those with no proven efficacy, then those with some proven efficacy. We will not venture into the minefield of unpublished treatments (although happy to discuss).

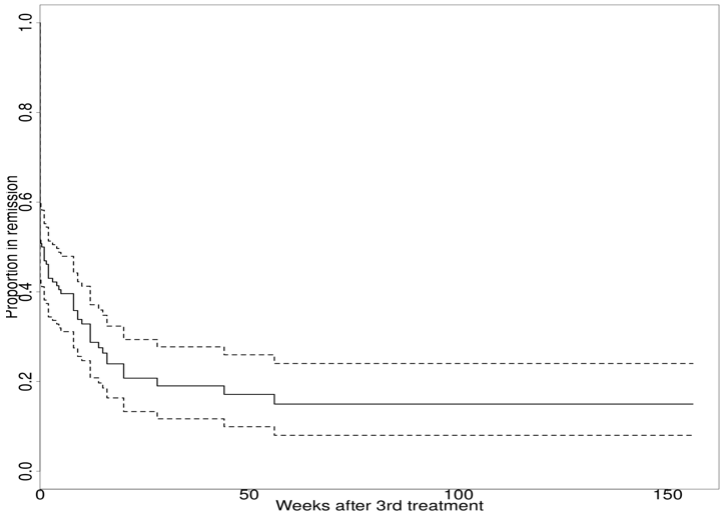
Treatments with no proven efficacy

Feed supplement
Talbot et al, 2013. Double-blinded placebo controlled trial of a popular headshaking supplement. There was no effect of the supplement or placebo as judged by vets blinded to whether the horse was pre-treatment, on treatment, and on which treatment. There was a significant placebo effect to the owner of both supplement and placebo.
Hormone vaccine
Pickles et al, 2011. Trialled a Gonadotrophin releasing hormone vaccine because some studies found more geldings affected. Trialled on15 horses with no actual improvement but 1/3 owners reported feeling horses improved. There were 4/15 vaccination reactions.
Pulsed high-dose dexamethasone
Tomlinson et al, 2013. No response, which is understandable now we have shown there is no inflammation in the nerve.
Various
Madigan and Bell, 2001. Owner survey with owner reported diagnosis and outcomes from various treatments. Despite 109 horses, nothing stood out from antihistamines (1/16 responded), antimicrobials (2/11 responded), corticosteroids (3/20 responded), nonsteroidal anti-inflammatory drugs (0/6 responded), melatonin (2/7 responded), chiropractic treatment (1/28 responded, 4/28 responded slightly), acupuncture (4/26 responded, 6/26 horses responded slightly).
Treatments with some proven efficacy
Nosenet
A nosenet is the first treatment to try, being cheap, non-invasive, risk-free, and can compete at most levels and in most disciplines (but definitely not all). Reported to provide up to 70% relief (not always enough in a badly affected competition horse) in 25% cases (Newton et al, 2000). I advise to try 3 types of nosenet although be aware of competition rules. The mechanism of action might be similar to gate control theory (but actually maybe gate control theory isn't right?). In gate control theory, the substantia gelatinosa in the dorsal horn of spinal cord acts as gate control system. This modulates the synaptic transmission of nerve impulses from peripheral fibres to the nervous system. Small nocioceptive fibres hold the gate in an open position. Stimulation of large mechanoreceptive fibres by touch, pressure or vibration, close the 'gate' and inhibit pain transmission to the brain. Small nocioceptive fibres have a higher activation threshold than larger mechanoreceptive fibres. Selective low-level stimulation of mechanoreceptors can prevent or reduce pain transmission. Activation of the inhibitory pathway results in release endogenous opioids and other neurocompounds. Use of a facemask is not published. Covering the eyes could reduce stimulation of the ophthalmic branch trigeminal nerve. If an eye mask alone effective, can try tinted contact lenses for (high level – expensive – need dispensation and have to place without sedation and local!) competition. In my experience rarely enough on its own. Try also a full face mask.
Pharmaceuticals
Madigan and Bell, 2001; Newton et al, 2000; Davis et al, 2007; Mullen at el, 2013. Cyproheptadine and / or carbamazepine have been published with mixed results. Gabapentin is published for neuropathic pain in the horse but not headshaking (and ? dose). Pre-gabalin pharmacokinetics is published. In people with neuropathic pain these drugs have inconsistent results with some individuals responding well to one drug, not to another. Results can be short-lived – but response to medication can aid diagnosis even if the results are short-lived. Some people have side-effects such as drowsiness (can wear off with time). In trigeminal-mediated headshakers these drugs have inconsistent results or have not been published. Results may be short-term. Pharmacology, and therefore dosing regimen, is uncertain in horses. Some horses may be affected by drowsiness (safe to ride/handle?). Some individuals can respond well. I do two week trials in appropriate cases – starting at the top dose rate is the most efficient.
Homeopathy
Mathie et al, 2010. 20 horses treated,15 horses had follow up,93.3% owners felt their horses were improved.

Sodium cromoglycate eye drops
Stalin et al, 2008. Mast cell stabiliser. Reported as effective in three seasonally affected horses, returning them to ridden exercise, leading to a proposal that allergic conjunctivitis is the cause of headshaking - but conventional allergy is not the cause in the majority of headshaking cases. Trialling treatment is low risk.
Magnesium (and boron)
Sheldon et al 2019. Oral magnesium (citrate) and boron supplementation reduced headshaking (in 6 horses judged in walk and canter for 5 minutes). Feeding concentrate had an effect although less so. This has led to a Platinum Performance supplement of magnesium and boron in the US. Magnesium acts on NMDA receptors, 'calms nerve firing'. Boron aids uptake of magnesium. Boron is not available as a foodstuff for animals in the EU or UK - but it is the magnesium we want. Magnesium supplementation appears to have a minor effect in my caseload, but this is a caseload of non-responders (or they wouldn't come to see me) with mostly severe signs.
Surgery
Mair, 1999. Bilateral infraorbital neurectomy resulted in success in 3/19 (15.8%). 42% cases had increased severity of headshaking with 84% self-trauma to muzzle for 3-8 weeks and 1 neuroma. So avoid, but good work to evidence nerve involved.
Roberts et al, 2009. Caudal ablation of the infraorbital nerve gave about a 50% success rate in 57 horses, but 26% relapsed with median remission 9 months (2 months to 5 years). Side effects of nose-rubbing in most. Mostly short-term but 4/58 horses euthanased due to severity and/or non-resolution of side effects. Avoid now we have better.
PENS neuromodulation
Percutaneous Electrical Nerve Stimulation PENS (Algotec Research and Development UK Ltd.) neuromodulation is a minimally invasive technique which can be used under UK NHS NICE guidelines for the management of neuropathic pain in people. There are blinded trials showing reasonably efficacy, variable between individuals, but no basic science work on the technique. The technique involves placing an electrically conductive probe over the offending nerve and stimulating it at various frequencies and voltages for a set period of time. Anecdotal advice is to try 3 treatments before being sure PENS neuromodulation does not help you. Importantly, people say once the probe is in place that the procedure is quite pleasant with no reported side-effects other than a bruise at the site of probe insertion.
Roberts et al, 2016. PENS neuromodulation trial. Found the technique was safe, could be performed under standing sedation and returned 5 of 7 horses back to ridden work in the short to medium term.
Roberts et al, 2020. EquiPENS ™ neuromodulation. 168 horses across the UK and Europe August 2013 – November 201, 530 procedures. All used the same equipment and technique, trained by the Bristol team. Complications were reported in 8%, all transient except possibly 1 (not clear if complication or disease progression).

	Complication					
Procedure number	Neuritis	Horse compliance	Haematoma	Dermatitis	Catheter complication	Colic
1	7	2	6	4	3	1
2	6	1	4	2	0	0
3	5	0	5	0	0	0
4	0	0	0	0	0	0
5	0	0	1	0	0	0
6	0	0	0	0	0	0

3.4% suspected neuritis (nerve inflammation), causing a worsening of signs, lasting a few days. Can speed recovery if give corticosteroid – consider if horse suitable for steroid. If have neuritis, tend to have it again at the next procedure. Can pre-treat with steroid next time. We found it was sensible to do 3 procedures before judging outcome. If no remission after 3 can do more, but with reducing odds of success. After 3 procedures there was 53% cases going into remission (judged as return to previous level of exercise), lasting from 2 days (!) to 5 years on-going. There was an average 9.5 weeks' remission (but a bit unfair as half of horses were still in remission at the time of follow up).



If the horse relapses after 3 procedures, they usually (but not always) go into remission after a 4th, usually but not always, for longer with the same pattern for 5th and 6th procedures. Six is the most we have done. There were no predictors for success.
At least until we know what causes trigeminal-mediated headshaking, we will struggle to treat it. We don't know how neuromodulation works, so that makes improving it difficult. Currently, EquiPENS™ is the safest procedure with the best results available for horses where a nose-net does not work but it is clearly not the solution to headshaking (will we ever get one?). Other challenges are that it is not available in the US and in some parts of the EU due to BREXIT.

Electroacupuncture

Devereaux, 2017. Electroacupuncture also provides percutaneous stimulation of the nerve. It has been published in 6 horses as an additional treatment along with face masks/nose nets, giving a median remission time to 3rd treatment of 2 ½ weeks.

Future

Unpublished data (hopefully not for too much longer?). Working with the Chiochetti group at University of Bologna, evidence of cannabinoid receptor expression in the trigeminal ganglion of normal horses, starting work to see if expression is different in affected horses. Could this be a future therapeutic target?

Summary

When managing a case: 1. be as sure as possible of a diagnosis. 2. Add in concentrate, magnesium as part of a holistic approach 3. Trial a nose net – try 3 types 4. Trial a face mask 5. Trial EquiPENS ™.

We need to know more about the disease to move forwards. Possibly we are making progress.

Contact

B&W Equine Vets, Breadstone, Berkeley, Gloucestershire GL13 9HG UK
00441453 811867 (Option 2 Hospital)
veronica.roberts@bristol.ac.uk

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NOTES

STREAM 2 - ROOM: RHONE 3

16.45 – 17.30 How to manage an obese equid - R. Morgan

Ruth Morgan, MA VetMB CertAVP(EM) DipECEIM PhD FHEA MRCVS
SRUC, Roslin Institute, Easter Bush Campus, EH25 9RG
Ruth.morgan@sruc.ac.uk

It is widely accepted that equine obesity is a growing problem in developed countries with estimates up to 40% of leisure horse populations presenting as overweight or obese. Managing the obese equid is challenging for vets and owners alike. In this session we will address some of the important unanswered questions that still remain in equine obesity. We will discuss the barriers to weight loss in the horse. We will address the different challenges of addressing obesity in the acute laminitic case and in the more routine consultation and we will discuss the role of the specialist in equine obesity management. We will explore how we might balance welfare with weight loss and the physiological impacts of weight loss in the horse.

Defining obesity

In this section we will start by addressing some of the quandaries facing our definition of equine obesity:

- Do we have an accurate definition of obesity i.e. when does fat accumulation impair health?
- How does obesity present and how can we assess different phenotypic presentations?
- What are the current limitations in measuring obesity and how can we address these?

What are the barriers to weight loss in horses?

In this section we will discuss the physical, psychological and environmental barriers to weight loss in the horse. Table 1 shows just some examples of these.

Physical	Environmental	Psychological
Laminitis/lameness	Nutrient content of grass	Owner's perceptions of obesity
Weight loss resistance	The livery environment	Owner's perceptions of weight loss
Behaviour changes	Limited control over grazing or stabling	Vet's communication about obesity
	Rugs	Behaviour changes in the horse

Managing obesity in different patients: treatment of the acute laminitic v preventative healthcare

Obese patients present to us in three main guises:

1. The acute endocrinopathic laminitic; first presentation
2. The chronic/sub-clinical laminitic or non-laminitic obese patient; routine visit or another health problem
3. An obese animal presenting to a different service for another health problem; obesity may impact the presentation or treatment of this other problem e.g. orthopaedic disease, requirement for steroid treatment

The acute endocrinopathic laminitic:

- Should we implement immediate dietary modification/restriction in these cases
- What is the aim of any such dietary modification; weight loss or reduction in insulin?

- What are the harmful effects of immediate dietary restriction in these cases?
- What is the best longer term management of these cases?

The chronic laminitic or non-laminitic obese patient; routine visit and the obese animal presenting to a different service:

- Laminitic risk evaluation; what tools can we use. When does it stop being an endocrine problem and just become a foot problem?
- What barriers to weight loss exist and can we overcome them
- When is the weight loss sufficient, how do we quantify our goals?

The physiological impact of managing the obese equid:

- Management changes, dietary restriction and pain as a stressors
- Activation of the hypothalamic-pituitary-adrenal axis and the impact on insulin regulation and laminitic risk

The behavioural/psychological impact of managing the obese equid:

- Management changes, dietary restriction and pain as a stressors
- Food motivation in obesity-susceptible animals

How can we balance weight loss and welfare?

- Enrichment methods for horses
- How can we determine dietary needs?
- Individualising plans for horse and owner

What is the role of the specialist in managing the obese equid?

- How do we market our services?
- Owner compliance and conflicts of interest
- Human behaviour change for animal welfare

NOTES

LECTURES: SATURDAY 28 OCTOBER 2023

STREAM 1 | ROOM: PASTEUR AUDITORIUM

09.45 – 10.15 IMMUNOLOGY: Immune dysregulation of the human skin - A. Nosbaum

Audrey Nosbaum, MD PhD, Hospices Civils de Lyon, Centre Hospitalier Lyon-Sud, Service d'Allergologie et Immunologie Clinique, Pierre-Bénite cedex F-69495, France
CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, F-69007, Lyon, France

Cutaneous hypersensitivity (HS) reactions are common in humans, and were first classified by Gell and Coombs in 1963. Although old, this classification remains the best (and simplest) classification of allergic and autoimmune diseases. It is organized into 4 types, depending on the immune effector involved. Type I to III reactions involve antibodies, while type IV involves lymphocytes, with an innate or adaptive origin. Actually, only HS reactions involving adaptive immunity are called allergic; the others are called non-allergic HS. Activation of the adaptive immune system induces the generation of an immunological memory, with increasingly severe reactions due to increased affinity for the allergen.

The Gell and Coombs classification has been updated to reflect advances in knowledge of the different types of lymphocyte-mediated inflammation. Type IV reactions now comprise 4 subtypes, from type IVa to IVd. Each type of inflammation is characterized by specific productions of cytokines (interleukin, IL), expressing clinically as specific skin diseases that are sometimes difficult to differentiate:

- Type IVa involves IFNa- and TNFa-producing lymphocytes, found in psoriasis.
- Type IVb involves lymphocytes producing IL-4, IL-5 and IL-13, found in atopic dermatitis.
- Type IVc involves lymphocytes producing cytotoxic molecules (granzyme B, granulysin), found in contact dermatitis.
- Type IVd involves lymphocytes producing IL-17 and IL-22, also found in psoriasis.

The concept of "endotype" is based on this characterization, defining the immunological mechanism underlaying a pathology, conversely to "phenotype", which is merely its clinical description. With the development of targeted biotherapies against different cytolokines or ILs, the current challenge is to improve endotype diagnosis to develop precision medicine for human cutaneous hypersensitivities.

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10.15 – 11.00 IMMUNOLOGY: equine immune-mediated skin diseases - D. Pin

Prof Didier Pin, Dermatology and Dermatopathology
VetAgro Sup Campus Vétérinaire de Lyon, 1, Avenue Bourgelat, 69280 – Marcy l'Etoile, France

In a broad sense, immune-mediated diseases include autoimmune diseases, autoinflammatory diseases and hypersensitivities (HSs) or allergies.
There are several controversies in equine dermatology, particularly about HSs or allergies. One of the mean to better understand equine immune-mediated is comparative pathology.

Hypersensitivities or allergic skin diseases
The most frequent hypersensitivies or allergies in the horse are insect bite hypersensitivity and urticaria. Other regularly mentioned HSs are atopic dermatitis, food allergy and pastern vasculitis. Very rare HSs, non described here, are allergic contact dermatitis and adverse drug reactions.

Urticaria and angioedema

According to the revised nomenclature for allergy, urticaria belongs to the type I HSs. Hypersensitivity is defined as reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose talerated by normal subjects. Then, allergy is a hypersensitivity reaction initiated by immunologic mechanisms (specific antibodies or T lymphocytes). The term nonallergic hypersensitivity is proposed when immunologic mechanisms cannot be proven (Johansson et al., 2001).
Urticaria is a condition characterized by the development of wheals (hives), angioedema, or both. Urticaria is classified based on its duration, as acute or chronic, and the role of definite triggers, as inducible or spontaneous. Acute urticaria is defined as the occurrence of wheals, angioedema, or both for less than 6 weeks. Chronic urticaria is defined as the occurrence of wheals, angioedema, or both for more than 6 weeks (Zuberbier et al., 2022).
Inducible urticaria is characterized by definite and subtype-specific triggers of the development of wheals, angioedema, or both. These triggers are definite because wheals, angioedema, or both always occur when the trigger is present and and never occur when it is absent. These triggers are specific because each subtype of inducible urticaria has its relevant trigger, for example cold in cold urticaria, and this trigger is not relevant in other forms of inducible urticaria (Zuberbier et al., 2022).
Urticaria is a predominantly mast cell–driven disease. Histamine and other mediators, such as platelet-activating factor (PAF) and cytokines released from activated skin mast cells, result in vasodilatation, plasma extravasation, and sensory nerve activation, as well as cell recruitment. Histologically, wheals are characterized by edema of the upper and mid dermis, with dilatation and augmented permeability of the postcapillary venules as well as lymphatic vessels of the upper dermis. Skin affected by wheals shows a mixed inflammatory perivascular infiltrate of variable intensity, consisting of T cells, eosinophils, basophils, and other cells. Vessel-wall necrosis, a hallmark of urticarial vasculitis, does not occur in urticaria Zuberbier et al., 2022).
Acute urticaria, when it is self-limiting (i.e. not associated with other organs symptoms), does not require a diagnostic workup apart from anamnesis for possible trigger factors. But acute urticaria could be part of anaphylaxis. Sensitized patients produce specific IgE which binds with strong affinity to FceRI receptors on the surface of mast cells. Some examples include generalized urticaria/anaphylaxis following bee stings in patients with hymenoptera allergy and some food- and drug induced urticaria/anaphylaxis. Because specific IgE binds to mucosa and skin mast cells and to blood basophils, IgE-mediated urticarias usually are associated with systemic symptoms involving the lung and the gastrointestinal tract and ultimately leading to anaphylactic shock (Zuberbier et al.,

2022). Therefore, in the absence of systemic symptoms, the probability that urticaria is IgE-mediated is very poor.
Although the pathogenesis of CSU is not yet fully understood, it is well established that its signs and symptoms are due to the activation of skin mast cells and release of mediators but not in the same way as in anaphylaxis (Hennino et al., 2006). Substantial progress has been made in dissecting the 2 main autoimmune mechanisms that drive the pathogenesis of CSU (Kolkhir et al., 2022). These CSU endotypes have been named on the basis of Gell and Coombs classification of hypersensitivity reactions. Because type I hypersensitivity is characterized by the aberrant production of IgE antibodies, patients harboring IgE autoantibodies have been classified into the type I autoimmune endotype of CSU (type I aiCSU), also called autoallergic CSU. This endotype is associated with IgE antibodies against autoantigens, for example, thyroid peroxidase and IL-24 in contrast to classical type I hypersensitivity and allergy, which involve exogenous allergens. Similarly, type IIb hypersensitivity is characterized by an antibody-dependent process in which specific IgG antibodies bind to autoantigens to create pathogenic states. Therefore, patients with CSU who harbor IgG autoantibodies have been classified into the autoimmune type IIb endotype, different to type IIa that involves cytolytic destruction of targeted cells. Type IIb autoimmune CSU is mediated by autoantibodies directed against IgE and FcεRI. A subpopulation of patients with CSU has both types. Both of these CSU endotypes share the same phenotype, that is, the recurrence of itchy wheals, angioedema, or both, without worsening, each day or several times a week (Kolkhir et al., 2022).
In humans, the first steps of standard treatment involve second-generation H1-antihistamines (sgAHs) followed by dose escalation up to 4-fold of the same sgAH in case of no or partial response (Zuberbier et al., 2022).

Atopic dermatitis

Our understanding of equine AD is largely an extrapolation of what was known about AD in the dog 2 decades ago (Marsella et al., 2023). Currently, in humans and dogs, AD, or atopic eczema, is defined as a common inflammatory skin disorder characterised by recurrent eczematous lesions and intense itch that spontaneously develops with nearly identical clinical phenotypes. Its pathogenesis is complex and involves a strong genetic predisposition, a dysfunctional epidermal barrier, skin microbiome abnormalities, and T-cell driven inflammation (Langan et al., 2020, Marsella et al., 2021). Although type-2 mechanisms are dominant, there is increasing evidence that the disorder involves multiple immune pathways (types 22, 1, and 17) (Olivry et al., 2016). Current pathophysiological concepts of AD highlight disruption of the epidermal barrier leading to increased permeability of the epidermis, pathological inflammation and percutaneous allergic sensitization. Functional disruption of the epidermal barrier is the primary pathogenic process in AD, thus facilitating the action of irritants and microbes and, rapidly, the recognition of environmental allergens, and initiation of an inflammatory cascade.
When this definition is used in the horse, we must notice that robust data are lacking for AD in horses, particularly no set of criteria has been developed in this species. In the absence of a typical clinical phenotype, it is impossible to diagnose AD in the horse while diagnosis of AD is based on clinical signs (characteristic eczematous lesions) and the exclusion of other diagnoses (especially insect bite hypersensitivity, ectoparasite infestation and some infectious skin diseases). Urticaria, urticaria with pruritus, or pruritus alone are the three common presentations of AD (Marsella et al., 2022). But AD, or atopic eczema, is not urticaria. Intradermal tests or serological testing for allergen-specific IgE are of no use in the diagnosis of AD. Furthermore, in humans and dogs, cases clinically indistinguishable from AD in the absence of detectable allergen-specific IgE are seen in a significant number of cases. This fact suggests, among others, that IgE play a minor role, if any, in the pathogenesis of AD. It may be that IgE formation is primarily an epiphenomenon that develops secondarily to severe skin inflammation (Tsakok et al., 2019). It is important to differentiate between sensitization, defined as

the detection of IgE antibodies to a specific allergen through either serum or skin-prick testing (SPT), and the presence of clinical allergic disease.
There is little evidence available in the equine literature on allergic diseases other than those associated with insects. After IBH has been strictly eliminated, cases with a chronic inflammatory disease must be divided, according to their clinical presentation, in urticaria or eczematous skin diseases. The aim is to define a typical phenotype of equine AD in accordance of what we know about AD in human and dog.

Food allergy

In humans, a 2010 Expert Panel Report sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), defined food allergy as « an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food » and food intolerance as « a nonimmune reactions that include metabolic, toxic, pharmacologic, and undefined mechanisms » (Boyce et al., 2010).
Food allergies are classified into those that are IgE mediated, those that are mediated by both IgE-dependent and IgE-independent pathways (eosinophilic gastrointestinal disorders, such as eosinophilic oesophagitis), and those that are not IgE mediated (food protein-induced enterocolitis syndrome, food protein-induced proctocolitis, and food protein enteropathy). IgE-mediated food allergy is characterized by the association of pruritus, urticaria, angioedema, abdominal pain, vomiting, wheezing, and hypotension, called anaphylaxis, and associated with the risk of severe or fatal reaction (Yu et al., 2016).
Classically, food allergy was thought to originate in the gut, through a failure in oral tolerance mechanisms. Recent studies have demonstrated that sensitization can occur through the skin or airway. Cutaneous sensitization explain the association between the disruption of the skin barrier in AD and food allergy. The dual allergen exposure hypothesis proposes that oral exposure to food allergens leads to tolerance, while cutaneous or airway exposure, in the absence of oral exposure, leads to the development of food allergy (Kulis et al., 2021).
All adverse events after food ingestion are called food allergy in the horse. Although food allergy is commonly suspected as the cause of urticaria in horses, it is rarely confirmed. Only a few case reports have been published and food allergy is reported in textbooks but well-defined cases or case series are lacking. Food allergy is described as both a pruritic disease as well as cause for urticarial lesions. Neither environmental nor food allergen extracts for intradermal testing in the horse are standardised and no study validates this technique to investigate a suspected case of food allergy (Littlewood and Jackson, 2022). Positive reactions to crude extracts likely represent irritant reactions. Furthermore, elevated specific IgE or a positive skin prick test to a food is an evidence for allergen sensitization but does not mean the subject is allergic to the food, unless that subject develops IgE-mediated symptoms with ingestion of that specific food (Dupont et al., 2016). To study food allergy in horses we need confirmed cases based on rapid recurrence of compatible signs on exposure to a given food, resolution of cutaneous signs after a period on a strict elimination diet, return of signs after provocative dietary challenge (in 1 hour) and subsequent resolution of signs back on the strict diet.

Insect-bite hypersensitivity

Insect bite hypersensitivity (IBH) is by far the most common and the best known allergy in the horse due to an allergic response to the bites of blood-feeding insects; most frequently midge species belonging to genus *Culicoides* although in some cases black fly of the genus *Simulium* have been implicated (Marti et al., 2023).
IBH is characterised histologically by mixed perivascular to diffuse cellular infiltrates of mononuclear cells and eosinophils, and a type 2 inflammation. Beside the Th2 cytokines IL-4 and IL-13, recent studies have shown the involvement of TSLP, IL-5 and IL-31 in the pathogenesis. The importance of type 2 inflammation, more precisely of IL-5 and IL-31 has been confirmed through targeting these cytokines by active immunisation of affected horses, resulting in a significant decrease of lesion (Fettelschoss-Gabriel et al., 2018 ; Olomski et al., 2020).

IBH can occur in horses as young as two years of age and are typically progressive over time so that each season the disease increases in clinical severity. Pruritus can be extreme and leads to severe self-trauma (broken hairs, extensive alopecia , crusting, alopecia, rugal folds, scars) and hyperaesthesia. Affected areas can be dorsal, ventral or both, depending on the feeding habits of the *Culicoides sp.* involved. Body regions classically affected are the ears, face, chest, legs, withers, rump, tail base, inguinal area and ventral midline (Marti et al., 2021). The clinical expression that is neither urticaria nor anaphylaxis suggests a minor role of IgE ad confirms that IBH is a type IVb hypersensitivity.
In practice, the diagnosis is based on history, consistent clinical signs, exclusion of other pruritic diseases, and a positive response to strict insect avoidance either through physical barriers or by use of insect repellents. The efficacy of antihistamines is very poor (Olsén et al., 2011).

Vasculitis

Classically, vasculitis is considered as a type III hypersensitivity, due to immune complex deposition in the bood vessel walls. However, the pathogenesis is more complex and, in horses as in humans, histopathological patterns of vasculitis are varied, including cell poor, lymphocytic or histiocytic, leukocytoclastic and eosinophilic (White et al., 2009). Furthermore, small vessels but also arteries could be involved.
Pastern cutaneous vasculitis is by far the most frequent form of vasculitis in the horse. It is defined as an inflammation of the cutaneous vessels, mainly the capillaries and the small venules of the superficial plexus, in the pastern area, characterized histopathologically by intramural inflammatory cells and hyalinization or fibrinoid necrosis of the vessel walls. There are no apparent age, brees, or sex predilections. The clinical lesions consist of erythema, purpura, erosions, ulcers, crusts, cutaneous thickening, necrosis, and oozing of one to four legs. Lesions are often painfull, sometimes pruritic. The aetiology is unclear and there are few published studies (Risberg et al., 2005 ; White et al., 2009 ; Panzuti et al., 2019). Several causes are recognized such as purpura haemorrhagica, infectious agents, drug reactions and idiopathic vasculitis. Among bacteria, *Staphylococcus pseudintermedius*, coagulase-negative staphylococci, *S. aureus*, *Proteus spp*. and *Pseudomonas aeruginosa* have been isolated in pastern vasculitis. The evolution and the prognosis are unpredictable. Treatment includes topical shampoos and moisturizers, antibiotic therapy, possibly based on a bacterial culture with antibiogram, then, in case of insufficient improvement, topical or systemic glucocorticoids could be added to the treatment.

<u>Autoimmune diseases</u>

The most frequent autoimmune dermatoses in horses are pemphigus foliaceus, cutaneous lupus erythematosus, alopecia areata, and vitiligo. Other very rare and not detailed here autoimmune dermatoses exist in this species, pemphigus vulgaris and pemphigoid, identified with certainty, and a paraneoplastic syndrome which resembles pemphigus and pemphigoid.

Pemphigus foliaceus

Although pemphigus foliaceus is an uncommon disease, it is the most common form of pemphigus and the most common autoimmune dermatosis in the horse (Vandenabeele et al., 2004; Zabel et al., 2005).
There are no apparent breed, sex, or age predilections, and the disease has been reported in horse 2-months to 25-years old. There is no clear triggering factor eventhough the season (fall, winter or summer) and drugs (vaccines, dewormers, supplement) have been suggested in anecdotal reports.
In humans and dogs, circulating autoantibodies target the epidermal transmembrane proteins, desmoglein or desmocollin, leading to subcorneal clefting, acantholysis and pustule formation. The nature of the antigen targeted by autoantibodies in horses remains, to date, unknown.
Skin lesions commonly begin on the face, neck, legs, or ventrum, and frequently become generalized within some days or weeks. In rare cases, the disease is localized to the face or coronary bands. Lesions usually exhibit a bilaterally symmetrical pattern. They consist

normally of pustules that evolve rapidly into more or less annular erosions, sometimes forming epidermal collarettes, covered with thick, multilayered, and adherent crusts. Oozing, matted hair coat, and scaling are frequent. Up to 50% of the cases have varying degrees of edema of the distal limbs and ventral abdomen and inflammation flares may be associated with systemic signs, including depression, lethargy, poor appetite, and fever.
The diagnosis is based on history, physical examination, direct smears, and skin biopsy. Skin biopsies reveal subcorneal, intragranular or subgranular, acantholytic, neutrophilic pustules.
The vast majority of horses require long treatment. It seems that younger horses (1-year old or less) may have a better prognosis. The initial treatment of choice is oral prednisolone (1-2 mg/kg per os, every 24 h). The induction dose should be maintained until the disease is inactive (usually 3 or 4 weeks). Then, the dosage is tapered very slowly. Too fast tapering of the dose is responsible of rapid reccurrence of the disease. Dexamethasone may be used but is not as safe as prednisolone. In generalized or severe forms, it could be advantageous to add azathioprine (1-2 mg/kg per os, every 24h) that allows to taper more rapidly the dose of prednisolone.

Systemic and cutaneous lupus erythematosus

Lupus erythematosus is a rare autoimmune disease in horses and donkeys. Actually, systemic lupus erythematosus in one horse, a lupus erythematosus-like syndrome in one horse and discoid lupus erythematosus in three horses have been documented (Vrins et al., 1983; Scott et al., 1990; Georg et al., 1990).
Systemic lupus erythematosus is a very rare, multisystemic disorder. No age, breed, or sex predisposition are recognized. Cutaneous changes associated with equine systemic lupus erythematosus are pododermatitis, perhaps due to vasculitis, panniculitis, mucocutaneous ulceration, alopecia, leukoderma and scaling of the face, neck, and trunk. Multisystemic signs include polyarthritis, anemia, thrombocytopenia, purpura, uveitis, fever, peripheral lymphadenopathy, depression, poor appetite, and weight loss.
In the dog, cutaneous lupus erythematosus (CLE) encompasses vesicular CLE (VCLE), the disease analogue of human subacute CLE, and chronic cutaneous lupus erythematosus (CCLE). CCLE include facial discoid LE (FDLE), mucocutaneous LE (MCLE), generalized DLE (GDLE) and exfoliative CLE (ECLE).
In the horse or donkey, VCLE has not been described but we have observed and diagnosed cases of FDLE, MCLE, localized and generalized DLE and ECLE.
In CCLE, neither breed nor sex predilection have been reported, age of affected animals ranged from 2- to 14- years of age. Lesions consist of erythema, alopecia, scaling, erosions or ulcers, covered with scales, scars and variable degrees of leukoderma and leukotrichia. Lesions are more or less circular, variably well-circumscribed, coin-shaped or annular, with a central ulcer or erosion covered with an adherent scale, and a annular ring of depigmented, erythematus skin, with a local, regional, multifocal or generalized distribution in localized or generalized DLE. Lesions are frequently more diffuse and ill-circumscribed in FDLE. They worsened with sunny weather. Lesions of the mucosae and mucocutaneous junctions, particularly depigmentation, ulcers and erosions, are predominant in MCLE. Lesions are mainly ulcers and erosions covered with scales in a generalized distribution in ECLE (Mosca et al., 2020). Lesions are generally non-pruritic but may be painfull. However, pruritus may be present in FDLE.
The diagnosis is based on history, physical examination, and skin biopsy. Treatment includes topical potent glucocorticoids or tacrolimus in case of localized forms, systemic glucocorticoids (cf pemphigus foliaceus) or methotrexate for generalized forms.

Alopecia areata

Alopecia areata (AA) is an acquired cell-mediated autoimmune alopecia, of unpredictable evolution. The immune reaction is directed against the follicular bulbs. Alopecia areata has been described in many species: humans, primates, dogs, cats, horses, cows, mice, rats and hens.
The cause is unknown. Genetic, endocrine and psychological factors are involved in humans. Autoantigens are derived from keratinocytes or melanocytes of the hair follicle.

No age, sex or race predisposition has been reported. Alopecia areata is clinically characterized by the sudden or insidious appearance of one or more non-inflammatory alopecic areas, well defined, more or less circular, of 2 to 25 cm in diameter. Alopecic skin looks normal. The alopecic areas can become coalescent, by centrifugal growth. The areas most often affected are the head, mane and tail, but the neck, trunk and limbs can also be affected. Alopecia areata is sometimes generalized (alopecia universalis) (Hoolahan et al., 2013). In horses, severe impairment of all 4 hooves has been described (Bruet et al., 2008). The lesions are neither itchy nor painful. Some cases of "mane and tail follicular dysplasia" are likely cases of alopecia areata.
The diagnosis is based on history and physical examination and confirmed by the histopathological examination of cutaneous biopsies of the center (lesions well established, absence of hair follicles) and the periphery (inflammatory area, lymphocytic bulbitis) of alopecic areas.
The vital prognosis is excellent. The aesthetic prognosis is variable depending on the distribution and extent of the lesions as well as the evolution. In case of spontaneous healing, hair regrowth takes place in a few months to 2 years. It may be finer and clearer than initially. Relapses are possible.
The only damage of alopecia areata being aesthetic, the interest of a treatment has to be discussed. Furtermore, it is only palliative. Minoxidil (indicated in human androgenetic alopecia), recommended by certain authors in localized forms, has not proved its efficacy. Glucocorticoid therapy could be used in extended forms but its efficacy has not been proven and its side effects are deleterious. The daily application of topical corticosteroid, tacrolimus or the monthly intradermal infiltration of a glucocorticoid can be tried in case of a few and small lesions. Ciclosporin should be effective but is not used in horses due to its cost. The alopecic areas must be protected from rubbing and sun.

Vitiligo

Vitiligo is an acquired autoimmune leukoderma of unpredictable evolution. The cause is unknown. In humans, genetic factors and cellular injury of internal or external origin may play a role. In humans, vitiligo is a cell-mediated autoimmune dermatosis, with an inflammatory infiltrate of autoreactive cytotoxic CD8+ T lymphocytes directed against melanocytes. Anti-melanocyte antibodies have been identified in the serum of a horse with vitiligo. Autoantigens are unknown. Vitiligo appears to be more common in the Arabian race but individuals of all races can be affected. Age of onset ranges from 1 to 23 years (Montes et al., 2008).
The lesion is an achromic macule of variable shape and size, well delineated. Lesions may coalesce giving rise to large depigmented areas. The surface of the depigmented skin is normal. Multiple lesions are generally bilateral and roughly symmetrical. Hair and mane can be depigmented. The most frequently affected areas are the muzzle, eyelids, lips, but the anus, vulva, sheath, hooves, neck, trunk, and limbs can be affected. The lesions are neither itchy nor painful.
Diagnosis is based on history and physical examination and may be confirmed by the histopathological examination of skin biopsies of the center (well-established lesions, absence of melanocytes) and the periphery of the achromic areas (inflammatory zone, interface dermatitis).
The vital prognosis is excellent. The aesthetic prognosis is variable depending on the distribution and extent of the lesions as well as the evolution. A few cases of spontaneous improvement or healing have been reported, in about a year. Relapses are possible.
The only damage of vitiligo being aesthetic, the interest of a treatment has to be discussed. Furthermore, it is only palliative. The remarks made about the use of systemic or topical glucocorticoid, or tacrolimus or ciclosporin in alopecia areata apply to vitiligo. Sun protection of achromic areas is essential.

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NOTES

11.30 – 12.15 IMMUNOLOGY: The mechanisms of equine "allergic lung diseases" – focus on equine asthma - S. Sage

Sophie Sage DVM, Dipl. ACVIM, Dipl. ECEIM
Swiss Institute of Equine Medicine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Foreword

What are equine "allergic lung diseases"?

In the veterinary literature, there is no recognized term or entity referred to as "allergic lung diseases". However, websites targeting horse *owners frequently use the terms "allergic lung diseases" or "respiratory allergies" to encompass mild-moderate equine asthma (MEA) and severe equine asthma (SEA). Is this language misuse? To answer this question, we need to go back to the definition of an allergy:*

- *Allergy sensu lato:* disease following a response by the immune system to an otherwise innocuous antigen, including different types of hypersensitivities, most commonly categorized as I to IV.
- *Allergy sensu stricto: type I hypersensitivity with Th2-mediated sensitization and production of allergen-specific IgE.*

Equine asthma (EA) occurs due to an exaggerated inflammatory response to airborne particles, thus warranting classification as an allergy *sensu lato*. Yet, the degree to which EA aligns to an allergy *sensu stricto* is unclear.

Why understanding the immunopathogenesis of EA is important

Understanding EA's immunopathogenesis is crucial for enhanced diagnosis and treatment. In human asthma, tailored treatments suit specific phenotypes; for instance anti-IL5 drugs are effective only for eosinophilic phenotypes. Understanding that EA is primary an inflammatory disorder justifies its treatment with corticosteroids, with bronchodilators and mucolytics as adjuncts. However, some horses do not respond to corticosteroids, or experience undesirable effects. It is thus important to better understand the immunopathogenesis of EA to find safer and more effective therapeutic options for each type of EA.

This presentation provides an overview informed by the latest insights into the pathophysiology of EA, delving into evidence both supporting and opposing the type 2 (Th2)-mediated disease hypothesis.

Phenotypes of equine asthma

Asthma is a chronic, non-infectious, inflammatory disease of the lungs, which occurs naturally in humans, cats and horses. Human asthma is a very heterogenous diseases, with two main categories identified: type 2 (or T2-high) and non-type 2 (or T2-low) asthma (27). Each category comprises several subtypes (27). Type 2 asthma, often referred to as allergic asthma, is characterized by airway eosinophilia. In contrast, non-type 2 asthma can be either neutrophilic or paucigranulocytic (1). In horses, two main disease categories are described: MEA (formerly inflammatory airway disease, IAD) and SEA (formerly recurrent airway obstruction, RAO, or heaves). Both forms are characterized by chronic lower airway inflammation, bronchial hyperreactivity, partially reversible airway obstruction and increased tracheal mucus production. Clinical signs consist in a variable combination of cough, nasal discharge, exercise intolerance, and abnormal breathing effort. Distinction between MEA and SEA in the field is clinical, with severely asthmatic horses displaying increased breathing effort at rest (1). It is still debated whether MEA and SEA represent two different pathological entities or whether they are part of a continuum. Indeed, some MEA-affected horses never develop SEA (2),

but horses with occasional coughing and/or mucous nasal discharge have an increased risk to develop severe signs (3). One argument in favor of two distinct pathophysiological entities was the presence of airway remodeling only in SEA. However, a recent study identified signs of bronchial remodeling in horses with MEA (4), fueling the hypothesis that MEA may represent an early stage of SEA.

A shift towards categorizing the different types of asthma using endotypes instead of phenotypes is now advocated. An endotype describes distinct pathophysiologic mechanisms at the cellular and molecular level (5), which might not necessarily align with the phenotype. Describing these endotypes would be highly beneficial, as it could enable more targeted therapy based on the underlying pathophysiology.

Determinants of equine asthma

Determinants are factors that contribute to or influence the occurrence of EA, which can include both elements that increase the risk (intrinsic and extrinsic risk factors) and those that directly lead to EA (causes). In horses like in humans, we know the triggers of asthma, but the exact cause remains elusive. EA seems to result from a combination of individual susceptibility and exposure to environmental risk factors.

Environmental exposure

EA stems from an exaggerated immune reaction triggered by environmental airborne particles, particularly hay dust, which includes mostly organic elements like endotoxins, fungal components and mites (2). EA is considered a disease of domestication associated with hay feeding (6) and stabling. A less common form of the disease, summer pasture EA, can also occur in response to outdoor mold spores and gras pollen inhalation (7).

Dust exposure is associated with airway neutrophilia in both healthy horses and SEA-affected horses (8). It has also been linked to the neutrophilic (1) and eosinophilic types of MEA (9,10). Endotoxins notably induce neutrophil influx and airway dysfunction, with hay dust challenge exacerbating inflammation and dysfunction beyond endotoxin effects alone, suggesting synergy with other dust elements (11). Stimulation of equine PBMCs and respiratory epithelial cells further substantiates the pivotal role of hay dust in triggering airway inflammation (12,13).

Although the evidence for an involvement of type I hypersensitivity remains conflicting, recent studies showed an association of EA status with heightened antigen-specific IgE levels targeting fungi, mites, pollens, or latex in serum and BALF (14,15). Exposure to mold spores, primarily Aspergillus and Alternaria species, is linked to airway inflammation, evidenced by elevated IgE in serum and BALF (14–18). However, this could merely reflect exposure to moldy hay, potentially also rich in other antigenic particles such as endotoxins. While fungal elements in tracheal wash cytology have been associated with neutrophilic EA (19), this association is absent when accounting for environmental factors (20). Therefore, it is plausible that aerosol exposure predominantly contributes to the fungal presence in the airways.

Infectious diseases

The contribution of bacterial and viral infections to the underlying etiology of EA remains debated. An argument supporting the contribution of respiratory infections to MEA is the reduced prevalence of the condition with advancing age in racehorses, potentially due to immunity development against common infectious agents. Additionally, an association between viral respiratory infections and the development or exacerbation was demonstrated (21), suggesting a potential role of viral infections in predisposing to MEA.

Genetic susceptibility

Individual susceptibility appears to be influenced, at least in part, by genetic factors. Genetic predisposition has been observed in Lipizzans and Swiss Warmbloods with SEA. The mode of inheritance appears to vary among families within the same breed (22). Equine PBMCs have also shown genetic heterogeneity in their response to allergens, further supporting the role of genetic susceptibility (23). Presently, there is no scientific proof regarding the hereditary nature of MEA.

Immune alterations associated with aging

The higher incidence of SEA in older horses might relate to immunosenescence, involving T cell dysregulation and reduced Treg cell proportion. Additionally, inflamm-aging, a chronic inflammation state marked by elevated systemic inflammatory cytokines, could contribute. Although no age-linked cytological trends exist in BALF profiles for MEA and SEA horses, an age-associated rise in IFN-γ-producing lymphocyte frequency in both BALF and PBMCs has been noted in healthy horses (24).

Immunopathogenesis of equine asthma

Immune defenses comprise innate and adaptive responses involving cellular and soluble components. Innate immunity is rapid and non-specific, triggering acute inflammation without memory. Adaptive immunity is slower, antigen-specific, and possesses memory for robust protection. While adaptive responses offer specificity and memory, innate responses initiate and buy time for adaptive defenses to develop.

In normal horses, inhaled particles are cleared from the lung rapidly by innate immune mechanisms, which consist of mucociliary escalator transport by airway epithelia, phagocytosis by neutrophils and macrophages, and drainage into the local lymph nodes via antigen-presenting cells.

Role of the bronchial epithelium

The bronchial epithelium serves as a physical barrier against pathogens. Integrity of epithelial tight junctions is essential to maintain barrier function. In SEA, genes related to tight junctions are downregulated, implying barrier disruption (25). This breach can trigger innate and adaptive immune responses. The bronchial epithelium expresses toll-like receptors (TLRs) and is thus able to recognize various damage- and pathogen-associated molecular patterns. Epithelial cells in SEA show increased expression of IL-8 and TLR-4 (13,26). These are essential for neutrophil recruitment into the lungs, suggesting that epithelial cells contribute to persistent airway inflammation.

Role of the mucociliary apparatus

Tracheal mucus accumulation is a characteristic of EA, stemming from increased mucus secretion and/or impaired mucociliary clearance. Genes associated with mucin synthesis has been found to be either upregulated (27,28), downregulated (25), or unaltered (29). The effect of EA on mucociliary clearance is also debated; while some propose decreased clearance in horses with respiratory disease (30), others note no difference from controls (31). BALF cells from horses with neutrophilic MEA exhibited reduced expression of genes associated with cilia assembly, suggesting compromised clearance. SEA showed more downregulated ciliary function-related genes compared to MEA, indicating inferior clearance in SEA, which correlates with clinical observations (25).

Role of alveolar macrophages

Alveolar macrophages (AMs) are the first responders in the alveoli. They are involved both in innate and adaptative immune responses, through phagocytosis and cytokines production. The proinflammatory cytokines TNF-α, IL-1β, and IL-8 were upregulated in AMs of horses with SEA, while IL-6 was downregulated; hay dust challenge induced the most significant change (32). BALF cell flow cytometry revealed increased CD163 and CD206 expression in SEA exacerbation (33), indicating increased phagocytic abilities.

CD206 also limit proinflammatory reactions against microorganisms. Moreover, increased gene expression of IL-10 was identified in the AMs of severely asthmatic horses, suggesting that at some point in disease exacerbation, an anti-inflammatory process is activated (34). AMs likely adopt an anti-inflammatory phenotype in EA to suppress inflammation during antigen exposure.

Role of neutrophils

Neutrophils play an important role in eliminating pathogens from the lungs through various mechanisms, including phagocytosis, production of cytokines, chemokines, proteases, reactive oxygen species, and neutrophil extracellular traps (NETs) formation. Prompt neutrophil apoptosis is essential to prevent excess inflammation and tissue damage.

The onset of pulmonary neutrophilia occurs as early as three hours after exposure to antigens (8). However, BALF neutrophilia is higher and persists longer in asthmatic horses (35), even when treated with glucocorticoids (36). This has been attributed to delayed neutrophil apoptosis in the asthmatic horses' airways (37,38).

NETosis, a specific form of apoptosis, involves the release of cellular DNA along with antimicrobial peptides and proteases. While their primary function is pathogen elimination, they can also induce tissue damage and sustain chronic inflammation. NET formation is enhanced in the BALF from horses with SEA (39–41). Single-cell RNA sequencing (scRNA-seq) of equine BALF cells found asthmatic horses' neutrophils displayed heightened migratory potential and NET generation capacity. Interestingly, some apoptotic neutrophils exhibited anti-inflammatory traits and reduced survival (41). This suggests that neutrophils play a role as effectors rather than initiators of asthmatic lung inflammation. This idea is also supported by the existence of non-neutrophilic asthma phenotypes, namely mastocytic or eosinophilic MEA.

Role of mast cells

The proportions of mast cells in BALF are linked to exposure to respirable β-glucans, suggesting mastocytic MEA is triggered by specific immunomodulators or allergens, potentially of fungal origin (42). Airway hyperresponsiveness is a prominent feature of mastocytic MEA (2). Mast cell stabilizers like sodium cromoglycate improve clinical signs and reduce bronchial hyperresponsiveness in mastocytic MEA (43), indicating a role of mast cells in airway hyperresponsiveness. Upregulation of genes tied to antigen presentation and complement activation in mixed mastocytic/eosinophilic MEA further support mast cells' inflammatory role (44). FKBP5 overexpression, related to glucocorticoid sensitivity, could contribute to corticosteroid resistance seen in mastocytic MEA (45).

Role of eosinophils

Eosinophilic airway inflammation is relatively uncommon among horses, and more frequently observed in young horses (<5 years old). This particular phenotype appears to correlate with respirable dust exposure (9,10). The presence of peripheral blood eosinophilia in horses with eosinophilic MEA is variable and does not correlate with internal parasitism (10). Since BAL eosinophilia has been linked to airway hyperresponsiveness (46), further investigations should aim to elucidate the role of eosinophils in the pathogenesis of MEA.

Immune response polarization

The three main immune pathways

The convergence of the innate and adaptive immune responses gives rise to three major types of cell-mediated effector immunity, known as Th1, Th2 and Th17-mediated diseases. Because these immune pathways do not rely exclusively on $CD4^+$ T helper (Th) cells, it has been proposed to label them type 1, type 2, and type 3 responses instead (47). Unfortunately, this terminology has not been widely adopted, and the terms have been used interchangeably.

T cell cannot recognize free antigen molecules. They can only respond to antigens bound to MHC molecules on antigen-presenting cells such as dendritic cells. The presence of co-stimulatory cytokines and activation of co-stimulatory receptors on the T cells are required for T cell differentiation. This will determine the differentiation pathways of the T cell (see Table 1).

Table 1: Characteristics of the three major types of cell-mediated effector immunity (*NB*: current knowledge is based mostly on experiments conducted on humans and mice. For example, ILCs have not been described in horses so far.)

	Type 1	***Type 2***	***Type 3***
Common names	T1, Th1, non-allergic	T2, Th2, allergic	T3, Th17
Defense against	Intracellular organisms (e.g., bacteria, viruses)	Helminths, hematophagous fluids (e.g., mosquitoes), venoms (e.g., bees), noxious xenobiotics (e.g., pesticides)	Extracellular bacteria, fungi
Implicated lymphoid cells	Th1, T_C1, ILC1	Th2, T_C2, ILC2	Th17, T_C17, ILC3
Other major effector cells	Macrophages, NK cells	B cells, mast cells, basophils, eosinophils	Neutrophils, fibroblasts, macrophages, endothelial cells, epithelial cells?
Hallmark transcription factor	T-bet	GATA3	ROR-γt
Major polarizing cytokines	IL-12	IL-4, (IL-1)	IL-23, IL-6, (TGF-β)
Major effector cytokines	IL-2, IFN-γ, TNF-α, TNF-β	IL-4, IL-5, IL-10, IL-13	IL-17A, IL-17F, IL-21, IL-22
Mechanisms of action	▪ Cytotoxicity (T_C1 and NK) ▪ Macrophage activation (phagocytosis and production of cytokines, MMPs and NO) ▪ Some IgG production	▪ IgE production ▪ IgA production ▪ Some IgG production ▪ Tissue regeneration ▪ Wound healing	▪ Mononuclear phagocytes recruitment and activation ▪ Neutrophil recruitment and activation ▪ Epithelial antimicrobial response induction
Exaggerated responses	Inflammation, autoimmunity	Allergies (HS1)	Inflammation, autoimmunity

ILC, Innate Lymphoid Cell; T_C, CD8+ cytotoxic T cell; Th, Thelper cell; HS1, type I hypersensitivities

Immune polarization in SEA

Studies investigating the predominant immune pathway in SEA have yielded conflicting outcomes, attributing the disease alternatively to type 2, type 1, type 3, or mixed immune responses (48). SEA has been historically regarded as a type 2 disease, a hypothesis supported by the observation that affected horses are more prone to other hypersensitivities such as insect bite hypersensitivity or urticaria (49,50). Yet an immediate-phase response is lacking in SEA. Instead, a delayed-phase allergic reaction to antigen challenge is seen, with neutrophil recruitment to the bronchial lumen. The Th17 pathway's role has grown prominent, as indicated by mediastinal lymph node analysis (51), serum miRNA dysregulation (52), and comprehensive miRNA-mRNA study in equine lung tissues (53). Upregulation of the Th17-associated CXCL13, a B cell chemoattractant, was observed in equine PBMCs and BALF cells from severely asthmatic horses (12,41). A recent flow cytometry study on equine BALF cells clearly identified a local type 3 response in SEA, marked by increased $CD4^+IL17A^+$ lymphocytes. Of note, modern single-cell techniques showed no elevation of Th1 or Th2-associated cytokines in relation to EA (41,54).

An additional argument against a type 2 response is the reduced fraction of activated plasma cells (capable of IgE production) in the BALF of severely asthmatic horses, despite a higher proportion of total B and plasma cells (41). The larger pool of B cells may foster secondary type 2 responses, predisposing asthmatic horses to hypersensitivities like urticaria. Based on these results, SEA could be primarily driven by a type 3 response characterized by an *IL17*-induced *CXCL13*-mediated recruitment of B cells into the lower airways. The resulting increase in B cell abundance may predispose asthmatic horses to secondary type 2 responses resulting in clinical hypersensitivities.

Immune polarization in MEA

MEA seemingly encompasses diverse subtypes characterized by a specific BALF inflammatory pattern. It is plausible that mastocytic MEA, eosinophilic MEA, neutrophilic MEA and mixed MEA represent distinct endotypes. Among MEA subtypes, horses with mastocytic inflammation (55,56) or eosinophilic inflammation (57) showed heightened expression of the type 2 cytokine IL-4. In contrast, neutrophilic cases displayed greater IL-17 and IL-23 expression (55,58). IL-4 stimulates B cells to switch immunoglobulin isotype to IgE, suggesting that IgE may play a role in the pathophysiology of mastocytic and eosinophilic MEA. This implies a type 2 polarization in mastocytic and eosinophilic MEA, while neutrophilic MEA may align more with a predominant type 3 response, resembling SEA.

Local and systemic responses

Asthmatic horses have activated PBMCs (12) and elevated levels of circulating markers of inflammation in both remission and exacerbation phases (59,60). It remains unclear whether the systemic inflammatory response is a primary factor predisposing horses to asthma or if it arises as a consequence of local pulmonary inflammation. BALF IgE profiles better correlate with asthmatic status than serum IgE profiles (15). Moreover, flow cytometric analysis of PBMCs and BALF cells in asthmatic and healthy horses revealed distinct responses among cell types (54), suggesting a disparity between local and systemic immune reactions. A recent study indicated increased $CD3^+$ T lymphocyte infiltration in duodenal and rectal biopsies of asthmatic horses, possibly due to systemic inflammation or local effects of ingested hay allergens (61).

Role of the microbiome

Lungs were once thought sterile, but the discovery of a respiratory microbiota in humans dispelled this belief. Imbalances in early-life microbiota are recognized as significant asthma risk factors. Notably, the respiratory microbiome has been correlated with specific asthma endotypes, prompting investigations in horses (48). Ongoing research explores the microbiome's role in horse asthma development or exacerbation. Past investigations detected differences in the lung, nasal and oral microbiomes between healthy and asthmatic horses, but only at the taxonomic family level, with an overrepresentation of the Pasteurellacea family. However, such disparities were not

present at higher classification levels, suggesting that these variations may be attributed to inflammation rather than constituting inherent microbiota characteristics (48).

In an experimental model of allergic airway inflammation in mice, administration of probiotics prompted the differentiation of regulatory T cells differentiation and subsequent suppression of Th2-mediated allergic responses. However, the fecal microbiota of severely asthmatic horses does not differ significantly from healthy horses, questioning the potential of probiotics in managing equine asthma (62).

Conclusion

Just like human asthma, EA appears to encompass several endotypes. Both innate and adaptive immune responses are activated in EA, both at the local and systemic levels. The cytokine profiles detected in the blood and BALF of horses with SEA has hinted to either a type 1 (Th1), type 2 (Th2), type 3 (Th17) or mixed immune response polarization, possibly reflecting different severity grades or disease stages. Immune response polarization likely changes over time, with external triggers and individual genetic differences contributing. Recent analyses at the single-cell level using flow cytometry or RNA-seq suggests the Th17 pathway is a key driver of neutrophilic SEA, challenging the Th2-driven (allergic) assumption. Consequently, allergen testing should not be relied upon in SEA. Evidence suggests the Th2 pathway is more relevant to mastocytic and eosinophilic forms of MEA, potentially making them "true" allergic diseases. This requires further investigation, especially at the single-cell level.

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NOTES

12.15 – 13.15 IMMUNOLOGY: Inflammatory bowel disease (IBD) in horses: from the immunological point of view. - D. Jean

Daniel Jean, DVM, PhD, DACVIM (LA)
Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal
(St-Hyacinthe, Canada)

Introduction

Inflammatory bowel disease (IBD) in horses is an idiopathic disorder, encompassing different types of intestinal inflammation. Intestinal involvement can be segmental to diffuse, and different histopathological forms have recognized, namely lymphoplasmacytic, eosinophilic and granulomatous. The exact cause of IBD is still unknown in horses as well as in humans. The pathogenesis of the disease in equine patients remains to be established. In human, IBD results from a complex series of interactions between susceptibility genes, the environment, and the immune system. The host microbiome, as well as viruses and fungi, play important role in the development of IBD either by causing inflammation directly or indirectly through an altered immune system. Epidemiological data suggest an association between IBD and a number of environmental factors, such as antibiotic use, microbial exposure both early and late in life, and possibly diet. In horses, in my knowledge, there is no similar epidemiological data.

In human, various components of the mucosal immune system are implicated in the pathogenesis of IBD and include intestinal epithelial cells, innate lymphoid cells, cells of the innate (macrophages/monocytes, neutrophils, and dendritic cells) and adaptive (T-cells and B-cells) immune system, and their secreted mediators (cytokines and chemokines). Either a mucosal susceptibility or defect in sampling of gut luminal antigen, possibly through the process of autophagy, leads to activation of innate immune response that may be mediated by enhanced toll-like receptor activity. The antigen presenting cells then mediate the differentiation of naïve T-cells into effector T helper (Th) cells, including Th1, Th2, and Th17, which alter gut homeostasis and leads to IBD. In my knowledge, little information is documented on the role and the interactions between the different immune cell populations in IBD horses.

Gut immune system

Inflammatory Bowel Disease (IBD) involves a complex interplay among host genetics, the immune system, intestinal microbiota, dietary constituents, and environmental triggers of intestinal inflammation. There is limited knowledge of these factors in equine IBD, and much of the information comes from studies evaluating human and canine IBD. The gastrointestinal mucosal immune system is highly complex and must be tolerant of food antigen and commensal organisms, but also must be able to respond rapidly and adequately to pathogenic microbes. There are innate physical factors that protect the GI mucosa: the mucosal epithelium and enterocytes, which are held together by tight junctions; mucus secreted by goblet cells, which coats the epithelium; and antimicrobial peptides, secreted by Paneth cells. In addition, there are the various parts of the local immune system, consisting of the gut-associated lymphoid tissue and lamina propria, Peyer's patches, lymphoid follicles, and mesenteric lymph nodes.

Antigen-presenting cells, most commonly dendritic cells, are continuously sampling antigens from the intestinal lumen through pattern recognition receptors. The antigen pattern determines the type of activation of the antigen presenting cell and, consequently, the direction of the adaptive immune response towards eradication or tolerance. For instance, if rotavirus is recognized, the T-helper 1 (Th1) pathway is favored, producing IFN-gamma, which leads to reduced rotavirus replication, the destruction of infected cells, and development of protective immunity. The Th2 pathway is initiated when parasite antigens, such as Parascaris equorum and small strongles, are recognized, and cytokines secreted (e.g., IL-4, IL-5), recruiting eosinophils, basophils

and mast cells. Bacteria such as Salmonella spp. may induce a proinflammatory response, characterized by Th17 and cytokines IL-17 and IL-23. In the case of commensal bacteria, the pattern recognition receptors are thought to stimulate the naïve T cells to differentiate into T regulatory cells, which counteract any proinflammatory cytokines produced by Th17 cells.
A delicate balance exists between inflammation and tolerance in the GI mucosal immune system. Any defect along the entire pathway from the intestinal barrier to the mesenteric lymph node can result in inappropriate and overacting responses. These responds result in intestinal inflammation, characterized by epithelial damage and ulceration, recruitment of inflammatory cells, and villous atrophy.
Mucosal secretory immunoglobulin A (IgA) is a major player of the GI immune system, but has not been extensively studied in the horse. IgA is the most abundantly produced immunoglobulin and accounts for about 80% of the total body immunoglobulin, with primary distribution in the mucosal surfaces. Gastrointestinal IgA is produced in a dimeric form (two IgA molecules joined by a J chain) by the Peyer's patches, lamina propria, and intestinal lymphoid follicles, and is transported trans-epithelially into the gastrointestinal lumen. The IgA intra-luminally binds to pathogens and prevents them from crossing the mucosal epithelial barrier. Equine IgA has been evaluated in serum, mammary secretions, nasal secretions, and gastrointestinal tissue sections.

Digestive Immune and Diet

The diet most certainly plays a role in GI disease and IBD, but limited information is available about this association in the horse. In a recent study, we evaluate the immune cell population in digestives biopsies collected in horses exposed to different diets. Despite not significant, horses fed with a high protein diet showed the highest values for T lymphocytes and T/B lymphocytes ratios in the duodenal and rectal lamina propria (personal data). It was reported a gluten-sensitive enteropathy in a horse suffering from IBD highlights the importance of the diet in the treatment and control of this disease. It was a Friesan stallion, which had originally a very high rhTGA concentration and, after a half a year of gluten-poor diet, showed an improvement in rhTGA antibody concentration, duodenal histopathology and total proteins. The real link between the diet and the development of IBD have to be better documented.

Digestive Immune and Microbiota

With the advent of technologies that sequence the 16S ribosomal RNA (rRNA) found in all bacteria, otherwise known as next generation sequencing, the complex ant diverse equine GI microbiome is being revealed. Most studies have utilized fecal samples, due to ease of sampling and clinical application, although few horses have been studied to date. Diet, stabling and management seem to have effects on the microbiota. Despite the diversity of bacteria found by next generation sequencing in the equine feces, the core microbiome defined as the microbiota consistently identified in healthy horses appears to be relatively small, and about 10-15% of the bacteria identified have been shared among all horses irrespective of feed or geographical location. Colitis horses have a shift in their microbiome towards less diversity, and antibiotics disrupt then normal GI microbiota.
No single pathogen has been implicated as the cause of IBD in horses to date. Mycobacterium paratuberculosis, which causes Johne's disease in ruminants, characterized by granulomatous inflammation of the ileum, has not been identified in the horse. Mycobacterium avium subspecies avium is a rare cause of granulomatous disease in the horse, and has only been reported in Europe. Further investigation is warranted into the equine intestinal microbiome and its relationship to IBD, which will hopefully improve our understanding of the disease and treatment plans for these horses.

Digestive Immune and Parasites

The horse is consistently exposed to parasites and is intermittently treated with anthelmintic compound. There may be a subset of horses that have a variable response to parasites due to deworming protocols or other factors. The role that parasites could play in IBD is not fully understood and probable overestimated.

IBD/Immune Digestive infiltration based on type of inflammatory cells

Lymphoplasmacytic Enteritis (LPE)

Lymphoplasmacytic enteritis is characterized by a mild to severe infiltration of lymphocytes or plasma cells in the lamina propria as well as the epithelium, that may be accompanied by varying degree of villus blunting, fusion or atrophy, and the presence of mucosal and/or submucosal edema. LPE is the most common type of idiopathic IBD in the dog and is presumed to represent a non-specific intestinal immune response to a variety of etiologic agents that causes epithelial damage. In horses, the most recent retrospective studies display large number of cases with LPE which dominates compared to all other types of infiltrations. These findings support the hypothesis that this infiltration type is a significant pathological change entity, a clinical reality and not just a pre-lymphomatous intestinal disease as mentioned before in the literature. We reported recently that lymphoplasmacytic infiltration were observed in 54% of duodenal biopsies and 37% of rectal biopsies. The causes remain unknown, and are similar to the other types of IBDs. LPE can occur at any age, and the disease has no breed or sex predilection.

Lymphocytes/Plasma Cells and IBD

The adaptive immune response is comprised of lymphocytes (T and B cells) that when activated generate effector responses (cytokines and antibodies). In contrast to the innate immune system, the adaptive immune system is highly specific and confers long lasting immunity (memory). In humans, it is generally thought that the adaptive immune system is the main contributor to disease pathogenesis in IBD, either through increased proinflammatory cytokines driven by the T-helper (Th) subsets or by ineffective anti-inflammatory regulatory T-cells (Tregs). Naïve T-cells (Th0) after activation are able to differentiate into Th1, Th2, or Th17 cells. In particular, Th1 responses have been thought to drive the pathogenesis of Crohn's disease, while ulcerative colitis is thought to be driven by Th2 responses. Recent advancements suggest that other cells, such as ILCs and Th17 cells, have emerged as important contributors to IBD pathogenesis. In horses, the literature is poor on the role of the adaptive immune system in IBD cases. Recently, we reported that asthmatic horses have greater infiltration of T lymphocytes in the duodenum and rectal mucosa than normal horses. Also, the duodenal and rectal epithelium of asthmatic and control horses contained exclusively T lymphocytes (In Press).

The inflamed gut of human patients with Crohn's disease or Ulcerative Colitis is massively infiltrated with B cells and IgA+ and IgG+ plasma cells, with a remarkable skewing toward IgG production, depending on the severity of inflammation. In horses, the role of B cells remains unknown. A study performed in cats with IBD revealed a chronic immune reaction in the diseased gut involving increased number of B and T lymphocytes.

Eosinophilic Enteritis (EE) and Multisystemic Eosinophilic Epitheliotropic Disease (MEEDs)

Eosinophilic enteritis is characterized by an inflammatory cell infiltration of intestinal mucosa, dominated by eosinophils. MEEDs is a rare equine disease affecting young to middle aged horses in a variety of horse breeds and is characterized by eosinophilic infiltration of the intestine and other organs. Also, a more focal eosinophil infiltrative disease has been reported more frequently at the end of the 20th century. It is characterized by localized circumscribed eosinophilic infiltrative lesions (also known as idiopathic focal eosinophil enteritis, IFEE) both in the small and large intestine and have reported to cause obstructive colic, resulting in exploratory laparotomy and resection of the affected segment. The etiology is unknown, although parasitic, allergic, toxic, and viral causes have been suggested.

Eosinophils and IBD

In human literature, the role of eosinophils in health and in disease remains unclear. Investigations into their function stem primarily from allergic diseases, asthma, and parasitic infections. It is more difficult to discern a role for the fascinating eosinophil in IBDs because, unlike the lung or the skin, eosinophils reside in normal intestine mucosa and increase in disease states; consequently, an intricate system must regulate their migration and numbers. These granulocytes are equipped with the machinery to participate in gastrointestinal (GI) inflammation and in the susceptible microenvironment, they may initiate or perpetuate an inflammatory response. A significant body of literature

characterizes eosinophils present in the GI microenvironment where they have the potential to interact with other resident cells, thus promoting intestinal remodeling mucus production, epithelial barrier, cytokines production, angiogenesis, and neuropeptide release. A number of lines of evidence support both potential beneficial and deleterious roles of eosinophils in the gut. Eosinophils are closely involved in the development of IBD, when their cytotoxic granule proteins cause damage to host tissues. However, their roles in Crohn's disease and ulcerative colitis appear to follow different immune response patterns. Eosinophils in IBD are especially important in altering the structure and protective functions of the mucosal barrier and modulating massive neutrophil influx to the lamina propria followed by trans-epithelial migration to colorectal mucus. In horses, the role of eosinophils in IBD is by far less understand and document.

Neutrophilic Enteritis (NE)

Neutrophilic enteritis is not well documented in literature as an IBD in horses and the clinical interpretation of this finding remains unknown. In our retrospective study from 2017 to 2021, this digestive infiltration type was the second most frequent pathological changes after lymphoplasmacytic enteritis. The cause of this inflammation remains unknown and could be related to local intestinal infection or a possible link with T lymphocytes as reported in human. Some of these cases were correlated to the presence of peritonitis evidence (neutrophilic exudate of abdominal fluid) and others not.

Neutrophils and IBD

In human, the hallmarks of IBD are dysregulated intestinal immune responses, in which neutrophils are accumulated in inflamed mucosa at the early stage of inflammatory response and play an indispensable role in the pathogenesis. Neutrophils are short-lived effector cells in innate immune system, which are the most abundant leucocyte population in the blood and also typically the first leucocytes to be recruited into the inflammatory areas. Neutrophils play a dual role in intestinal homeostasis and inflammation, playing an essential role in gut defense but also, upon excessive recruitment, being an important mediator of tissue damage in the inflamed mucosa. In human IBD, the neutrophil influence on adaptive immunity through interactions with T cells, and in particular Th17 cells, neutrophil antigen-presenting capacities, and their role in Il-23 in driven inflammation have been documented (neutrophil-T cell crosstalk). The ability of both neutrophils and T cells to infiltrate the intestine during homeostasis and inflammation is of particular importance for IBD. Also, the interactions between intestinal neutrophil influx mediated by T cells and neutrophils capacity of influence Th17 differentiation could be play a significant role in a wide range of clinical IBD contexts. The role of neutrophils in the pathophysiology of IBD have to be more documented in horses. It was suggested that Th17 cells are involved in active IBD, possibly through recruitment of neutrophils via IL-17A, in combination with inadequate suppression of the inflammatory response by Tregs.

Granulomatous Enteritis (GE) and Idiopathic Systemic Granulomatous Disease (ISGD)

Granulomatous enteritis is also a rare disease in horses and characterized by lymphoid and macrophages infiltration at the level of the mucosal lamina propria with variable numbers of plasma cells and giant cells. This type of infiltration has almost disappeared in more recent years. The cause is unknown, and it was hypothesized that that an abnormal host inflammatory reaction to intestinal bacteria is the likely basis for the development of this condition. The presence of hepatic and pulmonary granulomas has also been reported. Affected horses can be of any age, sex or breed, but young Standardbreds have been over-represented, and a familial predisposition has been been described in some cases. In a recent study, we described histiocytic enteritis in some cases in suspected cases to have significant immune digestive infiltration. The presence and the effect of the intestinal macrophages infiltration in horses have to be more documented.

Macrophages and IBD

In human, the macrophages play an important role in homeostasis and in the development of IBD, both in mouse models and in patients, by phagocytosis cellular

debris, producing multiple cytokines, regulating tissue repair and interacting with other cells. In the intestine, monocytes-derived macrophages are more abundant than tissue-resident macrophages of embryonic origin. Both perform phagocytosis, produces cytokines, and interact with other cells. In homeostasis, tissue-resident macrophages in the muscularis externa interact with enteric and myenteric neurons controlling intestinal secretion and motility, while in the lamina propria, macrophages provide signals to intestinal stem cells that give rise to goblet cells, Paneth cells, and intestinal epithelial cells. These macrophages also modulate T cell activities and functions, via the secretion of IL-10 for Tregs and IL-1B for Th17 cells. Macrophages also have an effect on the other innate and adaptive immune cells (T lymphocytes, eosinophils). During the resolution of intestinal inflammation, macrophages play a central role in the clearance of bacterial components and apoptotic cells. In IBD horses, the presence and the role of intestinal macrophages is not documented.

Conclusion

In human, it has been well documented that the adaptive immune system plays an important role in the development and perpetuation of the inflammatory cascades in IBD. In particular, T-cells have been shown to be key players in driving intestinal inflammation. However, a number of unresolved issues exist that need to be addressed in order to develop successful and appropriate therapeutic strategies. Recent advances have clarified the importance of the innate immune system in IBD pathobiology. The studies of the interactions between the different components of the innate and adaptive immune system, as well as the interaction with the intestinal microbiota, and how these interactions relate in the overwhelming context of an individual's genetics are areas that will open new horizons in the knowledge of mechanisms of gut inflammation. In horses, the knowledge on the role and the interactions of the different immune population cells in IBD is quite limited and should be developed to make treatment more specific.

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NOTES

14.15 – 15.00 IMMUNOLOGY: a review of immune maturation from the newborn foal to the aged horse – J. Felippe

M. Julia B. Felippe, MV, MSc, PhD, Diplomate ACVIM
Professor of Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

The immune system of the young horse

The immune system of the horse shows remarkable development during fetal life. The thymic lymphoid population is detected by approximately 80 days of gestation, with circulating lymphocytes observed by 120 days of gestation, and secondary lymphoid tissues populated soon after with primary lymphocyte follicles. Nevertheless, foals do not present organized lymphoid tissue in the lungs and gastrointestinal tract at birth.

By mid-gestation, B cells reach a mature stage in their development, and immunoglobulin isotype switching is present. At 100 days of gestation, mRNA expression of not only IGHM and the lambda light chain (IGLC) can be detected in the bone marrow, but also IGHD, IGHG1, IGH3, IGHG5, IGHG6, IGHG7, and IGHA isotypes. In the equine fetus, the diversity of immunoglobulin sequence content (i.e., the variation between immunoglobulin transcripts and germline sequences) significantly increases between 100 days of gestation and birth. Prior to 200 days of gestation, both IgM and IgG proteins can be measured at trace values in the fetal serum. In fact, antigen-specific antibody production, including antigen-specific IgG isotype switching has been measured in fetal infections. In physiologic conditions, foals at birth have low but detectable amounts of serum IgM, and traces of IgG. Altogether, the fetal humoral immune system repertoire undergoes expansion and limited-diversity in an antigen-independent manner during gestation.

Although a great part of the lymphoid tissue develops during gestation, the foal adaptive immune system is naive to environmental organisms at birth. The exposure to an abundant and diverse population of pathogens in early age induces a massive expansion of antigen-specific lymphocyte populations, reflected by 2 or 3 times increase in the number of circulating lymphocytes, and an increase in mass of secondary lymphoid tissues. Accordingly, there is an age-dependent increase in the peripheral blood lymphocyte subpopulation counts ($CD4^+$ T cells, $CD8^+$ T cells, and B cells), which also serve as a measure of immune competence. Foal peripheral blood lymphocytes proliferate in response to mitogen stimulation, with lower levels in the first few weeks of age, then progressively reaching adult horse levels by 4 weeks.

Exposure of the gastrointestinal tract and lungs to pathogenic organisms before lymphocyte priming and expansion creates a high risk of disease and sepsis in foals. In addition, elements that may affect the proliferation of the developing microbiome may affect or delay protective immune response. After birth, there is an increase in the presence of gut-homing T and B cells, which are likely dependent and driven by microbiome. Macrophages, T lymphocytes and plasma cells are virtually absent in lung tissues in the first week of age, and bronchus-associated lymphoid tissue is only observed by 12 weeks of age, also making the lungs an ideal target for infections.

The foal's immunity is highly dependent on the transfer of immunoglobulins through the colostrum, in addition to cytokines and other opsonins. A variable nadir of serum IgG levels occurs between 1 and 3 months of age due to the decay of colostrum-derived antibodies, with a half-life between 28 and 32 days. Appreciable endogenous serum IgM and IgG concentrations are detectable by 2-3 months of age, and levels increase at approximately 100 mg/dL (1g/L) per month in the first year, until it reaches adult-like concentrations; some studies indicate that production of $IgG_{4/7}$ subtype

develops more slowly than the other IgG subtypes. Failure of passive transfer of immunoglobulins through colostrum is classically defined by serum IgG concentration less than 800 mg/dL (8 g/L) in the initial 24 hours of age, and foals with serum IgG between 400 and 800 mg/dL (4 and 8 g/L) are considered to have partial failure; this condition predisposes the foal to infections and sepsis. However, levels greater than 1,000 to 1,200 mg/dL (12 g/L) would be more certain of protection for the first few months of age until endogenous levels become protective. Indeed, adequate transfer of immunoglobulins occurs naturally at levels much higher (> 2,000 mg/dL or 20 g/L). A quantitative assay, such as the turbidimetric or radial imumunodiffusion assay provides the actual blood immunoglobulin concentration achieved after passive transfer of immunoglobulins, which could be used for clinical planning and monitoring of foals at risk of developing infections in the first 5 months of age. Endogenous production of IgA can be measured between 1 and 2 months of age, whereas IgE production is delayed until 6 months of age, with robust serum IgE levels by 9 to 11 months.

Meanwhile, robust phagocytic and oxidative burst activities are present in neonatal phagocytes at birth, and foal neutrophils express greater integrin molecule CD18 during their initial 3 weeks of age when compared to adult horses, providing efficient diapedesis and robust cell-to-cell interactions. Nevertheless, phagocytosis and bacterial killing are highly dependent on the synergistic opsonization of the pathogen by circulating complement and antibodies. Opsonic capacity in foals becomes comparable to that of adults by 3 to 4 weeks of age, along with an age-dependent increase in complement concentration in serum. Opsonins can be quickly consumed during bacterial infection and/or sepsis, and intravenous plasma transfusion can be used as a source of opsonins in these conditions. Serum amyloid A (SAA), an acute phase protein produced in the liver and also present in colostrum (SAA3, produced in the mammary gland), induces chemotaxis, cell degranulation, opsonization, phagocytosis, and pathogen killing. SAA3 peak levels are detected on day 2 of age in foals, and SAA levels increase dramatically in foals with septicemia or focal infection.

Foal peripheral blood mononuclear cells show active cytokine production in the first month of age, including TNF-alpha, IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p35, IL-15, and IL-18. The proportion of IFN-gamma and IL-10 producing-cells increases with age, and IFN-gamma production reaches adult levels in the first year. Perhaps the most limiting factor in the equine neonatal immune response is the reduced capacity of antigen presenting cells (APCs, dendritic cells and macrophages) to respond to pathogen associated molecular patterns (PAMPs) through their Toll-like receptors, and prime and activate T cells. It is possible that foal APCs, like human neonates, require multiple stimuli (e.g., more than one TLR stimulation or a certain threshold of cytokine milieu) for competent activation. The functional capacity of APCs is achieved in foals around 3 months of age.

Some studies suggest that passively transferred antibodies have a suppressive effect on the foal's endogenous immunoglobulin production. This assertion stems from observations of accelerated onset of immunoglobulin production in colostrum-deprived foals and vaccine studies. However, there are as many examples indicating that foals can generate antigen-specific immune responses in early life despite circulating colostrum-derived antibodies, perhaps with different magnitude. Therefore, foals are immunocompetent but may require a specific immunogenic context to generate the desired immune response; nevertheless, memory response has not yet been characterized in the foal.

Foal vaccination has been an area of active research with strides of progress in recent years; yet refinement of optimal formulation and thorough assessment of protection is ongoing. An alternative approach is to vaccinate mares before foaling to maximize antigen-specific antibody transfer to the neonate. This approach has seen

success for some pathogens and environmental conditions; however, the quality of colostrum-derived antibodies and efficiency of transfer to/absorption by foals is variable, and individuals of a herd may be still not protected.

The paradox of neonatal vaccination is the need of immediate protection early in life with long-term memory, the perceived limitations of the immune system of the neonate, and the theory of maternal antibody interference. It is recommended to delay vaccination against influenza until 6 or 12 months of age to avoid maternal antibody interference. However, given the half-life of 30 days for IgG, it is likely that colostrum-derived antibodies reach low levels around 2 or 3 months of age; in addition, pathogen-specific cellular immunity is not efficiently transferred by colostrum; consequently, foals would be susceptible to disease between the decay of colostrum-derived antibodies and vaccine-induced immunity. Therefore, further studies are needed to better define vaccination strategies in foals.

Collectively, these studies suggest that vaccination of neonates can induce measurable immune responses, although effective administration strategies (product, adjuvant, route, boosters) and correlates of protection need still to be defined.

Transient hypogammaglobulinemia of the young is a developmental condition described in foals with recurrent infections, particularly respiratory, starting at the time when circulating colostrum-derived antibody levels decrease around 2 to 3 months of age (or earlier, if transfer of antibodies at birth was low). The transient hypogammaglobunemic condition may last a few months (5 to 10 months of age) or several months (18 to 24 months of age). Most foals maintain normal development when treated with antibiotics. The most challenging pathogens are encapsulated bacteria. It is not common practice to measure serum IgG and IgM concentrations in foals beyond the first month of age, and this condition is likely underdiagnosed. In foals with delayed antibody production, serum IgG concentration is often < 500 mg/dL (5 g/L), and IgM levels are decreased < 25 or < 50 mg/dL (0.25 or < 0.5 g/L). In general, peripheral blood B and T lymphocyte counts and distribution are normal in these foals; however, many cases have decreased CD4+ T cell distribution (< 50%), with a low CD4:CD8 ratio (< 2.0). It is unclear if all cases of transient hypogammaglobulinemia involve the same faulty immunologic mechanism or if the outcome (delayed immunoglobulin production) results from different dysfunctional immune processes, perhaps inadequate interaction and activation of antigen presenting cells and lymphocytes in secondary lymphoid tissues.

The immune system of the aging horse

In comparison to younger horses, horses older than 20 years of age present more frequent and more severe clinical conditions that affect gastrointestinal, musculoskeletal, and respiratory systems, often manifested by weight loss, dental problems, arthritis, and lameness. In addition, pituitary pars intermedia dysfunction (PPID) and increased risk for bacterial and viral infections of the upper and lower respiratory tract are conditions associated with changes in the immune system in aging horses.

Two simultaneous processes happen in older age that may explain a shift in the immune responses: immunosenescence and inflamm-aging. *Immunosenescence* is a progressive, age-related biological decline in the immune response due to cellular dysfunction. *Inflamm-aging* is a chronic, sterile, low-grade pro-inflammatory state with production of free radicals and activation of inflammasome. The balance of these two mechanisms influences immune responses and health in the aging horse. In fact, inflamm-aging have a protective effect when parts of the immune system develop senescence.

The negative effect of *immunosenescence* on the different mechanisms of the immune system explains, in part, the increased prevalence of cancer, autoimmunity, poor responses to certain vaccines, and increased susceptibility to common infectious

organisms in the aging horse. Although aging affects innate immunity in humans, the same effect is not obvious in the horse, given that neutrophil and monocyte counts, and lymphokine-activated killer cell activity do not differ from younger horses. Noteworthy is the effect of aging on T cell responses, starting at the thymic involution, and including decreased absolute lymphocyte counts, decreased distribution of T cells in blood, and decreased T cell proliferation *in vitro*. Potentially associated with these findings, aging horses have been shown to have a reduced humoral immune response to inactivated influenza virus vaccines when compared to younger horses, and a non-protective mild, short-term response to canarypox recombinant virus vector expressing the haemagglutinin antigen of influenza. In contrast, aging horses showed a primary humoral response to rabies vaccination like younger horses, but titers dropped faster than younger horses. It is not clear if humoral responses to other vaccines follow similar pattern, given that total serum IgG and IgA concentrations in the aging horse are increased or comparable to younger horses.

An imbalance between pro- and anti-inflammatory mediators, with increased pro-inflammatory cytokines (TNF-alpha, IL-6) contributes to the process of inflamm-aging and chronic diseases. Aging horses have higher levels of circulating inflammatory cytokines (IL-1b, IL-15, IL-18 and TNF-alpha), and higher frequency of blood leukocytes producing inflammatory cytokines (TNF-alpha and IFN-gamma) than younger horses. Moreover, it has been shown that horses with pituitary pars intermedia dysfunction have increased expression of IL-8, which is an inflammatory chemokine that amplifies inflammation.

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NOTES

15.00 – 15.25 INFECTIOLOGY: Equine piroplamosis: epidemiology of the disease in Europe L. Malandrin

Laurence Malandrin, UMR BIOEPAR, INRAE, Oniris, Nantes F-44300, France

1- Introduction

Equine piroplasmosis is a tick-borne disease of equids (horses, donkeys, mules, and zebras), and is caused by two protozoan parasites, *Babesia caballi* and *Theileria equi* (formerly *Babesia equi*). Equine piroplasmosis is the most prevalent tick-borne disease in equids and has a wide geographical distribution being endemic in most equine-habited parts of the world, with cases reported from Central and South America, Cuba, Europe, Asia and Africa. The few non-endemic countries (Australia, Canada, Great Britain, Ireland, Japan, New Zealand and USA) sometimes impose strict regulation on horses' importation to prevent the introduction of carrier animals (Wise et al. 2013, Tirosh-Levy et al. 2020). Equine piroplasmosis is responsible for important economic losses to the equine industry due to the treatments and their side effects, the decrease in performance of the animals or the negative impact on the horse international racing industry (Knowles 1996).
Clinical signs of equine piroplasmosis are not pathognomonic, and vary from sub-clinical (apathy, loss of appetite, poor exercise tolerance) to severe (fever, anemia, jaundice, haemoglobinuria, petechial hemorrhages of the mucous membranes). These symptoms are variable, non-specific, and infections with either *B. caballi* or *T. equi* cannot be distinguished clinically. The infection can also be asymptomatic. Equids may be infected without development of clinical signs. Infected animals recovering from acute or primary infection with *T. equi* remain life-long carriers, whereas horses infected with *B. caballi* may remain carriers for up to 4 years. Even with an appropriate treatment, only *B. caballi* can be cleared (de Waal 1992). This biological difference greatly influences the maintenance and spread of the disease as carriers represent a permanent source of infection for ticks.

2- Equine piroplasmosis: a vector transmitted disease

The two parasites responsible for equine piroplasmosis, *B. caballi* and *T. equi*, are transmitted by a very diverse range of *Ixodidae* tick species, depending on the geographical area. Equine piroplasmosis incidence therefore depends on the distribution of the vectors of each of the two parasites. About 33 tick species belonging to six genera have been implicated as vectors. In Europe, *Dermacentor reticulatus* / *D. marginatus* in the northern Europe and *Hyalomma marginatum / Rhipicephalus bursa* in the southern Europe are the main vectors. However, *Ixodes ricinus* (Italy), *Hyalomma detritum* (= *scupense*?) and *H. dromedarii* (Western Europe), *Rhipicephalus sanguineus* (Southern Europe), and *Haemaphysalis punctata* are also suspected to be vectors (Scoles and Ueti 2015, Onyiche et al., 2019). However, their vectorial competence needs to be experimentally proven, as the vectorial suspicion relies solely on parasite DNA detection in ticks.

3- Prevalence of equine piroplasmosis in Europe

3.1- Serological versus molecular detection to address *T. equi* and *B. caballi* prevalences

Serological tests are indirect tests, they detect antibodies produced by the infected animals. As antibodies persist after infection clearance, they do not distinguish infected from cured animals, leading to an overestimation of prevalence. Molecular tests are direct

tests, generally detecting parasite DNA. As the quantities of circulating parasites can be low in the case of asymptomatic carriage, these methods may underestimate prevalence.
Serological tests are very diverse in terms of technics and antigens used (CFT and IFAT with the use of non-standardized antigens, ELISA with specific but possibly variable antigens), that therefore influence differently their specificities and sensitivities. Molecular analysis, even if diverse technics are used (PCR, nested PCR, quantitative PCR, multiplex PCR, LAMP PCR, reverse line blot…), is based most of the time on the molecular amplification of the same gene target, the 18S ribosomal DNA. The presence of highly conserved and variable regions in this gene allows at the same time its amplification despite sequence heterogeneity, and further evaluation of this diversity through amplicon sequencing. The importance of this feature will become evident in the next section on the genetic diversity of both parasites.

3.2- A great diversity of equine piroplasmosis prevalences in Europe

In this section, the author will focus only on the molecular assessment of equine piroplasmosis prevalence.
Data on equine piroplasmosis prevalence are available from 15 countries in Europe (Nadal et al. 2021, Coultous et al., 2020). The first trend is a higher prevalence of *T. equi* (0 - 68%) compared to *B. caballi* (0 - 8.3%). This difference might be explained by the ability of horse immunity and treatment to clear *B. caballi* compared to the life-long persistence of *T. equi*. The second trend is the higher prevalence of both parasites in southern countries (Portugal, Spain, Italy, Romania, Hungary, Serbia with equine piroplasmosis prevalence higher than 30%) compared to the northern countries (Ireland, Netherlands, Poland, Slovakia with prevalence lower than 5%). The third trend is the great heterogeneity of prevalences within a country according to the study sites. For example, in Italy, equine piroplasmosis prevalence varies between 11.7 to 70.3 and in Spain between 3.9 to 66%. These differences may be related to horse management or geographical location, that influence tick contact or abundance respectively.

4- Genetic diversity of both parasites

With the first studies using molecular detection of equine piroplasmosis and subsequent sequencing of the 18S rDNA (Nagore et al. 2004; Criado-Fornelio et al., 2004), the unsuspected genetic diversity of both *B. caballi* and *T. equi* was revealed. After around 20 years of studies from different countries around the world, an ever-increasing quantity of 18S rDNA sequences available as well as a few complete sequenced genomes, a more accurate picture of this diversity is emerging (Tirosh-Levy et al., 2020).
B. caballi can be divided into 3 genotypes, named A, B1 and B2. Genotype A predominates worldwide.
T. equi is divided into 5 genotypes, A to E, distributed worldwide. A geographical repartition of these genotypes is not clear. Genotypes A and C are present worldwide, while genotype E seems to have an eurasian preference, and genotypes B and D an African preference (Tirosh-Levy et al., 2020). However, this distribution needs to be refined, and is blurred by the genetic analysis of parasites from infected horses that remain carriers for life and are moved geographically from one country to another.
Recently, a novel equine *Theileria* species, *T. haneyi*, has been proposed on the basis of genome sequencing (Knowles et al, 2018). This named species seems in fact to correspond to the genotype C described worldwide. The five *T. equi* genotypes may indeed correspond to different species, but at present, we have no life traits that we could be linked to these

cryptic species: neither pathogenicity, nor geographical distribution, nor vectorial transmission, nor any other aspect of the disease.
Conclusively, even if we have no evidence of it yet, genetic diversity may play an important role in influencing disease transmission and pathogenicity. However, it can greatly impact the degree of sensitivity of the diagnostic tools, especially the serological diagnostic based on highly variable antigens.

5- Are equids the only hosts of *T. equi*? Discussion about their epidemiological relevance.

T. equi is described as a parasite infecting equids (horse, zebra, mule and donkey). However, the use of molecular piroplasmosis diagnostic and subsequent sequencing have highlighted the presence of *T. equi* on non-equid hosts. The most frequently described host is the dog, symptomatic or asymptomatic, in very diverse countries in Europe as well as outside Europe (Croatia, France, Paraguay, Romania, Spain) (Oniyche et al, 2019). As a widespread domestic animal prone to canine piroplasmosis, the dog has been the subject of numerous studies, which may explain why *T. equi* has been described so frequently in this animal. However, studies have also shown *T. equi* to infect camel as well as waterbuck (*Kobus ellipsiprymnus defassa*) (Githaka et al., 2014). We may only be at the beginning of the discovery of the host range of *T. equi*, and for the moment we may have only uncovered the tip of the iceberg.
The epidemiological relevance of these hosts needs to be studied, as they often share the same vectors. For example, *Dermacentor reticulatus* and *Rhipicephalus* spp. are frequent tick species on dogs as well as on horses in Europe. The accidental infection of dogs by *T. equi* infected ticks may therefore occur in areas where both animals are present. However, it has yet to be demonstrated that dogs play an epidemiological role in the *T. equi* biological cycle. One of the most critical steps in the transmission of Piroplasmas is the production of presexual stages (gametocytes) in the vertebrate host. These stages can then be transmitted to the tick during the blood meal and are the only ones that can evolve within the vector to ensure vectorial transmission (transstadial as well as trans-ovarial) (Jalovecka et al., 2018). Some parasites may be able to multiply in erythrocytes of a vertebrate host, but not to differentiate into gametocytes, and therefore not to be transmitted by ticks. Such hosts constitute epidemiological dead ends.

6- Equine piroplasmosis and the PiroGoTick project in France

The PiroGoTick project has started in 2020 for at least 5 years (www.pirogotick.fr in French, and Pirogotick Facebook page). It is supported financially by three French founders: IFCE, Fonds Eperon and France Futur Elevage. With a central activity at Nantes in the UMR BIOEPAR, the project brings together staff from the 4 french national veterinary schools, veterinary students and uses citizen science to cover the whole of mainland France.
The aim of the PiroGoTick project is to gain knowledge at the same time on parasites and vectors involved in equine piroplasmosis. The project encompasses the evaluation of prevalence using molecular detection tools, the characterization of the genetic diversity of the French *T. equi* and *B. caballi* isolates on the basis of multiple molecular markers, and the inventory and spatio-temporal analysis of ticks from outdoor horses in different regions of France. It has been divided into several programs.
The PiroQuest program aims to assess and compare the prevalence of carriage of *T. equi* and *B. caballi* in 4 French regions and determine the 18S rDNA genotypes present in

France. This study involved 566 asymptomatic equids living at least part of the time outdoors and received at the 4 national veterinary schools for various reasons (lameness, reproduction, etc). The overall prevalence of equine piroplasmosis carriers was 38%, dominated by *T. equi* carriers (37%) and presence of a few carriers of *B. caballi* (3.4%). We highlighted a prevalence difference between regions with less impacted horses in the northern part of France (Paris 18.6%) compared to the Southeastern part (Lyon 56.1%). Most of the positive samples were sequenced generating 199 *T. equi* 18S rDNA partial sequences and 19 *B. caballi* partial sequences. The E genotype of *T. equi* is predominant in France (98% of isolates) with some A genotypes. For *B. caballi*, only genotype A has been detected (Jouglin et al., 2023).

The PiroTick and Pirosentinel programs focus on ticks from horses, to perform a combined inventory, spatio-temporal and abundances analyses of ticks linked to the horse carrier status (Malandrin et al., 2022). For these programs, we are using citizen science to collect ticks from all over the country and volunteer veterinary local practitioners to perform blood sampling. Two very different levels of involvement are proposed. The PiroTick program involves sending ticks on a one-off basis, while the PiroSentinel program involves monitoring over time. The combined two programs have enabled an inventory to be made of the ticks mainly found on horses in France and their period of activity to be pinpointed.

7- Conclusion and take-home messages

Even if equine piroplasmosis has been described for decades, we just begin to uncover the complexity of these parasites. There is still so much to discover on the two parasites, their genetic diversity, their interactions with the host target cells, their host range and their vectorial transmission in order to implement reliable and efficient diagnostic methods on a worldwide scale and to move forward on the road to a vaccine.

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NOTES

15.25 – 15.35 INFECTIOLOGY: Presentation of the R.E.S.P.E. - A Couroucé

Anne Couroucé [1, 2, 3] , Coralie Lupo [2]

[1] Université de Caen Normandie, BIOTARGEN UR7450, F-14000 Caen, France.

[2] RESPE, 3 rue Nelson Mandela, 14280 Saint Contest, France

[3]ONIRIS VetAgroBio, CISCO-ONIRIS, Route de Gachet, 44307 Nantes Cedex, France

Corresponding author: Anne COUROUCÉ; Tel.: +33 6 80 33 92 37; Email address:

anne.courouce@oniris-nantes.fr

Background

Equine health is important in regard to equine welfare, trade, economy, society as well as public health. Created in 1999, the RESPE is the first network of surveillance of equine diseases developed in Europe. Since 2008, it brings together veterinarians and the equine industry to monitor the epidemiological situation and evolution of equine diseases in France.

Objectives of RESPE

The RESPE has four main objectives:

1) Ensure appropriate vigilance of equine health by identifying key diseases;
2) Early detection of known and emerging equine diseases in France, for early warning of all the stakeholders of the equine industry
3) Share reliable information for disease management and prevention;
4) Minimize the consequences of equine disease outbreaks

Categories of focused diseases

The RESPE focusses on different groups of diseases:

• Contagious and transmissible diseases for which early detection is crucial to implement immediate and collective management measures

• Transmissible but not contagious diseases and metabolic diseases for which exposition factors are environmental and early detection is crucial for (1) treatment implementation at
the individual level, (2) prevention, by increasing stakeholders' awareness of the presence of favourable environmental conditions

• Non contagious diseases for which there is a lack of knowledge: centralising cases helps the development of research activities

The key role of the Sentinel Veterinarian

Currently there are more than 900 veterinarians participating in the sentinel veterinarian 'relay' network. This ensures rapid and accurate dissemination of critical information. One of the key roles of each veterinarian is to monitor diseases and to inform the RESPE office of any suspected cases. In the case of a suspected disease the sentinel veterinarian conducts appropriate testing previously defined by the RESPE. Samples are then submitted to a partner diagnostic laboratory using the standardized declaration form available on the RESPE reporting platform.

If lab results are 'positive' for a monitored disease the RESPE office is notified as soon as possible (phone/email). Subsequent to this, anonymous alerts are sent to appropriate contacts to ensure control and prevention measures.

When an epizootic disease agent is identified, RESPE ensures that there is a good transmission of key information to the industry and the Veterinary Authorities.

The RESPE surveillance

The RESPE conducts passive surveillance activities:
1. Syndromic surveillance based on clinical suspicions from the network of voluntary veterinarians, called «Sentinel Veterinarians»
Nine clinical syndromes are monitored, including acute respiratory syndrome, neurologic syndrome, abortion, atypical myopathy, undetermined fever previously called "piro-like" diseases, … in order to ensure appropriate disease control strategies during outbreaks.
2. Targeted surveillance on specific diseases based on positive laboratory diagnostic test results from the supporting laboratory
These two complementary surveillance modalities enable to monitor almost 25 different diseases. The complete list is presented in Figure 1.

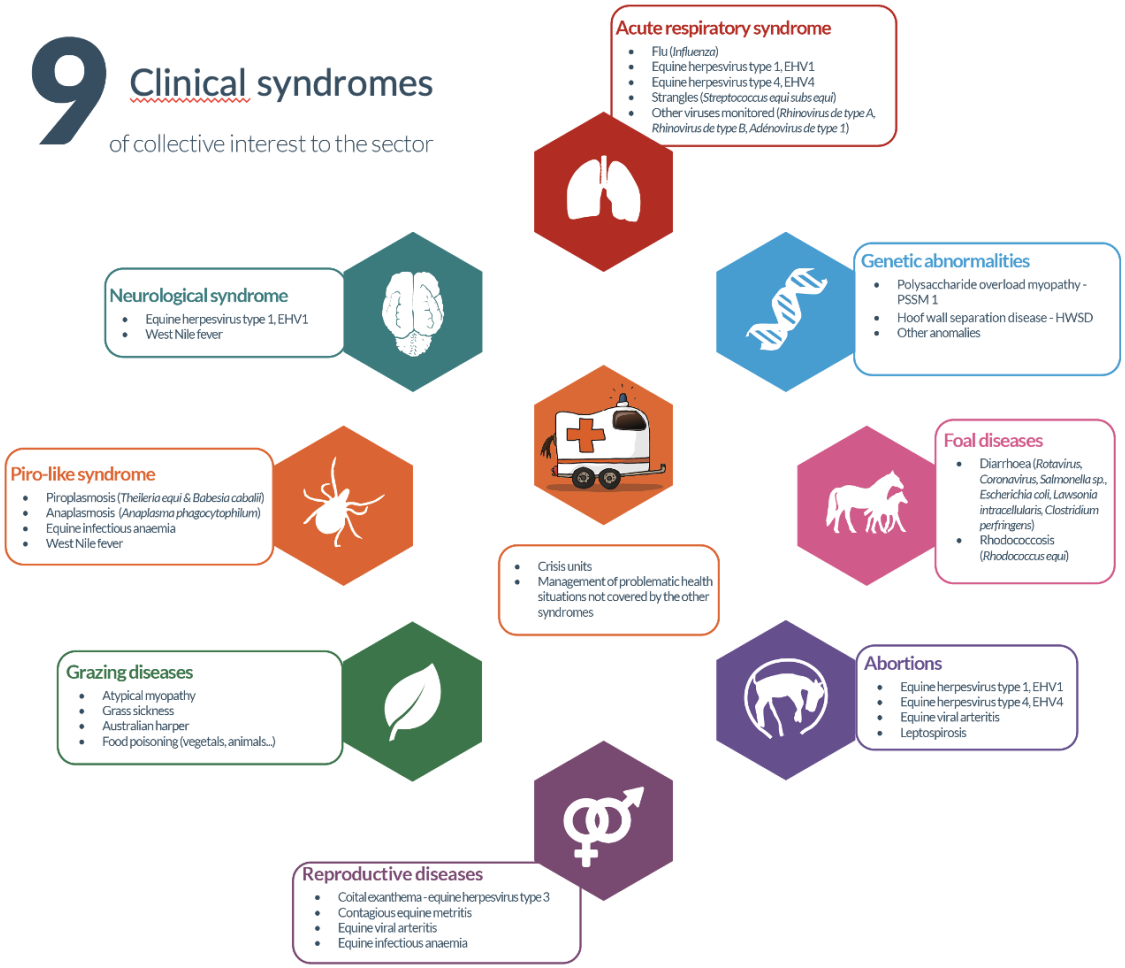

Figure 1. The nine clinical syndromes of the RESPE and corresponding diseases

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15.35 – 16.00 INFECTIOLOGY: Clinical variability and diagnostic controversies in equine piroplasmosis - A. Leblond

Prof. Agnès LEBLOND, Equine Internal Medicine, UMR EPIA, INRAE, VetAgro Sup, University of Lyon, Marcy l'Etoile F-69280, France

1. Introduction

Equine Piroplasmosis (EP) is the most prevalent tick-borne disease in equids (horses, mules, donkeys, zebras) in certain areas of the world and not only causes important economic losses but also leads to movement restrictions (Knowles 1996a). The disease is caused by two hemoprotozoan parasites of the phylum Apicomplexa: *Babesia caballi* (intraerythrocytic) and *Theileria equi* (intraerythrocytic and intralymphocytic). Both parasites are transmitted by ixodid ticks of the genera Rhipicephalus, Dermacentor, Haemaphysalis, Hyalomma, and Boophilus (Thompson, 1969; Klinkmann, 1981; Bautista, 2001; Battsetseg, 2001).
These two parasites have in fact very different life cycles (Mehlhorn and Schein 1984). Parasites of the Babesia species have a transovarial transmission in the vectors and then enters, as sporozoites, directly into the host red blood cells where they develop into piroplasms. *T. equi*, formerly called *Babesia equi*, was reclassified as *Theileria* species (Mehlhorn and Schein 1998) because of the transstadial transmission in the vector and because sporozoites do not infect red blood cells but penetrate a lymphocyte (or macrophage) where they develop into schizonts. The merozoites are released from the schizonts then enter the red blood cells where they grow into piroplasms.
EP is of importance for international trade, and a specific chapter in "The OIE Terrestrial code" chap. 12.7 (https://www.woah.org/en/disease/equine-piroplasmosis/) describes the standard procedure for EP control in horses. Detection of the areas where EP is endemic and of individual carriers before introduction in non-endemic countries or areas is of paramount importance and can be done using direct and indirect tests. In non-endemic countries like the USA, Canada, Australia, and Japan, only seronegative horses are allowed to be imported to prevent the introduction of carrier animals. For these reasons, in endemic countries, control of EP is critical for the equine industry to preserve the option of international movement of horses, because without it, horses cannot cross borders to compete in races or horse shows, be used for breeding purposes, or be sold abroad (Friedhoff, 1990).
In both situations, practitioners must keep in mind the 'suspicion of infection' criteria that will allow the early detection of cases, crucial in non-endemic regions. These criteria should rely both on clinical signs and epidemiological considerations. Considering the carrier state occurring with *T. equi* in endemic areas, here the issue is to assess whether the signs observed are related to clinical piroplasmosis or may be due to intercurrent disease (Leblond, 2019). The therapeutic decision should then be based on epidemiological considerations, whether the case is located in endemic or non-endemic region, the specific diagnosis of the parasite involved, and the balance benefit / risk.

2. 'Suspicion of infection' criteria

The clinical and epidemiological criteria for suspicion will be the same in endemic and non-endemic areas. In non-endemic areas, however, practitioners should be well aware of these criteria because the early detection of cases is crucial to implement treatment and biosecurity measures. In endemic areas, practitioners are generally inclined to readily suspect EP, which could lead to over-interpretation of positive diagnostic tests results, whereas healthy carrier status for *Theileria equi* should be considered.

2.1 Clinical signs and hematological changes

As highlighted by Coultous and others, EP should be included in the differential diagnosis of horses presenting with hyperthermia, lethargy and haemolytic anemia that live in (or come from) regions at risk of disease (Coultous, 2018).

EP can be acute, subacute, or chronic. During acute infection, both parasites cause severe hemolytic anemia with fever, icterus, hemoglobinuria, and edema in the distal limbs (Knowles, 1996). Subacute and chronic forms are associated with less specific clinical signs including inappetence, weight loss, exercise intolerance and depression (Camacho, 2005; Wise, 2013). In case of intrauterine infection by *T. equi*, abortion and neonatal death can occur (Potgieter, 1992). In naïve horses, the clinical disease is particularly aggressive producing a mortality rate of up to 50 % (De Waal, 1992). Infections with *B. caballi* are reported to be less severe than those with *T. equi*, but it is impossible to make the difference between the two parasites based on clinical signs only.
Once recovered from an acute episode, a horse could remain a carrier for up to 4 years with *B. caballi* and for life in the case of a *T. equi* infection (Bashiruddin et al. 1999), thereby serving as a source of infection for ticks. So, practitioners in endemic areas are faced with chronic carriers in which disease recrudescence can occur at times of increased stress and immunosuppression, such as may occur with increased handling, transport, co-infection or lactation.

2.2 Consideration of epidemiological criteria: endemic/non-endemic status, example of southern France

Since the clinical signs are not very specific, epidemiological criteria must also be taken into consideration in order to establish suspicion of infection. The awareness of practitioners is different depending the horse is located in an endemic or disease-free region. In the South of France and in Lyon, previous studies have shown that we are located in an hyperendemic region.
Retrospective studies conducted by the ANSES Laboratory (Maisons Alfort, France) have shown that out of 16,127 sera originating from all regions of France between 1997 and 2003 and analysed with Complement Fixation Test (CFT), 13.2% were positive for *T. equi*, 9.5% were positive for *B. caballi* and 4.1% were positive for both parasites (Nadal, 2022). The highest seroprevalences were observed in Auvergne-Rhône-Alpes (including Lyon) and Provence-Alpes-Côte d'Azur reaching 27% for *T. equi*, and 22% for *B. caballi* in Auvergne-Rhône-Alpes. This study addressed mostly a population of horses presenting clinical signs as hyperthermia, anemia and icterus and so, further studies are needed to estimate the prevalence of EP in the general population of horses in France.
Other retrospective studies were conducted in the south of France and included a representative sample of healthy horses living in the area. In 2002, a study has found that the Camargue (located in Provence-Alpes-Côte d'Azur) is a hyperendemic area for EP, where the seroprevalence of *T. equi* and *B. caballi* infection in horses has been estimated at 58% and 12.9%, respectively (Guidi, 2014). The CFT test was used in this study in 2002. A second study conducted in the same area in 2015-2016 used real time PCR tests on whole blood for the diagnosis of EP in horses (Rocafort-Ferrer, 2022). The prevalence of *T. equi* and *B. caballi* was found to be 68.6% and 6.3%, respectively. Only 1.7% of the horses were coinfected.
Besides, within the equine university hospital of Lyon, we conducted several studies to estimate the prevalence of EP in horses presented at our clinic, aiming at identifying the criteria that would lead to a suspicion of infection.
The first study was retrospective, conducted between January 2011 and December 2013, and included 173 horses admitted at the Lyon Vet School. Horses were tested by PCR due to clinical or hematological findings leading to a suspicion of piroplasmosis (Deberge, 2014). Seventy-one horses (41%) were positive with *T. equi* solely, 10 (11%) were positive to *B. caballi* and 6 (9%) were positive to both parasites. The positive horses were initially admitted for various reasons: colic (36%), weight loss (21%), exercise intolerance, depression, dyspnea, recurrent fever, icterus… They were tested following the observation of anemia, fever, oedema and/or icterus. In this study, no age, sex, nor breed predisposition was observed. History of recent surgery or previous diagnosis of piroplasmosis were also not linked to a greater risk of being positive. There were statistically more positive horses detected during hospitalization rather than among outpatients.

Amongst signs reported on horses' examination, no individual sign could be linked to positive cases, but a combination of anemia, abnormal mucous membranes and edema was significantly associated with positive piroplasmosis PCR results (p=0.009). Regarding hematological findings, anemia (based on RBC count, hemoglobinemia or hematocrit) was significantly correlated with PCR-positive cases. Hyperbilirubinemia, uremia or increased GLDH serum levels were also positively correlated with positive cases ($p < 0.05$).
This retrospective study was biased as the selected population was not representative of the general population of horses presented at our clinic. Therefore, a second prospective study was implemented, aiming at evaluating prevalence and risk factors for EP in horses referred at VetAgro Sup. This study was part of the study PIROQUEST, part of the PiroGoTick project, implemented in the four French veterinary schools from 2020 (Jouglin, 2023; Muzard, 2021).
All horses presented to the clinic, over 1 year old and spending more than 8 hours a day out of the stall were eligible for inclusion in the study. A nested PCR test was used for the diagnosis (Jouglin, 2023). Finally, out of 88 horses included between September 2020 and May 2021, 48 were positive to *T. equi* only (58%), 4 were positive to *B. caballi* only (8%) and 3 were positive for both haemoprotozoa (3.4%). In comparison, for the study as a whole, including the 4 university hospitals, the prevalence of *T. equi* was 37% and of *B. caballi* 3%. In our clinic, *Babesia*-positive horses were significantly diagnosed more frequently in spring and summer, whereas *Theileria* cases were recorded all the yearlong. Analysis of clinical and paraclinical parameters did not reveal any significant difference between PCR-positive and negative horses, either in terms of reason for consultation, complication rate, red blood cell parameters on admission (haematocrit, red blood cells, haemoglobin) or routine blood biochemistry (GGT, urea, total bilirubin).
No complications of general anesthesia were reported in the 30 positive horses that underwent surgery. However, 12 cases of complication during hospitalisation were reported, including oedema, fever, anemia, neutropenia and/or lymphopenia. Of these 12 horses, 8 were positive for *Theileria* and 1 for *Babesia*. However, given the small number of cases, this trend does not appear to be significant.
Finally, the prevalence of EP positive horses received in our clinic is quite high, above 50%, but these studies have not yet allowed us to reach a consensus on the criteria for suspecting a case of EP. Our clinical experience has led us to develop a clinical suspicion of piroplasmosis based on clinical signs that are both very diverse and sometimes remote from classical signs of piroplasmosis. These signs are more often observed during the hospitalisation of the horse, leading to prescribe a PCR test for the diagnosis. Once the diagnosis of EP is established, different ways of management are possible.

3. Diagnostic criteria and management

3.1 Diagnosis methods, sensitivity and specificity

As clinical signs are indistinguishable for the two parasite infections but the treatment and prognosis are different, the differential diagnosis should always be laboratory-based.
Serological tests are widely accepted as surveillance tools because of their ease of use. The Complement Fixation test (CFT) was developed in 1945 and officially recognised as the official test for equine piroplasmosis in 1969 (Friedhoff, 1982). However, despite its good specificity, this test lacks sensitivity (47% for *T. equi* and 88% for *B. caballi*), especially in the case of latent infections (Wise, 2013). The IFA test for *T. equi* was considered more sensitive than that of the CF test (89 % vs. 63 %), while the estimated specificity was similar (96 %) (Ogunremi et al. 2007). The IFA test for *B. caballi* was more sensitive than that of the CF test (92 % vs. 28 %) but less specific (95 % vs. 99 %) (Ogunremi et al. 2008). Problems associated with the IFA test included cross-reactivity, subjective judgment of the reader, the high cost of the antigen (Bakheit et al. 2007) and, more recently, a lack of availability. Consequently, the indirect fluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA) and the competitive inhibition ELISA (cELISA) tests have now become the tests of choice for indirect diagnosis of *B.*

caballi and *T. equi* infections and have replaced the CFT as the official tests for certifying horse movements (Wise, 2013).
More recently, qPCR assays have been developed for the diagnosis of EP (Rocafort-Ferrer, 2022). These highly sensitive molecular tests are useful in detecting cases with low parasitaemia or even for monitoring the parasitaemia after treatment. Moreover, these tests enable genetic characterisation. However, validation of these PCR assays is needed as there is genetic variation between isolates of *T equi* and *B caballi*.
Coultous and others report that diagnostic testing is not routinely undertaken in disease-free areas, even in horses showing haemolytic anaemia (Coultous, 2018). Surprisingly, practitioners in endemic areas often establish the suspicion of EP based on clinical and epidemiological criteria only and implement a treatment regimen without undertaking laboratory confirmation tests (Leblond, 2019).
In endemic areas, practitioners are often used to perform a blood smear to detect acute cases. However, even though light microscopy can be used to identify the organisms within the erythrocytes during the acute stage of infection, its low sensitivity precludes its use in cases of chronic or subclinical infection. Therefore, PCR and/or serological testing should be conducted to confirm chronic and/or atypical cases, especially if a relapse from chronic carrier status is suspected.

3.2 Controversies in the management: examples of clinical cases in endemic areas

The treatment practices and objectives of endemic and non-endemic countries are also quite different. While it is important to completely clear horses of the parasite in disease-free areas to limit transmission, in endemic areas it is common practice to question the benefit/risk ratio before starting treatment. Moreover, complete elimination of the parasite from a horse in an endemic area could be detrimental to the immune protection assumed to develop during parasite persistence. While treatment can completely clear *B caballi* from infected horses, it is impossible to completely clear a horse of *T equi*. Therefore, the identification of these chronic carriers is of paramount importance to prevent EP introduction into disease-free areas.
Treatment usually consists of intramuscular administration of two to four doses of imidocarb dipropionate (2.2 to 4.4 mg/kg every 48 hours), but it must be borne in mind that the complete eradication of parasites is much more difficult than the simple disappearance of clinical signs (Wise, 2013). Treatment can also have detrimental effects that should be explained to the owner before starting the treatment protocol. Local injection site swelling and muscle inflammation are very frequent, especially since the administration is repeated, and injection sites should, therefore, be rotated with each dose to minimise this adverse effect. Adverse reactions with agitation, diarrhoea, sweating or colic are frequent. However, the last can be prevented with the administration of anticholinergic drugs such as n-butylscopolamine. Imidocarb dipropionate undergoes hepatic and renal clearance, so there is also a risk of toxic injury to these organs.
To discuss management of horses diagnosed positive to one of the parasites *Theileria* or *Babesia* in an endemic area, we choose to present a selection of cases admitted in our equine clinic at VetAgro Sup. The horses presented here were all diagnosed as positive by PCR, carried out at the VetAgro Sup veterinary laboratory between February 2021 and July 2023. Based on these cases, we will be able to consider all the situations seen in the clinic, and attempt to draw up proposals for implementing treatment.
For the management, we will first differentiate between the diagnosis of the species, *B. caballi* or *T. equi*, and then consider cases where the horse is symptomatic or not. Given the frequent latent and asymptomatic carriage of *T. equi*, the practitioner has to rule out other conditions that could be linked with the clinical presentation. Investigations should help to establish a causal link between the clinical signs and the PCR positivity and will impact the decision to initiate treatment.
Finally, we will attempt to propose a decision tree for recommending treatment of the horse to the owner.

4. Conclusion and take-home messages

In conclusion, there is probably a widespread lack of awareness regarding EP risk among equine practitioners in non-endemic countries. On the other hand, practitioners in endemic countries seem to 'deal with' the parasite, or rather seem not to consider that the issue should be further addressed. However, to secure the international horse trade, it is crucial to obtain reliable and timely estimates of the numbers and location of EP cases. Moreover, the related economic losses should be fully evaluated. Finally, actualized recommendations for the diagnosis, prevention and treatment of the disease should be formulated to provide workable solutions for stakeholders and owners.

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NOTES

STREAM 2 - ROOM: RHONE 3

09.00 – 09.45 HOT TOPIC: How to review a paper - C. Marr and G. Hallowell

Celia M Marr, Equine Veterinary Journal & Rossdales Veterinary Surgeons, editor@evj.co.uk
Gayle D Hallowell, Veterinary Medicine and Science, Wiley & IVC Evidensia Referrals, gayle.hallowell@ivcevidensia.com

(Adapted from guidelines for Medical Education)

What is peer review
Peer review is designed to assess the validity, quality and often the originality of articles for publication. Its ultimate purpose is to maintain the integrity of science by filtering out invalid or poor quality articles. In clinically orientated journals, editors may also expect peer reviewers to provide insight on whether the article makes a useful contribution towards improving clinical practice. Peer review is intended to be constructive and add value to articles and readers rely on peer review to ensure that methods and analytical approaches used in the article are robust. If a paper you are reviewing is really good and an excellent addition to the existing literature, do not be afraid to say so.
Journal editors will usually approach at least two experts in the field and they may also invite additional reviewers to look specifically at statistics and analysis. Most of all, journal editors appreciate promptness: if you cannot deliver a review in the requested time-frame, then it is better to decline an invitation.

Ethical issues

Peer review should be confidential and you should never share a manuscript you have been sent for review with others except where you are mentoring a less experienced reviewer (see below). Most high quality veterinary science journals adhere to the guidance and practices issued by the Committee for Publishing Ethics (COPE). If you feel it may not be appropriate for you to review a specific article, perhaps because you have been indirectly involved in planning or executing the study, then either decline the invitation to review, or discuss your concerns with the editor. If you feel there may be reason for concern about matters relating to publishing ethics, conflict of interest, duplicate publication, or "salami slicing" (i.e., submitting the smallest publishable unit from a larger study) then you should also bring these to the Editors attention.

Double anonymous review

Some veterinary science journals have double anonymised review process. In order to achieve this, authors are encouraged to insert the phrase "details to be added on acceptance" or “masked for peer review” where they believe that the details would assist a reviewer in identifying them. Occasionally, reviewers feel that details of previously published methods or results are compromised to the point that they cannot assess the work properly. These details are made available to editors and therefore, if you have concerns relating to this phrase and feel that you need more information, contact the editor who may be able to reassure you.

Writing the review

It is essential that you write your review in constructive language bearing in mind that some authors may be new to the peer review process and deserve support and

encouragement. On the other hand, please do not feel that you should spend a large amount of time on a review (the quality of a review is not indicated by its length). If you feel that the study is unlikely to be of interest to the journal's readers, or you consider it to be fatally flawed, a brief but polite statement of your reasoning should be provided. Remember you can give additional information in the confidential report to the editor if appropriate.

You are **not** required to copy edit the submission or produce a word-by-word and sentence-by-sentence list of minor modifications. You should remember that it is the author's paper. If there is a consistent or recurring error in nomenclature or use of language then it may be helpful to give advice. It is helpful if you flag up where language is poor, particularly if this renders some or all of the paper difficult to understand. Journal editors will often provide authors with information on language services which can help them.

When writing your review try to limit yourself to 3 or 4 major concerns, and distinguish major and minor corrections. Try to be objective and succinct. If there is a single fatal flaw you should state that clearly but, if so, it is not then necessary to produce an extensive list of minor points. Try to be clear issues where you feel strongly about an issue (i.e. must be changed) from matters where you think the paper could be improved but are happy to leave a final decision to the Editor or the authors.

Reviewers are encouraged to consider the following when assessing a manuscript:

Overall Evaluation and General Comments

What do you consider to be the key messages in this paper? Are the authors using chance findings as key messages? Did you learn anything from reading it that you would consider important information for the field, given existing understanding? Is the paper original? Does the paper inform veterinary science and/or improve clinical practice.

Title and Abstract

These are arguably the most important pieces of a paper as they will determine whether or not a potential reader chooses to read the full text. Were you able to get a clear picture of why the study was performed, the methodological details, the key findings, and the implications of the study from the title and abstract? Would you recommend any changes?

Introduction and Conceptual Framework

Does the paper establish a clear conceptual framework, laying out what is already known on the topic, and identifying the gaps in the literature that this paper is attempting to fill? Is it clear that the work is relevant to a broad, international readership? Is the purpose of the study made clear by the inclusion of a research question or hypothesis? Is the hypothesis testable? Are specific aims outlined to address the hypothesis? The introduction is not expected to be an exhaustive account of the field but should not be excessively selective or subjective.

Methodological Rigour

The sole requirement of the Methods section is to describe the study design in sufficient detail to allow a similarly qualified individual to repeat the study. It may be necessary to ask for greater detail or clarity if this has not been achieved. Is the study population

appropriate in size and characteristics, are the assessment methods robust and valid, are the outcome measures clearly defined and applies?

No study design is perfect and you should consider the practicalities of implementing the 'perfect' study design. Consider: are the methods appropriate for the stated research question? Is the data analysis appropriate given the problem the authors are trying to address and given the data available? Are the methods adequate to address the hypothesis?

Results

Are the results clearly presented? Are they consistent with both the methods used and the problem the authors are trying to address? Do they yield a clear answer to the research question (hypothesis)? Is there a logical balance in presentation of results in the text versus figures or tables? Is there duplication of material in the text, figures and tables? Figures are generally used to highlight interesting results while tables are used to summarise results. Legends for figures and tables should provide sufficient detail that a reader can understand the content without reference to the text.

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Every study has limitations; the authors should point these out and discuss how this may have impacted results and influenced the conclusions.

Discussion and Conclusions

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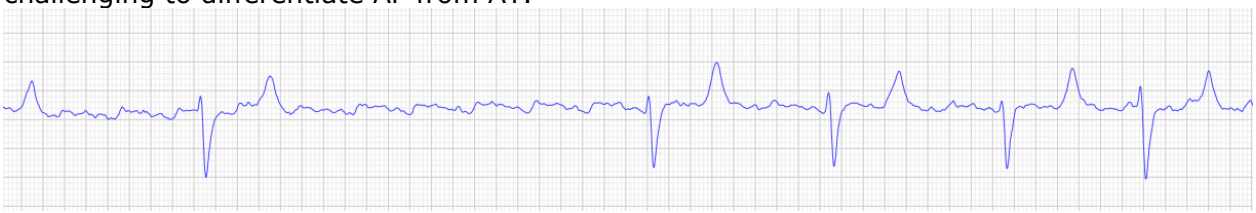
NOTES

09.45 – 10.15 CARDIOLOGY: New insights into Atrial premature depolarizations, atrial tachycardia, atrial fibrillation - G. van Loon

Gunther van Loon, DVM, PhD, Dipl ECEIM, Assoc Member ECVDI
Equine Cardioteam Ghent, Ghent University, Merelbeke, BELGIUM

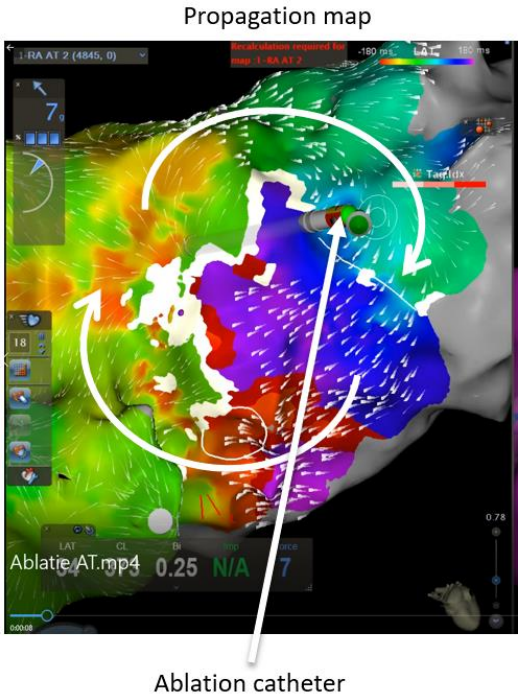
Atrial fibrillation (AF) is the most important arrhythmia affecting performance in horses. The arrhythmia is based upon reentry circuits, a mechanism which requires a premature depolarization start it and a substrate (myocardium) with an area of slow conduction, an area with conduction block and spatial differences in refractoriness. Indeed, atrial fibrillation is known to be initiated by atrial premature beats or rapidly firing foci. Maintenance of AF than depends upon atrial size, presence of fibrotic areas and electrophysiological properties of the myocardium. Larger atria more easily maintain AF because more reentry circuits can co-exist at the same time, which reduces the statistical likelihood that all circuits simultaneously terminate. Fibrotic areas are associated with slower conduction which promotes fibrillation because less tissue surface (smaller atria) is necessary to harbor a reentry path. In addition, in areas with 'islands' of fibrosis, reentry waves can more easily meander through the tissue creating more stable reentry circuits. Finally, the local electrophysiological properties of the myocardium determine the susceptibility to AF. Besides conduction velocity (see above), the local atrial refractory period has an important effect on AF susceptibility. Short refractory periods allow shorter reentry pathways and this in turn means that less atrial tissue is needed to result in self-sustaining, persistent AF. On top of that, rapid myocardial depolarizations (atrial depolarizations during AF are typically around 300-450 per minute which means an AF cycle length (AFCL) of around 135-200 ms) fairly quickly (minutes, hours) result in progressive shortening of the atrial refractory period, which means that AF quickly becomes more and more stable. As AF continues it also induces fibrosis, which in turn further stabilizes the arrhythmia. Finally, during exercise, atrial pressures in a horse with AF are likely to be higher, leading to stretch and further stimulation of fibrosis.
Recent studies in horses have shown a huge spatial dispersion in atrial refractory period within individuals (personal data), even within left or right atrium, and even between pulmonary veins of the same horse. This means that there is no 'overall' atrial refractory period but that it really depends on anatomical location in that animal. Similarly, recent data show considerable spatial variation of AFCL within the same horse and therefore certain areas show much faster activation patterns than others. Based upon a limited number of horses with spontaneous AF, the area between the caudal right atrium (where it changes to caudal vena cava) often shows a slower AF (longer AFCL) due to a different refractoriness and/or conduction velocity (personal data).
When a horse with AF is presented it is very difficult to know how and from which area the arrhythmia started, because once initiated, AF per definition is chaotic in nature. It has been reported that atrial premature beats and runs of focal or macro-reentry atrial tachycardia (AT) post-cardioversion represent a risk factor for recurrence of AF. Indeed, surface ECG recordings have shown that these arrhythmias can initiate AF. Multiple-lead surface ECG recordings post cardioversion combined with vectorcardiography have documented that in some horses these arrhythmias originated from the caudal right atrium. It remains however difficult to document the origin of ectopy with a catheter-based electrophysiological study as the catheter itself can induce ectopy when it touches the endocardium. Therefore, during cardiac catheterizations we have observed atrial ectopy from any location in left and right atrium, including pulmonary veins, but when based upon 1 catheter, it is impossible to know whether the ectopic rhythm is spontaneous or, more likely, induced by the catheter. Occasionally, healthy horses can develop AF, even sustained AF, just from catheter manipulations in the left or right atrium.
Sustained atrial tachycardia shows similarities with AF (etiology, clinical signs, diagnosis, …) but consists of a rapid, regular activation of the atria. On the surface ECG rapid

(about 160-220/min), regular P' waves, with variable RR intervals are visible. Occasionally, depending on the type of AT and surface electrode positions, it can be challenging to differentiate AF from AT.



Surface ECG recording of a horse with sustained atrial tachycardia.

With consistent atrioventricular conduction (for example one QRS for every 4 P' waves), this rhythm may sound almost regular on auscultation, especially at slightly elevated heart rates. When vagal tone is high, however, atrioventricular conduction usually becomes more irregular. Three-dimensional electro-anatomical mapping (3D EAM) has provided new insights into the mechanism of AT in horses. The 3D EAM procedure, uses catheters with multiple electrodes that are manoeuvred along the endocardial surface. A powerful computer collects all intracavitary electrograms and measures the amplitude and compares the timing with a reference electrode. At the same time, the precise 3D location of the catheter in the heart is determined based upon impedance measurements or upon a magnetic field created over the heart. The 3D EAM data from horses with spontaneous sustained AT have shown that the underlying mechanism usually is a macro-reentry circuit, located in the caudomedial right atrium, just caudal to the fossa ovalis.



3D EAM of a horse with sustained AT (right side of the image is caudal vena cava; left side is cranial). The re-entry circuit, which circulates around a line of block and shows slow conduction at the turnin point, is shown in the caudomedial right atrium. Ablation was performed at the isthmus.

This caudomedial region of the right atrium shows myocardial sleeves, myocyte fibre disorientation, fibrosis and fatty infiltrates, all factors that promote reentry. Sustained AT has been treated by transvenous electrical cardioversion or quinidine sulphate, but the latter treatment shows a lower success rate compared to AF treatment, and recurrence rate of AT after medical or electrical treatment is higher compared to AF. A totally new treatment of AT consists of visualizing the precise anatomical pathway of the reentry circuit with 3D EAM and subsequently interrupting this circuit with radiofrequency catheter ablation (RFCA). Radiofrequency energy is delivered to the myocardium via a catheter, which heats the myocardium and thereby terminates local conduction. By delivering point-by-point ablation through the reentry circuit, the arrhythmia can be terminated by interrupting the reentry circuit. This permanent alteration of the myocardium results in electrical isolation of the arrhythmogenic myocardium, thereby terminating the arrhythmia, but also preventing its recurrence.
Since recently, there is accumulating evidence that AT can deteriorate into AF and that horses successfully treated for sustained AT, by for example transvenous electrical cardioversion, can subsequently develop AF. Also, it has been shown that quinidine sulphate treatment in some horses with AF brings them into sustained AT. Therefore, at

least in some horses, there can be a close relation between AT and AF and this might also have an impact on recurrence. These findings suggest that these horses might benefit from ablation of the caudal medial right atrium in order to reduce the risk for AF recurrence. Clinical data are needed to determine whether this is the case or not.
Finally, atrial premature depolarizations (APDs) play a role in the initiation of AF. In some situations, however, it might be challenging to differentiate APD from sinus arrythmia or from a sinus rhythm with a different impulse exit point from the sinus node (eg. lateral versus medial exit). Twelve lead ECG recording is helpful to determine the presumed anatomical location of ectopic rhythms. More detailed information could be obtained from 3D EAM. This technique was recently used in a horse with APDs and allowed precise localisation of the origin of the APDs in the craniolateral right atrium. Radiofrequency ablation of such APDs is a potential next step for treatment. Clinical studies are needed to determine efficacy and safety of this treatment.

NOTES

10.15 – 11.00 CARDIOLOGY: Review on equine myocarditis - G. van Loon

Gunther van Loon, DVM, PhD, Dipl ECEIM, Assoc Member ECVDI
Equine Cardioteam Ghent, Ghent University, Merelbeke, BELGIUM

The typical definition of myocarditis is based upon histopathology of conventionally stained myocardial sections that show cellular infiltration with or without myocyte necrosis. Newest guidelines include immunohistochemical staining for improved sensitivity and prognostic value. In human patients viral infection is the most common cause of myocarditis and recent SARS Cov-2 infections are an example of that. But other causes have also been described.
The etiology of myocarditis in horses is often not precisely known but includes a long list of possibilities. Infectious causes include viral (EHV1, EIA, EVA, EI, EEE, African horse sickness), bacterial (streptococcus, staphylococcus, clostridia, Borrelia, babesia, leptospirosis, Neorickettsia, endotoxemia), or parasitological (strongylus, onchocerca) causes. Vitamin E and/or selenium deficiency has been reported repeatedly as a cause of myocarditis in young but also adult horses. This type of myocarditis will usually also affect (some) skeletal muscles. There are quite some reports on toxic causes of myocarditis. Cardiac glycoside intoxication results from drug overdosing or from ingestion of plants containing cardiac glycosides such as foxglove, oleander, adonis aestivalis, lilly of valey, … Hypoglycin A intoxication, which causes atypical myopathy due to ingestion of Acer seeds, has been shown to result in myocardial damage. Cantharidin intoxication due to ingestion of blister beetles can result in direct myocardial damage. Although not common, these beetles do occur in Europe (European blister beetle). Ionophores are used as coccidiostatics in poultry or growth promotors in cattle but are very toxic to horses resulting in severe, potentially lethal, myocardial damage. Intoxication usually results from accidental mixing of food (eg during transport). Snake envenomation has also been shown to result in severe myocardial necrosis in horses. Different types of neoplasia may invade the myocardium and cause an inflammatory reaction. Also chronic hypertension due to pain, pheochromocytoma, chronic renal failure, and sustained ventricular tachycardia can lead to inflammation of the myocardium. Generalized or local hypoxia and severe blood loss can be associated with myocardial damage. Severe colic and endotoxemia can cause myocardial injury and increased levels of cardiac troponins. Myocarditis can be generalized or focal. Healing of severely inflamed myocardium often results in fibrosis.
Clinal signs are very variable: from no clinical signs, to reduced performance, up to weakness, collapse or sudden death. Beside the signs due to underlying disease, signs associated with myocarditis are the result of an impaired myocardial function on the one hand and/or arrhythmias on the other hand. Therefore, echocardiography and ECG are essential diagnostic tools. Due to inflammation the myocardial wall can become thicker than normal, with an abnormal (heterogenous) ultrasound appearance. Poor myocardial function can lead to hypotension and over time to progressive cardiac dilatation in the affected chamber, often with thinner walls. In case of intoxication (for example cardiac glycosides), myocardial inflammation and wall thickening is associated with dehydration. On ultrasound the clinician should try differentiate myocarditis (with potential dehydration) from hypovolemia alone, which also results in thick walled, small sized chambers. A wide range of arrhythmias can be associated with myocarditis. Underlying disease and hypotension can lead to sinus tachycardia. Myocardial damage itself is often associated with ventricular (sometimes atrial) arrhythmias. Often these consist of isolated, multimorphic ventricular (sometimes atrial) premature beats, paroxysmal or persistent ventricular tachycardia or torsades-de-pointes. The latter carry the risk to deteriorate into fatal ventricular fibrillation. Clinicians should be aware that myocarditis often is associated with arrhythmias but that arrhythmias not necessarily mean that (active) myocardial injury is present. Indeed, from human medicine it is known that atrial and ventricular premature beats, tachycardia and even fibrillation often occur due to

alterations in electrophysiological properties or presence of fibrosis in the absence of active myocardial inflammation.
Cardiac troponins are the most important biomarkers to confirm active myocardial damage. Both cardiac troponin I (cTnI) and cTnT can be used in horses. cTnI is more commonly used and more readily available compared to cTnT. The disadvantage is that many types of cTnI tests are available and these can produce different results. So results from different labs are not necessarily comparable. Also, follow-up of patients should ideally be done with the same type of test. Preferentially, one should use validated tests. Occasionally, a cTnI test can produce increased cTnI results in an otherwise healthy horse, without any indication of myocardial injury. In such circumstances another type of cTnI test or a cTnT test may show a normal value. The precise reason for the falsely elevated values is unknown but the same occurs in human medicine and is thought to be due to allo-antibodies. This means that, when increased troponin I values are found that do not 'fit' with the healthy status of the horse, one should use a different cTnI and a cTnT test in order to assess whether or not both indicate increased values. The increase in cTnT values in case of myocardial injury is slightly less pronounced compared to cTnI but the major advantage of cTnT is that only one company (Roche) produces the test. This means that cTnT values can be easily compared between labs.
It is not indicated to rely on CK-MB or LDH iso-enzyme 1 as these biomarkers are much less specific and sensitive to myocardial injury. Also nt-proBNP should not be used as long as no proper validation has been performed. The nt-proBNP molecule is also different between species and therefore a horse specific tests needs to be developed.
Endomyocardial biopsy is a technique that can be used in standing horses. After placement of a steerable sheath in the right atrium or right ventricle, multiple biopsies can be taken from right atrium or right ventricle. The procedure should be carefully guided by ultrasound in order to sample the desired region and to avoid structures such as valves, chordae, moderator band. The technique has been safely used in an experimental setting but only limited in clinical cases. One should be aware that myocardial injury can be generalized but also focal. In the latter case a targeted biopsy from an affected area is needed to confirm the diagnosis. Also, the biopsy technique has been described for the right heart. Left heart biopsy would require to first perform a transseptal puncture through the oval fossa and place a steerable sheath into the left atrium. This transseptal technique is feasible in horses and has been used for mapping and ablation of the left heart, but has not been applied for biopsy procedures so far.
Besides treatment of underlying disease, myocarditis treatment consists of anti-inflammatory treatment, treatment of arrhythmias and support of overall cardiac function and output. Steroids are often used for the treatment of myocarditis but are not indicated in case of a bacterial infection. The clinician should be aware that arrhythmias, such as atrial or ventricular premature beats or tachycardia, with normal cTnI concentrations and no obvious abnormalities on ultrasound are relatively frequently found in horses. Often these ectopic rhythms remain present for a long period of time supporting the fact that these horses show no indication of an active myocarditis. Therefore, there is no evidence that steroid treatment would be beneficial in these cases and very often, empirically given steroid treatments have no effect. Similar as in human medicine, these ectopic rhythms can originate from areas with altered electrophysiological properties in the absence of an active inflammation. In human patients, inactivation of these parts of the myocardium by ablation are the most effective treatment. It is very likely that ablation of such arrhythmogenic regions might be effective in horses, but so far, this has not been investigated yet. The thickness of the equine myocardium also impedes creating transmural lesions and ablation in the ventricle might risk to induce ventricular fibrillation.
Anti-arrhythmic treatment is indicated whenever the arrhythmia impairs cardiac output and blood pressure, and when the arrhythmia is potentially dangerous (multi-morphic QRS complexes, shortly coupled QRS complexes, high rates, torsades…). Finally, reduced myocardial function can lead to low output and hypotension, and should be treated accordingly by inotropes and vasopressors. Obviously, stress and physical activity should

be avoided because of the potential risk of collapse and fatal arrhythmia. Caretakers and owners should be well aware of this.
The prognosis for mild episodes of myocarditis is often fairly good as it is reversible and horses can return to their previous level of activity. However, severe injury can result in substantial damage and myocardial fibrosis, leading to reduced myocardial function and presence of arrhythmias. Such horses cannot return to competition and should be considered unsafe to ride or drive. One should be aware that some arrhythmias occur due to an altered substrate (electrophysiology, fibrosis, high excitability) in the absence of active inflammation and that these arrhythmias may recur after successful medical treatment. Close follow-up might therefore be needed.

NOTES

11.30 – 12.15 INFECTIOLOGY: PCR testing : new tools and rapid testing - A. Waller

Andrew Waller
Chief Scientific Officer, Intervacc AB, Sweden

Aim of presentation:
The accurate and rapid detection of pathogenic agents is critical to the prevention and mitigation of outbreaks of infectious disease in horses and other animals. The availability of genome sequences of pathogenic agents including equine influenza virus, equine herpes virus and *Streptococcus equi* has provided new opportunities to improve the speed of diagnosis, and the sensitivity and specificity of agent detection.
This presentation will focus on the application of these assays, old and new, in order to improve the diagnosis of infection with *Streptococcus equi*. When these assays are best employed, and the sampling methods utilised according to the phase of infection, testing can greatly assist the prevention and management of strangles.

Features of strangles and their importance for diagnostic testing:
Streptococcus equi subsp. *equi* (*S. equi*) is the causal agent of strangles, a highly contagious disease that is endemic worldwide. *S. equi* is typically the most frequent cause of infectious disease in horses, but the type of samples taken, the method employed to test for agent detection and interpretation of the results all have implications on the successful outcome for both patient and client.
Strangles can spread rapidly through populations of horses, assisted by sharing of drinking water, feed, riding equipment and other materials. 100% of horses at an affected premises can develop signs of disease if biosecurity measures are not rapidly implemented and strictly adhered to. Fatality rates range from 1 to 10%, depending on the level of exposure to *S. equi* and prior history of the horse, including previous recovery from strangles, or vaccination status.
The implementation of biosecurity restrictions, cost of diagnostic testing, treatment and lost income due to closure of facilities are such that Strangles causes profound disruption and economic losses to equine industry and is undoubtedly one of the most challenging equine infectious diseases to manage.
S. equi is an obligate pathogen that does not survive well outside the horse. Therefore, the eradication of *S. equi* infection from affected premises should be a realistic aim, and provide confidence to others in the community where the disease is managed appropriately. In some countries, including the USA and Sweden, strangles is a reportable or notifiable disease.
A major factor in *S. equi's* success as an equine pathogen is its ability to survive in, and spread from, horses that are not exhibiting clinical signs. Transmission from apparently healthy horses is likely to be of greater importance than transmission from horses with clinical signs in triggering new outbreaks of disease and their diagnosis and treatment, before they come into contact with naïve horses, is vital to disease prevention strategies.

Pathogenesis and timing the collection of diagnostic samples:
Following the exposure of horses to *S. equi*, there is a latent period with incubation typically lasting up to 14 days. The duration of the incubation period is affected by pre-existing immunity in the horse, from prior infection or vaccination, and the size of the dose of *S. equi* received. For example, in experimental challenge studies, a dose of 1000 *S. equi* cells sprayed directly into the nasopharynx of naïve horses was sufficient to establish infection over a 21-day period. A dose of 100 million *S. equi* cells induced clinical signs in naïve horses in as little as three days post-challenge and a dose of 1 billion *S. equi* cells induced disease within 24 hours.
The first clinical sign of strangles is a rise in rectal temperature to 38.5 ^{o}C or higher. At this time *S. equi* is multiplying in the lymph nodes of affected animals and may not begin to be shed via the nasopharynx for up to several days after the onset of fever. Therefore,

the isolation of a horse at that time can prevent exposure of other horses and minimise the impact of disease. However, because of the low levels of *S. equi* being shed, no diagnostic samples collected at that time are 100% certain to facilitate the detection of *S. equi*. The optimal sample in these early stages of infection are nasopharyngeal swabs or lavages, but a horse with fever should remain in isolation even if a negative test result is reported. Note that even the more sensitive quantitative polymerase chain reaction (qPCR) assays may not be sufficient to detect the presence of *S. equi* at this time.

Infected lymph nodes develop abscesses containing billions of *S. equi* cells, and if these are accessible, they can be sampled by taking a needle aspirate. This is the optimal sample for the detection of *S. equi* using the culture test as it is less likely to contain *S. zooepidemicus*, a very closely related bacterium that may otherwise confound the diagnosis of *S. equi* using the culture assay. Note that the presence of *S. zooepidemicus* does not affect the ability of PCR, qPCR or LAMP assays to detect *S. equi* within a clinical sample.

Abscesses typically burst between around 1 to 2 weeks after the onset of fever. At this point the horse will also typically display clinical signs of profuse mucopurulent nasal discharge. A swab sample of a discharging submandibular lymph node, or a nasopharyngeal swab/wash at this time is highly likely to facilitate the detection of *S. equi* by culture, PCR, qPCR or LAMP assays. One millilitre of purulent material can contain up to a billion *S. equi* cells. Affected horses are most infectious at this point and their isolation is essential to minimise the exposure of other horses to *S. equi*. Higher doses of *S. equi* received by other horses induce more rapid, and potentially, more severe signs and so the isolation of affected horses is important to mitigate the impact of a strangles outbreak.

Over 90% of horses recover from infection with *S. equi* over a period of two to four weeks. However, horses that have shown clinical signs of disease may remain infectious for several weeks after the cessation of clinical signs as they continue to shed *S. equi* from draining lymph nodes. This presents a risk for onward transmission if freedom from infection is not demonstrated prior to lifting isolation measures. The improved sensitivity of PCR, qPCR and LAMP assays in comparison to the culture test makes these assays the most appropriate for the demonstration of freedom of infection.

Approximately 10% of horses become persistently infected "carriers" horses in which *S. equi* may persist within one or both guttural pouches for months or years. The shedding of *S. equi* from guttural pouches is often intermittent and of a low level, again favouring diagnosis by the more sensitive PCR, qPCR and LAMP assays. Whilst sampling healthy horses to establish whether they are persistently infected can be performed using nasopharyngeal swabs and washes, the gold standard remains lavage of the guttural pouches regardless of the diagnostic testing method employed.

Serological screening of horses, particularly at the end of an outbreak, can also be a useful tool to determine if some unaffected horses were also exposed to *S. equi*. Guttural pouch lavage samples from seropositive horses can then be tested by PCR, qPCR or LAMP assays to determine if they are persistently infected.

Diagnostic testing methodologies:

Traditional bacterial culture methods have been used to confirm the presence of streptococci for over a century. They employ selective agar to grow streptococci, then beta-haemolytic colonies are examined further by using sugar fermentation tests to confirm the species present. *S. equi* will not ferment lactose or sorbitol, whilst its close relative *S. zooepidemicus* will usually ferment both of these sugars. However, traditional culture methods have a number of disadvantages: i) bacterial culture is far less sensitive than PCR, typically requiring the equivalent of at least 1000 copies of *S. equi* DNA per ml before a positive result can be obtained. ii) false negative results may occur if organisms present in samples are not viable when they reach the laboratory, such as if the sample is exposed to warm temperatures during the summer months. iii) *S. zooepidemicus* is a common opportunistic pathogen and if mixed β-haemolytic streptococcal infections are present, *S. equi* may be easily overlooked. iv) it takes a number of days to obtain results. On rare occasions, culture may identify the presence of *S. equi* when PCR has failed and

false negative PCR results have been obtained; however, this scenario is so infrequent that culture is increasingly omitted when concurrent PCR testing is being performed.
PCR usually provides results within 24 hours (and potentially within one hour), which represents a significant advantage over traditional culture results in preventing disease transmission. Early PCR methods targeted the gene encoding the SeM protein, however this gene exhibits variation, with target-site segments being truncated and lost in isolates infecting some carrier horses. Other targets have since been identified including the genes *seeI*, *seeH*, *eqbE* and SEQ2190. Some strains recovered from persistently infected carrier horses contain gene deletions that can result in negative PCR tests. Therefore, some assays include 2 gene targets, such that both would be required to be deleted in order for a false negative result to be obtained. Some assays also include internal control targets, such as *sodA* or a synthetic DNA sequence, SZIC, which enable verification of negative results. As PCR methods become less expensive to develop and use, alternatives are being brought to the market which will be advantageous to disease control, provided that they are appropriately validated and are accurate. These include point-of-care assays that have the potential to enable vets to obtain a diagnosis within an hour of collecting a clinical sample, and before leaving the affected premises. Such assays have great potential in mitigating the transmission of *S. equi*. However, challenges remain in the preparation of the sample, microfluidics and sample throughput.
Loop-mediated isothermal amplification (LAMP) assays enable DNA amplification and detection in a single isothermal step. LAMP assays have been developed for rapidly detecting *S. equi* gene targets SeM and *eqbE*. These assays are appealing, particularly for development for use in point-of-care settings; however in a comparative study the *eqbE* LAMP assay had 11% lower sensitivity and 21% lower specificity when compared against the *eqbE* PCR assay. Therefore, whilst point-of-care testing has significant future potential, particularly where the speed of diagnosis is of critical importance, confirmation of the test result using a laboratory-based assay remains prudent. LAMP assay technology continues to improve and further advances in this field are expected in the coming years.
Anyone submitting samples for *S. equi* PCR should be aware of the diagnostic accuracy of the test that is being used and should be aware that not all tests are the same. The accuracy of PCR methods is dependent upon scrupulous technique and the elimination of any possibility of cross contamination. Interpretation of PCR results is also important as the results generated are not dichotomous and careful consideration has to be given to results with low DNA copy, or high cycle threshold (CT), numbers that suggest the presence of low levels of target organism that *might* not be relevant clinically or indeed accurate if attributable to cross-contamination. The inclusion of a positive control DNA target provides greater reassurance that negative results are genuine and are not the result of a failure of the laboratory process. A quality assurance scheme is available for strangles diagnosis by *S. equi* detection (http://apha.defra.gov.uk/ahvla-scientific/vetqas/PT0193.html).
Measurement of the antibody response to *S. equi* enables the identification of horses that have been exposed to *S. equi*. This knowledge is particularly useful at the end of an outbreak so as to facilitate further diagnostic testing, such as qPCR testing of guttural pouch lavage samples to identify persistently infected horses. Not all persistently infected horses necessarily display clinical signs during an outbreak and so screening apparently unaffected horses can be a valuable approach to minimise the risk of recurrent cases of disease. Serological testing was initially developed to identify horses with high levels of SeM specific antibodies indicating potential complications such as metastatic abscesses, or the risk of developing purpura haemorrhagica if vaccinated with SeM-containing vaccines. However, cross reactivity between the SeM protein and the homologous SzM protein of *S. zooepidemicus* can lead to false positive diagnoses of exposure to *S. equi*. A dual antigen ELISA was therefore developed targeting antibodies to the N-terminal portion of SEQ2190 surface protein (antigen A) in addition to the N-terminal portion of the SeM protein which is unique to *S. equi* (antigen C). Both the dual antigen ELISA and an SeM ELISA are available commercially in Europe. A quality assurance scheme is also available for strangles serology (http://apha.defra.gov.uk/ahvla-scientific/vetqas/PT0175.html).

When performing diagnostics for *S. equi* in horses with signs of strangles, the possibility of infection with pathogenic strains of *S. zooepidemicus* expressing superantigens or other virulence proteins always needs to be considered. In this situation, with horses showing signs of strangles, the differentiation of *S. equi* and *S. zooepidemicus* is academic as virulent strains of *S. zooepidemicus* with a propensity to produce lymph node abscessation should be managed in the same way as *S. equi*.

Vaccination of horses with live attenuated vaccines can lead to the return of positive culture, PCR, qPCR, LAMP and iELISA diagnostic test results as these vaccines contain live *S. equi*, *S. equi* DNA and produce the SeM and SEQ2190 antigens. Horses vaccinated with live attenuated vaccines that test positive should be examined further in case the result reflects incomplete protection against a wild-type strain, which could be transmitted to other horses.

A new fusion protein vaccine for intramuscular vaccination is now available in Europe. This protein-based vaccine does not contain live *S. equi*, *S. equi* DNA, or the SeM or SEQ2190 antigens. Therefore, vaccinated horses test negative by culture, PCR, qPCR, LAMP and iELISA diagnostic tests unless exposed to *S. equi* and so can be differentiated from those with natural infection using all of the above-described diagnostic tests. Initial reports from the use of the fusion protein vaccine in the face of strangles outbreaks in the field identified horses that remained healthy, but tested seropositive for exposure to *S. equi*, indicating that a potential protective effect had been induced and facilitating the further monitoring of exposed horses.

Conclusions:

The enhanced sensitivity and specificity of modern diagnostic tests for strangles combined with good equine management practices and the use of vaccines that can be used alongside diagnostic testing have tremendous potential to reduce the prevalence of strangles in horse populations. The timing and type of diagnostic sampling employed, the diagnostic test utilised, and the disease and vaccination history of the horse are all important factors to understand in order to correctly interpret and act upon diagnostic test results.

NOTES

12.15 – 13.00 INFECTIOLOGY: Understanding Next Generation Sequencing technology and metagenomics in equine infectiology - A. Waller

Andrew Waller
Chief Scientific Officer, Intervacc AB, Sweden

Aim of presentation:
The agents of infectious disease continuously evolve over time to become 'fitter', i.e. more able to transmit to other animals. Next generation sequencing technology, and the analysis of the data generated, is a tool that can be utilised to examine and chart this process of evolution. The data generated can be applied to monitor outbreaks, for example, of equine influenza virus (EIV) and to guide decisions on whether updates to the EIV vaccines are required in order to maintain their protective effect. This presentation will examine the evolution of *Streptococcus equi* subspecies *equi* (*S. equi*), the causative agent of strangles in horses, from it's origins as an ancestral strain of *Streptococcus equi* subspecies *zooepidemicus* to the latest variants affecting horses around the world today. The application of next generation sequencing technology facilitates the tracking of transmission events, enhancing our understanding of 'risk' events so that interventions such as enhanced biosecurity and vaccination can be implemented to mitigate the risks of transmission.

The worldwide population of *S. equi*:
Strangles, caused by *Streptococcus equi* subspecies *equi*, is one of the most prevalent diseases of horses around the world. Only the geographically isolated population of horses in Iceland remains free of *S. equi*, which is due to a self-imposed ban on the import of horses that has been in place for over a thousand years. The economic consequences of strangles can be serious with some individual outbreaks involving hundreds of horses leading to associated costs exceeding £300,000. The disease also has important health and welfare impacts with up to 100% morbidity and up to 10% mortality. Horses of any age, breed and fitness can be affected. For example, a recent outbreak in European Sport Horses affected around 100 animals and led to the death of six of these high-performance athletes.
Equine strangles is characterised by pyrexia and the development of abscesses in the lymph nodes of the head and neck. As the disease progresses, abscesses in the retropharyngeal lymph nodes rupture and drain into the guttural pouches, and then into the environment via the nasopharynx. Incomplete drainage of abscess material from the guttural pouches results in a proportion of convalescent animals becoming persistently infected with *S. equi*. These outwardly healthy 'carrier' animals can harbour *S. equi* for years, intermittently shedding bacteria into the environment where the organism can be taken up by naïve animals, triggering new outbreaks of disease.
S. equi was first identified in 1888. The first genome sequence of this pathogen and a strain of the closely related bacterium *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) strain were completed at the Animal Health Trust, UK, in 2009. Comparison of the genomes of *S. equi* and a *S. zooepidemicus* identified a host of genetic differences, which have shaped the evolution of *S. equi*, enabling it to cause strangles. Gene decay events were identified that restrict the ancestral capabilities of *S. equi* and these explain how *S. equi* only infects horses, and only causes strangles. In contrast, *S. zooepidemicus* strains have a much more diverse genome, infect a very wide range of different animals, including horses, pigs, sheep, dogs and humans, leading to a huge variety of different diseases such as pneumonia, endometritis, keratitis, nephritis and septicaemia. Gene gain events enabled *S. equi* to further misdirect the equine immune response and acquire nutrients from equine tissue that help it to grow and form abscesses. For example, a key 'speciation event' was the acquisition of an iron uptake system, which resembles that utilised by the pathogen *Yersinia pestis* – the causative agent of bubonic plague in humans!!

Although strangles is an ancient disease, next generation sequencing provided evidence that a global population replacement occurred at the end of the 19th/beginning of the 20th Century. During this period of history, the international transport and mixing of horses that accompanied a series of global conflicts is thought to have led to the emergence of a 'fitter' strain of *S. equi* from which apparently all contemporary strains are derived. An estimated 8 million horses died in World War I, leading to the establishment of initiatives such as the National Stud in Ireland during 1915 in order to accelerate the breeding of more cavalry horses that were needed by the military and to replace those sent to Europe. These conditions provided the perfect situation for the emerging 'fit' strain of *S. equi* to be transported to nations around the world from where it continued to evolve into distinct European (called: BAPS 2, BAPS 5 and BAPS 6), North American (called BAPS 1), South American (called BAPS 4) and Oceanic (called BAPS 3) lineages.
Horses continue to be transported all over the world for trade, training and competition and in 2016 there were over 150,000 horse trade events between EU member states registered. To minimise the risks of diseases such as shipping fever, where the prolonged transport of horses from one country to another via rail or sea can lead to respiratory disease and, occasionally, the death of the affected animal, horses are transported long distances by air. This rapid movement of horses provides new opportunities for the transmission of infectious diseases. To minimise the risk of pathogen transmission, horses are examined by veterinarians pre- and post-transport. However, whilst veterinary examinations identify animals suffering from acute disease, such as equine influenza, *S. equi* infections can incubate for several weeks in apparently healthy horses, and this pathogen can also persist in healthy animals that have recovered from strangles. The lack of quarantine of newly transported horses provides an opportunity for the transmission of *S. equi* to new populations of horses, sometimes even across different continents. However, despite the global prevalence of strangles, and the severity of this disease, strangles is conspicuously absent from the Office International des Épizooties or World Organisation for Animal Health list of diseases, infections and infestations of the horse and no diagnostic testing for strangles is typically conducted pre-purchase, export or import of horses.

Applying next generation sequencing towards preventing outbreaks of strangles:
The lack of effective control continues to facilitate the emergence and dissemination of ever fitter strains of *S. equi*. New data from isolates of *S. equi* recovered between 2016 and 2022 from horses in the UK identified a 'fastbaps 3' sub-variant of *S. equi*, which accounted for over 90% of outbreaks in the UK during 2022. Incredibly, this variant accounted for less than 10% of UK outbreaks in 2016, highlighting an unexpectedly rapid change in the population of *S. equi* causing strangles in horses in the UK. These data provide evidence supporting the hypothesis that the vast majority of UK outbreaks occur via transmission from recently infected horses, or those that have recently recovered from strangles, but continue to shed *S. equi*. Of particular note, very few *S. equi* strains that were recovered from horses with strangles in the UK during 2022 were of an older *S. equi* lineage, suggesting that long-term persistently infected horses triggered relatively few outbreaks of strangles in the UK during this period. These data have important implications regarding the control of strangles, as the movement of horses and the containment of outbreaks are highlighted as key 'at risk' events.
In Sweden, SVA, SLU and Intervacc AB sequenced 42 isolates of *S. equi* that were recovered from different outbreaks between 2021-2022. All of these isolates belonged to the BAPS-2 (European) cluster. However, at least 13 distinct subvariants were detected, including the fastbaps 3 sub-variant that is the dominant type of *S. equi* in the UK horse population. Detailed analysis of the circumstances surrounding each outbreak revealed that most outbreaks were linked to the introduction of a new horse to the facility, often one that was recently imported. The most prevalent subvariant was found in 31% of the tested samples.

In a trotting stable with unvaccinated horses, where new cases emerged over an extended period while new horses continued to be introduced, it was found that they were initially affected by one strangles variant and later by another variant a few months later, highlighting two independent introductions of *S. equi*. When DNA testing revealed different bacterial subvariants at different facilities, the possibility of cross-contamination between them could be dismissed. When tests indicated the same subvariant, the infection might have been directly transmitted between the facilities, or there could have been a common source of infection. This common source could involve a widely spread subvariant, and the shared link might have existed much earlier and several steps away, potentially even in a different country. Examples of the same infectious subvariant being transferred to different facilities via infected horses transported in shared horse carriers, or through inadequately cleaned horse transports where the infection persisted between different trips were also identified. The protein sequences in the available new fusion protein-based strangles vaccine matched the variants of *S. equi* in Sweden with an impressive 99.9% level of identity. However, of note, none of the affected horses on the affected farms had been vaccinated against strangles.

The genetic analysis of strangles outbreaks represents a pioneering area of research. By meticulously tracing infections and transmission routes it is possible to identify key risk events in order to implement targeted preventive measures, such as vaccination and quarantine. For example, the vaccination of horses prior to competition, the vaccination of new horses prior to transport, or whilst within quarantine at a new farm, and the vaccination of resident horses at a farm prior to the arrival of a new horse.

If an outbreak does occur, then reacting quickly can mean the difference between one index case or a whole infected farm. An increase in temperature to ≥38.5 ^{o}C is usually the first clinical sign. Isolating an affected horse into a 'red' group at this time can often prevent other horses being exposed to *S. equi*. Horses in contact with the affected horse should be isolated into an 'amber' group in case they were exposed and may be incubating the disease, and horses that have not had contact with the infected horse should be isolated in a 'green' group (so-called red/amber/green, or 'RAG' management of strangles). The rectal temperature of horses should be checked daily, operating from the green, to amber, to red groups to minimize the chance of spreading *S. equi* via human contact. Any horse in the green or amber groups that has a temperature of ≥38.5 ^{o}C should be moved to the red group. The testing of blood samples from horses in the green and amber groups at 4 to 6 weeks after resolution of the last clinical case using the dual antigen ELISA for strangles can be utilised to identify which of these horses were exposed to *S. equi* during the outbreak so that their infection status can be checked by the attending veterinarian.

Early indications from the field suggest that the vaccination of horses in the green group, and horses in the amber group (provided their rectal temperatures have remained normal for two weeks after removal of the index case) has been beneficial. For example, following the identification of three horses with strangles at a farm in Sweden, none of the remaining 17 healthy horses that were vaccinated in the face of this outbreak developed signs of strangles despite eight of them subsequently testing seropositive in the strangles blood test, indicating that they had been exposed to *S. equi* during the outbreak.

Conclusions:

The use of next generation sequencing techniques is shedding unprecedented light on the evolution and transmission of *S. equi*. By analysing isolates from different outbreaks critical 'at risk' events can be identified, facilitating new interventions such as improved biosecurity and vaccination strategies. Together, the effective use of biosecurity and vaccination has the potential to dramatically reduce the transmission of *S. equi* and minimise the number of affected horses, reducing the prevalence of this disease and improving the health of our horses.

NOTES

14.15 – 15.00 INFECTIOLOGY: Overview of arthropod-borne infectious diseases in horses - E.J. Cunilleras

Eduard Jose-Cunilleras LV PhD DECEIM
Dept. Animal Medicine and Surgery, Universitat Autònoma de Barcelona

Vector-borne infectious diseases pose a global threat to human and animal health. According to the WHO, vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually. They can be caused by parasites, bacteria, or viruses. In humans, the most prevalent infections are Malaria, caused by the protozoa *Plasmodium* spp transmitted by *Anopheline* mosquitoes, and Dengue, caused by a flavivirus transmitted by *Aedes* mosquitoes. In horses, the most important arthropod-borne infectious diseases may vary geographically but mosquito-borne viral diseases are the most common infection of the equine central nervous system globally and are increasingly more common as the spatiotemporal distribution of the vector increases as a result of global warming.

This overview focuses on existing and emerging arthropod-borne infectious disorders in Europe, with an emphasis on evidence from recent studies. Piroplasmosis (*Babesia* and *Theileria* spp) will not be discussed because it will be covered by other speakers in this ECEIM Congress.

The **main arthropod vectors of diseases** in equids are mosquitos (*Culex* spp., *Aedes* spp, and others), biting midges (*Culicoides* spp.), sandflies (*Phlebotomus* spp.), and ticks (*Ixodes* spp., *Hyalomma* spp, and others). A partial list of pathogens, vectors, and diseases affecting equids is presented in Table 1, including their zoonotic potential and major hosts. Other pathogens do not replicate in the body of the insect and are, therefore, distinct from arboviruses. Such pathogens are only transmitted with a mechanical mode (e.g. equine infectious anemia virus is transmitted on or in the mouthparts of insects without replication).

The following includes an overview and main novelties of arthropod-borne infectious diseases over the past few years discussed according to the main presenting clinical signs.

ARBOVIRAL EQUINE ENCEPHALITIDES

In addition to traumatic, compressive myelopathy ("Wobblers"), and certain viral infections (herpesvirus, rabies, etc.), viruses transmitted by arthropod vectors should be considered in horses presenting signs of central nervous system disease (ataxia, altered mental status, etc.).

<u>West Nile and other flavivirus (Usutu and Tick-borne encephalitis)</u>

WNV is a member of the Japanese encephalitis virus (JEV) antigenic serogroup. Viruses in this serogroup cause similar diseases across the world, including nonspecific systemic symptoms and less frequently, neurologic disease in birds, equids, and humans. These include the JEV (Asia), St. Louis encephalitis (Americas), Kunjin encephalitis (Oceania), Murray Valley encephalitis (MVEV; Australia/South Pacific), and WNV (Asia, Africa, Europe, Americas) viruses. Usutu virus infects birds and rarely humans, and seroconversion has recently been demonstrated in horses. Tick-borne encephalitis is also a flavivirus that rarely infects horses, results in fever and neurologic clinical signs is distributed in central Europe and Asia, and is transmitted by Ixodes ticks.

WNV was historically restricted to Africa and the Middle East, however, it is currently also in Europe, Asia, Oceania, and the Americas. It was first introduced to North America in 1999 where it encountered naïve human and animal populations, and the virus spread rapidly across the United States over a decade. In Europe, WNV resurfaced from 1996 to 2000, mainly lineage 1. However, in recent years, there has been an increase in human and equine cases, in particular in Central and Northern Europe. In 2018 the number of human and animal cases and deaths was 8-10 times higher than in previous years, and 2020 was particularly more severe in Spain with up to 10 times higher cases and deaths in humans and horses.

The virus is maintained in an enzootic cycle involving culicine mosquitoes (Culex spp., Aedes spp.) and birds. In birds, WNV causes high viremias, which favor mosquito infection and viral dissemination to susceptible animals and people. Horses and humans are dead-end hosts because of their low and short-lived viremia. Only about 10% of infected horses and about 1% of infected humans develop neurologic signs. The virus enters the CNS through a combination of disruption of BBB, transported by mononuclear cells that migrate into the CNS and retrograde axonal transport, and after 5-15 days of incubation period develop clinical signs.
Clinical signs: Symptomatic horses may only develop fever, but typically neurologic signs are characterized by ataxia, proprioceptive deficits, muscle fasciculations/tremors that are more pronounced on the face and neck, somnolence, blindness, cranial nerve deficits and/or recumbency. Compared with other viral diseases, muscle fasciculations, tremors, and behavioral changes are consistent findings in horses with WNVE, and spinal cord signs are more evident with WNV than with alphavirus encephalomyelitis.
Diagnosis: Laboratory confirmation in live neurologic horses is often reached by positive serum capture ELISA (IgM) results, compatible cerebrospinal fluid cytology (mild mononuclear pleocytosis), and detection of viral RNA by RT-PCR in blood, CSF, or tissues. Competition ELISA tests (IgG) are very sensitive but highly sensitive but not very specific because of possible previous exposure or cross-reactions with other flavivirus.
Treatment of WNV and equine alphavirus infections relies on supportive treatment to reduce cerebral and spinal cord inflammation intracranial pressure, edema, and hemorrhage, controlling neurologic signs, and treating secondary infections. A combination of NSAIDs (flunixin meglumine, 1.1 mg/kg, IV, twice daily), glucocorticoids, dimethyl sulfoxide, hypertonic saline solution, and/or mannitol are often used, but solid evidence is lacking as to their effectiveness.
Recovery can take several months and not all horses will fully recover. It is reported that 10-40% of horses will have residual neurologic deficits. Mortality from WNE is about 30% and approximately a third of horses may experience a relapse of signs after initial improvement.
Prevention is centered on vaccination in endemic areas and mosquito control. Several vaccines are commercially available in Europe and the US, with significant evidence of being highly effective at reducing their incidence. Reducing mosquito breeding areas by eliminating standing water (e.g., tires, buckets, cans, and ponds) is important. Approved insecticides should be considered to control adults and larvae. Biological pesticides (e.g., *Bacillus thuringiensis*) are effective as larvicides, widely used, and are safe for animals, people, and plants.

<u>Eastern/Western/Venezuelan equine encephalitis</u>

Equine togaviral encephalomyelitis is caused by viruses of the Alphavirus genus that includes four viruses: Eastern equine encephalitis virus (EEEV), Madariaga virus (MV), Western equine encephalitis virus (WEEV), and Venezuelan equine encephalitis virus (VEEV). These viral encephalitides are restricted to the Americas and affect horses, donkeys, and humans as dead-end hosts. Depending on the virus, the biological cycle includes mosquitoes, birds, small mammals, and equids. These alphaviruses are not a problem in Europe, but recent studies classify them as potential emergent diseases for humans and horses in our continent.

Eastern equine encephalitis (EEE) is a neurologic condition of equids and people transmitted by mosquitoes, and it's the deadliest alphavirus for humans and horses (mortality rates up to 50% and 90%, respectively). After recent gene sequencing analysis studies, South American lineages of EEEV (II, III, and IV) have been reclassified as the Madariaga virus which typically causes neurologic disease in horses and donkeys, and occasionally humans. In addition, recently Ross River virus in Australia and Papua New Guinea and Semliki Forest Virus in Africa have both been reported to cause neurologic disease in horses.
Western equine encephalitis virus (WEEV) is restricted to North-western, Central and South America. Reports of large outbreaks in equids and humans in the 1930s and 1960s have

not occurred more recently, and the number of cases declining, but sporadic human cases occur in South America. Within the same WEEV antigenic serocomplex, there are multiple other alphaviruses, including the Sindbis virus (SV). SV was recently reported to cause fever and neurologic disease in horses in South Africa and was also detected in Sweden by PCR in hibernating mosquitos and serologic response in Swedish horses.
Venezuelan equine encephalitis has been reported in central and South American countries, but certain outbreaks have also involved Mexico and Texas, most of them in the first half of the XX century. After a 20-year quiescent period, the virus resurfaced in Venezuela in 1992 to lead in 1995 to the second largest outbreak in Venezuela and Colombia, with 100,000 human and many more equine cases.
Clinical signs: Horses, donkeys, and mules develop similar clinical signs with alphaviral infections. Initially, there is fever, anorexia, and lethargy that are nonspecific and associated with viremia. It progresses to obtundation, incoordination, hyperexcitability, dysphagia, muscle tremors, impaired vision, head pressing, circling, leaning against walls, paresis, paralysis, recumbency, convulsions, comma, and death.
Diagnosis: given the nonspecific neurologic signs, the differential diagnoses include West Nile virus (WNV) encephalitis, rabies, equine protozoal myeloencephalitis (EPM), equine herpesvirus 1 (EHV-1) myeloencephalopathy (EHM), toxicities (e.g., leukoencephalomalacia), metabolic disorders (eg, hepatic encephalopathy), and others (e.g., aberrant parasite migration, tumors, abscessation). Evaluation of cerebrospinal fluid may reveal neutrophilic pleocytosis is a consistent and unique finding with EEE, whereas VEE and WEE typically have increased CSF protein and mononuclear pleocytosis, but CSF can be unremarkable. Confirmatory laboratory testing includes detection of IgM-specific antibodies with capture ELISA can be valuable early in the course of disease, seroconversion in paired samples, or RT-PCR to viral genomic material in blood, nervous, and peripheral tissue.

TICK-BORNE PATHOGENS RESULTING IN FEVER, STIFFNESS AND OTHER UNSPECIFIC SIGNS

Among the plethora of disorders to include in the differential diagnosis of fever of unknown origin, stiffness, limb edema, and other unspecific clinical signs, we should consider certain bacterial pathogens transmitted by ticks.

Equine granulocytic anaplasmosis

Horses infected by *Anaplasma phagocytophila* may present initially with fever, depression, anorexia, and icterus, and anorexia. Later in the course of the disease, petechiation and distal limb edema may appear, alongside gait stiffness and myalgia. Rare neurologic forms of the disease have also been reported, manifesting as mild hind limb ataxia or more rarely as severe ataxia. Characteristic changes of hematology include mild to moderate thrombocytopenia.
This obligate intracellular bacterium has a wide range of hosts, and it is transmitted by Ixodes ticks. The prevalence of anaplasmosis seems to be spreading geographically following increasing climactic temperatures and prolongation of the vector season. There is significant variation in the epidemiology of this disease in North America and Europe. The same pathogen causes Human Granulocytic Anaplasmosis (HGA), which is common in humans in the United States, with 5,000 to 6,000 cases reported to the Centers for Disease Control and Prevention annually, yet it is rare in Europe.
In horses, diagnosis relies on detection by PCR and direct observation of morulae within neutrophils in blood smears, and more recently it has been demonstrated that rapid antibody tests have high sensitivity and specificity for diagnostic confirmation. Effective treatment includes tetracycline antibiotics and supportive therapy.

Lyme disease

Borrelia burgdorferi is an spirochete that is transmitted by *Ixodes ricinus.* These ticks are observed across Europe and prefer deciduous woodlands and mixed forests. In Europe, the regions with the highest tick infection rates (nymphs > 10%; adult ticks > 20%) are located in central Europe and include Austria, Czech Republic, southern Germany, Switzerland, Slovakia and Slovenia. The highest incidence rates of human cases of Lyme

disease are reported in Estonia, Lithuania, Slovenia, and Switzerland, followed by France, Poland, Finland, and Latvia.
Case definition in humans varies across countries, and there are still controversies in the disease definition in equids. According to the ACVIM Consensus statement on equine borreliosis, the best-documented, naturally occurring syndromes attributed to *B. burgdorferi* infection include the rare cases of neuroborreliosis, uveitis, and cutaneous pseudolymphoma; and the commonly reported association of *B. burgdorferi* infection with stiffness and lameness in horses is not well documented and there is no evidence of the infection causing laminitis. Proposed criteria for antemortem diagnosis of Lyme disease in horses include: (i) cytology: pleocytosis or increased protein in CSF or ocular fluids, (ii) abnormal ratio serum:CSF *B. burgdorferi* antibodies (evidence of intrathecal antibodies), and (iii) positive antigen testing by PCR or immunohistochemistry in affected samples. Cases with one of these 3 criteria are considered possible cases, and those with 2 of 3 criteria are considered probable cases. Evidence for prior or current infection with *B. burgdorferi*, as demonstrated by positive serology, is present in most Lyme cases but the positive predictive value is very low because of the high exposure rate in endemic areas.
Borrelia burgdorferi is transferred from the tick gut to animals during blood meals. After tick attachment, several hours are believed to be required to successfully transfer the organism to a mammalian host. Early removal of ticks is considered effective in preventing the development of Lyme disease in humans.
Treatment recommendations have been based on in vitro *B. burgdorferi* antibiotic susceptibility, available antibiotic pharmacokinetic data, and a single treatment trial in experimentally infected ponies, as well as extrapolation from human treatment guidelines. Some authors recommend a month-long course of IV tetracycline or oxytetracycline for suspect cases of equine Lyme disease. An alternative might be treatment of similar durations with either doxycycline (10 mg/kg PO q 12 h) or minocycline (4 mg/kg PO q 12 h) with less risk of an adverse event. Minocycline might be a better alternative compared to doxycycline for neuroborreliosis or cases with ocular involvement given that minocycline has better blood-brain barrier and ocular penetration in healthy horses. However, evidence of efficacy in equine Lyme disease cases is lacking.

AFRICAN HORSE SICKNESS, TRYPANOSOMIASIS AND OTHER EXOTIC DISEASES

African Horse Sickness

The present geographic distribution of African horse sickness includes several Sub-Saharan African countries (Nigeria, Cameroon, Ethiopia, Eritrea, Namibia and South Africa). The disease severely affects horses, while mules, donkeys, and zebras are less susceptible.
The clinical signs may vary depending on the species of equid and four clinical forms have been described: (i) subclinical/febrile form; (ii) subacute, edematous or cardiac form: swelling of the supraorbital fossa, eyelids, facial tissues, neck, thorax; (iii) peracute, respiratory form: fever, intense dyspnea, and pulmonary edema, and (iv) acute, mixed form: mild/moderate pulmonary signs, edematous swellings and effusions.
Laboratory diagnostic confirmation is achieved by one of several methods: (a) virus isolation in cell cultures or embryonated eggs, (b) virus detection by RT-PCR or ELISA for rapid detection of AHSV in blood, or (c) serological diagnosis: virus neutralization, blocking or indirect ELISA.
Recent studies using ecological niche modeling have suggested that OIE's disease-free areas, like India, Australia, and Brazil, are predicted suitable for the disease. Recently, in the early months of 2020, an unexpected outbreak of AHS appeared in Thailand, affecting to Malaysia too, and AHS continues to be a potential threat to the equine industry worldwide.

Equine Trypanosomiasis

The diseases caused by these trypanosomes are called "surra" (*T. evansi*), "dourine" (*T. equiperdum*), and "nagana" (*T. brucei, T. congolensis* and *T. vivax*). *Trypanosoma brucei* and *T. congolensis* are the only species that are confined to the distribution of tsetse flies (the vector) in sub-Saharan Africa. *Trypanosoma equiperdum* is transmitted sexually, and *T. evansi* is transmitted mechanically by blood-sucking flies, vampire bats, and possibly

sexually. Infection of working equids by *Trypanosoma brucei*, *T. vivax*, and *T. congolensis* in Central Africa is endemic and common.
Clinical signs: Donkeys can carry an infection without developing classical signs of the disease. Typical signs in horses are pyrexia, anemia, ventral edema, conjunctivitis, keratitis, etc. In the neurological phase of infection with *T. brucei, T. equiperdum*, or *T. evansi*, ataxia and paralysis of the hind quarter and lips usually precede death.

Diagnostic confirmation of suspected cases often relies on PCR detection of the Trypanosomes. Alternatives include direct observation of trypanosomes in blood smears or serologic tests. The OIE recommended serologic tests vary according to the species involved:
- *T. equiperdum*: complement fixation test (CFT) and the indirect fluorescence antibody test (IFAT)
- *T. evansi*: IFAT, an ELISA (RoTat 1.2), and a card agglutination test
- *T. brucei*, *T. vivax* and *T. congolense*: IFAT and ELISA
Treatment: Specific trypanocide drugs include isometamidium, diminazene, or melarsomine dihydrochloride. Based on recent studies, melarsomine dihydrochloride appears less effective compared to isometamidium and diminazene.
Recent surra and dourine outbreaks in Europe highlight the risks and consequences of importing equine trypanosomosis through infected animals into non-endemic regions. Introduction of *T. brucei* into the Canary Islands by importation of infected camels, and sporadic equine cases in mainland Spain have been reported.
Crimean-Congo hemorrhagic fever (CCHF)
CCHF virus is an emergent pathogen that belongs to the Bunyaviridae family, which is transmitted by *Hyalomma* ticks. *Hyalomma* ticks prefer a Mediterranean climate (hot and dry), most species are present in southern and eastern Europe, and its distribution is expanding into Germany and other central European countries. This tick is a known vector of a wide variety of pathogens of medical and veterinary importance, including CCHF virus, West Nile, and *Babesia caballi*.
Among tick-borne zoonoses in Southern Europe, CCHF is one of the most concerning because of the severity of its clinical signs and high fatality rate in humans. Adult stages usually of *Hyalomma* ticks feed on large ungulates such as horses, cattle, sheep, goats, deer or wild boar, and occasionally humans. In a recent study in the south of France, about 1/3 of the ticks sample from horses at pasture were *Hyalomma*. In addition, sporadic cases of CCHF have been detected in Spain, Bulgaria, and Macedonia.
Experimental CCHF infection in horses results in fever, somnolence, and weakness which quickly resolves after 2 days. Donkeys are not susceptible to CCHF infection, but seroconversion in horses and donkeys has been detected and the role of equids as amplifiers or contributors to the spread of this tick is unknown.
Leishmania
Human Leishmaniosis is caused by *L. infantum* in Mediterranean countries and *L. infantum* and *L. braziliensis* in Central and South America. A cutaneous and a visceral form has been described. Dogs and other canids are considered the main reservoir of *Leishmania*, and it is transmitted by sandflies (*Phlebotomus*). However, in endemic areas horses and donkeys may be alternative hosts for sandflies, and in certain studies, seroprevalence to Leishmania is higher in donkeys relative to dogs. In endemic areas, equids infected with *L. braziliensis*, have been suggested as food sources for phlebotomines in the peridomiciliary environment that could play a role in the human cutaneous leishmaniasis cycle.
Symptomatic equine leishmaniasis is less severe than in other hosts (dogs, humans). The few reported equine cases showed typical external symptoms, with equids developing single or multiple papules or nodules in areas where sand flies usually bite, such as the eyes, muzzle, neck, ears, scrotum, and legs. Confirmatory diagnosis has been reached by PCR or immunohistochemistry of suspected lesions.

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Table 1. Selected list of arthropod-borne infectious diseases in equids, adapted from Chapman et al 2018 and Onmaz et al 2013

Classification	Virus	Abbr.	Major vectors	Zoonotic	Major hosts involved in transmission
Alphavirus	Eastern equine encephalitis	EEEV	*Culiseta melanura, Aedes taeniorhynchus*	Y	Passerine birds, rodents
	Western equine encephalitis	WEEV	*Culex tarsalis, Culiseta melanura*	Y	Passerine birds
	Venezuelan equine encephalitis	VEEV	Enzootic form, *Culex melanoconion* spp Epizootic form – wide vector range including *Psorophora* and *Ochlerotatus* spp.	Y	Rodents
Flavivirus	Japanese encephalitis	JEV	*Culex tritaeniorhynchus*, *vishnu complex* spp., *gelidus*	Y	Waterbirds, swine
	Murray Valley encephalitis	MVEV	*Cx. annulirostris*	Y	Waterbirds
	West Nile virus	WNV	Many *Culex* spp. some of the most important include *Culex pipiens, tarsalis, modestus,* quinquefasciatus	N	Birds (rodents, and reptiles)
	Usutu	USUV	*Culex pipiens*	(Y)	Birds
	Tick-borne encephalitis	TBE	*Ixodes ticks*	Y	mainly small rodents (voles, mice)
	Louping ill	LIV	*Ixodes ricinus*	(Y)	Sheep, red grouse
Orbivirus	African horse sickness	AHSV	*Culicoides imicola, bolitinos*	N	Equids
	Equine encephalosis	EEV	*Culicoides imicola*	N	Equids
Orthonairovirus (Bunyavirales)	Crimean congo hemorrhagic fever	CCHF	*Hyalomma* ticks	Y	wild and domestic animals (e.g. livestock, horses, dogs, chickens, camels, ostriches, swine, hares, deer, buffalo and rhinoceroses)
Ricketsia	*Anaplasma phagocytophila* (Equine granulocytic anaplasmosis)	EGA	Ixodes ticks	Y	Dogs, cats, ruminants, small mammals, birds, and lizards
Borrelia (Spirochete)	*Borrelia burgdorferi* (Lyme disease)		Ixodes ticks	Y	Deer and other large mammals
Piroplasmidae	*Babesia caballi, Theileria equi* (Piroplasmosis)		Ixodes ticks (*Dermacentor*, *Hyalomma* y *Rhipicephalus* spp)	N	
Trypanosomatida	*Leishmania infantum, L. braziliensis*		Phlebotomus	Y	Dogs
Trypanosomatida	*Trypanosoma brucei, T. vivax, T. congolense*		*Glossina* spp. (Tse-tse flies)	Y	Camels and other large mammals

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15.00 – 15.45 HOT TOPIC: An update on simulation-based learning in equine veterinary medicine - S. Bailie

Sarah Baillie, Emeritus Professor
Bristol Veterinary School, University of Bristol, UK

The presentation will start by defining simulation, including its relevance and applications in veterinary education, will describe the benefits of its use for learners and patients (humans and animals), and will illustrate how simulation is currently embedded in undergraduate veterinary degrees and the potential opportunities in post-graduate training.

Simulation involves the use of an artificial representation of a real-world task for training, and provides a safe and controlled environment to practice clinical and surgical skills, decision making, problem solving and teamwork. The level of difficulty and complexity varies; for example veterinary students (novice learners) can practice basic skills e.g., a simple interrupted suture on a silicon skin pad, while clinical teams can rehearse complex procedures and in challenging environments e.g., new surgical techniques, emergency situations in a mock theatre.

Simulation has a long history in aviation and medicine. One of the first manikins was designed by Madame du Coudray, a midwife who travelled across France in the 18th century providing simulation-based training. Aviation is an industry that has embraced simulation for pilot training using a simulated cockpit with scenarios from basic flight skills to handing emergencies, and for routine retraining or learning to fly new aircraft. Simulation is now widely embedded in undergraduate and post-graduate medical education, from simple skills such as venipuncture on an artificial arm to minimally invasive surgery and theatre team's practicing scenarios and debriefing.

There are well established benefits of simulation-based education in the health professions, and these can be extended to veterinary education. Simulation supports hands-on learning in a safe, 'trial & error' environment, and allows for repeated, deliberate practice, which is crucial as the learner progresses from being a novice to the development of expertise and mastery. One specific benefit for veterinary students is that after developing skills on models, they will be better prepared for learning opportunities with live animals, which will reduce student anxiety and improve animal welfare.

There are various factors to consider when including simulation-based education in a veterinary training programme, whether at undergraduate or post-graduate level. Over the last 20 years a wide range of models have been developed for veterinary students and a growing number are becoming available for post-graduate and specialty training. Models may be quite simple, particularly for basic skills, and where possible should be reusable, affordable, and validated i.e. shown to equip trainees with the required skills. The veterinary education community have been resourceful and innovative in developing models for core skills (e.g., injection techniques and suturing) and more complex clinical and surgical procedures (anaesthesia, ultrasound, ophthalmology, bitch spay). For equine skills, models are available for basic handling techniques such as placing a headcollar, tacking up and bandaging a limb or tail; injection techniques including intramuscular, intravenous, catheter placement and blood sampling; and other models include suturing a wound on a limb, nerve blocks, castration, dentistry, rectal palpation for reproduction and colic examination, nasogastric intubation and abdominocentesis.

Another recent and growing development is the introduction of clinical skills laboratories at veterinary schools. These facilities have a building or set of rooms dedicated to taught classes, self-directed learning, and assessments. In addition to having plenty of space for teaching, the clinical skills facility needs enough storage, a room to make and repair models, somewhere for students to change and leave their bags, and an office for staff. One of the most important factors for success are the staff; a team dedicated to clinical skills, which typically includes an academic lead, veterinarians and veterinary nurses,

other technical staff, and possibly peer tutors. The team will support and manage the facility, and prepare for and deliver teaching and assessment activities.
Practice on a model, whether during a taught class, with a peer or during self-directed learning, is greatly enhanced if good quality supporting learning resources are also available. Videos and instruction booklets for many clinical skills have been developed by members of the veterinary education community, some of which are open access. Before coming to the practical class, it is helpful if the trainee has prepared e.g., by completing pre-work in an online flipped classroom. The pre-work typically covers the underpinning knowledge and includes videos that clearly show all the steps of a skill or procedure. However, even with models, a clinical skills laboratory and supporting learning resources, the development of skills and Day One Competences depends on the associated teaching being fully integrated across the entire curriculum – from animal handling (e.g., placing a bandage on a model horse's leg before repeating the skill on a live horse), to basic clinical and surgical skills (e.g., gloving, making and closing an incision, otoscopy, ophthalmology), to more complex procedures (e.g., neutering), and simulations that integrate skill performance with problem solving and decision-making (e.g., role-play of a colic scenario with rectal palpation on a model and communication with a simulated client, while making a diagnosis and discussion of treatment options).
As well as formal taught classes and providing opportunities for practice with models in a clinical skills laboratory, the skills must be assessed to demonstrate the trainee has reached the required level of competence e.g. before handling live animals or starting clinical rotations. Assessments are typically undertaken using Objective Structured Clinical Examinations (OSCEs) where a set of stations (each testing a different skill) are combined in a circuit. Trainees demonstrate the technique or procedure, usually on a model. At the next level, competences are learned and practiced in the workplace, and then assessed using one or more of a variety of workplace-based assessment tools (WBAs).
In support of the emerging discipline of simulation in veterinary education, there are an increasing number of associated research studies. These contribute to the evidence-base; informing the use of models in teaching and demonstrating the benefits of clinical skills and simulation-based training initiatives. The field is growing rapidly, from just a few studies 20+ years ago to regular publications in veterinary education and specialty journals.
Although the focus of simulation-based education has been to improve undergraduate veterinary student education, there are various opportunities to increase the use of models and simulators in post-graduate training. For example, simulators are available in medical training for minimally invasive surgery, endoscopy and diagnostic imaging techniques, and some of the underpinning skills are similar enough to be applicable to veterinary training. There are specific times when simulation-based training could be useful e.g., when a new graduate is making the transition into equine practice, negotiating the first year or two, and could benefit from learning some procedures under supervision on a model. Another opportunity is team-based learning, where a simulated scenario allows clinical teams to explore, discuss and practice their roles, role-play new, challenging or life-threatening scenarios, and engage in constructive debriefing sessions.
In summary, whether in undergraduate or post-graduate veterinary training, there are key features of simulation-based education that enhance learning and skill development. These include being able to practice with models and simulators repeatedly and in a timely fashion e.g., in preparation for a clinical workplacement or before performing a new procedure. Simulation-based environments are safe, and mistakes have no serious consequences for the trainee or patient/animal. Importantly, the instructor must support the learner's active engagement in the task and provide feedback to the trainee or team. This will contribute to the reflective process and planning for the next practice session or real situation. Another consideration, especially when getting enthusiastic about using a new simulator or model, is whether the model has been validated i.e., is there evidence of the benefits, do trainees learn the required skills and develop confidence, and do they go on to perform better in the real-world setting?

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Vet Ed Research Resource Repository: Clinical skills + simulation research papers https://docs.google.com/spreadsheets/d/1v9AK-bBfnpFbOs8LocPDKlIFewr3DPfb16BQRBYtox8/edit#gid=938388233
Veterinary Clinical Skills + Simulation online community: https://www.clinicalskillsandsimulation.com/

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15.45 – 16.30 HOT TOPIC: Stem cell and blood product use in equine internal medicine: where are we? - L. Berg

L. Berg
Associate Professor - Applied Biomedical Science, Large Animal Sciences. Faculty of Health and Medical Sciences, University of Copenhagen

Cell therapy and biologicals are increasing in popularity both in veterinary and human medicine. There are several reasons for this; more supporting evidence for clinical efficacy, improved availability, lower cost, good biocompatibility, fewer side effects, increasing familiarity with the products, and few restrictions for sport.
But first, what do we mean by stem cells and blood based products? It may seem obvious, but the distinctions are not always clear, and the differences really matter when we consider applying these products in clinical practice. They are all based on biological material from the patient itself (autologous) or donor horses (allogenic), but each product has a unique profile and proposed mode of action, and even stem cells are not just stem cells. The tissue source, donor, preparation, priming/activation, etc. all influence a potential outcome.

For blood products, blood is sampled and treated to separate and/or upregulate specific components that are considered desirable. Most are autologous, which may be a limiting factor, but new advances may make allogenic application possible. The products can be plasma- or serum-based, and bench-top / stall-side devices for preparation of blood-derived therapeutics have been commercially available for several years. There are several products available, but the most well-known examples include platelet-rich plasma (PRP) where platelet concentration is increased using centrifugation or filtration methods, and autologous conditioned serum (ACS) where serum is incubated in a special device to increase the content of interleukin-1 receptor antagonist protein (IL-1RA) (this product is also known as iRAP). Platelets contain a large volume and variation of growth factors, while IL-1RA blocks the intracellular proinflammatory effects of IL-1 at the level of the cell surface receptors.

For stem cell-enriched products and 'pure' stem cell products, tissue samples (e.g. bone marrow, fat or blood) are either treated directly for immediate use, or stem cells are isolated and expanded in culture for future application. Instant treatments include concentrated products (e.g. bone marrow aspirate concentrate) or mononucleated cell fraction isolates (e.g. stromal vascular fraction (SVF)). These products have been shown to contain a variable number of stem cells together with a mixture of other tissue components including growth factors and cytokines. 'Pure' stem cell treatments contain stem cells isolated from tissue samples in a laboratory and expanded to a cell number considered sufficient for therapy, so the concentration of stem cells is higher. They are often resuspended in serum or similar eludates to add growth factors etc. This concentrated stem cell option has been less accessible, because it requires local on-site cell culture facilities or the shipping of tissue samples to external service providers. Around 2021-2022 the availability changed significantly, and three commercial stem cell products are approved for horses for the European market, thus making stem cell based therapies more widely available to veterinarians outside of larger hospitals with cell culture facilities.

As mentioned above there are multiple advantages to blood-derived and stem cell products. However, since individual horses (even when matched for age and gender) will have differences in the composition of their blood or the characteristics of their stem cells, the precise contents and thus expected efficacy of any one treatment is harder to control than for more traditional pharmaceutical options.
Biologicals including stem cells have mainly been used to treat musculoskeletal conditions including joint disease and tendon and ligament injuries. However, in recent years more applications have been explored crossing into the field of internal medicine.

Why are biologicals potentially an attractive treatment option? Inflammation is an essential part of tissue healing. Our traditional options for regulating inflammation include medical blocking of all or parts of the inflammatory signalling pathways, but studies have shown that tissue healing and regeneration is dependent on intricate interactions occurring during inflammation resolution which are strongly affected, when we interfere with the inflammatory pathways. A more nuanced regulation and resolution of inflammation using immunomodulatory therapies could therefore potentially improve clinical outcomes. We know that both blood derived products and stem cells have immunomodulatory effects, mainly anti-inflammatory, but for stem cells additional immunoregulatory effects have been shown both in response to direct cell-to-cell contact between stem cells and host immune cells or through stem cell secretomes or exosomes. These include changes in polarisation of macrophages and alterations in T- and B-cell activity. We also know biological treatments contain a large number of growth factors and signalling molecules. In addition, stem cells are believed to have properties related to anti-scarring, revascularisation, regeneration, and trophism. This could all improve clinical results. A final less scientific reason for exploring these new treatment modalities is when we do not have any other treatment options, or our current options fail either immediately or the treatment effects diminish over time.

So what clinical cases would be candidates for treatment with blood-derived or stem cell products? In what groups of cases are we currently struggling to keep disease processes under control or achieve inflammation resolution rather than just symptom relieve? Obvious candidates would be inflammatory diseases in different organ systems including respiratory, gastrointestinal, and musculoskeletal problems, ophthalmological diseases, and wounds.

In a recent review by Drs Barrett and MacDonald (2023), they present a thorough summary of our current state of evidence in the use of stem cells in inflammatory diseases. A general conclusion is that we need stricter reporting criteria (many reports do not detail source of stem cells, treatment dose, treatment time, patient inclusion and exclusion criteria, etc.), and more research especially in horses to determine not only efficacy, but also ideal treatment time, dose, choice of stem cell source, and whether pre-injection stem cell activation or priming is required. But they also conclude that the available evidence from studies in horses, humans, and/or rodents show promising results in inflammatory bowel disease, gastric ulcers, peritonitis, colitis, abdominal adhesions, and recurrent airway obstruction/asthma despite the limited data available. Other studies have explored the use of biologicals and stem cells in wound healing in horses with promising results of both PRP and stem cell treatments on quality of repair tissue, but the number of replicates are small, and the findings have to be repeated in larger trials before we can assess the true value of biological therapies in skin wounds. The same applies to laminitis, where studies in small numbers of horses have shown improved vascularisation and clinical outcome.

There are still a lot of unanswered questions. We need a more detailed understanding of the modes of action of the different therapeutic options, and the best choices for type of therapy, when, how often, delivery method, dose, and so on. But if we consider the inherent roles of blood components and stem cells in maintaining, and if necessary re-establishing equilibrium in body systems, and then consider injury and disease in part as a failure of those local components and stem cells to sustain the equilibrium, then the concept of supplementing and thus boosting the body’s own defence system is attractive and worth pursuing.

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17.00 – 17.45 HOT TOPIC: Patient safety and quality of care. - C. Sanchez

L. Chris Sanchez, DVM, PhD, DACVIM (LAIM) and Linda S. Allen, MHA
College of Veterinary Medicine
University of Florida, Gainesville, FL, 32610

Overview

As veterinarians, we are committed to providing patients with the highest quality of care within the spectrum of care appropriate for the client and setting. Providing a culture of safety is an important component of quality and safety, especially in a hospital environment. This talk will touch upon implementation of a quality and patient safety program in an academic hospital setting and important components of its success.

Medical Error Reporting and Just Culture

While patient safety and medical error reporting have been recognized in human medicine for over 20 years[1-3] and are requirements for accreditation in many jurisdictions, such programs are only recently gaining traction in veterinary medicine.[4-8] Important components of a reporting system are ease of use, ability to retrieve and analyze data readily, and incorporate aspects of investigation. At the University of Florida Veterinary Hospitals, we have adapted a web-based medical error reporting system (IDInc, International Developers, Inc.) used in the UF Health system of hospitals for veterinary use. The "Just Culture" model encourages staff and students to report errors without fear of retribution. The "Just Culture" creates an environment of learning and improving, not one of blame and punishment.[9] The framework of a just culture ensures balanced accountability for individuals and the organization. As such, evaluation of the event includes staff intent as well as action. In most cases, staff do not intend to make a mistake and are therefore managed based upon their intention. The leadership response to medical errors is critical to the success of a Culture of Safety. The main barriers to reporting in veterinary and human medicine include fear, underlying attitudes and uncertainty.[10] Thus, emphasizing process improvement rather than individual performance is key to program success. To increase individuals' comfort, anonymous reporting should be permitted, though it can also be used as a measure of system success. For example, at the UF Veterinary Hospitals, anonymous reporting decreased by 96% over the first 5 years of program implementation.

Team Communication, Handoffs and Checklists

Because breakdowns in communication have been frequently identified as a source of medical errors in human and veterinary medicine, many strategies for improvement focus on team communication, handoffs and checklists.[2, 5, 7, 8, 11, 12] One example from the human side is TeamSTEPPS®, which was designed to improve collaborative work within human healthcare teams in collaboration between the US Department of Defense and the Agency for Healthcare Research and Quality (AHRQ).[12-14] A systematic protocol (I-PASS) has been shown to reduce errors associated with handoffs, or transitions of patient care between providers, in human medicine[15] and a version of this tool has been recently evaluated in a small animal hospital setting.[16]

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NOTES

ORAL ABSTRACTS

Oral presentation
Friday 27 October 2023, 09.00-09.15

Retrospective case control study based on magnetic resonance imaging reveals asymmetry of the trigeminal nerve in horses with headshaking

F. Heun, J. Delarocque, M. Hellige, K. Feige
Clinic for Horses, University of Veterinary Medicine Hannover, Hannover, Germany

Introduction:
Idiopathic trigeminal-mediated headshaking (ITMHS) in horses may be associated with trigeminal nerve (TN) neuropathy. In human medicine, TN asymmetry was revealed by magnetic resonance imaging (MRI) correlating with nerve atrophy.

Methods:
MRI examinations of twenty adult horses diagnosed with ITMHS were retrospectively compared to six horses that underwent MRI of the head for other reasons. All horses presented to the equine hospital of the University of Veterinary Medicine Hannover, Foundation in 2021 and 2022. MRI studies were obtained with a 3 Tesla MRI scanner (Philips Achieva 3T dStream). Standardized cross-sectional area measurements of branches of the TN were performed bilaterally at 4 defined locations (1: rostral to the trigeminal ganglion, 2: close to the pituitary gland, 3: close to the optic chiasm, 4: rostral to the foramen rotundum). Bilateral cross sectional transverse area differences (TN asymmetry) were calculated and compared between the two groups using a generalized additive model of the Tweedie family.

Results:
Horses with ITMHS had 2–8.7 times higher TN asymmetry than controls ($F(1) = 22.093$, $p < 0.001$). The difference in TN asymmetry was most pronounced rostral to the trigeminal ganglion – area 1 (back-transformed estimated marginal mean ratio and standard error: 8.66±3.975, $t(212) = 4.700$, $p < .0001$). There was no effect of age of the horse or duration of ITMHS on the TN asymmetry.

Discussion and Clinical Relevance:
The asymmetry of the TN in horses with ITMHS indicates a unilateral enhanced neuropathy similar to findings in humans. Few previous studies provided evidence of atrophy or dysfunction of the TN in horses diagnosed with ITMHS, moreover, this MRI study revealed morphological changes of the TN in horse and provides directions for further studies and diagnostic approaches.

Oral Presentation
Friday 27 October 2023, 09.15-09.30

Detection of EqHV RNA in Stomoxys calcitrans in eastern Austria

V. Frisch[1], S. Ramsauer[2], I. Preining[1], M.S. Unterköfler[1], T. Borysova[1], M. Haller[1], J. Wigger[1], H.-P. Führer[1], J.-M.V. Cavalleri[1]
[1]University of Veterinary Medicine Vienna, Vienna, Austria
[2]University of Zurich, Zurich, Switzerland

Introduction:
The global prevalence of Equine Hepacivirus (EqHV), a member of the family *Flaviviridae*, is discussed to range from 3.6% to 16.1%. The EqHV antibody prevalence in eastern Austria in the horse population is high with 45.9%. These numbers suggest a horizontal way of transmission, however only vertical and iatrogenic viral transmission were detected to date. Mechanical transmission routes by vectors are discussed, but have not been thoroughly investigated. This is the first study investigating the detection of EqHV RNA in *Stomoxys calcitrans* (stable fly), a member of the family Brachycera.

Methods:
In 2021, 606 *Stomoxys calcitrans* were caught in two horse stables in the surroundings of Vienna and at the University Equine Hospital of the Veterinary University Vienna. Maximum 5 flies´ heads and thoraxes including legs and wings were pooled and analyzed for the presence of EqHV RNA by reverse transcription quantitative polymerase chain reaction (RT-qPCR). The minimum infection rate (MIR) was calculated ([number of positive pools / total number of flies tested] x100), to determine the infection rate of the analyzed stable flies.

Results:
In 7 out of 135 pools, collected in September at one stable in eastern Austria, EqHV RNA could be detected. The MIR of *Stomoxys calcitrans* in 2021 was 1.2%. The viral RNA was detected in females as well as in male flies.

Conclusions:
This is the first study detecting the presence of EqHV RNA in *Stomoxys calcitrans* and reporting a potential, possibly mechanical vector for this virus.

Clinical relevance:
The detection of EqHV RNA in *Stomoxys calcitrans* suggests a possible horizontal way of transmission. Action concerning husbandry management against viral infection can only be initiated after identification of transmission routes.

Oral presentation
Friday 27 October 2023, 09.30-09.45

The role of insulin clearance in hyperinsulinemia and its association with non-alcoholic fatty liver disease in insulin-dysregulated horses : a preliminary study

R. Ruth [1], H. Holly[1], L. Scott[1], M. Dosi[2], J.A. Keen[2], B. McGorum[2], A. Alexandra[2]
[1]Scotland Rural College, Edinburgh, United Kingdom
[2]University of Edinburgh, Edinburgh, United Kingdom

Introduction:
Horses with endocrinopathies often have sustained hyperinsulinemia. In humans, reduction of insulin hepatic clearance participates to the hyperinsulinemia and has been associated with non-alcoholic fatty liver disease (NAFLD), although this is not known in horses.

Methods:
Tissue samples were collected at post-mortem from horses with and without (n=14) hyperinsulinemia associated with EMS (n=10) or PPID (n=10). Healthy horses were euthanized for unrelated diseases and included if they did not have liver pathologies. Equine Metabolic Syndrome (EMS) was defined as a body condition score >3.5/5, fasting basal insulin >20mlU/L, history of laminitis. Pituitary Pars Intermedia Dysfunction (PPID) was defined according to ACTH seasonal cut-off, and histological changes to the pituitary. Liver tissue was fixed in 10% formalin, paraffin-embedded and 5 µm sections stained with haematoxylin and eosin (H&E) and immunohistochemically for CEACAM1, an insulin degrading protein. Each section was scored by two blinded observers using the equine liver disease scoring system (Durham et al 2003) and a human NAFLD scoring system (Bedossa et al 2012). In frozen liver sections, triglycerides and Insulin Degrading Enzyme (IDE) activity were quantified.

Results:
Although the cumulative NAFLD scores and hepatic triglyceride content were not significantly different between groups, the most severe changes were seen in horses with endocrine disease (3/20). There was no correlation between NAFLD score and basal insulin measurement. CEACAM1 was identified in all horses by immunohistochemistry, but the study was underpowered to show differences between groups. Quantitative tests are required to confirm these results. IDE activity was significantly decreased in horses with hyperinsulinemia compared with controls (controls 3 IQR4.50, EMS 2.18 IQR1.57 activity/mg protein).

Discussion:
While NAFLD is not a feature of equine hyperinsulinemia, there are differences in insulin clearance proteins suggesting that reduced clearance is likely a contributing factor and could represent a therapeutic target.

Oral presentation
Friday 27 October 2023, 09.45-10.00

Retrospective Analysis of Equine Sarcoid Treatment Protocol and Factors Associated with Recurrence

K.S. Offer, D.G.M. Sutton
University of Glasgow, Glasgow, United Kingdom

Introduction:
Treatment methods reported for equine sarcoids (ES) include laser excision and cryotherapy. Concurrent 5-fluorouracil (5-FU) may aid in cell death at the periphery of lesions treated with cryotherapy. This study aimed to compare laser excision with a combination of laser excision, cryotherapy, and 5-FU chemotherapy for ES treatment. Factors associated with sarcoid recurrence were also investigated.

Methods:
Medical records were reviewed for horses treated at Glasgow University for ES using the above two protocols (2013 – 2022). Inclusion required histological confirmation of sarcoids. Treatment outcome was determined by owner communication. Univariate and multinominal logistic regression was performed to identify variables associated with recurrence. Cox's proportional hazards model was employed to identify variables influencing time to recurrence.

Results:
84 horses and 168 sarcoids were suitable for inclusion. Overall recurrence rate was 23%. Previous sarcoid treatment (OR 7.6 (2.0-33)), individual sarcoid diameter ≥100 mm (OR 5.6 (1.1-30)), requirement for general anaesthesia (OR 5.0 (1.4-19)) and total number of sarcoids (OR 1.2 (1.0-1.5)) were associated positively with recurrence, in contrast to confirmation of surgical margins (OR 0.40 (0.005-2.3)). For time to sarcoid recurrence, lower limb (HR 0.2 (0-1.6)) and first ES episode (HR 0.3 (0.1-0.7)) decreased risk, which was increased by ≥1 mixed sarcoids (HR 9.9 (3.3-30)) and urogenital location (HR 3.6 (1.3-10)). Treatment category was not associated with either sarcoid recurrence or time to recurrence.

Conclusions:
In this retrospective study, the individual characteristics of the sarcoid, number and location of lesions and previous treatment attempts were of greater influence on recurrence rate than individual treatment modality.

Clinical relevance:
Sarcoid masses should be treated at the first opportunity, with particular attention to surgical margins. Mixed sarcoids affecting the urogenital region merit increased care. Multimodal treatment may be of benefit in ES treatment, but prospective intervention studies are required to determine efficacy.

Oral presentation
Friday 27 October 2023, 10.00 – 10.15

Infections with Extended Spectrum Beta-Lactamase Producing Enterobacterales in hospitalized neonatal foals: association with rectal colonization, risk factors and outcomes

A.S.T. Shnaiderman-Torban[1], L.M. Meltzer[2], T.Z.D. Zilberman Daniels, S.N.V. Navon-Venezia[3], G.A.S. Abells Sutton[1], A.K. Kohen[1], S.B. Blum[4], S.A. Amit[2], A.S. Steinman[1]
[1]KSVM Israel, Rehovot, Israel
[2]Sheba medical center, Ramat Gan, Israel
[3]Ariel University, Ariel, Israel
[4]Kimron Veterinary Institute, Rishon LeZion, Israel

Introduction:
Infections with extended spectrum beta lactamase producing *Enterobacterales* (ESBL-PE) contribute to morbidity and mortality among newborn human infants. In equine neonatal medicine, data is scarce.

Methods:
This prospective, single center cohort study, assessed 67 neonatal foals presented to a veterinary teaching hospital. Foals were screened for ESBL-PE rectal colonization on admission. From admission onwards, bacterial isolates from blood, umbilicus, IV catheter and joint were tested for ESBL production. Medical data were analyzed for risk factors and clinical outcomes.

Results:
The prevalence of ESBL-PE colonization and infections were 46% (n=31/67) and 52% (n=35/67), respectively. Colonization on admission was significantly associated with an ESBL-PE infection during hospitalization (p=0.018). On multivariable logistic regression analysis, colonization on admission was associated with the Arabian breed (p=0.014, OR=14.5). Clinical signs of an umbilical infection on admission were associated with an ESBL-PE infection during hospitalization (OR=4.8, p=0.004). In an outcome analysis, an ESBL-PE infection was associated with surgery during hospitalization (OR=5.2, p<0.001) and with a longer length of stay (OR=8.1, p<0.001).
The main ESBL-PE species isolated from both rectal screening and clinical samples was *Escherichia coli* (52%, n=27/52 and 44%, n=18/41, respectively). Concordant ESBL-PE species was recovered from a rectal screening sample and at least one clinical sample in 16% of foals (n=11/67).

Conclusions:
On-admission ESBL-PE rectal colonization is associated with ESBL-PE infections in neonatal foals. Such infections are related to surgery during hospitalization and a longer length of stay.

Clinical relevance:
This study demonstrates an alarming prevalence of ESBL-PE colonization and infection, and the significance of rectal screening on admission as a predictor for infection with ESBL-PE during hospitalization.

Oral presentation
Friday 27 October 2023, 10.15-10.30

Use of a point prevalence survey to measure antibiotic use in equine veterinary hospitals

E. K. Leus[1], C.H. Lyle[1], N. Collins[2], M. Gruyaert[3], R.N. Kennedy[1], E. McConnell[4], B.C. McGorum[5], D. Luethy[6], M. Sanz[7], A. Versporten[8], A. Viljoen[9]
[1]Vetscape equine Referrals, Paarl, South Africa
[2]Scone Equine Hospital, Scone, Australia
[3]Faculty of veterinary medicine Ghent university, Merelbeke, Belgium
[4]Murdoch University, Perth, Australia
[5]Edinburgh university, Edinburgh, United Kingdom
[6]University of Florida, Gainesville, United States
[7]Washington State University, Washington, United States
[8]University of Antwerp, Antwerp, Belgium
[9]University of Pretoria, Pretoria, South Africa

Introduction:
Antibiotic resistance is increasingly recognised in equine medicine. Antibiotic use is a key driver of antibiotic resistance. This study piloted a point prevalence survey (PPS), based on the Global-PPS used in human hospitals, to obtain data on antibiotic prescribing in equine hospitals. The aim was to determine if the PPS could be used as an antibiotic stewardship tool to identify targets for improvement in antibiotic use.

Methods:
Eight equine hospitals located in Australia, Belgium, South Africa, the United Kingdom and the United States of America were recruited. Data on antibiotic use were collected from all in-patients on antibiotic treatment at 8 am on four selected study days throughout the study year (2022). Data collected included patient details, antibiotics prescribed, indication, use of a treatment stop/review date and adherence to local guidelines.

Results:
A total of 742 patients, 310 (41.8%) surgical and 432 (58.2%) non-surgical cases, were evaluated. A total of 58.7% of the surgical patients were on antibiotic treatment, compared to 25.9% of the non-surgical patients. The most prescribed antibiotics were penicillin, gentamicin, and trimethoprim sulphonamides. In 45.2% of treatments, use was prophylactic (33.2% surgical prophylaxis and 12% non-surgical prophylaxis). Therapeutic use was based on a biomarker in 48% of the treatments. A sample was submitted for culture in 56.9% of therapeutic treatments. An antibiotic use review/stop date was not recorded in 59.5% of treatments. Local guidelines did not exist for 27.5% of treatments.

Conclusions and clinical relevance:
The PPS identified multiple ways in which antibiotic use could be optimised. Targets identified for stewardship interventions included the high prevalence of prophylactic use and the lack of use of a stop/review date. The survey could be used as a repeatable tool to assess the impact of stewardship interventions in equine hospitals.

Oral presentation
Friday 27 October 2023, 10.30-10.45

Comparison of tracheal wash and bronchoalveolar lavage for cytological diagnosis of exercise-induced pulmonary haemorrhage in horses

P. Barbazanges[1], E.A. Richard[2], L.C. Lemonnier[1], M.P. Toquet[2], A. Couroucé[1]
[1]Oniris, Nantes, France
[2]LABEO, Saint-Contest, France

Introduction:
Various methods are reported for diagnosis of exercise-induced pulmonary haemorrhage (EIPH). Cytological evaluation of airway samples is a sensitive method, but the correlation between tracheal wash (TW) and bronchoalveolar lavage fluid (BALF) findings for diagnosis of EIPH is unknown.
The objective was to determine whether diagnosis of EIPH, using haemosiderophages/macrophages (H/M) ratio, differs when based on samples from TW and BALF collected concomitantly from the same racehorse.

Methods:
Prospective cross-sectional study on 102 Standardbred horses in active training. TW and BALF from each lung separately were collected from all horses at rest. Smears were stained with May-Grünwald-Giemsa (MGG) and H/M ratio calculated. Diagnostic cut-off values were set at 17% for individual (left and right) BALF and 9% for pooled BALF. H/M ratio in TW samples were scored as none (0%), occasional (<10%), small (10-25%), moderate (25-50%) or large proportions (>50%).

Results:
In BALF, 21 horses met the cytological inclusion criteria for EIPH diagnosis from individual and/or pooled samples. In TW, 20 horses had occasional proportions of haemosiderophages, and respectively 9, 1 and 3 horses had small, moderate and large proportions. Poor correlations between TW and respectively pooled, left and right BALF were found for H/M ratio. Among the 13 horses with at least small proportions of haemosiderophages in TW, 8 (61.5%) had no cytological evidence of EIPH in any BALF.

Conclusion and clinical relevance:
No association between TW and BALF was found for the cytological diagnosis of EIPH. A large number of horses has cytological evidence of pulmonary bleeding in BALF with none or occasional proportions of haemosiderophages in TW. In addition, finding small to large proportions of haemosiderophages in TW is mostly not associated with evidence of pulmonary haemorrhage in BALF.
Based on H/M ratio, BALF remains the sample of choice for cytological diagnosis of EIPH.

Oral presentation
Friday 27 October 2023, 10.45-11.00

Long-term follow-up of colic recurrence and athletic performance in horses after explorative celiotomy

A.C. Calewaert,
Ghent University, Merelbeke, Belgium

Introduction:
Exploratory celiotomy is a critical and expensive intervention for treating colic in horses. Insight into the long-term effect on athletic performance and recurrence of colic is important for decision-making.

Methods:
An email survey was conducted and answered by 96 horse owners whose horses had undergone colic surgery at Ghent University between 2013 and 2019. The questionnaire aimed to gather long-term (>3 years) follow-up information, focusing on predisposition to new colic episodes and changes in athletic performance after the surgery.

Results:
The majority (72%) of horses were still owned by their previous owners, while 13% had been sold. 15% of the horses had died, out of which 78% causes of death were related to a recurrent colic episode. Among all the horses included in this study, 42% had initial small intestinal colic, while 58% had initial large intestinal colic. According to the owners' reports, 10% of horses with initial small intestinal colic and 21% of horses with initial large intestinal colic seemed more prone to redeveloping colic signs. Regarding athletic performance, owners reported that 89% of the horses maintained or even increased their pre-surgery performance level, 5% decreased the level of performance due to the owners' decision and 12% performed at a lower level. The latter group consisted of 30% small intestinal and 70% large intestinal colic cases.

Conclusion and Clinical Relevance:
Based on the owners' perception, only a small percentage of horses exhibited a renewed colic episode following surgery. Most of the horses were able to maintain or even improve their performance levels. These findings emphasize the positive long-term outcomes for athletic performance after an exploratory celiotomy in horses with colic.

Oral presentation
Friday 27 October 2023, 11.30-11.45

Activin A concentrations as a potential biomarker for detecting insulin dysregulation and predicting laminitis risk in ponies

C.J. McGuire[1], E.J. Knowles[1], P.A. Harris[2], N.J. Menzies-Gow[1]
[1]Royal Veterinary College, Hertfordshire, United Kingdom
[2]Waltham Petcare Science Institute, Leics, United Kingdom

Introduction:
Basal plasma Activin A (AA) concentration has recently been shown to positively correlate with insulin concentrations 60 minutes following an oral sugar test (OST) in ponies with equine metabolic syndrome, therefore there is potential for this to be used as a marker for insulin dysregulation. Insulin concentrations at baseline (T0) or 60 min (T60) post OST best quantify the risk of future laminitis in non-laminitic ponies. The study aim was to explore the relationship between AA concentrations at T0 and T60, in addition to other metabolic markers in ponies that subsequently develop laminitis and those that remain non-laminitic.

Methods:
Case-control study design**.** Forty-two ponies that developed laminitis during a 4-year surveillance period were selected from a cohort of 374 non-laminitic animals; 42 ponies from the same cohort that remained non-laminitic were selected as controls. Plasma AA concentrations were measured using a validated ELISA at T0 and T60 post OST. Serum insulin, blood glucose and plasma triglyceride, adrenocorticotrophin hormone and adiponectin concentrations had been previously measured. Plasma AA concentrations were compared between groups at T0 and T60 using Kruskal-Wallis one-way ANOVA and correlations with other metabolic markers investigated using Spearman correlations.

Results:
There was no significant difference between Plasma AA and Insulin concentrations at either time point, nor between Plasma AA concentrations and any other metabolic markers or pony parameters between the two pony groups. There was a significant positive correlation between Plasma AA T0 and T60 ($P < 0.001$, $r^2 = 0.67$) and a significant positive correlation between the increase in Plasma AA and the increase in insulin concentrations between T0 and T60 in the control ponies ($P = 0.009$, $r^2 = 0.399$), but not the laminitic pony group.

Clinical Relevance:
Plasma AA concentration is not a useful marker for ID and does not appear to be associated with laminitis development.

Oral presentation
Friday 27 October 2023, 11.45-12.00

Quantification of immune cell populations in equine intestinal biopsies of healthy horses

M. Robel[1], P. Grest[1], A. Schoster[2]
[1]University of Zurich, Zurich, Switzerland
[2]Ludwig-Maximilians-University Munich, Oberschleissheim, Germany

Introduction:
There are little data on normal inflammatory cell counts (ICCs) in the intestinal wall of healthy horses. Often only minimally invasive obtained mucosal biopsies are available for evaluation. The objective was to describe and compare inflammatory cells in different locations and biopsy types of healthy horses.

Methods:
Full-thickness and endoscopic forceps obtained mucosal biopsies were taken post-mortem from the duodenum and rectum of fifteen healthy horses slaughtered for meat production. After fixation in 10% buffered formalin, samples were stained with hematoxylin and eosin (HE) and immunohistochemistry (IHC; CD3, CD20, Iba-1) was performed. Four-ten fields with 0.01mm^2 or 250-500 cells or 4-10 High Power fields were evaluated per sample. ICCs between biopsy types and locations were compared with the Steel-Dwass test.

Results:
Depending on biopsy type, median lymphocyte counts in the epithelium and lamina propria of the duodenum (LP) on HE stains were 4.9-9 and 3.8-8.5 and on IHC$_{CD3}$ 7.6-15.2 and 7.35-15. In the duodenum ICCs were significantly higher in the epithelium and LP of full-thickness biopsies compared to mucosal biopsies (all $p < 0.04$) except for macrophages ($p = 0.3$). There was no difference in ICC between biopsy types in the rectum (all $p > 0.3$). In full-thickness biopsies ICCs were significantly higher in the duodenum (Lamina propria (LP)-plasma cells$_{HE}$ $p = 0.003$, LP-B-cells$_{CD20}$ $p = 0.01$). In mucosal biopsies LP-eosinophils were significantly higher in the rectum ($p = 0.005$).

Discussion:
Higher ICCs were present in full thickness biopsies of the duodenum, likely due to the greater biopsy depth. Higher ICCs were present in the duodenum, except for eosinophils which were higher in the rectum. IHC increased the detection of immune cells.

Clinical Relevance:
ICCs need to be interpreted based on intestinal segment and biopsy type.

Oral presentation
Friday 27 October 2023, 12.00-12.15

8-hydroxy-2'-deoxyguanosine as a Potential Marker of Oxidative Damage in Horses with Neuroaxonal Degeneration

M. Palmisano
Large animal internal medicine department, University of Pennsylvania's New Bolton Center.

Background
Equine neuroaxonal dystrophy/equine degenerative myeloencephalopathy (eNAD/EDM) is increasingly recognized as a cause of neurologic disease in adult horses. Diagnosis requires postmortem examination, with no accurate antemortem diagnostic test available. 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative damage utilized in human neurodegenerative disease, has potential to correlate with postmortem diagnosis of eNAD/EDM.

Hypothesis/Objectives
We hypothesized that 8-OHdG will be increased in CSF and serum from eNAD/EDM horses compared to horses with other neurologic diseases and a control group of neurologically normal horses. 8-OHdG will be increased in CSF compared to serum of eNAD/EDM horses.

Animals
50 client-owned horses with postmortem diagnoses: 20 eNAD/EDM, 10 CVSM, 10 EPM, and 10 control horses. Serum and CSF samples were obtained from November 2010 through March 2022.

Methods
Case-control study using biobanked samples was performed and commercial competitive ELISA kit (Highly Sensitive 8-OHdG Check ELISA) utilized. Concentration of 8-OHdG was quantitated in both CSF and serum and compared between groups.

Results
The median concentration of 8-OHdG amongst all groups was 156.9 pg/mL (41.5-635.4) in CSF and 125.3 pg/mL (36.8-857.5) in serum. Poisson regression showed no significant difference ($P > .05$) once confounding variables (breed, age, sample age, vitamin E concentration) were considered.

Conclusions
8-OHdG is detectable in equine CSF and serum, although does not aid in antemortem diagnosis of eNAD/EDM based on this population of horses. The findings support that at the time of diagnosis horses with eNAD/EDM do not have ongoing oxidative stress. Further studies are needed to improve our ability to obtain an antemortem diagnosis.

Oral presentation
Friday 27 October 2023, 12.15-12.30

Influence of N-butylscopolammonium bromide and metamizol on echocardiographic variables in Warmblood horses with aortic and mitral valve regurgitation

A. Dufourni, M. Demeyere, G. Loon, A. Decloedt
Ghent University, Merelbeke, Belgium

Introduction:
N-butylscopolammonium bromide (NBB) causes transient tachycardia and hypertension, which has anecdotally been associated with intensified cardiac murmurs. Administration of Buscopan compositum (NBB and metamizol sodium) might be useful as a pharmacological stress test for evaluating valvular regurgitation. We hypothesized that the regurgitant jet area increases with higher heart rates and blood pressure.

Methods:
Regurgitant jet areas, cardiac dimensions and function were measured in horses with aortic (AR, n=10) and mitral valve regurgitation (MR, n=10) by 2D-, M-mode and colour Doppler echocardiography before and after intravenous administration of 0.2 mg/kg N-butylscopolammonium bromide and 25 mg/kg metamizol sodium. Measurements were performed by a blinded observer. Data were analyzed using repeated measures analysis of variance.

Results:
Compared to rest, Buscopan compositum administration resulted in similar increases in mean heart rate in horses with AR and MR (38±5 vs. 71±12 bpm, $p<0.001$). The regurgitant jet area increased in horses with AR and MR ($p=0.017$). Left ventricular end-diastolic area (174±20 vs. 148±12 cm^2, $p=0.002$), volume (1435±273 vs. 1107±158 ml, $p=0.004$) and internal diameter on M-mode (12.1±0.9 vs. 11.0±1.3 cm, $p=0.036$) decreased significantly in horses with AR, but not significantly in horses with MR. Left atrial fractional area change increased significantly in horses with AR (24±10 vs. 41±6 %, $p=0.001$) and MR (23±6 vs. 31±7 %, $p=0.012$).

Conclusion:
Buscopan compositum administration results in an increased regurgitant jet area in horses with aortic and mitral valve regurgitation.

Clinical relevance:
Buscopan compositum exacerbated valvular regurgitation and may be useful as pharmacological stress test in horses with AR and MR. With a similar increase in heart rate, Buscopan resulted in a much more pronounced decrease in left ventricular dimensions in horses with AR compared to MR. This finding emphasizes the importance of heart rate while evaluating cardiac dimensions during follow-up exams in horses with AR.

Oral presentation
Friday 27 October 2023, 12.30-12.45

Comparison of sequential versus average R-R intervals for evaluation of the degree of prematurity and pause length in arrhythmias in adult horses at rest

E. Williams Louie[1], B. Suter[2], C.C. Schwarzwald[2], K. Mitchell[1]
[1]Cornell University, Ithaca, United States
[2]University of Zurich, Zurich, Switzerland

Introduction:
Criteria for arrhythmia classification in horses is lacking. This study investigates the %R-R interval change using comparison of sequential and average R-R intervals in atrial (APC) and ventricular (VPC) premature complexes and sinus arrhythmia (SA).

Methods:
Arrhythmias were classified as APC, VPC or SA, with 9 R-R intervals recorded; the premature interval at position 4 and pause at position 5 in each sequence. The %R-R deviation between sequential and average (intervals 1-3) intervals were calculated. Comparison between arrhythmia classification and sequential vs average R-R intervals were analyzed by ANOVA.

Results:
30 ECGs were included; 10 each classified as APC, VPC and SA.
The sequential % prematurity was significantly different between APC vs VPC (mean diff: 95% CI; 17%: 3-32%, P=0.013) and VPC vs SA (-28%: -42 to -13%, P<0.0001). The sequential % pause was significantly different between all groups: APC vs VPC (-133%: -147 to -119%, P<0.0001); VPC vs SA (178%: 164-192%, P<0.0001); APC vs SA (45%: 31-60%, P<0.0001).
When comparing to the average of R-R intervals all groups were significantly different; in % prematurity, APC vs VPC (16%: 12-21%, P<0.0001), VPC vs SA (-27%: -31 to -23%, P<0.0001) and APC Vs SA (-10%: -14 to -6%, P<0.0001); and in the % pause, APC vs VPC (-34%: -38 to -30%, P<0.0001), VPC vs SA (52%: 48-56%, P<0.0001) and APC Vs SA (18%: 14-22%, P<0.0001).

Discussion:
Comparing the average (intervals 1-3) to the premature interval and subsequent pause offers greater differentiation between APCs, VPCs and SA than sequential R-R intervals. More investigation using P-P intervals in place of R-R intervals and evaluating arrhythmias at exercise is needed.

Clinical Relevance:
Sinus arrhythmia can be differentiated from APCs by the duration of the pause with both sequential and average R-R interval analysis. Compared to APCs, VPCs are more premature with longer pauses.

Oral presentation
Friday 27 October 2023, 12.45-13.00

Study into an improved Einthoven's triangle around the equine heart: proposition of the Delta (Δ) configuration.

E.P. Paulussen[1], G.V.S. Glenn[1], A.D.C. Decloedt[1], B.H. Hermans[2], T.D. Delhaas[2], G. van Loon[1]
[1]Ghent University, Merelbeke, Belgium
[2]Maastricht University, Maastricht, Netherlands

Introduction:
The absence of a standardized, data-driven electrode configuration for equine electrocardiogram (ECG) recording hinders the advancement of arrhythmia diagnostics and data exchange between equine clinicians. Commonly used configurations are effective for rate and rhythm diagnosis but remain limited in identifying the origin of arrhythmias.

Objectives:
The study aimed to assess various Einthoven's triangle electrode configurations to enhance ECG recordings in horses. Optimal ECG recordings are characterized by a large P and QRS amplitude, along with electrodes positioned on both sides of the body, to differentiate between left- and right-sided ectopy. We hypothesized that a base down triangle (called Delta Δ configuration) would yield increased diagnostic information to fulfill these criteria.

Animals:
Seventy-five healthy warmblood horses aged 4 to 20 years were examined. Thirty electrodes were placed and a 5 minute ECG recording at 1000Hz sampling rate was made using a wearable physiological signal amplifier system.

Methods:
We compared the modified base-apex configuration with the Dubois, Copenhagen and our own proposed Δ configuration (Fig.1). A weighted score was calculated based upon the following normalized criteria: P and QRS amplitude, P/R ratio, Q/T ratio and coherence. All signal processing was done with custom scripts in Matlab.

Results:
The modified base-apex configuration, which does not include left-right information, scored 16.5 ± 3.5. For systems with Einthoven's triangle around the heart scores were 16.0±3.6 for Δ, 13.7±3.6 for Copenhagen, and 13.1±2.8 for Dubois.

Conclusions and clinical relevance:
The Δ configuration is promising because of sufficiently large P and QRS amplitudes, and best overall scores. Having the base of Einthoven's triangle along the ventral part of the heart is likely to result in better left-right differentiation of ectopic rhythms and improve vectorcardiography results. Implementing this new configuration could lead to improved arrhythmia diagnosis in horses.

Oral presentation
Friday 27 October 2023, 14.00-14.15

Epidemiology of equine herpesvirus 1 myeloencephalopathy outbreak in Valencia 2021.

E.J.C. Jose-Cunilleras[5], M.C.T. Cuesta-Torrado[1], I.S.L. Santiago-Llorente[2], L.A.R. Armengou[3], A. Velloso Alvarez[1], F.N. Nieto[4], J.R. Ríos[5], F.C.L. Cruz-Lopez[2]
[1]Universidad Cardenal Herrera CEU, Valencia, Spain
[2]Universidad Complutense, Madrid, Spain
[3]Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain
[4]Equihealth Veterinarios, La Roca del Vallès, Barcelona, Spain
[5]Universitat Autonoma de Barcelona, Bellaterra (Barcelona), Spain

Introduction:
An EHV-1 myeloencephalopathy (EHM) outbreak occurred in Valencia, Spain, in 2021. The aim of this study was to evaluate risk factors, effective reproduction rate (Rt) and long-term outcomes of this severe outbreak.

Methods:
Retrospective epidemiological study based on clinical records from the veterinary team at the competition site and three referral equine hospitals. All quarantined (n=160) and referred (n=31) horses were included in this study. All horses had at least one EHV-1 PCR test performed in blood/nasal swabs. Association of risk factors (sex, vaccination, age, breed, viral load on PCR) with mortality, development of EHM, and odds of returning to competition by 2 years, were estimated by odds ratios [95% CI]. Rt was estimated by the Robert Koch's Institute method.

Results:
Out of a total of 191 horses, 11 died or were euthanized, 78 developed EHM and survived, 50 were febrile, 14 were asymptomatic (positive PCR) and 38 remained healthy. Vaccination rate for EHV was 30%. There were 84 mares, 80 geldings, and 27 stallions. Previous EHV vaccination was associated with development of EHM (4.4 [2.2-8.7]; $p<0.0001$). Mares were over-represented among non-surviving horses: 8/11, 3/11, and 0/11 (mares, geldings, and stallions, respectively). The likelihood of returning to competition was similar in horses recovered from EHM (73%; 57/78) and infected horses without neurologic disease (76%; 47/62). Rt was 5.3 early on and decreased to 1.9 by 2 weeks from onset of outbreak.

Conclusions:
Sex and vaccination status appeared to play a role in development of EHM or increase the risk of mortality after EHV1 infection. Horses recovered from EHM could be as likely to return to competition as other EHV-1 infected horses.

Clinical relevance:
Further studies are necessary to establish the role of individual risk factors, vaccination and level of herd immunity on development of EHM.

Oral presentation
Friday 27 October 2023, 14.15-14.30

Study of the survival of equine herpesvirus 4 (EHV-4) as a source of environmental contamination

C. Normand[1], A. Guenoux[2], C. Fortier[2], E. Hue[2], P.H. Pitel[3], S. Pronost[2]
[1]LABÉO - BIOTARGEN , Saint-Contest, France
[2]LABÉO - BIOTARGEN, Saint-Contest, France
[3]LABÉO, Saint-Contest, France

Introduction:
Equine herpesvirus 4 (EHV-4) is responsible for respiratory diseases, called equine viral rhinopneumonitis, in horses and in rare cases for abortions or neonatal deaths. This disease is highly contagious and transmitted mainly through infected nasal discharge. However, studies of the survival of EHV-1 in water and on inanimate surfaces highlight a potential source of infection for susceptible horses.

Methods:
Water was spiking with EHV-4 and incubated at 4 temperatures (4°C, 20°C, 27°C and 34°C) for up to 21 days (34°C) or 105 days (20°C, 27°C and 34°C). The survival of EHV-4 in water was analyzed by qPCR and an innovative culture cellular method: Real-Time Cell Analysis. The detection of EHV-4's genome in the environment was investigated during episodic EHV-4 in stud farms.

Results:
Depending on the temperature, EHV-4 is able to remain infectious in water. For example, at 4°C, EHV-4 is infective for up to a quarantine period (21 days). EHV-4 was also detected on surfaces like troughs, feeders, metal grids, and others.

Conclusions:
These results suggest that the environment could be an abiotic source of contamination by EHV-4 for susceptible horses. Also, this study highlights the necessity to improve preventive strategies, through biosecurity measures (disinfection), during outbreaks.

Clinical relevance:
This work highlights the risk of environmental contamination, which can cause individual contamination leading to an epizootic. EHV-4 and rhinopneumonitis provide a good model for studying the survival of equine viruses in the environment.

Oral presentation
Friday 27 October 2023, 14.30-14.45

Meta-analysis of naturally occurring Equine Herpesvirus-1 (EHV-1) outbreaks finds no evidence for a significant effect of vaccination on the effective reproduction number.

R.M.A.C. Houben[1], C. Maanen[2], J.R. Newton[3], J. Broek[1], M.M. Sloet[1], J.A.P. Heesterbeek[1]
[1]Utrecht University, Utrecht, Netherlands
[2]Royal GD, Deventer, Netherlands
[3]University of Cambridge, Cambridge, United Kingdom

Introduction:
EHV-1 infection is the cause of high impact disease syndromes, affecting the global horse industry. The effect of vaccination on transmission dynamics of EHV-1 in naturally occurring outbreaks is insufficiently quantified. Our objective was to estimate R_0 for EHV-1 in equine herds from outbreak reports and evaluate the effect of herd vaccination status.

Methods:
Systematic review, model-based estimations, meta-analysis. A literature search for outbreak reports was carried out. Depending on available data, the early epidemic growth rate (GR) or final attack rate (AR) approach was used to estimate the basic reproduction number for that outbreak. Herd vaccination status and strain type, and use of antivirals were also recorded. Only outbreaks in herds where either none or all horses had been vaccinated within six months prior to the outbreak were included. An overall estimate for R_0 (non-vaccinated herds) and R_v (vaccinated herds) was computed by meta-analysis and the two groups were compared using a mixed effects model.

Results:
Ten outbreaks met the inclusion criteria, of which five occurred in non-vaccinated herds and five in vaccinated herds. One R_0 calculation derived from a report describing empirical determination of a herd immunity threshold was also included. We did not detect a significant effect of vaccination status of the herd on the effective reproduction number in the included outbreaks: R_0 = 3.3(2.6 – 4.1) and R_v = 2.8(2.1 – 3.5), p = 0.285. Insufficient (discordant) data were available to investigate the influence of strain type, vaccine type or antivirals on this result.

Conclusions:
We were unable, with the available evidence, to support the assumption that herd vaccination significantly decreases transmission of EHV-1. The lower limit of the R_v confidence interval was >1.

Clinical Relevance:
Herd vaccination as a sole mitigating measure may have insufficient effect on transmission of EHV-1 to prevent major outbreaks.

Oral presentation
Friday 27 October 2023, 14.45-15.00

Efficacy of an active vaccination against il-5 for the treatment of equine recurrent urticaria

Birkmann, K.[1,2], Waldern N.[1], Jebbawi F.[1,3,4], Canonica F.[1,3,4], Fettelschoss-Gabriel, A.[1,3,4]

[1]Evax AG, Im Binz 3, 8357 Guntershausen, Switzerland.
[2]Equine Clinic, Ludwig-Maximilians University Munich, Sonnenstrasse 14, 85764 Oberschleißheim, Germany.
[3]University Hospital Zurich, Department of Dermatology, Wagistrasse 18, 8952 Schlieren, Switzerland.
[4]Faculty of Medicine, University of Zurich, Switzerland.

Introduction:
Equine recurrent urticaria is a common clinical sign with various causes. Pathophysiology of equine urticaria is still poorly understood. However, a significant increase in inflammatory eosinophils in the dermis of lesional skin was confirmed. IL-5 is the key-cytokine for development, attraction and activation of eosinophils and might play a major role in the pathophysiology of equine recurrent urticaria. Therefore, the aim of our placebo-controlled double blinded randomized study was to evaluate the efficacy of a previously described active vaccination against IL-5 (eqIL-5 vaccine) for the treatment of equine recurrent urticaria.

Methods:
36 client-owned horses with recurrent urticaria were enrolled into the ongoing study. At screening visits, lesional and non-lesional skin punch biopsies were collected for gene expression analysis by qPCR. Horses were randomly assigned to treatment or placebo group and received a basic vaccination either with the eIL-5 vaccine or placebo. After completion of the basic vaccination, horses entered a blinded follow-up, where all horses received the eqIL-5 vaccine either as a re-vaccination or as a basic vaccination. During both study phases, urticaria lesions were scored according to an urticaria activity score, every 4 weeks at live visits (UAS live), in weekly intervals from photographs (UAS photo). UAS scores of subgroups were compared by repeated measures ANOVA.

Results:
Gene expression confirmed role of eosinophilic genes in lesional skin punch biopsies. An interim analysis showed statistically significant improvement of UAS live and photo scores in vaccinated horses.

Conclusions:
These results suggest that IL-5 plays a major role in the pathogenesis of equine recurrent urticaria and active vaccination against IL-5 is an effective treatment.

Clinical relevance:
Since identification and elimination of the inciting antigen is often difficult and other treatment options carry the risk of severe side effects, this therapeutic vaccine targeting IL-5 offers an effective treatment for equine recurrent urticaria.
Keywords: Recurrent Urticaria, IL-5, Active Vaccination

Oral presentation
Friday 27 October 2023, 15.00-15.15

The hypothalamic-pituitary-adrenal gland axis response to vasopressin (AVP) stimulation test in healthy and critically ill foals

K.A. Dembek, E.M. Elder
North Carolina State University, Raleigh, United States

Introduction:
Sepsis remains the leading cause of death in foals. The hypothalamic-pituitary-adrenal gland axis (HPAA) dysfunction is a common complication of sepsis resulting in decreased survival. HPAA dysfunction can be diagnosed with AVP stimulation test in other species. The goal of this study was to evaluate HPAA response to AVP stimulation in healthy and hospitalized foals. We hypothesized that AVP would stimulate a rise in ACTH and cortisol in healthy foals. We also proposed that cortisol and ACTH response would be decreased in critically ill foals compared to healthy foals, and that the diminished response would be associated with disease severity and outcome.

Methods:
HPAA function was assessed in 12 healthy foals utilizing 2 doses of AVP (2.5, 5 IU), administered at 48h of age. Hospitalized foals (n=18) were <7-days old and received 2.5 or 5 IU of AVP on admission. Cortisol and ACTH were measured at 0, 15, 30, 60, and 90 minutes after AVP administration with immunoassays. A fold increase 15 and 30 minutes from baseline was calculated for cortisol and ACTH concentrations.

Results:
All doses of AVP resulted in a significant increase in cortisol concentration and a dose-dependent increase in ACTH concentration over time in both groups. ACTH and cortisol concentration increased 15 and 30 minutes after all doses of AVP compared to baseline in healthy and hospitalized foals ($P<0.01$). Cortisol and ACTH response to AVP administration (2.5 and 5 IU) at 30 and 15 minutes was lower in critically ill foals compared to healthy foals suggesting HPAA dysfunction ($P<0.05$).

Discussion:
Administration of AVP is safe and results in a significant rise in ACTH and cortisol in both healthy and hospitalized foals.

Clinical relevance:
A stimulation test with 2.5 and 5 IU of AVP can be considered for HPAA assessment in critically ill foals

Oral presentation
Friday 27 October 2023, 15.15-15.30

Increased serum thymidine kinase 1 activity is mostly not diagnostic for equine malignant lymphoma

J. Zeppenfeld[1], J. Marchewski[2], A. Gläsel[3], K.A. Roscher[1]
[1]Equine Clinic, Internal Medicine, Giessen, Germany
[2]Institute of Veterinary Pathology, Giessen, Germany
[3]Clinical Pathophysiology and Clinical Pathology, Giessen, Germany

Introduction:
Lymphomas are the most common malignant haematopoietic neoplasia in horses, but antemortem diagnosis is often challenging for clinicians. Measurement of serum thymidine kinase 1 (sTK1) activity as an inexpensive and almost non-invasive biomarker is described in the literature revealing controversial results. The aim of the study was to evaluate the performance of a commercial assay for the measurement of sTK1, in particular in patients whose cytology was non-diagnostic for lymphoma.

Methods:
Activity of sTK1 was measured in 19 equids with lymphoma, 11 equids with non-lymphoid neoplasia, 15 equids with non-neoplastic diseases and 27 clinical healthy equids. Seventeen lymphoma patients underwent at least one cytological examination antemortem (blood smear; aspirate of lymph node, bone marrow, spleen or abdominal tumor; abdominal or thoracic fluid). With the exception of the healthy control group all patients underwent a complete post-mortem examination.

Results:
Median (range) sTK1 activity was 2.50 (0.49-382.0) U/L in lymphoma, 2.90 (0.49-18.4) U/L in other neoplasia, 1.28 (0.49-28.5) U/L in non-neoplastic diseases and 0.63 (0.49-1.95) U/L in controls. Only differences between control and lymphoma (P=0.0002) and control and neoplasia (P=0.024) achieved significance. Lymphoma was diagnosed antemortem by cytology in 12 equids. To distinguish lymphoma from all other patients, a maximum likelihood ratio of 6.42 could be achieved for sTK1 > 23.45 U/L (sensitivity 10.53 %, specificity 98.36 %). However, only 2 horses with lymphoma reached these levels and both of them were diagnosed by antemortem cytology (blood smear and lymph node aspirate respectively).

Conclusions:
Compared to healthy controls, sTK1 activity was increased in equids with lymphoma and non-lymphoid neoplasia. However, elevated levels are usually not a definitive sign of lymphoma.

Clinical relevance:
The study highlights the value of cytological examination for the diagnosis of lymphoma in equids.

Oral presentation
Friday 27 October 2023, 16.00-16.15

Equine asthma severity correlates with neutrophil extracellular traps in bronchoalveolar lavage fluid

L.K. Meiseberg, J. Delarocque PhD, R. Imker, M. Köckritz-Blickwede, B. Ohnesorge, N. Buhr PhD
University of Veterinary Medicine Hannover, Foundation, Hannover, Germany

Introduction:
Neutrophil extracellular traps (NETs) are released by activated neutrophils and consist of extracellular chromatin-histone-components, granule proteins, enzymes, and anti-microbial agents. They are described in equine and human asthma patients, where they can cause and feed-forward inflammation. The aim of this study was to investigate clinical relevance of NETs in horses affected by equine asthma (EA).

Methods:
26 horses underwent a complete respiratory workup including exercise testing, arterial blood gas and bronchoalveolar lavage fluid (BALF) analysis. EA severity was determined based on the consensus statement criteria. NET-activated cells in BALF were quantified using immunofluorescence confocal microscopy and blood-derived neutrophil reactivity was assessed *ex-vivo* by cathelicidin stimulation.

Results:
EA was absent in 8 horses, mild in 4, moderate in 6 and severe in 8. Ordinal regression revealed a positive association between NET-activated cells in BALF and blood-derived neutrophil reactivity with EA severity ($p = 0.009$ and 0.02, respectively). As indicated by Welch's ANOVA, the latter was also associated with pO_2 at rest ($F(3, 11.894) = 8.365$, $\omega^2 = 0.582$, $p = 0.003$), which was significantly lower in horses with severe EA compared to all other groups (mean difference: -19 to –14 mmHg, $p \leq 0.031$). Nevertheless, the negative correlation between NET-activated cells in BALF and pO_2 at rest was weak to moderate ($r = -0.37$).

Discussion:
Proportion of NET-activated cells in BALF and peripheral neutrophil reactivity increased with asthma severity, indicating that EA may be associated with local as well as systemic immunologic changes.

Clinical relevance:
Increasing our understanding of EA pathophysiology may help to develop new therapeutic approaches. For example, NET-formation inhibitors may be investigated. Moreover, quantification of blood-derived neutrophil reactivity may be considered as screening tool to identify horses at risk for severe EA.

Oral presentation
Friday 27 October 2023, 16.15-16.30

A new scoring system to evaluate mucosal surface pathology in equine glandular gastric disease

V.H.L Scott, D.G.M. Sutton, N.P. Evans, D.E.F. McKeegan
University of Glasgow, Glasgow, United Kingdom

Introduction:
Both ordinal scale and descriptive systems have been reported for assessment of equine glandular gastric disease (EGGD). A new point scoring system (NPSS) is presented that aims to quantify specific features of glandular mucosal pathology and facilitate assessment of chronicity and healing.

Methods:
Sequential gastroscopy videos were reviewed from Thoroughbreds (n=26) diagnosed and treated for EGGD as part of a larger research study. Lesions were first (T_0) categorised qualitatively as 'mild', 'moderate', or 'severe' by specialists in internal medicine. The change in appearance of glandular lesions during 4-12 weeks of treatment was reviewed. NPSS was developed by assigning numerical values to lesion features of erythema, fibrin, and haemorrhage. Total NPSS score was scaled according to lesion surface area. The proportion contribution ($\hat{p}$) of each feature score to NPSS was calculated and feature profiles were generated for disease severity. In horses with improving or deteriorating EGGD, changes in feature profiles were calculated.

Results:
Features with the highest mean proportion in NPSS for mild, moderate, and severe EGGD at T_0 were erythema ($\hat{p}$=0.45), fibrin ($\hat{p}$=0.4) and haemorrhage ($\hat{p}$=0.53) respectively. Modal category change for horses with worsening EGGD was mild to moderate. Within this group, increasing lesion fibrin was the greatest feature change ($\Delta\hat{p}$=0.18) and a feature profile similar to moderate EGGD at T_0 was observed. Modal category change for horses with improving EGGD was moderate to mild and decreased lesion haemorrhage was the greatest feature change ($\Delta\hat{p}$=-0.2); a feature profile different to mild EGGD at T_0 was observed.

Discussion and Clinical Relevance:
NPSS highlights quantitative variability in mucosal surface pathology for differing severities of EGGD. Horses with worsening lesions show progression of surface pathology consistent with spectrum of disease at initial diagnosis. Feature profiles for horses with improving lesions do not reflect a reverse sequential, facilitating assessment of healing stages.

Oral presentation
Friday 27 October 2023, 16.30-16.45

Secretomic profiles to distinguish adult horses with non-complicated acute gastro-intestinal disease from those with sepsis

A. Blangy--Letheule[1], M. Bouaud[1], B. Lauzier[1], A.A. Leroux[4], S. Bourgoin-Voillard[2], D. Habert[3], J. Courty[2], M. Seve[2], B. Rozec[1]
[1]Université de Nantes, CHU Nantes, CNRS, INSERM, l'institut du thorax, Nantes, France
[2]Univ. Grenoble Alpes, TIMC, PROMETHEE Proteomic Platform, Grenoble , France
[3]University of Paris-Est Créteil (UPEC), Inserm U955, Equipe 21, UMR_S955, Créteil, France
[4]Oniris, Nantes, France

Introduction:
Sepsis is a life-threatening organ dysfunction resulting from a deregulated host response to infection. Like humans, adult horses are prone to sepsis development secondary to gastro-intestinal disease (GID). The secretome is a set of proteins secreted by a cell at a given time and under certain conditions. Easily accessible from plasma and analyzable by proteomic approach, it represents an opportunity to identify biomarkers in sepsis. This study aims to improve early detection and management of equine sepsis of digestive origin by identifying a combination of biomarkers that might help in clinical decision-making.

Methods:
Sample and clinical data of healthy horse (HH, n=12), horses hospitalized for GID without signs of sepsis until discharge (n=12) and horses with signs of sepsis (SH, n=11) were collected upon admission to the equine emergency department and over the first 2 days of hospitalization. Samples were analyzed using large-scale tandem mass spectrometry based on a label free quantification to allow identification of deregulated proteins (DEPs). The receiver operating characteristic curve (ROC) of the DEP were then analyzed with R software.

Results:
Protein selection identified 469 proteins. The study of DEPs over time in the SH group compared with the HH and GID groups revealed 46 and 20 DEPs only upon admission compared with the HH and GID groups respectively. Among these proteins, 5 were common between HH *vs* SH and GID *vs* SH analysis. ROC curves were established for different protein combinations in order to characterize the pathophysiological signature of SH. A combination of 4 DEPs that distinguishes sepsis from GID with an area under the curve of 99.6% was identified.

Clinical relevance:
This study identified a promising combination of diagnostic biomarkers of sepsis. These data allow us to distinguish molecular alterations between these two conditions and may help delineate treatment strategies.

POSTER ABSTRACTS

Poster presentation 2

A prospective, randomised study into the complications and outcomes of upper or lower eyelid subpalpebral lavage treatment systems in 66 equine eyes (2015-2023)

A.E. Graham, H. Carslake, F. Malalana
University of Liverpool, Neston, United Kingdom

Introduction:
Subpalpebral lavage systems (SPLs) are commonly used in horses for administration of topical ophthalmic medication. Evidence for the optimal location is lacking. The aim of this prospective, randomised treatment trial was to compare the rate and type of complications of SPLs located in the central upper- compared to the medial lower eyelid in hospitalised patients.

Methods:
Horses admitted for ophthalmic treatment using an SPL from February 2015 to March 2023 were included if the ocular pathology did not necessitate placement of the SPL in a specific location. A coin toss was used to randomly determined the SPL location. SPLs were monitored at least daily, and complications defined as major (displacement of the footplate from the fornix +/- corneal ulceration; loss of footplate; eyelid infection/abscess formation) or minor (loss of suture / tape; palpebral cellulitis; leakage or tube rupture; loss of injection port; subcutaneous swelling / abscess at suture site).

Results:
Sixty-six SPLs in 65 horses were included, with 36 (54.5%) located in the upper-, and 30 (45.5%) in the lower eyelid, for a median (IQR) duration of 10 (8-16.2) days. Fifty-nine complications occurred in 38/66 SPLs (57.6%) (32 upper eyelids (32/59; 54.2%) and 27 lower SPLs (27/59; 45.8%)). Major complications occurred in 2 lower SPLs (2/59; 3.4%) and 8 upper SPLs (8/59;13.6%). The most common major complication was displacement of the lavage footplate from the conjunctival fornix (6/66;9.1%). The most common minor complication was loss of the suture or butterfly tape (19/66;28.8%). Univariable and multivariable logistic regression failed to demonstrate an association between SPL location and any, or major complications.

Discussion and clinical relevance:
The biggest limitation was small study size. Although not significant, results may suggest that although the upper eyelid is not significantly more likely to see complications, any complications may be more serious.

Poster presentation 3

Single-cell transcriptome profiling of bronchoalveolar cells identifies a Th17 signature in severe equine asthma

S.E. Sage[1], P. Nicholson[2], L.M. Peters[1], T. Leeb[1], V. Jagannathan[1], V. Gerber[1]
[1]Vetsuisse Faculty, University of Bern, Bern, Switzerland
[2]Next Generation Sequencing Platform, University of Bern, Bern, Switzerland

Introduction:
Severe equine asthma (sEA) has been attributed in turn to a Th2, a Th1, a Th17 or a mixed immune response type. This conundrum stems in part from the current experimental and technological limitations. Using the cutting-edge single-cell mRNA sequencing (scRNA-seq) technology, we profiled the transcriptome of equine bronchoalveolar lavage fluid (BALF) at cellular resolution to elucidate the underlying immune mechanisms of sEA.

Methods:
Cryopreserved BALF cells from 11 Warmblood horses (5 control, 6 sEA) underwent droplet-based scRNA-seq. Horses were selected based on history, clinical score, and BALF cytology (neutrophils >10% for sEA). Data pre-processing was performed with the Cell Ranger standard workflow and downstream analysis with the R package Seurat. Differential gene expression (DGE) analysis used the mixed model method Nebula.

Results:
A total of 60,262 bronchoalveolar cells recovered after quality control and filtering could be grouped into six major cell types: B cells, T cells, monocytes-macrophages, dendritic cells, neutrophils, and mast cells. With the exception of mast cells, all cell types displayed significant heterogeneity, with previously and newly described cell subtypes. Monocyte-lymphocyte complexes were identified. We detected a strong Th17 signature in sEA, with upregulation of the B cell chemoattractant *CXCL13* in intermediate monocytes. The B cells were six times more abundant in sEA horses, with a lower fraction of switched plasma cells. Naïve CD4+ T, Treg and γδT cells also presented Th17-polarization, with upregulation of *IL-17A*, *IL-17F*, *IL-21* and *CCL20*. Several genes involved in T cell function were dysregulated. Neutrophils presented an enhanced capacity for migration and neutrophil extracellular trap formation.

Discussion:
Single-cell profiling of BALF cells supports a predominant Th17 immune response driven by monocyte and T cell gene dysregulation in neutrophilic sEA. The reproducibility of these results should be investigated in other breeds.

Clinical relevance:
The dysregulated genes identified with scRNA-seq are potential biomarkers of sEA and promising therapeutic targets.

Poster presentation 4

Immunohistochemical expression of cyclooxygenase-2 (COX-2) in equine melanomas

J. Pimenta, J. Prada, I. Pires, M. Cotovio
University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

Introduction:
Melanomas are one of the most common types of skin cancer in horses. These tumors present an uncommon benign behavior in comparison to other species, with low invasiveness and metastatic rates, but tumoral mass growth is usually a concern. COX-2 is related to pathologic conditions such as oncogenesis promoting neoplastic cell proliferation, invasion and metastasization, however studies regarding its expression in horse's tumors are scarce.
The aim of this study was to evaluate the immunohistochemical expression of COX-2 in equine melanomas.

Methods:
38 equine melanomas were processed by immunohistochemistry to COX-2 and classified by extension of labelled cells in 0) negative; 1) 1-5%; 2) 6-20%; 3) 21-50%; 4) >50% and intensity of labelling in 0) negative, 1) weak, 2) moderate, 3) strong. A final score was calculated by multiplying the extension by intensity of labelling with ≤6 being classified as weak and >6 as strong expression of COX-2.

Results:
Concerning final score, 27.8% of melanomas had high COX-2 expression (>6) and 72.2% had low expression (≤6). Regarding extension of labelling 15.8% presented a score of 4; 50% of 3; 18.4% of 2; 5.3% of 1 and 10.5% of 0. Regarding intensity 21.1% were scored as 3; 28.2% as 2; 39.5% as 1 and 10.5% as 0.

Discussion:
The overall low COX-2 expression in equine melanomas is in accordance with biological behavior of these tumors. The low levels of COX-2 may contribute for the typical mass growth of equine melanoma instead of contributing to invasiveness that is related to high COX-2 levels.

Clinical Relevance:
COX-2 selective nonsteroidal anti-inflammatory drugs could be a possible initial therapeutical approach for dermal melanomas/melanomatosis that are achieving concerning dimensions and invasiveness and that can make difficult a future surgical excision. NSAID will act by reducing the proliferation rates and thus the mass growth.

Poster presentation 6

Determination of a mathematical score of survival in newborn foals: Retrospective study on foals admitted to intensive care at the Lyon Equine Vet Hospital (Clinéquine) between 2007 and 2020

A.A. AMAR, A. BENAMOU-SMITH
VETAGROSUP Lyon, MARCY l'ETOILE, France

Introduction:
During the neonatal period, foals are very susceptible to disease. Their state of health can deteriorate suddenly, and appropriate medical care requires time-consuming and expensive intensive care. This warrants an accurate assessment of the vital prognosis on admission based on clinical exam and ancillary tests, for ethical reasons as well as economical considerations.

Methods:
During this retrospective study, the medical records of 226 foals less than 21 days old, for which the outcome "survival" or "non-survival" was known, were examined. Twenty-five variables including information from history, physical examination and laboratory findings were examined for their association with survival. Variables associated with survival were entered into a multivariable logistic regression model to determine which ones would be included in the survival score. Of these, 3 variables were retained in the final model.

Results:
15 mortality risk factors used as prognostic tools, collected at admission, have been identified as statistically significant. Factors in the final model included inability of the foal to stand, hematocrit, and neutrophils count. The highest (2,45) and the lowest (-2,25) scores represented 92% and 9% probability of survival, respectively. Sensitivity, specificity, positive and negative predictive values for the survival score were 83,7%, 63,6%, 79,4%, and 70%, respectively.

Conclusions and Clinical Relevance:
This study provides strong correlations between mortality and a reasonable number of usual clinical and clinical pathology parameters, directly usable in the neonatal period. Based on our foal population presenting a wide array of affections and breeds, we show that a core list of parameters involved in usual neonate assessment allows us to provide useful prognostic values and the survival score established in our study can be easily implemented using data available at the admission. Further evaluations of this scoring system in a prospective study are needed.

Poster presentation 8

Effect of dopamine on glucose-stimulated insulin production in the equine pancreas in vitro

F. Li[1], E. Green[1], R. Spence[1], M. Laat[1], S. Bailey[2], N. Galinelli[2], P. Harris[3], J. Sonntag[4], T. Warnken[4], M. Sillence[1]
[1]Queensland University of Technology, Brisbane, Australia
[2]The University of Melbourne, Parkville, Australia
[3]Waltham Petcare Science Institute, Melton Mowbray, United Kingdom
[4]Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany

Introduction:
Insulin dysregulation (ID), a key risk factor for hyperinsulinaemia-associated laminitis (HAL), is often seen in older *Equidae* with pituitary *pars intermedia* dysfunction (PPID), that show a decline in dopamine production. We have recently shown that pergolide can attenuate the insulin response to a glycaemic meal in animals with concurrent PPID and ID, but the link between dopamine and insulin requires further investigation. In other species, insulin secretion can be decreased or increased, by activating D_2 or D_3 dopamine receptors, respectively. This study aimed to determine the dominant effect of dopamine on glucose-stimulated insulin secretion in healthy equine pancreata *in vitro*.

Methods:
Samples of pancreas were collected from 12 mixed-breed horses slaughtered for human consumption. Pancreas explants (50 – 100 mg) were incubated for 1 h in Kreb's buffer containing either 2.5 mM glucose (low glucose control), or 10 mM glucose (high glucose) plus dopamine at 0, 0.1, 1, 10, or 100 µM. Insulin concentrations were measured in samples of the incubation medium, using an enzyme-linked immunosorbent assay. The data were adjusted for tissue weight then analysed using repeated measures ANOVA and Bonferroni's t-test.

Results:
Dopamine had a bi-phasic effect on glucose-stimulated insulin production ($P < 0.05$). Compared with the high glucose control, insulin output was doubled at 10 µM dopamine ($P < 0.05$), but no increase was seen at 100 µM dopamine.

Conclusions:
Dopamine can augment glucose-stimulated insulin production in isolated tissue from healthy horses, potentially acting via D_3 receptors, but this effect is counteracted by high dopamine concentrations, suggesting the activation of D_2 receptors.

Clinical relevance:
A better understanding of the interaction between dopamine and insulin production could lead to new insights into PPID and ID, and new approaches for the prevention of HAL.

Poster presentation 10

Obesity and Associated Metabolic Disease Conditions in Connemara Ponies in Ireland

A. Al Ansari, N. Walshe, E. Golding, V. Duggan
University College Dublin , Dublin 4, Ireland

Introduction:
Equine obesity and insulin dysregulation (ID) are major risk factors associated with endocrinopathic laminitis. This study was conducted to investigate the prevalence of obesity, increased adiposity and associated endocrine/metabolic disease conditions in Connemara ponies in Ireland.

Methods:
Registered Connemara ponies from Ireland, were recruited through public and veterinary social media posts. Ponies underwent a clinical exam and information on the management and clinical history was obtained via an owner questionnaire. Body condition score (BCS) was measured using the Henneke system; cresty neck score (CNS) and regionalised adiposity were recorded. Blood glucose, triglycerides and basal insulin concentration (BIC) were measured in all ponies and an oral sugar test (OST) was performed in 102 ponies. To differentiate Pituitary Pars-intermedia disfunction (PPID) as a cause of ID, plasma Adrenocorticotrophic hormone (ACTH) concentration was measured in ponies ≥ 10 years old.

Results:
287 ponies were included; 77 ponies (27%) had BCS ≥ 7, 74 (26%) had CNS ≥ 2.5 and 179 (62%) had regionalised adiposity. Owner reported history and/or clinical evidence of chronic laminitis found in 149 ponies (52%), with divergent rings observed in 130 ponies (45%). Twenty of 214 (9.3%) ponies ≥10 years old had plasma ACTH concentration above the seasonal reference range. Current ID was confirmed in 24% of 99 ponies in which OST results were available. Hypertriglyceridemia was observed in 15 (5%) ponies and hyperglycaemia in 14 (4.9%) ponies. The multivariable model illustrated that the odds of showing ID-OST were significantly associated with generalised obesity ($p < 0.0001$).

Conclusions:
Obesity, increased adiposity, laminitis and metabolic derangements are prevalent in this native Irish pony breed.

Clinical Relevance:
ID and associated metabolic conditions are major risk factors for endocrinopathic laminitis in this breed; owners and vets should be alerted to these risks.

Poster presentation 11

Breath Characteristics and Adventitious Lung Sounds in Healthy and Asthmatic Horses : Assessment of a novel digital auscultation-based method in equine asthma

E. Greim[1], J. Naef[1], S. Mainguy-Seers[2], J.-P. Lavoie[2], S. Sage[1], G. Dolf[1], V. Gerber[1]
[1]Swiss Institute of Equine Medicine (ISME), Bern, Switzerland
[2]Faculty of Veterinary Medicine, St-Hyacinthe, Canada

Introduction:
Thoracic auscultation is essential in the diagnostic work-up of equine asthma (EA), but is limited by low sensitivity for transient or localized lung sounds, high subjectivity and the lack of standardized terminology.

Methods:
This prospective multicenter case-control study investigated normal and pathological breath sounds in clinically healthy horses (12 controls) and horses with mild-moderate EA (12 mEA) and severe EA in remission (5 sEAr) or in exacerbation (5 sEAe) using a novel digital auscultation device. Group assignment was based on clinical and tracheal mucus scoring, bronchoalveolar lavage fluid cytology, and lung function testing. Each horse was auscultated in eleven locations simultaneously for one hour, resulting in 5'478 to 27'852 breath recordings per horse. Per recording, 100 breaths were randomly selected, visually and acoustically assessed, and blindly scored for breathing intensity (normal/increased/decreased), abnormal sounds (wheezes, crackles or rattles) and coughs.

Results:
Most (85.9%) of analyzed breathes were good quality, allowing sound characterization. Cough episodes appeared as wide vertical bands with high intensity (>2000 Hz), wheezes as thin horizontal bands (200-1000 Hz), and crackles and rattles as series of high-frequency peaks. Preliminary analyses showed that pathological sounds were significantly more frequent in sEAe, but not in sEAr or mEA, when compared to the control. Wheezes were associated with clinical score and tracheal mucus score, while breathing intensity was associated with clinical score and BALF neutrophil percentage.

Discussion:
While this pilot study demonstrated the capacity of a digital auscultation device to detect and quantify normal and abnormal respiratory sounds in horses, additional analyses on a larger sample are required to determine its ability to discriminate mildly asthmatic from healthy horses.

Clinical Relevance:
This digital auscultation device may ultimately improve the diagnostic value of auscultation in horses. This is the first step towards developing a user-friendly auscultation device with automated real-time diagnostic feedback.

Poster presentation 22

Neonatal piroplasmosis, an underestimated problem?

L.M.H. Hermans[1], A.L. Leblond[2], A.J. Josson[3], J.G. Gasco[3], C.B. Bonsergent[4], L.M. Malandrin[4]

[1]Equine department, VetAgro Sup, University of Lyon, Marcy l'étoile, France
[2]UMR EPIA, INRAE, VetAgro Sup, University of Lyon, Marcy l'étoile, France
[3]Equine Department, VetAgro Sup, University of Lyon, Marcy l'étoile, France
[4]Oniris, INRAE, UMR BIOEPAR, Nantes, France

Introduction:
Clinical cases of piroplasmosis have been reported rarely in neonates, resulting in jaundice. The question is to estimate the valuability of including piroplasmosis in the differential diagnosis of jaundice in the neonates. The objectives of our study were to estimate the frequency of vertical transmission of piroplasmosis from infected asymptomatic broodmares to their foals and observe the symptoms in positive newborns.

Methods:
Mares spending more than 6 months/year on pasture, in their last trimester of gestation were included, as well as their foals of less than 72 hours of age and born in a box/paddock. Blood smears were evaluated, and nested PCR (nPCR) were performed on collected blood samples.

Results:
Seventy-one mares and their foals were included. Among the mares, a prevalence of 35,2% (25/71) and 2,8% (2/71) was detected by nPCR for *Theileria equi* and *Babesia caballi* respectively. Blood smear evaluation of 61 samples revealed presence of T. *equi* in 6 smears (9,8%) and in none, B. *caballi*. No mare showed symptoms at the time of sampling. Among the foals born from an infected mare, a prevalence of 8% (2/25) and 0% (0/2) for *T. equi* and *B. caballi*, respectively, was detected by nPCR. Parasites were detectable in the blood smears of both infected foals. None of the infected foals had symptoms at the time of collection nor in the following days.

Discussion:
These results confirm that vertical transmission of *T. equi* from infected broodmares to their foals may occur with a low prevalence (8%). Foals born from asymptomatic mares did not show clinical signs, which is consistent with previous publications. Nevertheless, symptomatic piroplasmosis may be sporadically observed in this age group.

Clinical Relevance:
Vertical transmission of piroplasmosis and correlation with symptoms in neonates may be of importance for breeders and warrants further research.

Poster presentation 24

Salmonella shedding amongst colic cases presenting to an equine referral hospital in Qatar

S. Baillie[3], TF. Moreira Fernandes[1], MR. Robin[1], C. Jamieson[2], BU. Uberti[1]
[1]Equine Veterinary Medical Center, Doha, Qatar
[2]Purdue university, 625 Harrison Street, West Lafayette, IN, United States
[3]EVMC, Doha, Qatar

Introduction:
Sub-clinical shedding of Salmonella is known to be a cause of disease outbreaks in equine hospitals . Consequences include increased patient morbidity and mortality, economic loss, reputation damage and, potentially, zoonotic infection. Horses with gastrointestinal disease are more likely to shed salmonella whilst hospitalised than horses with other types of illness and the rapid identification of infected horses can help reduce the spread of nosocomial infection

Methods:
A prospective observational study was carried out between June 2021 and May 2023. All horses presenting with colic had samples submitted for Salmonella PCR and culture within 24hrs of arrival at the hospital. Horses were then kept at an increased biosecurity level until results were available. Data were analysed to identify any association with clinical pathology data on arrival.

Results:
321 horses were tested during the two-year period. Twenty-four cases tested positive (7.5% prevalence). No clinically significant association with clinicopathological data was identified.
Of the positive cases, 18 were PCR and culture positive, 3 were PCR negative and culture positive, 2 were PCR positive and culture negative and 1 was PCR positive with no culture submitted.
Of the positive cases, 14/24 underwent surgery and 10/24 were treated medically. Of the negative cases, 111 /297 underwent surgery and 185/297 were treated medically.

Conclusions:
The prevalence of detectable salmonella infection in horses presenting for colic was 7.5%.

Clinical Relevance:
The data support the hospital's ongoing biosecurity protocols for colic patients, although no comparison can be drawn with non-colic patients. There was no evidence of any additional clinical data that could be used to modify biosecurity protocol risk factors. The use of both PCR and culture to detect subclinical salmonella shedding should continue.

Poster presentation 28

Evaluation of symmetric (SDMA) and asymmetric (ADMA) dimethylarginines in healthy and in systemic inflammatory response syndrome (SIRS) negative or positive colic horse

F. Bindi[1], I. Nocera[2], G. Vallesi[3], V. Meucci[3], F. Bonelli[3], A. Spadari[4], R. Rinnovati[4], M. Sgorbini[3]
[1]Department of Veterinary Sciences, University of Pisa, Pisa, Italy
[2]Institute of Life Sciences, Sant'Anna School of Advanced Studies, Pisa, Italy
[3]Department Veterinary Sciences, University of Pisa, Pisa, Italy
[4]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

Introduction:
The aim of this study was to compare plasmatic concentrations of ADMA and SDMA in healthy vs. SIRS-positive/negative colic horses over-time and to evaluate the correlation between ADMA/SDMA and SIRS score to determine the effectiveness of these biomarkers (BIOs) for the evaluation of SIRS severity.

Methods:
The study was approved by Ethical Committee (Pisa University 2825/14) and involved 2 veterinary teaching hospitals. A total of 66 horses were enrolled: 17/66 were healthy and 49/66 were colic horses. At admission (T0), and then after 24(T24), 48(T48), 72(T72), and 96(T96) h, each horse underwent a complete physical exam and SIRS score evaluation. Blood samples were collected once in healthy and at each sampling times for colic horses to assess SDMA and ADMA concentration using the Ivanova's method. Data distribution was analyzed using the Komolgorov-Smirnov test. Kruskal-Wallis and Dunn's multiple comparisons test were applied to verify differences for ADMA and SDMA between healthy and colic horses for all the sampling time. Correlation between SIRS score and ADMA/SDMA at T0 was assessed using Spearman test ($p<0.05$).

Results:
Of the 66 horses enrolled, 17/66 were healthy, 15/66 colic SIRS-negative and 34/66 colic SIRS-positive horses. Statistical differences were found ($p<0.0001$) for SDMA between healthy vs. SIRS-negative (T24-T96) or SIRS-positive (T0-T96) horses, while no differences were obtained for ADMA ($p=0.1487$). No correlation was observed between SIRS score and SDMA at T0 ($p=0.584$).

Discussion:
SDMA shows potential as a biomarker for distinguishing between healthy horses and sick colic horses both SIRS-negative and SIRS-positive; ADMA might not demonstrate comparable discriminatory efficacy. However, the potential of SDMA as a biomarker for assessing the severity of SIRS has not yet been established.

Clinical relevance:
Although further research is needed to determine the diagnostic and prognostic role, our results suggest that SDMA might be a promising biomarker for equine colic research.

Poster presentation 29

Efficacy of intramuscular omeprazole in horses with ESGD and EGGD, with or without IBD, previously treated unsuccessfully with oral omeprazole

L.C. Kranenburg, C. Ludin, R. Boom
Utrecht University, Utrecht, Netherlands

Introduction:
The aim of this study was to investigate the success rate of intramuscular omeprazole in horses with ESGD and EGGD, with and without concurrent IBD, that had been previously treated unsuccessfully.

Materials and methods:
Twenty horses received 20ml long-acting intramuscular omeprazole (IMOM), 4 times with a 7-day interval, following unsuccessful treatment during 28 days with oral omeprazole, combined with sucralfate in case of EGGD. Of the 20 horses 4 horses were diagnosed with ESGD, 15 horses were diagnosed with both ESGD and EGGD and one horse was diagnosed with EGGD. In 10 horses a duodenal biopsy was obtained, and a histological diagnosis of IBD was made in 9 cases.

Results:
The ESGD lesions improved in 18/19 (94.7%) and healed in 11/19 (57.9%) cases. In case of EGGD 10/16 (62.5%) of the lesions improved and 8/16 (50.0%) of the lesions had resolved.
In horses with ESGD the lesion score after treatment was significantly lower than before treatment, both in horses with IBD ($p=0.022$) and those without IBD ($p=0.005$). For EGGD there was only a significant change in lesion score in the group without IBD ($p=0.018$), but not in the group with IBD ($p=0.345$). In one horse out of 9 with IBD the glandular lesions were more severe after the treatment, while none of the lesions deteriorated in the group without IBD.

Discussion:
Superior results are reported for the treatment of EGUS with intramuscular omeprazole, compared to oral administration. Intramuscular omeprazole led to significantly decreased lesion scores for both ESGD and EGGD in horses without concurrent IBD, even when these lesions had not responded to previous treatment with oral omeprazole.

Clinical relevance:
The efficacy of IMOM is lower than previously reported when initial oral treatment was not successful. The concurrent presence of IBD negatively influences the healing of EGGD.

Poster presentation 30

Evaluation of a new smartphone-based digital stethoscope featuring phonocardiography and electrocardiography in adult horses

F. Bindi[1], T. Vezzosi[2], E. Zucca[3], D. Caivano[4], F. Freccero[5], M. Sgorbini[2]
[1]Department of Veterinary Sciences, University of Pisa, Pisa, Italy
[2]Department Veterinary Sciences, University of Pisa, Pisa, Italy
[3]Department of Veterinary Clinical Sciences, University of Milan, Milano, Italy
[4]Department of Veterinary Medicine, University of Perugia, Perugia, Italy
[5]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

Introduction:
The aim was to evaluate a novel smartphone-based digital stethoscope (DS) designed for simultaneous auscultation and recording of phonocardiogram and one-lead ECG in horses.

Methods:
This prospective, multicenter study, approved by the Ethical Committee (Pisa University-3/22), included 94 adult horses. Each animal underwent conventional auscultation with an acoustic stethoscope, phonocardiographic recordings, standard base-apex ECG, and recordings with the DS. The audio and phonocardiographic recordings, standard and DS ECG traces were blind reviewed. Cohen's κ was used to calculate the agreement between conventional auscultation and standard ECG vs. DS. The Cohen's kappa coefficient was interpreted as follows: ≤0 indicated no agreement, 0.01–0.40 slight, 0.41–0.60 moderate, 0.61–0.80 substantial, 0.81–1.00 optimal agreement. The agreement between standard ECG and DS ECG tracings was assessed using the Bland Altman plot.

Results:
Twentyone/94 had a heart murmur (13 systolic; 8 diastolic), 16/94 sinoatrial or second-degree atrioventricular block, 7/94 atrial fibrillation and 3/94 premature complexes; in 7/94 horses, both arrhythmias and murmurs were present; 44/94 were healthy horses. All the audio recordings were considered interpretable and optimal agreement in the diagnosis of heart murmurs (k=1) and arrhythmias (k=0.98) was found between conventional auscultation and DS. A suboptimal (k=0.68) and moderate (k=0.48) agreement was observed for P and QRS polarity. All the ECG traces recorded with the DS were deemed interpretable. The bias (95% limits of agreement) between standard ECG and DS was 0.08 (-4.34-4.51 bpm) for heart rate, 0.02 (-0.01-0.05 sec) for P wave, -0.37 (-7.40-6.66 sec) for PR, 0.008 (-0.03-0.04 sec) for QRS duration, -0.02 (-0.22-0.18 sec) for QT, -0.005 (-2.23-2.22 sec) for artifacts durations.

Discussion:
The DS exhibited good feasibility and diagnostic accuracy in detecting both heart murmurs and arrhythmias in adult horses.

Clinical relevance:
The DS could be a useful device for equine cardiac screening, especially in field conditions.

Poster presentation 32

Prevalence and role of gluten intolerance in 52 horses suspected of Inflammatory Bowel Disease

L.C. Kranenburg, C.L. Tijssen, A.J.Brom-Spierenburg, R. Boom
Utrecht University, Utrecht, Netherlands

Introduction:
Food allergies have been suggested to play a role in the pathogenesis of inflammatory bowel disease (IBD). The aim of this study was to investigate the prevalence of gluten intolerance and its role in IBD in horses.

Materials and methods:
The serum of 52 horses with a histological diagnosis of IBD was tested for the presence of antibodies against transglutaminase-2 (TGA). The test was considered negative in the case of a TGA IgA-titer lower than 35 AU/ml, dubious between 35-55 AU/ml and positive above 55 AU/ml. Follow-up data regarding outcome, the effect of a gluten free diet/management and general well-being were obtained for horses with a positive or dubious TGA titer, 6 months to 6 years after the serological test.

Results:
A positive result was obtained in 7/52 (13,5%) horses, a dubious result in 13/52 (25%) horses and a negative result in 31/52 (59,6%) horses. Follow-up data were available for 14 horses, 6 with a positive test result and 8 with a negative result. Of those 14 horses, 3 had been euthanized for reasons related to IBD and 2 because of a strangulating intestinal lesion. For 12/14 (85.7%) horses the owner considered a gluten free diet/management to be of benefit to the horse and the clinical signs improved: appetite increased, abdominal discomfort and sensitivity were reduced, performance improved and faecal consistency improved. In some cases, all clinical signs disappeared. The diet had no noticeable beneficial effect in one horse and a questionable effect in another horse.

Discussion:
A histological diagnosis of IBD may be associated with a positive or dubious gluten serology result and gluten intolerance can play a role in some cases of IBD. A gluten free diet and management appear to be beneficial.

Clinical relevance:
Testing for gluten intolerance can be relevant in managing IBD cases.

Poster presentation 36

The effects of detomidine infusion with and without vatinoxan on blood glucose and insulin concentrations in horses

N. Jantunen, M. Raekallio, H.Tapio, B. Obrochta, L. Gracia Calvo, R. Rivera Pöyhönen, K. Hagman, N. Karikoski
University of Helsinki, Helsinki, Finland

Introduction:
Alpha-2 adrenoceptor agonists are widely used for equine anaesthesia regardless of their side effects. The aim of this study was to investigate the effects of detomidine infusion with and without a peripherally acting alpha-2 adrenoceptor antagonist, vatinoxan, on blood glucose (BG) and insulin concentrations.

Methods:
Eight Finnhorses were assigned to two 4-hour infusions: detomidine (0.01 mg/kg + 0.015 mg/kg/h IV) (DET) and a combination of DET and vatinoxan (0.15 mg/kg + 0.05 mg/kg/h IV) (DET+VAT) using cross-over design. Blood samples were taken before, during and for 4 hours after the infusion at one hour interval. Blood glucose was analyzed with a portable glucometer (AlphaTRAK2) and serum insulin concentration with ELISA (Mercodia equine insulin ELISA).

Results:
Mean BG peaked (17.0±2.2 mmol/L) at the end of DET infusion and was higher than with DET+VAT (10.0±1.6 mmol/L, $p<0.001$). During DET infusion, median insulin concentration was lower than limit of quantification (1.15 µIU/mL, min-max <1.15-1.49 µIU/mL) and lower than with DET+VAT (2.24 µIU/mL, min-max <1.15-4.58 µIU/mL, $p=0.018$). With neither treatment, insulin concentration reached the baseline within 4 hours after the infusion.

Discussion:
Vatinoxan alleviated the detomidine-induced increase in BG and decrease in serum insulin concentration during 4-hour infusion and may be a beneficial addition to equine anaesthesia protocols.

Clinical Relevance:
Perioperative hyperglycemia is known to be associated with adverse events in humans but the role of transient hypoinsulinemia is less well recognized. In horses, both phenomena warrant more research.

Poster presentation 37

Urethrolithiasis in Equids: a Retrospective Study of 7 Cases (2013-2022)

D. Jean, L. Kamus, G. Fouquet, P. Ruzickova
University of Montreal, St-Hyacinthe, Canada

Introduction:
Urethrolithiasis in equids is poorly documented and usually associated with a low survival rate. The objectives are to describe the clinical findings, alterations in renal function and short-term survival rate of equids with urethrolithiasis.

Methods:
Medical files from 2013 to 2022 with urethrolithiasis as a primary or secondary diagnosis in seven equids presented to the Centre Hospitalier Universitaire Vétérinaire (CHUV) were reviewed. Follow-up was obtained by phone call.

Results:
Seven equids (6 horses of various breeds and one donkey including 6 geldings and one mare) between 11 and- 36 years old were included. Urethral calculi were removed digitally via the urethral orifice (4 cases), surgically via urethrotomy (2 cases) or not removed (1 case). The short-term survival rate was 100% with a hospitalisation duration from 3 to 42 days. Hypercreatininemia (recorded highest value during hospitalization ranging from 134 - 924 µmol/L) was observed in 6/7 patients and renal function was considered normalized in 4/7 patients at discharge. Ultrasonographic evaluation showed anomalies of kidneys in all patients including nephromegaly (4 cases), renal pelvis dilation (5 cases) and abnormal corticomedullary junction definition (3 cases).Cystoscopy revealed: urethritis and cystitis (7 cases), ureteral opening abnormalities with edema and dilation (5 cases), sabulous urolithiasis bladder (3 cases) and, bladder necrosis (2 cases) including one case resulting in a rupture. Urolithiasis was identified in several anatomic location in 5/7 patients, including bladder (3 cases) and kidneys (5 cases). Follow up was documented for 4 patients (1 to 5 years post discharge) without recurrence and 2/4 patient showing signs of chronic renal disease.

Discussion and Clinical Relevance:
In the present study, equids with urethrolithiasis had a better short-term survival rate than previously described in the literature. Improved survival was described with or without altered renal parameters, or ultrasonographic changes in the renal parenchyma.

Poster presentation 45

Clinical findings of eosinophilic keratoconjunctivitis in horses

Z. Bakos[1], R. Lorenz[1], J. Toth[2]
[1]University of Veterinary Medicine Budapest, Budapest, Hungary
[2]Tierärztliches Kompetenzzentrum Karthaus GmbH, Dülmen, Germany

Introduction:
Eosinophilic keratoconjunctivitis (EKC) is an immune-mediated disease of the cornea and conjunctiva of the equine eye. The aim of the study was to analyse the clinical records of horses diagnosed with EKC between 2012 and 2020 at a private equine hospital in Germany.

Methods:
Medical records of 114 horses were collected. Retrospective data analysis included age, breed, gender, year and month of admission, affected part of the eye, clinical signs, laboratory results, presence of secondary infections, treatment, recovery time, and recurrences.

Results:
Warmbloods were overrepresented (79%). The disease showed seasonality, the highest number of cases were diagnosed in July, August, and September. Based on the appearance of the lesions, a new classification has been suggested consisting of granulomatous, ulcerative, diphtheroid/pseudomembranous, and mixed forms of EKC. Only the cornea was affected in 59 patients (51.8%). Only the conjunctiva was involved in 17 cases (14.9%), and 33 horses (28.9%) showed lesions on both the cornea and the conjunctiva. Five horses (4.4%) had lesions on the cornea and additionally on the third eyelid. The disease was bilateral in 43% of the cases. Treatment regimens were chosen individually, and in 90 horses (78.9%) it was multimodal including laser therapy, diamond burr debridement and keratectomy besides medical therapy. Recovery time was extremely variable ranging from 5 to 330 days (median ± interquartile range: 30±39 days). The affected eye was surgically removed in 8 horses (7%). Recurrence occurred in two horses (1.8%).

Conclusions:
Our results demonstrate that EKC is an emerging ocular disease with variable clinical manifestations. Successful treatment often requires individualisation, and a multimodal approach.

Clinical Relevance:
To our knowledge, the current study population is the largest in the literature, thus our results may provide a deeper insight into this ocular condition to better understand its clinical features.

Poster presentation 49

The effect of high-carbohydrate feeding and body condition on pancreatic histomorphometry in mixed-breed ponies

K. Timko[1], M. Watts[2], J. McCutcheon, P. Weber, G. Ray, .J. Belknap, T. Burns
[1]The Ohio State Univeristy , Columbus, United States
[2]The Ohio State University , 614-292-6661, United States

Introduction:
Hyperinsulinemia-associated laminitis is a debilitating disease that affects many equids. The endocrine pancreas is the source of endogenous insulin and presumed to be dysfunctional in hyperinsulinemic animals. The objective of this study was to assess pancreatic insulin, glucagon, and somatostatin expression in ponies fed a high non-structural carbohydrate (NSC) diet.

Methods:
Twenty-one adult mixed-breed ponies were divided into four experimental groups based on body condition scoring (lean: BCS4, obese: BCS7) and diet (low NSC [1.8 g/kg/day NSC], high NSC [8 g/kg/day NSC]): lean, low-NSC (n=5); obese, low-NSC (n=5); lean, high-NSC (n=5), and obese, high-NSC (n=6). Ponies received their respective diets for 7 days. Pancreas samples were collected following the feeding protocol and evaluated for immunohistochemical expression of insulin, glucagon, and somatostatin within islets. Insulin and glucose dynamics in this population were previously evaluated.

Results:
The mean percent surface area of pancreatic islet cells expressing insulin, glucagon, and somatostatin in ponies fed the low-NSC diet were 63%, 21%, and 5%, respectively and 50%, 20%, and 3% in ponies fed the high-NSC diet. Insulin and somatostatin expression were decreased in ponies fed the high-NSC diet compared to low-NSC diet ($P = 0.044$, 0.046), whereas glucagon expression did not differ. There was a negative correlation with somatostatin expression and serum [insulin] ($P = 0.018$, $r = -0.55$), but no correlations with insulin or glucagon expression were observed. There were no differences among groups associated with BCS.

Conclusions:
Insulin and somatostatin expression were reduced in ponies fed a high-NSC diet, indicating a reduction in -cell and -cell surface area, respectively.

Clinical Relevance:
Ponies fed a high-NSC diet for 7 days demonstrate altered pancreatic histomorphology. Somatostatin expression was lower, which may contribute to hyperinsulinemia. A reduction in pancreatic insulin expression despite elevated serum [insulin] suggests the possibility of reduced insulin clearance.

Poster presentation 50

Contact force-guided 3D electro-anatomical mapping and radiofrequency ablation (CARTO®3) for improved diagnosis and treatment of sustained atrial tachycardia in 9 horses

E. Buschmann, G. Van Steenkiste, I. Vernemmen, M. Demeyere, S. Schauvliege, A. Decloedt, G. Loon
Ghent University, Merelbeke, Belgium

Introduction:
Treatment of atrial tachycardia by three-dimensional electro-anatomical mapping (3D EAM) and radiofrequency catheter ablation (RFCA) has been described using a non-contact force system, but recurrence was still seen in some patients. This could be caused by inadequate catheter-tissue contact during RFCA, resulting in incomplete ablation lesions. Real-time assessment of the contact force (CF) between catheter and tissue might improve procedural success and decrease arrhythmia recurrence rate. Contact force-guided ablation has not yet been used to treat arrhythmias in clinical patients.

Methods:
Records from nine horses with sustained atrial tachycardia treated by RFCA, using a CF-guided mapping and ablation system (CARTO®3), were reviewed.

Results:
3D EAM of the right atrium was performed in a mean time of 50±27min and revealed a clockwise re-entry (n=6), a counter clockwise re-entry (n=2) and a focal source (n=1), all located in the caudomedial aspect of the right atrium. Point-by-point RFCA was performed in power-controlled mode, with a mean of 18±8 applications in 41±16min. A median power of 35[24-45]W for a median duration of 19[8-45]s was delivered, with a median CF of 11[3-49]g and irrigation rate of 30ml/min. A median ablation index of 439[312-1018] was reached. Sinus rhythm was restored in all nine horses. To date, 3-24 months post-ablation, none of the horses showed recurrence.

Discussion:
Contact force-guided RFCA with the CARTO®3 system was feasible and effective to permanently treat the cause of sustained atrial tachycardia in horses. CF monitoring ensured efficient lesion creation, thereby minimizing the risk of recurrence.

Clinical relevance:
Compared to atrial fibrillation, treatment of atrial tachycardia using quinidine sulphate or transvenous electrical cardioversion can be more difficult, with a higher recurrence rate. 3D EAM and RFCA using real-time CF measurement not only allows to restore normal sinus rhythm, it might also minimize the risk for recurrence of atrial tachycardia.

Poster presentation 52

Dust generation and microbiological air quality with different bedding materials in a horse stable

C.U.P. Herholz[1], L. Wicki[1], J. Siegwart[1], P. Küng[2], A. Burren[1]
[1]Bern University of Applied Science, Zollikofen, Switzerland
[2]MUUTU AG, Bern, Switzerland

Introduction:
The stable climate is of paramount importance to the respiratory health of horses

Methods:
Four bedding materials (deep straw mattress, daily cleaned straw, dedusted soft wood granulate and bio-compost) were compared with respect to the amount of airborne particular matter (PM2,5 and PM10) at two different heights in a horse box (50 cm and 120 cm from the ground) and to the microbiological air quality of the air as colony forming units per cubic meter, (CFU/m^3) of total bacteria, mold spores, total actinomycetes and the proportion of thermophilic actinomycetes. The bedding materials were tested for 10 days each. Dust was recorded continuously with two SDS011 sensors and microbiological air sampling was performed on days 1, 5 and 10 using an air sampling system (MBASS30v3, Holbach GmbH, Germany). The air temperature and humidity as well as the work in the barn were considered in the statistical analysis (R Core Team 2019, level of significance $p<0.05$).

Results & Discussion:
The differences between the bedding materials, in terms of dust were considered significant ($p<0.001$), except for PM10 between soft wood granulate and bio-compost ($p>0.05$). The soft wood granulate presented the lowest values in terms of total germs (average 3'552 CFU/m^3), total actinomycetes (average 528 CFU/m^3) and the proportion of thermophilic actinomycetes (average 176 CFU/m3). The lowest values in mold spores were found with the deep layer straw litter (average 1'776 CFU/m^3). The highest values were found with bio-compost for all types of germs (average: total germs 21'097 CFU/m3; mold spores 38'860 CFU/m^3; total actinomycetes 4'440 CFU/m^3, the proportion of thermophilic actinomycetes 777 CFU/m^3). Airborne particular matter (PM 2.5 and PM 10) and microbiological air quality were not correlated ($r = 0.01$).

Clinical Relevance:
The type and management of bedding influences microbiological air quality and thus lung health

Poster presentation 55

Association between fungal detection and diagnosis of moderate equine asthma (mea) according to sampling site and methodology

P Barbazanges[1], A Couroucé[1], L.C. Lemonnier[1], G. Le Digarcher[1], J.M. Cardwell[2], M.P. Toquet[3], E.A. Richard[3]
[1]Oniris, Nantes, France
[2]Royal Veterinary College, Hatfield, United Kingdom
[3]LABEO, Saint-Contest, France

Introduction:
Poor agreements were previously described between tracheal wash (TW) and bronchoalveolar lavage fluid (BALF), as well as fungal detection by cytology and mycology culture. The link between moderate equine asthma (mEA) and detection of fungal elements in the airways remains controversial.
Objectives: To determine the prevalence of fungal detection in TW and BALF and its association with diagnosis of mEA.

Methods:
Prospective study on 120 horses in active training or referred for respiratory disease. Horses were classified as "control" or "mEA" based on clinical examination, airway endoscopy and BALF. A sample was considered positive if at least one colony was identified by culture or at least one fungal element was observed on cytology.

Results:
Respectively, 35 and 85 horses were classified as "control" and "mEA". No significant difference was observed between groups for fungal detection by cytology, regardless the sampling site. Prevalence of positive mycology culture was significantly higher for TW (89.4%) and BALF (31.8%) of mEA horses compared to controls (respectively 68,6%and 8.6%). Diagnosis of mEA was significantly associated with positive mycology culture on both TW (OR = 3.9) and BALF (OR = 5.0) Mycology culture on BALF exhibited high specificity (0.90) and high positive predictive value (0.91), unlike mycology culture on TW (respectively 0.76 and 0.31).

Conclusion and clinical importance:
Despite a significant association with asthma diagnosis, the high prevalence of fungal detection in TW of control horses precludes its clinical relevance.
However, positive mycology culture on BALF represents a significant risk-factor of suffering mEA.

Poster presentation 59

Improvement of gastric ulcer and ridden horse pain ethogram scores with diet adaptation in sport horses

V. G. M. PINEAU[1], F. ter Woort[1], F. Julien[2], M. Vernant, S. Lambey, E. Y. van Erck
[1]ESMP, Waterloo, Belgium
[2]Lambey SA, Torpes, France

Introduction:
Gastric ulcers are highly prevalent in sport horses and may lead to poor performance, changes in behaviour and impact horse welfare. We wanted to assess whether sole dietary changes affect gastric health and pain ethogram scores in ridden horses.

Methods:
Nine showjumpers trained at the same stable receiving a pelleted diet high in sugar and starch (>30%) were examined at T0 and after 12 weeks (T12) of changing to a cooked, muesli-type low-starch (11%) diet. At each examination, the horses underwent a filmed standardized exercise test (SET) with the same rider. A ridden pain score (RHpE, out of 24) was calculated by two blinded observers watching the videos. The day after the SET, horses underwent a gastroscopy and ulcers were blindly scored using a proprietary score (out of 11, hyperkeratosis on 3, squamous ulcers on 4 and glandular lesions on 4). No antiulcer medication was administrated, horses were housed on shavings and received free choice hay. Horses were checked monthly for lameness. Results were analysed with Wilcoxon and Spearman tests.

Results:
After 12 weeks of the low starch diet, there was a significant improvement of ulcer scores (4.62.5 at T0 vs 1.01.0 at T12, P=0.006) and of the RhpE scores (6.92.9 at T0 vs 2.92.0 at T12, P=0.009). Total ulcer scores and glandular disease scores were positively correlated with RhpE scores (respectively, r=0.436, P=0.07, and r=0.564, P=0.015). Heart rate and blood lactates measured during SET were not significantly different at T0 and T12.

Conclusion:
There is a positive correlation between ulcer scores and pain scores in ridden horses. A low starch diet significantly reduces the incidence of gastric ulcers and associated pain score during riding in horses.

Clinical relevance:
It is possible to mitigate gastric ulcers and to increase the equine athlete comfort during riding with dietary adjustments.

Poster presentation 60

Serial investigation of seroprevalence and faecal shedding of Lawsonia intracellularis in Thoroughbred foals in the first year of life

C. Ribonnet, L. Palmer, S. Bate, M. Cubitt, C. Bolton, A. Foote, C. Mackenzie, E. Floyd
Rossdales Veterinary Surgeons, Newmarket, United Kingdom

Introduction:
Equine Proliferative Enteropathy is an intestinal disease of young horses caused by *Lawsonia intracellularis*. Seroprevalence is documented in several countries. This is the first UK-based study with the aim to report seroprevalence, timing of seroconversion, frequency of faecal shedding and relationship with clinicopathological findings in Thoroughbred foals during their first year of life.

Methods:
Clinical examination, haematology, biochemistry, serum immunoperoxidase monolayer assay (IPMA) for analysis of antibodies against *L.intracellularis* and faecal PCR were recorded for each foal at 24-48 hours, 3, 6, 9 and 12 months of age. A blood sample was collected from the dams at timepoint 0 for IPMA analysis.

Results:
47 foals from six different farms were enrolled and 37 completed the entire study. At timepoint 0, 77% of mares and 64% of foals were seropositive. There was a linear relationship between mare and foal serum titres ($r=0.75$; $p=<0.001$) and foals with failure of passive transfer had a significantly lower *L.intracellularis* titre ($p=0.0004$; 95% CI 29.5-93.6). 8/37 foals (22%) did not seroconvert during the study period. Peak timing for seroconversion was between 6 – 9 months of age. At 9 months, seropositive foals had significantly lower serum albumin concentrations ($p=0.029$, 95% CI 0.048 - 1.029) than seronegative foals. Only 2 faecal PCR samples tested positive for *L.intracellularis*, and no clinical signs of disease were recognised in any foal.

Discussion:
This study indicates common exposure to *L.intracellularis* in UK Thoroughbred stud farms. Results correlate with the previously described timeframe for bacterial exposure and the highest risk for development of clinical disease. Lower albumin levels in 9-month-old seropositive foals suggests potential presence of subclinical disease.

Clinical Relevance:
This information can help optimise screening policies, targeted treatments and vaccination schemes. Further investigation is needed to evaluate the subclinical effects on foal growth, development and future sales value.

Poster presentation 64

Effects of tight nosebands on the upper airways of horses

D. Scholler[1], A. May[2]
[1]Equine Hospital of Ludwig Maximilians University, Munich, Germany, Oberschleissheim, Germany
[2]Equine Hospital of Ludwig Maximilians University, Munich, Germany

Introduction:
The public perception of animal welfare in equestrian sports depends on training methods and presentation of horses at equestrian events. In this context, the often very tightly buckled nosebands, which are intended to prevent the horse from opening its mouth in response to a hard hand impact, also attracted a lot of attention. Various studies have evaluated the impact of tight nosebands on so-called stress parameters – whereas the situation inside the pharynx has not yet been further looked at. Therefore, the main aim of the study was to evaluate the response of the pharyngeal structures to tight versus loose nosebands using overground endoscopy.

Methods:
In this study, 16 warmblood horses were ridden with loose and tight nosebands while an overground endoscope was inserted. The animal study was approved by the government of Bavaria, Germany (approval AZ ROB-55.2-2532.Vet_02-21-100). For video analysis, five freeze frames each were prepared and analyzed at the beginning of expiration phase at rest after and during maximum exercise. The pharyngeal diameter was measured using a ratio of epiglottic width and a perpendicular line to a fixed point at the dorsal nasopharyngeal wall. Other findings such as swallowing, pharyngeal collapse, soft palate movements and secretion were also evaluated.

Results:
While the pharyngeal-epiglottic-ratio (PE) did not change significantly in horses ridden with loose vs. tight nosebands, there was a significant increase in parameters associated with discomfort in the pharyngeal region, e.g. accumulation of secretions ($p = 0.001$) and pharyngeal collapse ($p = 0.04$) in horses ridden with tight nosebands.

Discussion/Clinical relevance:
The results show that tight nosebands do not only cause stress reactions visible from the outside, but also contribute to discomfort and adverse reactions in the pharyngeal region. These results may provide objective evidence for future decisions of equestrian sports organizations concerning further regulations on nosebands.

Poster presentation 76

Dynamics of training and acute exercise-induced shifts in muscular glucose transporter (GLUT) 4, 8 and 12 expression in locomotion (M. vastus lateralis) versus posture muscles (M. pectoralis profundus) in healthy horses.

C. Vidal de Moreno de Vega[1], D. Lemmens[1], C. Constance[1], B. Boshuizen[2], L. Mare[1], L. Leybaert[3], K. Goethals[1], J.E. Oliveira[4], G. Hosotani[4], D. Deforce[3], F. Nieuwerburgh[3], L. Devisscher[3], C.J.G. Delesalle[1],
[1]Ghent University, Faculty of Veterinary Medicine, Ghent, Belgium
[2]Ghent University, Faculty of Veterinary medicine, Ghent, Belgium
[3]Ghent University, Ghent, Belgium
[4]Cargill, Vilvoorde, Belgium

Introduction:
Important changes in muscle GLUT protein expression are expected if glucose influx plays a pivotal role in fuelling metabolic pathways in response to exercise. Our aim was to assess dynamics of equine muscle GLUT4, GLUT8 and GLUT12 protein expression in response to training and acute exercise.

Methods:
Sixteen untrained Standardbred mares (3-4y) performed an incremental SET at the start and end of 8 weeks harness training. Pectoralis (PM) and vastus lateralis (VL) muscle biopsies were taken before and after each SET, providing rest and acute samples in untrained and trained conditions, using Western blot for GLUT quantification and Image Pro1 for blot analysis. Data were normalized against GAPDH. Data was not normally distributed (Shapiro-Wilk's test) therefore non parametric tests were used. Basal GLUT-levels were analysed with the Wilcoxon matched-pairs. The effect of acute exercise and training on GLUT expression was assessed using the Friedman test with post-hoc Dunn's.

Results:
Basal GLUT4 and GLUT12 protein expression was significantly higher in the VL compared to the PM (P_{GLUT4} = 0.031; P_{GLUT12} = 0.002). Training had no effect on rest GLUT4 expression, neither in the VL, nor the PM (P_{VL} > 0.999; P_{PM} > 0.999). However, acute exercise in trained condition decreased GLUT4 expression in the VL (P = 0.015). No GLUT8 expression changes were recorded. Training decreased GLUT12 in rest VL biopsies (P = 0.036). This decrease was even more prominent in the VL after acute exercise in the trained condition (P_{VL} = 0.003).

Discussion:
The important GLUT12 downregulation, both in answer to training and acute exercise; the GLUT4 downregulation after acute exercise in trained horses; and the lack of GLUT8 changes in any of the studied conditions, question the importance of glucose in equine muscle metabolism.

Clinical Relevance:
These findings encourage to further explore alternative fuel involvement in equine muscular energetics.

Poster presentation 77

NT-proBNP as a potential biomarker for diagnosis, prognosis and monitoring of cardiac disease in horses

M. Demeyere, A. Dufourni, G. Loon, A. Decloedt
Ghent University, Faculty of Veterinary Medicine, Merelbeke, Belgium

Introduction:
N-terminal pro-brain natriuretic peptide (NT-proBNP) is currently the biomarker of choice in human and canine cardiology for diagnosis, prognosis and follow-up of cardiac disease. This preliminary study aimed to investigate whether NT-proBNP can be used as a diagnostic marker for cardiac dilation in horses.

Methods:
Serum samples were collected from 20 healthy horses and 19 horses with left atrial (LA) and/or left ventricular (LV) dilation, aged 14.0±5.2 years. Cardiac dilation was diagnosed when 2D and M-mode echocardiographic measurements exceeded the upper reference range. Horses with cardiac dilation showed a grade 2-5/6 systolic or diastolic heart murmur caused by mitral or aortic valve regurgitation (n=16), or a ventricular septal defect (n=3). Clinical signs of congestive heart failure were present in one horse. Healthy horses did not show a murmur greater than 1/6. Serum was stored at -20°C until analysis. NT-proBNP concentration was determined using the Horse NT-proBNP ELISA kit MBS014699 (MyBiosource, Inc., San Diego, USA).

Results:
NT-proBNP concentrations were significantly higher in horses with cardiac dilation (n=19) compared to healthy horses (n=20) (72.7±31.3 vs. 51.9±23.4 pg/ml, p=0.023). NT-proBNP concentrations were also significantly higher in horses with LV dilation (n=16) compared to horses with cardiac disease without LV dilation and healthy horses (n=23) (75.3±32.3 vs. 52.9±23.2 pg/ml, p=0.016). No significant difference in NT-proBNP concentrations was found between horses with LA dilation (n=15) and horses with cardiac disease without LA dilation and healthy horses (n=24) (68.7±33.9 vs. 57.9±25.6 pg/ml, p=0.267).

Conclusion:
NT-proBNP concentration differed significantly between horses with and without LV dilation. Further research in a larger group of horses with cardiac disease is needed to establish cut-off values for cardiac dilation in order to estimate long-term prognosis.

Clinical relevance:
NT-proBNP might have an added value as diagnostic biomarker in the follow-up of horses with structural heart disease.

Poster presentation 78

Baselining physiological parameters in posture versus locomotion muscles across breeds. Towards tailored dietary and training management.

C. Vidal Moreno de Vega[1], C. Delesalle[1], C. De Meeûs d'Argenteuil[1], B. Boshuizen[1], L. De Maré[1], Y. Gansemans[2], F. Van Nieuwerburgh[2], D. Deforce[2], K. Goethals[1], W. De Spiegelaere[1], L. Leybaert[3], L. Verdegaal[4]
[1]Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
[2]Ghent University, Ghent, Belgium
[3]Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium
[4]Roseworthy Campus, University of Adelaide, South Australia, Australia

Introduction:
Translating the physiological meaning of specific morphophysiological properties of specific muscle groups to their individual metabolic blueprint contributes significantly to a better understanding of the complex adaptations of muscle tissue to stimuli. The aim of the study was to map out and compare the fiber type composition, fiber type and mean fiber cross-sectional area (fCSA, mfCSA) and metabolic blueprint of three muscles in 3 different breeds.

Methods:
Muscle biopsies (m. pectoralis (PM), m. vastus lateralis (VL) and m. semitendinosus (ST)) were harvested of 7 untrained Friesian horses, 12 Standardbred and 4 Warmblood mares. Immunohistochemistry was analyzed using Image Pro software. Untargeted metabolomics was performed on the VL and PM of Friesian and Warmblood horses and the VL of Standardbreds using UHPLC/MS/MS and GC/MS. Overall effect of breed on fiber type percentage and fCSA and mfCSA was tested with Kruskall-Wallis. Breeds were compared two-by-two with Wilcoxon rank-sum test, with Bonferroni correction. Spearman correlation explored the correlation between the breed-metabolites and the morphometric muscle-parameters.

Results:
Standardbreds had a significantly higher proportion of type IIA fibers in the PM and VL ($p=0.0003$) with a bigger mfCSA ($p=0.0017$) in the VL when compared to Friesians. Friesians showed significantly more type IIX fibers ($p=0.0047$) in their PM. No significant differences in fCSA were seen across breeds. The lipid and nucleotide superpathways were significantly more upregulated in Friesians. Standardbreds showed highly active xenobiotic pathways and within the lipid superpathway, long and very long chain acylcarnitine upregulation. Amino acid metabolism was similar, except for increased branched chain amino acid and aromatic amino acid sub-pathways in Friesians. The carbohydrate, amino acid and nucleotide superpathways and carnitine metabolism showed higher activity in Warmbloods.

Discussion:
Results show important muscular metabolic breed differences and specificities.

Clinical relevance:
Results provide an essential basis for formulating breed-specific dietary and training protocols.

Poster presentation 81

Evaluation of the delta neutrophil index (DNI) in equine neonatal sepsis

N. Ellero, F. Lunetta, S. Fasoli, A. Mannini, C. Castagnetti, J. Mariella, F. Dondi, F. Freccero
University of Bologna, Bologna, Italy

Introduction:
Delta neutrophil index (DNI) represents the fraction of circulating immature granulocytes in peripheral blood automatically calculated by ADVIA-series haematology analysers and has been associated with sepsis in human neonates. This study aimed to describe the diagnostic and prognostic potential of DNI in equine neonatal sepsis.

Methods:
All foals undergoing a complete physical and haematochemical evaluation at birth and during the first 5d of life or, in association with blood culture sample collection, at hospital admission were reviewed. One-hundred and sixteen foals less than 5d old were enrolled and divided into: healthy (H group; n=17); septic (S group; n=23), in the presence of both positive blood culture and SIRS; sick-nonseptic (NS group; n=76). DNI was calculated by ADVIA®2120i hematology system as the leukocyte difference between myeloperoxidase channel and nuclear lobularity channel count. Differences in leukocytes, DNI, SAA, outcome and neutrophils changes between groups were analyzed with Mann-Whitney test, while Spearman's correlation coefficient was evaluated among different variables.

Results:
In group H, based on a general linear model, no time-dependent changes in DNI were detected and no differences were found between the three groups. Leukocyte and neutrophil counts were lower ($p<0.001$), while SAA concentration was higher ($p<0.001$) in S group than in NS group. Overall in sick foals, DNI was able to discriminate between survivors (n=69) and non-survivors (n=30; $p=0.01$), was higher in foals with the presence of neutrophils toxic changes on blood smear evaluation than in foals without alterations ($P=0.03$) and was weakly correlated with SAA concentration ($r=0.3$; $P=0.003$).

Discussion:
DNI could anticipate the morphological evaluation of blood smears to detect the left shift, which, together with SAA concentration, is an indicator of inflammation.

Clinical relevance:
DNI deserves further insights for its prognostic potential and for leukogram interpretation in sick foals, which, if done manually, may be operator-dependent and time-consuming.

AUTHORS INDEX

Leroux, Aurélia
France

Leus, Emilie
South Africa

van Loon, Gunther
Belgium

Malandrin, Laurence
France

Marr, Celia
United Kingdom

McGuire, Carly
United Kingdom

Meiseberg, Lia Kristin
Germany

Morgan, Ruth
United Kingdom

Normand, Camille
France

Nosbaum, Audrey
France

Offer, Katie
United Kingdom

Palmisano, Megan
United States of America

Paulussen, Ellen
Belgium

Pimenta, José
Portugal

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France

Pineau, Violaine
Belgium

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United Kingdom

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Switzerland

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United Kingdom

Roscher, Katja A.
Germany

Sage, Sophie
Switzerland

Sanchez, Chris
United States of America

Scholler, Dominik
Germany

Scott, Victoria
United Kingdom

Shnaiderman-Torban, Anat
Isreal

Sillence, Martin
Australia

Timko, Kathryn
United States of America

Touzot-Jourde, Gwenola
France

Waller, Andrew
Sweden

Williams Louie, Elizabeth
United States of America

Wong, David
United States of America

Welcome to the 17th ECEIM Congress 2024

It is a great pleasure to invite you to the ECEIM 2024 congress in Copenhagen, the capital of Denmark.
The main scientific congress is scheduled for November 15th and 16th, following a series of pre-congress workshops held in the evening on the 13th and throughout the day on the 14th of November.
Her Royal Highness, Princess Benedicte, will serve as the patron of the ECEIM 2024 Congress.

Copenhagen has repeatedly been named 'World's most Livable City' and the Danes ranked 'World's Happiest People'.
Copenhagen's 1,000 year history is reflected in buildings, museums, sights and attractions, but also with innovation as one of the hallmarks. Modern design and daring architecture underline the city's progressive approach. The city´s restaurant scene is among the world's most famous and innovative, and it caters to all budgets, tastes, and situations.

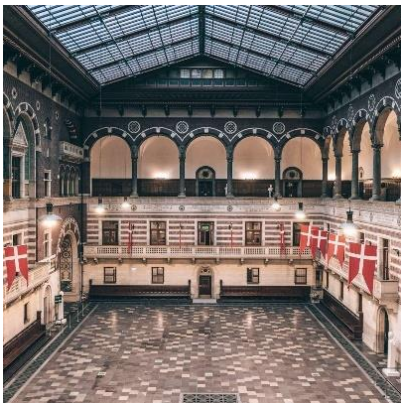
The congress venue is the Frederiksberg campus of the University of Copenhagen, the former site of the Royal Veterinary School. The congress venue is easily accessible by public transport or and a direct metro link connects the venue to the airport. Additionally, the main congress hotel is a mere 5-minute walk from the venue.

The scientific program will feature state-of-the art lectures as well as abstract and poster presentations.
Before the main congress, a series of workshops will be available, catering to various interests, including hands-on clinical training and exploration of subjects like scientific design, among others. These workshops will be hosted both at the large animal university hospital located outside the city and the primary congress venue.

No ECEIM congress would be complete without a vibrant social program, and the 2024 Congress is no exception. It offers abundant opportunities to immerse yourself in the Danish concept of "hygge," delve into the city's rich history and remarkable design, and, for those who are inclined, savor the legendary nightlife.

We hope to see you in Copenhagen next year!

Charlotte Hopster-Iversen
Chair of the 2024 ECEIM Congress Organizing Committee



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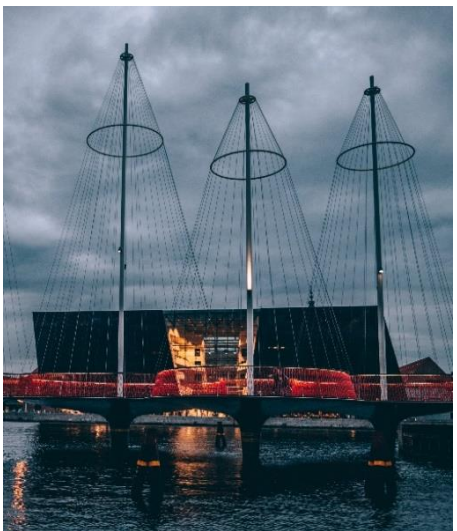

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