Volume 56 | Supplement 60 | September 2024 | ISSN 0425 1644

Equine Veterinary Journal

A journal of the
British Equine Veterinary Association

Abstracts from the 12th
International Equine Infectious
Diseases Conference

View this journal online at
www.wileyonlinelibrary.com/journal/evj
www.beva.org.uk/education/journals

WILEY

BEVA

Abstracts from the 12th International Equine Infectious Diseases Conference 30th September – 4th October 2024

WILEY

BEVA

CONTENTS

Abstracts from the 12th International Equine Infectious Diseases Conference 30th September – 4th October 2024

Volume 56 • Supplement 60 • September 2024

BACTERIOLOGY 5: GENOMICS

Michel D'Ornano Wednesday 09.00–10.30

BACTERIOLOGY 6: CLINICAL

Michel D'Ornano Wednesday 11.00–13.00

BACTERIOLOGY 7: DIAGNOSTICS 1

Lexington Wednesday 15.00–16.00

BACTERIOLOGY 8: DIAGNOSTICS 2

Lexington Wednesday 16.30–17.30

BACTERIOLOGY 9: ANTIMICROBIALS 2

Michel D'Ornano Thursday 11.00–12.00

BACTERIOLOGY 10: DIAGNOSTICS 3

Michel D'Ornano Thursday 12.00–13.00

PARASITOLOGY 1: CESTODES

Lexington Tuesday 11.00–12.00

PARASITOLOGY 2: PROTOZOANS AND TICK BORNE PARASITES

Lexington Tuesday 12.00–13.00

PARASITOLOGY 3: STRONGYLIDS

Lexington Wednesday 11.00–13.00

PARASITOLOGY 4: PIROPLASMOSIS

Lexington Thursday 09.00–10.30

VIROLOGY 1: INFLUENZA

Michel D'Ornano Tuesday 09.00–10.30

VIROLOGY 2: AFRICAN HORSE SICKNESS AND OTHER VIRUSES

Lexington Tuesday 09.00–10.30

VIROLOGY 3: CORONAVIRUS, ROTAVIRUS AND ARTERITIS VIRUS

Michel D'Ornano Tuesday 15.00–17.30

VIROLOGY 4: OTHER VIRUSES

Lexington Wednesday 09.00–10.30

VIROLOGY 5: EPIDEMIOLOGY

Michel D'Ornano Wednesday 15.00–17.30

VIROLOGY 6: DIAGNOSTICS

Michel D'Ornano Thursday 09.00–10.30

VIROLOGY 7: ANTIVIRAL IMMUNOLOGY 1

Michel D'Ornano Thursday 15.00–16.00

VIROLOGY 8: ANTIVIRAL IMMUNOLOGY 2

Michel D'Ornano 16.30–17.30

VIROLOGY 9: HEPATITIS

Michel D'Ornano Friday 09.00–10.30

VIROLOGY 10: SURVEILLANCE

Michel D'Ornano Friday 11.00–12.00

VIROLOGY 11: EQUINE INFECTIOUS ANAEMIA

Michel D'Ornano Friday 12.00–13.00

POSTER PRESENTATIONS

DOI: 10.1111/evj.14215

Equine Veterinary Journal WILEY

Abstracts of the 12th International Equine Infectious Diseases Conference International Conference Centre (CID), Deauville, France 30th September–4th October 2024

Foreword and Acknowledgements

The 12th International Equine Infectious Diseases Conference (IEIDCXII) will be held in the elegant resort of Deauville in Normandie, France from 30th September to 4th October 2024. Originally scheduled to take place in 2020, the SARS Covid-2 pandemic necessitated the transition of IEIDCXI into an on-line format in 2021 and IEIDCXII in Deauville was therefore postponed until 2024. So everyone is very enthusiastic to meet in-person for the first time since IEIDCX in Buenos Aeres, Argentina in 2016.

Perhaps as a consequence of this enthusiasm, >190 scientific abstracts have been submitted to IEIDCXII 2024 from colleagues located around the world. These abstracts are published in this supplement and cover the latest research in the disciplines of Bacteriology, Mycology, Parasitology and Virology. See https://ieidc.org/ for the full programme.

Regardless of global events, equine infectious disease outbreaks continue to occur internationally. Clearly these have negative impacts on equine welfare but also affect people's livelihoods, whether via working equids or sports and pleasure horses or bloodstock. Furthermore with the increasing public awareness of social licence relating to the use of horses for sport or other activities, it is incumbent on the equine infectious disease community to promote and apply the highest health and welfare standards to all equids, regardless of their financial value. Thus the declarations including ethics and owner informed consent which accompanied all abstracts have also been reviewed.

Since 2021, notable events in equine infectious diseases include the launch of a subunit vaccine with DIVA (differentiating infected from vaccinated animals) capability against *Streptococcus equi*, the pathogen responsible for strangles and the confirmation of *Taylorella equigenitatlis* and Contagious Equine Metritis (CEM) in the USA in 2024. This is the first report of CEM in the USA since 2013. In Parasitology, the global occurrence of macrocyclic lactone resistance in Cyathostomin parasites and the first cases of apparent pyrantel and praziquantel resistance in *Anoplocephala perfoliate* are noteworthy. The importance of monitoring horses attending sports horse events was emphasised by the detection and confirmation of a clinical case of piroplasmosis among the horses attending the Olympic games in Tokyo, Japan in 2021. The same year there was a major outbreak of equine herpesvirus myeloencephalopathy (EHM) in Valencia, Spain which tragically resulted in the deaths of several horses. This year, the American College of Veterinary Internal Medicine also released an updated consensus statement on equine herpesvirus-1 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11099706/). Also in Europe, West Nile Virus now appears to be endemic amongst equids in the more northerly parts of Germany. Aside from vector borne pathogens, the widespread international transport of equids also increases the risk of disease outbreaks. For example African Horse Sickness was detected then eradicated in Thailand, returning the country to AHS-free status. Phylogenetic typing indicated a close relationship with isolates from South Africa but the European Union has now agreed to the resumption of direct imports of horses from South Africa. In 2023, several countries in South America experienced outbreaks of Western Equine Encephalitis Virus and Eastern Equine Encephalitis Virus was detected in Ecuador. Thus the importance of ongoing surveillance accompanied by fundamental scientific research cannot be overemphasised. This is achieved through the application of verified and state-of the art diagnostic tests and as well as learning from and characterising events in historical outbreaks and thereby maximise preparedness for future incursions.

The abstracts published in this supplement record many of the current research efforts in the understanding, diagnosis and treatment of equine infectious diseases. Whether you are a basic scientist or practising clinician, there should be something for everyone. In addition, due to the recent hiatus in normal life, for many graduate students and early career researchers, this will be their first abstract presented at an international scientific conference, so we have tried to promote their attendance and presentation of an abstract—please welcome them.

In closing, this conference would not be possible without the generous financial support of our sponsors, so thank you to them all and please visit their stands in the commercial exhibition and their websites. Furthermore, the organisation of IEIDCXII is only possible due to the efforts of many friends and colleagues. I would like to extend my heartfelt thanks to you all, only some of whom can be mentioned here. In particular, thanks must

go to all members of the IEIDCXII International Committee, chaired by Professor James Gilkerson and the Local Organising Committee Co-chaired by Professors Stéphane Pronost and Stéphan Zientara. Their substantial efforts in organising the conference, reviewing abstracts and recruiting generous sponsorship from regional governments and commercial exhibitors is much appreciated. It was a pleasure to work with you all. Hopefully this team effort will result in a memorable IEIDCXII—from scientific, clinical and social points of view. May many new collaborations and friendships result.

All that remains is for me is to wish you a successful conference—I hope you enjoy it!

Dr Julia Kydd
Editor

DOI: 10.1111/evj.14406

THANK YOU TO ALL OUR SPONSORS:

Platinum sponsors:

JRA

RÉGION NORMANDIE

Centaure

Fonds Éperon

Gold sponsor:

Calvados LE DÉPARTEMENT

Silver sponsors:

zoetis

The Hong Kong Jockey Club

ORIENTGENE 东方基因

NEOGEN

IRT Your horse. Our passion.

Boehringer Ingelheim

Bronze sponsors:

EDS Equine Diagnostic Solutions

ID.vet

LABMASTER LUCIA VET www.labmaster.fi

ENALEES Le Nouvel Art du diagnostic

intervacc

KRA Korea Racing Authority

Dechra

IDEXX

HORSERACE BETTING LEVY BOARD HBLB

Fluxergy

Grassroots sponsors:

RESPE

NEH NEWMARKET EQUINE HOSPITAL

anses

LABÉO Pôle d'analyses et de recherche interdépartemental de Normandie

pôle hippolia RECHERCHE ET INNOVATION ÉQUINE

RÉPUBLIQUE FRANÇAISE Liberté Égalité Fraternité

INRAE

Friends sponsors:

EQUIWAYS Biosecurity solutions to outsmart the risks

UNICAEN UNIVERSITÉ CAEN NORMANDIE

NORMANDIE ÉQUINE VALLÉE CAMPUS INTERNATIONAL DU CHEVAL

SOCIÉTÉ DU TROTTEUR FRANÇAIS

FRANCE GALOP

Société Française de Virologie

Accepted: 14 August 2024
DOI: 10.1111/evj.14215

Equine Veterinary Journal WILEY

Oral Presentations

Bacteriology 1: *Rhodococcus equi*, *Salmonella*, *Streptococcus equi*

Michel D'Ornano Tuesday 11.00–12.00

1 | Dirt-bound battle: phage vs. *Rhodococcus equi*

G. O'Reilly[1], K. Muscat[1], C. Giles[2], M. Barton[3], C. Venturini[1,4] and G. Muscatello[1]

[1]*School of Life and Environmental Sciences, University of Sydney, NSW, 2006, Australia;* [2]*Centre for Aquatic Animal Health and Vaccines, Department of Primary Industries, Parks, Water and Environment, TAS, 7249, Australia;* [3]*UniSA Clinical and Health Sciences, University of South Australia, SA, 5000, Australia and* [4]*Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research, NSW, 2145, Australia*

Email: grace.oreilly@sydney.edu.au

Background: Virulent *Rhodococcus equi* is a soil saprophyte known to cause bronchopneumonia in young foals, recognised as an economically significant pathogen of the global equine breeding industry. The lack of a vaccine, emergence of multi-drug resistance and the soil–animal lifestyle of *R. equi* necessitates novel approaches to disease control. The specificity of bacteriophages (phages) offers a promising opportunity to manage *R. equi* burdens in soil.

Objectives: To create phage cocktails and assess their capacity for *R. equi* burden control in soil.

Study design: *In vitro* laboratory and soil intervention study.

Methods: Five phage candidates isolated from faecal and soil samples from Thoroughbred farms were screened against historical and contemporary virulent *R. equi* isolates. Cocktails made up of these phages were inoculated into sterile and natural soil contaminated with virulent *R. equi* to assess the efficacy of the phages over 48 h.

Results: The five phages were collectively able to lyse 20/22 historical (1991–2004) and 17/21 contemporary (2021–2023) virulent isolates, including 7/8 clinical drug-resistant strains. Tri-valent phage treatments in sterile soil against a single *R. equi* host demonstrated up to 7-log suppression of growth. Cocktails were tested in sterile soil with mixed *R. equi* hosts, achieving a 5.8-log suppression of *R. equi* and 3.3-log suppression of the drug-resistant isolates. Although when applied in natural soil the reduction was to a lower magnitude, phages were still able to suppress up to 90% of *R. equi* growth.

Main limitations: Cocktail experiments were conducted in sand-based soil. Small collection of lytic phages.

Conclusions: Collectively, the phages were able to infect and lyse 86% (37/43) of *R. equi* isolates and significantly suppressed the growth of virulent *R. equi* in soil. Phage cocktails may be a viable control strategy for virulent, drug-resistant *R. equi* in soil, potentially reducing the risk of *R. equi* pneumonia.

Ethical animal research: Approved by the Animal Ethics Committee (AEC), University of Sydney (Project number 2018/1319).

Informed consent: Farm managers signed a consent form for the non-invasive collection of soil and faecal samples.

Competing interests: None declared.

Funding: Agrifutures.

2 | Novel small molecules for the control of *Rhodococcus equi* infections in foals

Y. A. Helmy[1]*, B. Lamichhane[1], K. A. Shaaban[2], L. V. Ponomareva[2] and J. Thorson[2]

[1]*Maxwell H.Gluck Equine Research Center, Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky, Lexington, Kentucky, USA and* [2]*Center for Pharmaceutical Research and Innovation, and Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40 536, USA*

Email: yosra.helmy@uky.edu

Background: *Rhodococcus equi* is an equine pathogen causing bronchopneumonia in foals below 6 months of age. The infection is treated using rifampin and macrolides. However, rising antibiotic resistance necessitates the development of alternative therapeutics. Small molecules (SMs) are low molecular weight compounds that can exhibit broad-to-narrow spectrum activities, potentially targeting specific bacterial cellular processes.

Objectives: To screen and identify the novel SMs with high efficacy against *R. equi*.

Study design: *In vitro* experiments

Methods: After screening the SMs' libraries, we assessed the impact of the selected SMs on growth, pre-formed biofilms, and intracellular survival of *R. equi in vitro*. Primarily, 1900 SMs were screened for their effect on growth of *R. equi*. SMs with 100% inhibition were further assessed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The impacts on preformed biofilm and intracellular survivability in murine macrophage (J774A.1) cell lines were also evaluated. Data were analysed using two-way ANOVA for multiple comparison using Tukey analysis.

Results: Ten SMs demonstrating 100% growth inhibition were selected for further evaluation. The selected SMs demonstrated high growth inhibition against multidrug-resistant *R. equi* strains. The MIC and MBC of the SMs were found to be as low as 0.078 μM and 0.156 μM, respectively. Five out of 10 SMs demonstrated 100% inhibition of preformed biofilms at the concentration of 10 μM. Similarly, all the selected SMs significantly inhibited ($p < 0.05$) surviving *R. equi* inside the macrophage cells.

Main limitations: The study only focuses on *in vitro* evaluation and lacks *in vivo* validation. The long-term stability and safety profiles of the identified SMs require further evaluation.

Conclusion: Our study demonstrated that novel SMs can be used as alternatives to antibiotics for controlling *R. equi* infections. In the future, we will continue the evaluation of the SMs *in vitro* as well as test their efficacy on *R. equi* infected foals.

Ethical animal research: Not required: analysis of microorganisms

Informed consent: Not applicable.

Competing interests: None declared.

Funding: American Quarter Horse Foundation; Center for Pharmaceutical Research and Innovation (CPRI, NIH P20 GM130456).

3 | Successional assessment of bacterial counts and histologic findings in organs of pregnant mice after intraperitoneal inoculation with *Salmonella* Abortusequi

D. Kishi, A. Ochi, Y. Kinoshita, H. Niwa and T. Ueno

Equine Research Institute, Japan Racing Association, Tochigi, Japan

Email: daikikishi219@equinst.go.jp

Background: *Salmonella enterica* subsp. *enterica* serovar Abortusequi (*S.* Abortusequi) can cause infectious abortions in equids. Outbreaks of equine abortion have been reported in the past in Europe and Argentina and more recently in Asia. However, the detailed mechanism behind the abortion caused by *S.* Abortusequi remains unclear.

Objectives: To investigate successional histologic changes in organs and bacterial distribution in pregnant mice inoculated with *S.* Abortusequi.

Study design: Experimental challenge study.

Methods: Twenty mice (gestation day 14.5) were inoculated intraperitoneally with *S.* Abortusequi (2×10^3 CFU/mouse). The mice were allocated to four groups ($n = 5$) that were euthanised at 12, 24, 36, or 48 h post-inoculation (hpi). The heart, lung, liver, spleen, kidney, uterus, placenta, and fetus were collected. As a control group, five mice (gestation day 14.5) were inoculated intraperitoneally with sterile saline and euthanised at 48 hpi for sample collection. Bacterial counts in each sample were measured by the dilution plate method, and histopathological and immunohistological analyses were performed.

Results: Intraperitoneal inoculation resulted in appearance of the organisms in the uterus, placenta, and foetus. The number of organisms peaked between 24 and 36 hpi. At 24 hpi, 80% (4/5) of the mice showed neutrophilic infiltration of the endometrium, decidua, and visceral yolk sac. Immunostaining of these same areas with anti-O4-specific antibody revealed the presence of the organisms. In some mice, the amnion and foetus were positive for O4 antigen, indicating the presence of *S.* Abortusequi. No histopathological changes attributable to *S.* Abortusequi inoculation were observed in the other organs.

Main limitation: The infection route was not natural.

Conclusion: Intraperitoneally inoculated *S.* Abortusequi could infect, and cause inflammation in the uterus, decidua, and visceral yolk sac within 24 hpi. Furthermore, these results suggest that *S.* Abortusequi has tissue tropism in the genital tract.

Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute with Accession number 23–22.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Japan Racing Association.

4 | Sorbitol and lactose improve the biofilm production in genetically diverse strains of Argentinian *Streptococcus equi* subsp. *zooepidemicus*

G. Retamar[1], F. Ferretto[1], S. Frosth[2], H. Wilson[3], L. Frykberg[2], B. Guss[2], A. Waller[2,4], A. Muñoz[1], M. Mesplet[1] and C.P. Bustos[1,5]

[1]Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Cátedra de Enfermedades Infecciosas, Argentina; [2]Swedish University of Agricultural Sciences, Sweden; [3]University of Cambridge, United Kindom; [4]Intervacc AB, Sweden and [5]Consejo Nacional de Investigaciones Cientificas y Tecnicas, Argentina

Email: carlabustos@fvet.uba.ar

Background: *Streptococcus equi* subsp. *zooepidemicus* is an opportunistic pathogen of the respiratory and reproductive tracts of healthy horses. *S. zooepidemicus* has the capability to ferment lactose and sorbitol. Biofilm formation is associated with chronic and recurrent diseases, and the ability to form biofilm is also related to surviving in harsh environments. The process of biofilm formation is complex and may be influenced by sugar utilisation.

Objectives: To study the role of lactose, sorbitol and trehalose on biofilm production, as well as to explore the molecular diversity among Argentinian *S. zooepidemicus* strains.

Study design: *In vitro*.

Methods: Nine Argentinian *S. zooepidemicus* strains isolated from both healthy horses and those with disease, were included in the study. Biofilm formation was measured using a microplate assay with crystal violet staining. The culture medium consisted of Todd Hewitt broth supplemented with horse serum or plasma, along with lactose, sorbitol, and/or trehalose. All strains had previously been Illumina sequenced, and the genes associated with sorbitol and lactose metabolism identified. Additionally, *in silico* Multilocus Sequence Typing (MLST) was performed. Wilcoxon test ($p < 0.05$) was used to compare biofilm production.

Results: The highest levels of biofilm production were observed in the presence of plasma supplemented with sorbitol, followed by plasma

with lactose. Trehalose did not influence biofilm formation. Sequence analysis revealed a substantial number of SNPs in genes associated with sugar metabolism, such as *srlM* (sorbitol operon activator protein gene) and *lacR_1* (lactose phosphotransferase system repressor). Furthermore, high molecular diversity was uncovered through *in silico* MLST, identifying eight sequence types: *ST-5*, *ST-211*, *ST-302*, *ST-409*, *ST-410*, *ST-427*, *ST-430*, and *ST-431*.

Main limitation: Absence of gene expression analysis.

Conclusions: Sorbitol and lactose were found to enhance the biofilm formation of *S. zooepidemicus*, and the strains exhibited high molecular diversity.

Ethical animal research: Not required: analysis of microorganisms

Informed consent: Not stated.

Competing interests: A.S. Waller is employed by Intervacc AB.

Funding: Universidad de Buenos Aires (UBACYT 20020190200042BA and UBACyT-20020220400023BA) and Agencia Nacional de Promocion de la Investigación, el Desarrollo Tecnológico y la Innovacion, Argentina (PITC-2018-0242).

Bacteriology 2: Surveillance

Michel D'Ornano Tuesday 12.00–13.00

5 | Equine Veterinary Surveillance Network (EVSNET): Occurrence and resistance patterns in *Escherichia coli* isolated from equine clinical submissions

A.L. Lawson[1], N.J. Williams[1], D. Timofte[1], D.A. Singleton[1] and G.L. Pinchbeck[1]

Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Leahurst Campus, Chester High Road, Neston, CH64 7TE, UK

Email: aprillilylawson@gmail.com

Background: *E. coli* is an excellent indicator for antimicrobial resistance (AMR) surveillance owing to its pathogenicity, public health importance, ubiquity, and adaptable genome (AMR acquisition and spread).

Objectives: To describe the acquired AMR patterns of *E. coli* isolated in horses from UK veterinary diagnostic laboratories (VDLs).

Study design: Observational study.

Methods: Bacterial culture and antimicrobial susceptibility tests were assessed from 6 UK VDLs submitting data to EVSNET between January 2012 and May 2023 (VDLs submission time periods varied). Multidrug resistance (MDR) was defined as acquired non-susceptibility to ≥1 agent in ≥3 classes. Descriptive proportions and confidence intervals were adjusted for clustering within veterinary practice sites. A mixed effect logistic regression model (VLDs random effect) was performed to investigate the odds of MDR *E. coli*. Univariable analysis considered categorical explanatory variables, which were retained for multivariable analysis if $P \leq 0.20$ and a subsequent stepwise backward elimination performed.

Results: In total, 1004 *E.coli* isolates from 963 submissions from skin/hair/wound/abscess ($n = 210$), respiratory ($n = 27$), urogenital ($n = 231$), orthopaedic ($n = 14$), ocular ($n = 4$), unknown ($n = 487$) and other sites ($n = 31$) were examined. MDR was detected in 24.79% (18.88–30.70). Higher prevalence of resistance was observed to potentiated sulphonamides (42.77%, 35.86–49.68), tetracyclines (42.08%, 35.32–48.84), aminoglycosides (33.18%, 27.18–39.17) and extended spectrum penicillins (48.97%, 36.65–61.29), and lower prevalence for cephalosporin (9.57%, 3.88–15.27), amphenicol (17.37%, 10.51–24.23) and fluoroquinolone (15.27%, 10.05–20.48) resistance. The multivariable model identified females had lower odds (OR 0.57, 0.36–0.92), horses ≤2 years had increased odds (OR 4.39, 1.57–12.24), samples submitted in the earlier years (2012–2017) had lower odds (OR 0.54, 0.32–0.91), and urogenital and orthopaedic samples had lower odds (OR 0.41, 0.23–0.72 and OR 0.11, 0.01–0.87, respectively; skin/hair/wound/abscess reference category) for MDR *E. coli*.

Main limitations: Lack of harmonised laboratory methodology.

Conclusions: Acquired AMR is prevalent amongst *E.coli* in horses. AMR surveillance is essential to identify potential threats to animal and public health.

Ethical animal research: Approved by the University of Liverpool Central Ethics RETH001078.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Horse Trust (Registered Charity No: 231748).

6 | *Streptococcus equi* infections in Sweden are preceded by introducing new horses to the premises

B. Lakic[1], A. Ekmann[2], J. Lindahl[1] and G. Gröndahl[1]

[1]*Swedish Veterinary Agency, Uppsala, Sweden and* [2]*District Veterinary Organization, Sweden*

Email: branislav.lakic@sva.se

Background: Strangles, infection with *Streptococcus equi spp equi*, is an important equine infectious disease. It is notifiable in Sweden, with 667 reported outbreaks during 2014–2023.

Objectives: To study risk factors and impact of strangles in Sweden.

Study design: Descriptive.

Methods: Stable managers/owners were contacted for structured interviews to collect data for an investigation of outbreaks of *S. equi* infection in horses reported in Sweden from January 2022 to December 2023.

Results: The 76 interviewed case farms had in total 256 horses with clinical signs of strangles. Fifteen horses, representing 5.9% of all case horses, were euthanised due to complications from the infection according to the owners. Arrival of new horses preceded the strangles outbreak in 75 of 76 case farms, within 2 weeks ($n = 37$), 2–4 weeks ($n = 20$), and/or 4–8 weeks ($n = 18$). In 49 case farms (64.5%) one or more of the new horses were imported. The most common origin was Ireland (in 15 case farms). 27 of the premises (35.5%) did not have any routines for isolation upon arrival of new horses. Some horses were

vaccinated to *S. equi* in 6 case farms (7.8%). Estimates for the total cost of the outbreak per case farm varied from 170 to 111 874 Euros, with a median of 2582 Euros.

Main limitations: Recall bias was mitigated using clear, specific questions, prompt timing, and standardised formats. Estimates on economic loss were based on best guesses of the horse owners.

Conclusions: Almost all Swedish strangles outbreaks were preceded by new horse introductions, a critical risk factor needing careful management. Our study revealed inadequate biosecurity routines and low vaccination rates, despite high costs associated with outbreaks. Enhanced biosecurity and improved vaccination coverage are essential to mitigate this risk.

Ethical animal research: Approved by the juridical department of the Swedish Veterinary Agency.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: Swedish Veterinary Agency.

7 | Aetiological and serological surveillance of equine abortus salmonellosis in equids in China from 2017 to 2022

K. Guo[1*], J.H. Wang[1*], Z.N. Zhang[1], Y. Yang[1], W.G. Zhang[1], X.Y. Chu[1], Y.X. Wang[1], D.Q. Liu[1], W. Guo[1], Z. Hu[1**] and X.J. Wang[1,2**]

[1]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China and* [2]*Institute of Western Agriculture, the Chinese Academy of Agricultural Sciences, Changji, China*

*These authors contribute equally.

Email: guokuiking@163.com

Background: *Salmonella enterica* subsp. *enterica* serovar Abortusequi is commonly responsible for abortion in mares, neonatal septicaemia, and multiple abscesses, orchitis and polyarthritis in equine hosts. The high abortion rate associated with *S.* Abortusequi infection in equids has re-emerged over the past ten years and has caused serious economic losses to China. However, the prevalence of *S.* Abortusequi infection in China has not been comprehensively described.

Objectives: To investigate the prevalence of equine abortion salmonellosis in China.

Study design: Cross-sectional

Methods: A total of 377 clinical samples from sick equines, including tissue, vaginal swab, and joint effusion samples, were collected from 2017 to 2022, and these samples were cultured and evaluated for the presence of *S.* Abortusequi. The isolates were identified by biochemical tests and then serotyped according to the Kauffmann-White-Le Minor scheme. A total of 1584 equine sera from 55 farms in 19 provinces were tested by the tube agglutination test (TAT).

Results: A total of 243 isolates were obtained from 2017 to 2022 and identified as *S.* Abortusequi. All isolates were serotyped and found to be *S.* Abortusequi 4,12:-:e,n,x. A total of 176 (11.1%, 95% CI, 11.06%–11.14%) *S.* Abortusequi-positive sera were detected.

Main limitations: The sensitivity of the TAT is not high, and more sensitive, specific and rapid serological assays are required.

Conclusion: *S.* Abortusequi is an important pathogenic factor causing abortion in equids in China in recent years. Serological findings suggested that the disease is currently found in Heibei, Shandong, Inner Mongolia and Xinjiang provinces. Considering that the disease is spreading, equine practitioners and breeders should be aware of the risks and take appropriate preventive measures to reduce the risk of *S.* Abortusequi infection in equids.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (approval no. 210519–03).

Informed consent: Not stated.

Competing interests: None declared.

Funding: Heilongjiang Provincial Natural Science Foundation of China (TD2022C006), and the National Key Research and Development Program of China (No. 2021YFD1800500).

8 | Reining in strangles: absence of disease in horses vaccinated with a DIVA compatible recombinant fusion protein vaccine following natural exposure to *Streptococcus equi* subspecies *equi*

G. Gröndahl[1*], F. Righetti[2], A. Aspán[1], J. Bjerketorp[3], S. Frosth[3], L. Frykberg[3], K. Jacobsson[3], B. Guss[3], J.-I. Flock[2], B. Henriques-Normark[2,4] and A.S. Waller[5]

[1]*Department of Animal Health and Microbial Strategies, Swedish Veterinary Agency, Uppsala, Sweden;* [2]*Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solna, Sweden;* [3]*Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [4]*Clinical Microbiology, Karolinska University Hospital Solna, Stockholm, Sweden and* [5]*Intervacc AB, Stockholm, Sweden*

Email: gittan.grondahl@sva.se

Background: Strangles, caused by *Streptococcus equi* (*S. equi*), is a prevalent infectious disease of horses. This is the first report on protection conferred by a new vaccine[1] against natural infection.

Objectives: To measure the effects of vaccination in the face of an outbreak of strangles at a Swedish farm.

Study design: Retrospective cohort study.

Methods: Healthy horses ($n = 17$) were vaccinated on day 0, which was 23 days after the first confirmation of strangles in three unvaccinated horses on the same farm. As part of clinical management during the outbreak, blood serum samples were collected on day 0 ($n = 17$) and day 28 ($n = 19$). A combined iELISA was used to measure total antibody titres towards vaccine antigens. Specific antibody levels in sera to *S. equi* were also quantified using the antigen A and antigen C iELISAs, which can differentiate infected from vaccinated animals (so-called DIVA). Clinical signs were monitored.

Results: All vaccinated horses showed an increased total antibody titre to vaccine components from day 0 (2.50 ± 0.28) to day 28 (3.63 ± 0.31); $p < 0$.

001, students t-test). Seropositivity in the antigen A/C iELISA was noted in 5/17 healthy horses on day 0 and 8/16 vaccinates on day 28, and in 3/3 unvaccinated clinical cases on day 28. Two mild adverse reactions for 1–2 days post-vaccination were noted, including fever ($n = 1$) and injection site swelling ($n = 1$). None of the vaccinated horses developed strangles.

Main limitations: Aside from the three unvaccinated clinical cases, no unvaccinated control group was available.

Conclusions: Despite adherence to recommended biosecurity measures, serological evidence of exposure to *S. equi* was demonstrated in half of the vaccinated horses. Notably, all vaccinated horses responded to the vaccine components and remained healthy, supporting the protective effects of vaccination of horses with Strangvac[1] in the face of natural *S. equi* exposure.

Key Manufacturer: 1. Strangvac, Intervacc https://intervacc.se/en/research/pipeline/strangvac/.

Ethical animal research: Not required: retrospective case series.

Informed consent: Not stated.

Competing interests: A Waller is CSO at Intervacc.

Funding: Intervacc.

Bacteriology 3: Antimicrobials 1

Lexington Tuesday 15.00–16.00

9 | Identification and antimicrobial susceptibility of bacterial isolates obtained from Thoroughbred horses in Hokkaido, Japan, with endometritis

H. Niwa[1], Y. Kinoshita[1], Y. Sawa[2], S. Fujii[2], D. Miyakoshi[3], M. Sato[3] and T. Ueno[1]

[1]*Equine Research Institute, Japan Racing Association, Tochigi, Japan;* [2]*Hokkaido Agricultural Mutual Aid Association, Hokkaido, Japan and* [3]*Hokkaido Hidaka Livestock Hygiene Service Center, Hokkaido, Japan.*

Email: niwa@equinst.go.jp

Background: Infectious endometritis is an important and major cause of infertility in Thoroughbred horses. It is usually treated with antimicrobial agents. Selection of adequate antimicrobials is important for early recovery, especially because the Thoroughbred breeding season is limited.

Objectives: To clarify the antimicrobial susceptibility of recent endometritis-causing bacteria.

Study design: In vitro.

Methods: Two hundred and four bacterial isolates obtained from 174 endometritis cases in Hokkaido, Japan were used. These isolates were identified by MALDI-TOF MS. Minimum inhibitory concentrations of ampicillin (AMP), cephalothin, enrofloxacin (*Escherichia coli* only), doxycycline (DOX), gentamicin, penicillin (PEN), and sulfamethoxazole–trimethoprim were measured by Etest (bioMérieux). Susceptibilities were determined according to Clinical and Laboratory Standards Institute criteria when available. PCR was used to detect tetracycline-resistance genes and extended-spectrum ß-lactamase (ESBL) genes.

Results: The isolates were classified into 24 bacterial species. *Streptococcus equi* subsp. *zooepidemicus* (122/204) and *E. coli* (32/204) were the most common isolates, followed by *Klebsiella aerogenes* (9/204). Approximately 40% of *S. zooepidemicus* isolates were resistant to DOX. Highly DOX-resistant isolates had the tetracycline resistance gene *tet(O)* or *tet(W)*. All *E. coli* isolates were resistant to AMP, PEN, and DOX. Three *E. coli* isolates had the ESBL gene and tended to be resistant to other agents. *Klebsiella aerogenes* isolates differed in their susceptibilities. No isolates of the remaining species showed multidrug resistance.

Main limitation: No data on the clinical outcomes of the horses were available.

Conclusion: Although antimicrobial resistance among endometritis-causing bacteria overall has not progressed, a small number of multidrug-resistant organisms, such as ESBL-producing *E. coli*, were observed. Continuous surveillance of antimicrobial susceptibility and prudent use of antimicrobials are necessary for preventing the dissemination of these bacteria in this region.

Ethical animal research: Not required: analysis of microorganisms

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Japan Racing Association.

10 | Clinical audit on antimicrobial stewardship effectiveness to reduce antimicrobial resistance bacteria colonisation rates in an equine veterinary teaching hospital

K. Mazzola[1], A. Lanci[1], R. Scarpellini[1], S. Piva[1], L. Pusinich[1], J. Mariella[1], F. Freccero[1], M. Farnè[2], V. Schisa[2], E. De Carolis[2] and C. Castagnetti[1]

[1]*Department of Veterinary Medical Sciences, University of Bologna, Italy and* [2]*Department of Statistical Sciences, University of Bologna, Italy*

Email: carolina.castagnetti@unibo.it

Background: Antimicrobial stewardship (AMS) is mainly focused on rational antimicrobial use (AMU) and biosecurity measures to reduce antimicrobial resistance (AMR) [1].

Objectives: To perform clinical audits to evaluate the effectiveness of an AMS programme on reducing AMR bacteria colonisation rates in an Equine Veterinary Teaching Hospital.

Study design: Clinical audit.

Methods: From January to July 2022, the first audit cycle was carried out and data collected to assess AMR bacteria colonisation rates and related risk factors in foals, mares and other hospitalised adults. Then, based on A1 results and AMS principles, previous AMU and biosecurity policies were reviewed and implemented. From January to July 2023, the second audit cycle was conducted to check the effectiveness of the changes adopted. From each horse, rectal and nasal swabs were collected at admission/birth and at discharge/death and processed for bacteriological culture on selective media for AMR bacteria.

Rectal swabs were cultured for third generation Cephalosporins-resistant Enterobacterales (3GCR-E) and for Carbapenems-resistant Enterobacterales (CR-E), while nasal swabs were cultured for Methicillin-Resistant Staphylococci (MRS). All the isolates were identified by MALDI-TOF. A logistic model was employed to investigate the effect of the year of hospitalisation on the colonisation rate and to evaluate risk factors.

Results: 328 horses were included, and 1096 samples collected. In 2023, a significant ($p < 0.05$) reduction in the odds was observed for AMR bacteria (61%), MRS (63%) and 3GCR-E (47%) colonisation rates in the full sample set, whereas for MRS in foals ($p < 0.05$, 70%–73%) and for 3GCR-E (87%) and AMR bacteria (89%) in mares hospitalised after parturition ($p < 0.001$). The main risk factors for AMR bacteria colonisation in general was being a foal, whereas in foals it was amikacin treatment duration and in mares, dystocia.

Main limitations: Isolated bacteria were not genotyped.

Conclusions: The AMS programme was effective for AMR bacteria colonisation rate reduction in hospitalised horses.

Ethical animal research: The study protocol was approved the University of Bologna Animal Care and Use Committee (Approval number, 302315; Approval date, 10/11/2022).

Informed consent: Not required: clinical audit

Competing interests: None declared.

Funding: None.

Reference:

[1] Prescott JF. Outpacing the resistance tsunami: antimicrobial stewardship in equine medicine, an overview. Equine Vet Educ 2021;33:539–545.

11 | Antibiotics in strangles?

J. Pringle

Department of Clinical Sciences, Swedish University of Agricultural Sciences, 750 07, Uppsala, Sweden

Email. john.pringle@slu.se

Background: Antibiotics are often unnecessary in clinical strangles,[1] and overuse may promote development of antimicrobial resistance.

Objectives: Review the reported antibiotic use in strangles in relation to current consensus guidelines and contrast results in relation to sensitivity testing of *S. equi* to penicillin.

Study design: Evidence review.

Methods: An electronic search of the literature over the past 50 years which included natural outbreaks of strangles and data on morbidity, mortality and antibiotic use was conducted. Descriptive statistics collected regarding animal numbers, morbidity, proportion of animals treated with antibiotics, as well as indications for antibiotic use, mortality, and principle antibiotic used. Results were compared to recent reports on testing for antibiotic sensitivity of *S. equi*.[2,3]

Results: Sixteen reports were identified, including outbreaks of 20 to 2173 equids (median $n = 58$). Most reports had similar age groups. Morbidity ranged from 13% to 100% (median 53%) with clear differences in age related morbidity. Seven studies (4 of which had no antibiotic use) had no mortalities. In studies with mortalities, the proportion of animals treated with antibiotics varied from 25% to100%; with case-based mortality at 1%–10%, (mean 4.8%). Most reports lacked pertinent clinical data to facilitate comparison of antibiotic treatment in relation to clinical severity and complications such as mortality. Penicillin was the most commonly used antibiotic. Despite one outlying report[2] penicillin remains the drug of choice [3] for treatment of infection by *S. equi*.

Main limitations: Most reports lacked data regarding the clinical threshold for antibiotic use.

Conclusions: In contrast to clinical evidence of recovery from strangles without antibiotics, they continue to be used in many outbreaks. Penicillin remains the antibiotic of choice.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: FORMAS (Swedish Research Council for Sustainable Development).

References:

[1] Boyle AG, Timoney JF, Newton JR, Hines, MT, Waller AS, Buchanan BR. Streptococcus equi infections in horses: Guidelines for treatment, control, and prevention of strangles – revised consensus statement. J Vet Intern Med 2018;32(2):633–647.

[2] Fonseca JD, Mavrides DE, Morgan AL, Na JG, Graham PA, McHugh TD. Antiiotic resistance in bacteria associated with equine respiratory disease in the United Kingdom. Vet Rec 2020;187(5):189.

[3] Johns IC, Adams E-L. Trends in antimicrobial resistance in equine bacterial isolates: 1999–2012. Vet Rec 2015;28;176(13):334.

12 | Update on macrolide (multidrug) resistant *Rhodococcus equi*

J.A. Vazquez-Boland, J. Val-Calvo, M. Scortti, and the International MDR *R. equi* Surveillance Consortium

Microbial Pathogenesis Laboratory, College of Medicine and Veterinary Medicine, Edinburgh BioQuarter IRR bldg. South, University of Edinburgh, Edinburgh EH16 4UU, UK

Email: v.boland@ed.ac.uk

Background: Since 1987 treatment of *Rhodococcus equi* infection in foals has relied on a combination of rifampicin and a macrolide (erythromycin, later azithromycin or clarithromycin). Dual macrolide-rifampicin resistant *R. equi* began to be detected in the late 1990s on horse farms in the USA linked to mass antibiotic prophylaxis.[1] Work in our laboratory determined that the emerging macrolide-rifampicin resistant *R. equi* was a clonal population (designated MDR-RE 2287) carrying a conjugative resistance plasmid, pRErm46, and a unique *rpoB*S531F mutation. pRErm46 harbours *erm*(46) encoding MLS_B resistance in a transposable element, Tn*RErm46*, plus a class I integron conferring resistance to streptomycin, spectinomycin, sulfonamides and tetracycline/doxycycline.[2]

Objectives: To monitor the potential spread of MDR-RE across and between horse breeding countries.

Study design: genomic surveillance

Method: An International MDR *R. equi* Surveillance Consortium involving laboratories in different continents has been established. Harmonised PCR-based tests allow the specific detection of MDR-RE with the University of Edinburgh ensuring reference laboratory support. Whole genome sequences are subjected to detailed phylogenomic and microevolution marker analysis.

Results: MDR-RE likely emerged in 1993 and has been spreading across horse breeding farms in the USA. In 2016 it was identified in Ireland,[3] and the same genomic profile again in 2021 and 2023 suggesting local circulation. Recent genomic analyses indicate its presence in Asia.

Main limitations: The study is not systematic and therefore is likely to underestimate the true scale of MDR-RE's international spread.

Conclusions: MDR-RE renders the only clinically proven antimicrobial therapy for clinical disease associated with *R equi* infection in foals largely ineffective, complicating the clinical management of this difficult-to-treat equine infectious disease. MDR-RE also poses a public health risk due to its potential zoonotic transmission. MDR-RE can become globally disseminated with horse travel unless active international surveillance and measures to prevent its spread are not implemented.

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Horserace Betting Levy Board.

Acknowledgements: We gratefully acknowledge HBLB, the Irish Equine Centre, all of our international collaborators, and Richard Newton and the Equine Infectious Disease Surveillance team.

References:

[1] Anastasi E, Giguère S, Berghaus LJ, Hondalus, Willingham-Lane JM, MacArthur I, Cohen ND, Roberts MC, Vazquez-Boland JA. Novel transferable *erm*(46) determinant responsible for emerging macrolide resistance in *Rhodococcus equi*. J Antimicrob Chemother 2015;70:3184–3190.

[2] Alvarez-Narváez S, Giguère S, Anastasi E, Hearn J, Scortti M, Vázquez-Boland JA. Clonal confinement of a highly mobile resistance element driven by combination therapy in *Rhodococcus equi*. MBio 2019;10:10–1128.

[3] Val-Calvo J, Darcy J, Gibbons J, Creighton A, Egan C, Buckley T, Schmalenberger A, Fogarty U, Scortti M, Vazquez-Boland JA. International spread of multidrug-resistant *Rhodococcus equi*. Emerg Infect Dis 2022;28:1899.

Bacteriology 4: Vaccines

Lexington Tuesday 16.30–17.30

13 | An alternative vaccination regime against strangles in sport horses

U.O. Hedenström

Wången AB in association with Inst. Equine studies at SLU, Uppsala, Sweden

Email: ulf.hedenstrom@wangen.se

Background: A new strangles vaccine[1] has reached the market, but is not yet fully implemented by itself or in conjunction with existing vaccination regimes for sport horses. Studies suggest a possible efficient regime with less frequent vaccinations.[1]

Objectives: To determine if standard protocols for influenza vaccination are applicable for Strangvac[1] to aid effective synchronous vaccination against strangles.

Study design: Descriptive clinical.

Methods: First (V1) and second (V2) vaccinations were administered by intramuscular injection into the neck at a 6-week interval to 35 adult sport horses, 10 of which received a combination of vaccines against influenza, tetanus and strangles. A third vaccination (V3) against strangles was administered after 5 or 12 months (regime one ($n = 20$) or two ($n = 15$), respectively). Horses were monitored closely for increased body temperature, the injection site was clipped and horses were checked for signs of swelling, stiffness of the neck and decreased appetite. Blood samples were collected to assess the routine vaccination programme immediately before and 14 days after V3 to quantify antibody responses by ELISA.

Results: 10% of injection sites showed a local swelling. A temperature rise of $\sim$0.8°C occurred post-vaccination in most horses for 1 day. Health status returned to normal within 48 h. The 21 horses on regime one had a mean ELISA reading (reflective of antibody titres) of 3.34 ± 0.31 against strangles at 5 months after V2, which increased significantly to 3.9 ± 0.29 at 14 days post-V3 ($p < 0.001$, students *t*-test).

Main limitations: A matched unvaccinated control group were unavailable. The antibody titre required for protection against strangles is currently unknown.

Conclusions: A normal vaccination program against influenza was equally efficient for strangles vaccination. A Scandinavian minimum protocol is being evaluated this spring. Negative side effects were as expected or less than stated in the SPC.

Key Manufacturer: 1. Strangvac, Intervacc https://intervacc.se/en/research/pipeline/strangvac/

Ethical animal research: Approved by Umeå djurförsöksetiska nämnd (A4-2022)

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: None.

Reference:

[1] Robinson, C, Waller, AS, Fryberg, L, Flock, M, Zachrisson, O, Guss, B, Flack, J. Intramuscular vaccination with Strangvac is safe and induces protection against equine strangles caused by *Streptococcus equi*. Vaccine, 2020; 38 (31) 4861–4864. https://www.sciencedirect.com/science/article/pii/S0264410X20306915

14 | Effects of *Streptococcus equi equi* status on the upper respiratory bacterial microbiota of horses

A.G. Boyle, K. Narayan, N. Indugu, R. Kashyap, T. Webb, T. Schaer, J. Woodrow, E. Nelson and D. Pitta

Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Pennsylvania, USA
Email: boylea@vet.upenn.edu

Background: Exploring the guttural pouch bacterial microbiota during *Streptococcus equi equi* (*S. equi*) infection may determine indicators that can lead to biofilm formation.
Objectives: To assess the effects of *S. equi* status on the equine upper respiratory microbiota.
Study design: Prospective observational clinical study.
Methods: 10 healthy university-owned (*S. equi* PCR negative), 9 strangles convalescent client-owned (*S. equi* PCR negative), and 9 strangles convalescent client-owned (*S. equi* PCR positive) horses were enrolled. Oral wash (OW), nasopharyngeal lavage (NPL), and guttural pouch lavage (GPL) samples were collected and processed for genomic DNA extraction. PCR amplification and Illumina sequencing was performed followed by data analysis using QIIME2 pipelines.
Results: Observed species and Shannon diversity metrics showed significant differences between OW, NPL and GPL samples ($P = 0.001$). GPL had much higher diversity compared to OW and NPL ($P = 0.005$). The extent of interaction between commonly present bacterial populations (beta diversity) resulted in a separation by sample type ($P = 0.001$) and a difference between *S. equi* positive and negative samples ($P = 0.05$). Firmicutes was the most abundant phylum across all sample types. Proteobacteria were reduced in GPL *S. equi* positive samples. *S. equi* negative OW and NPL samples had more *Gemellaceae* whereas *Streptococcus* was proportionally increased in *S. equi* positive GPL samples.
Main limitations: Low sample size.
Conclusions: While genera such as *Gemellaceae* are commonly shared between the upper respiratory tract microbiota, the microbiome associated with the guttural pouch appear distinct with a rich diversity of *Streptococcus* related genera. *S. equi* positive guttural pouch microbiome differed from negative horses.
Ethical animal research: Approved by the University of Pennsylvania Institutional Animal Care and Use Committee Protocols: #805825 and POAP (Privately Owned Animal Protocol) #807073.
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: None declared.
Funding: Boehringer Ingelheim Advancement in Equine Research Award.

15 | Serological responses of horses in response to vaccination in a farm associated with a natural outbreak of strangles

E. Rask[1]*, F. Righetti[2], A. Ruiz[3], J. Bjerketorp[4], S. Frosth[4], L. Frykberg[4], K. Jacobsson[4], B. Guss[4], J.-I. Flock[2], B. Henriques-Normark[2,5], E. Hartman[6], A. Gustafsson[6] and A.S. Waller[6]
[1]*Veterinär Erika Rask AB, Kungälv, Sweden; 2Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solna, Sweden;* [3]*Mybac, Stockholm, Sweden;* [4]*Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [5]*Clinical Microbiology, Karolinska University Hospital Solna, Stockholm, Sweden and* [6]*Intervacc AB, Stockholm, Sweden*
Email: erikarask@icloud.com

Background: Exposure to *Streptococcus equi*, the cause of strangles in horses, generates antibody responses to SEQ_2190 (antigen A) and SeM (antigen C), which can be differentiated from antibody responses measured post-vaccination with Strangvac.
Objectives: To measure antibody levels in response to vaccination and exposure to *S. equi* at a Swedish farm.
Study design: Retrospective cohort study.
Methods: Thirteen of 18 healthy horses at a farm in Sweden received their first vaccination[1] on 09/12/23, which was 11 days before the onset of strangles in one of the vaccinated horses. Vaccination was then employed to control the transmission of *S. equi* through the population. Blood samples were collected from all horses on 11/02/24 and 12/04/24 for clinical purposes. Total antibody titres towards vaccine antigens were compared with an iELISA using a single 1:10,000 dilution. Exposure to *S. equi* was determined using the antigen A/C iELISA. Clinical signs were monitored.
Results: One vaccinated horse (#1) developed a cough and nasal discharge from 11 days after first vaccination, which subsequently tested positive for EHV-4 and *S. equi*. Two other horses developed fever for one day at 22 and 23 days post-first vaccination. Second vaccinations were administered from 04/01/24. No further clinical signs of strangles were observed. All vaccinated horses had high antibody titres to vaccine components, which corresponded with those measured in the single dilution assay. Six and seven of the 18 horses were seropositive for exposure to *S. equi* on 11/02/24 and 12/04/24, respectively.
Main limitations: Only blood samples from two horses were collected prior to first vaccination.
Conclusions: The single dilution iELISA is an effective assay with which to measure responses to Strangvac vaccination. Whilst three of 18 vaccinated horses developed mild signs of strangles after first vaccination, serological data support the effectiveness of this vaccine for the prevention of strangles.
Key Manufacturer: 1 Strangvac, Intervacc https://intervacc.se/en/research/pipeline/strangvac/.
Ethical animal research: Not required: retrospective case series.
Informed consent: Explicit owner informed consent not stated.
Competing interests: A. Waller, E. Hartman and A. Gustafsson are employed by Intervacc. A. Ruiz is employed by Mybac. F. Righetti, J. Bjerketorp, S. Frosth, L. Frykberg, K. Jacobsson, B. Guss and B. Henriques-Normark are supported by Intervacc. J-I. Flock is on the board of Intervacc.
Funding: Intervacc.

16 | Investigation of a natural episode of *Streptococcus zooepidemicus* respiratory disease and the potential for cross-protection with a vaccine against *Streptococcus equi* subspecies *equi*

F. Whitlock[1], J.R. Newton*[1], S. Frosth[2] and A. Waller[3]

[1]University of Cambridge School of the Biological Sciences, Department of Veterinary Medicine, Cambridge, UK; [2]Swedish University of Agricultural Science, Faculty of Veterinary Medicine and Animal Science, Department of Biomedical Sciences and Veterinary Public Health, Uppsala 750 07, Sweden and [3]Intervacc AB—Research, Stockholm S-129 44, Sweden
Email: fw371@cam.ac.uk

Background: *Streptococcus equi* subspecies *zooepidemicus* is a highly diverse opportunistic pathogen of horses, associated with respiratory disease and endometritis.
Objectives: To identify *S. zooepidemicus* isolates recovered from young ponies during a natural episode of respiratory disease, and to determine if vaccination with Strangvac,[1] a vaccine against *Streptococcus equi* subspecies *equi*, conferred cross-protection.
Study design: Retrospective clinical study.
Methods: Thirty-two research ponies in a double-blinded placebo-controlled experiment (16 vaccinated[1] and 16 given an adjuvant-only placebo) experienced a natural episode of respiratory disease. Ponies were monitored daily for clinical respiratory signs with nasopharyngeal swabs taken from affected animals to identify equine pathogens by qPCR and culture. The genomes of 23 *S. zooepidemicus* isolates were sequenced. Clinical differences between vaccine and placebo groups were assessed using non-parametric rank-sum methods.
Results: None of 15 clinical samples were qPCR positive for equine influenza virus or equine herpes virus-1, 1/15 (7%) was qPCR positive for EHV-4, 13/15 (87%) were qPCR positive for *S. zooepidemicus* and 18/18 (100%) were culture positive for *S. zooepidemicus*. Seven different *S. zooepidemicus* sequence types (ST-43, ST-49, ST-103, ST-118, ST-300, ST-366 and ST-418) were identified, encoding between four and six Strangvac vaccine antigens, with 71%–100% amino acid identity. The median total cough score for the vaccinated group was 1 and 3 for the placebo group (rank-sum $P = 0.066$). The total number of days coughing was 20 for the vaccinated group and 50 for the placebo group (rank-sum $P = 0.06$). Vaccinated ponies experienced a total of two days with a marked cough, which was significantly fewer than the 13 days of marked coughing in the placebo group (rank-sum $P = 0.03$).
Main limitations: Small group size.
Conclusions: This study provides preliminary evidence in support of a cross-protective effect of Strangvac for the reduction of clinical signs associated with natural infection with *S. zooepidemicus*.
Key Manufacturer: 1 Strangvac, Intervacc. https://intervacc.se/en/research/pipeline/strangvac/.
Ethical animal research: The study was licensed by the UK Home Office and approved by the AHT's AWERB Committee.
Informed consent: Not applicable.
Competing interests: Dr Waller was contracted by Intervacc AB to conduct the work at the Animal Health Trust (AHT), UK and is currently employed by Intervacc AB.
Funding: Intervacc AB.

Bacteriology 5: Genomics

Michel D'Ornano Wednesday 09.00–10.30

17 | Genetic characterisation of *Klebsiella aerogenes* isolated from horses

J. Gibbons and C. Egan
Irish Equine Centre, Johnstown, Naas, Kildare 291 RH93, Ireland
Email: jgibbons@irishequinecentre.ie

Background: *Klebsiella aerogenes* is isolated frequently from samples of reproductive origin in horses but little is known about the population structure, antimicrobial resistance or virulence status of this bacterium or its association with uterine infection.
Objectives: The aims of this study were to characterise a collection of *K. aerogenes* via whole genome sequencing (WGS) and bioinformatic analysis and to determine its association with cytological evidence of uterine infection.
Study design: Descriptive study.
Methods: All *K. aerogenes* isolated from diagnostic samples in the period 2019–2023 and associated data (submitting vet, sample type, other bacteria isolated, and cytology results) were identified. Whole genome seuqencing and bioinformatic analysis was carried out on a subset of isolates to identify population structure, antimicrobial resistance and virulence genes.
Results: 155 samples of reproductive origin were *K. aerogenes*-positive; in 32 (20.65%) of these samples, *K. aerogenes* was isolated in pure culture. Eighty-four *K. aerogenes*-positive samples had cytology results available; 38 (45.34%) were positive for the presence of neutrophils. Twenty-five isolates were sequenced and 24 were typed as Sequence Type (ST)93 or related variants. Two distinct clusters of *K. aerogenes* were identified on phylogenetic analysis; there was no evidence of clustering by sample type, time, veterinary practice or virulence score. Tetracycline, aminoglycoside and sulphonamide resistance genes were identified most frequently; no ESBL, carbapenamase or colistin resistance genes were detected. Genes for yersinibactin and colibactin production were detected in 18 and 13 genomes respectively. Genes associated with aerobactin production and hypermucoidy were not detected.
Main limitations: The number of samples with cytology results available was small and a small number of isolates were available for WGS.
Conclusions: *K. aerogenes* can act as an opportunistic pathogen in the equine reproductive system with 2 dominant clones in circulation. However, equine *K. aerogenes* appears to lack the antimicrobial resistance and virulence genes which have made this an important pathogen in human medicine.
Ethical animal research: Not required: retrospective analysis of laboratory data.
Informed consent: Not applicable.
Competing interests: None declared.
Funding: Department of Agriculture, Food and the Marine Equine technical and Breeding scheme 2022.

18 | Genetic diversity and capsule locus typing of *Klebsiella pneumoniae* isolates from the equine reproductive tract

A. Kawarizadeh[1,2], K. Bailey[1,4], L. Hardefeldt[1,4], K. Mitchell[3], M. Marenda[2], C. Chicken[3], A. Blishen[3], R. Bushell[2] and J. Gilkerson[1,4]
[1]*The Centre for Equine Infectious Disease, Melbourne Veterinary School, Faculty of Science, University of Melbourne, Parkville, VIC 3010, Australia;* [2]*Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, University of Melbourne, Parkville, VIC 3010, Australia;* [3]*Scone Equine Hospital, Scone, NSW 2337, Australia and* [4]*National Centre for Antimicrobial Stewardship, Peter Doherty Institute for Infection and Immunity, Melbourne, Australia*
Email: a.kawarizadeh@unimelb.edu.au

Background: *Klebsiella pneumoniae* is a capsule forming Gram negative bacterium with great potential for acquiring antimicrobial resistance (AMR) genes and is associated with equine reproductive disease. The polysaccharide capsule is one of the most important virulence factors located on the surface of this bacterium.

Objectives: To study genetic diversity, molecular identification and capsule locus typing of *K. pneumoniae* isolates from the equine reproductive tract.

Study design: Genomic surveillance

Methods: Reproductive samples collected from Thoroughbred horses in Australia, were cultured between 2020 and 2022 (inclusive) as part of a routine surveillance programme. Colony morphology, biochemical tests, and negative motility tests were used to presumptively identify *K. pneumoniae* isolates. The genomes of the isolates were sequenced and assembled to confirm the taxonomic identification using FastANI, and to determine the capsule locus types using Kaptive 2.0. The assembled genomes were also used for phylogenetic analysis.

Results: Of the 117 isolates (mare: 99, stallion: 18) initially identified as *K. pneumoniae*, genomic analysis revealed 14 misclassifications (seven *K. variicola*, four *K. quasipneumoniae*, one *Raoultella terrigena*, one *Raoultella planticola*, and one *Escherichia coli*). Twenty-four capsule locus types were identified, and preliminary phylogenetic analysis suggests that 73 out of 103 isolates can be grouped into seven main clades showing significant genetic diversity.

Main limitations: Available *K. pneumoniae* assemblies of equine sources in the GenBank were limited in number and geographical area.

Conclusion: This study identified a diverse population of *K. pneumoniae* in the equine reproductive tract, with a significant proportion being misclassified using conventional biochemical methods. This finding highlights the importance of accurate identification and characterization of *K. pneumoniae* for effective disease control and management.

Ethical animal research: Not required: analysis of microorganisms

Informed consent: Consent for research was obtained

Competing interests: None declared.

Funding: Centre for Equine Infectious Disease.

19 | *Streptococcus equi* whole genome sequencing data shed new light on endemic persistence of strangles in the United Kingdom

A.A. McGlennon[1,2], A.S. Waller[3], J.R. Newton[2] and K.L. Verheyen[1]
[1]*Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, AL9 7TA, UK;* [2]*EIDS, Department of Veterinary Medicine, University of Cambridge, CB3 0ES, UK and* [3]*Intervacc, Hägersten, Sweden*
Email: aam94@cam.ac.uk

Background: Strangles, caused by *Streptococcus equi (S. equi)*, remains endemic in the United Kingdom (UK) horse population. Sub-clinically infected long-term carrier horses that intermittently shed *S. equi*, infecting naïve horses, are considered the principal driver of strangles endemicity. Monitoring genomic changes in circulating strains may provide valuable information on how the pathogen is adapting within horse populations and driving its endemic persistence.

Objectives: Utilise whole genome sequencing (WGS) data to determine the population structure and diversity of UK field-derived *S. equi* bacterial isolates.

Study design: Retrospective cross-sectional genomic surveillance.

Methods: *Streptococcus equi* isolates ($n = 510$) from samples submitted to six UK diagnostic laboratories between 2016 and 2022 underwent DNA extraction prior to WGS. Assembled WGS sequences were compared with the *S. equi* 4047 reference genome to identify genomic differences and determine the collection's population structure via Bayesian Analysis of Population Structure (BAPS).

Results: Nine BAPS groups were identified, although 82% of strains belonged to only two groups (BAPS3, $n = 230$, 45%; BAPS5, $n = 189$, 37%). There was a statistically significant association ($p < 0.001$) between year of recovery and the relative proportion of BAPS groups, with linear trends over the study period for increasing proportions of BAPS3 isolates (7% in 2017 to 93% in 2022) and decreasing proportions of BAPS5 isolates (69% in 2017 to <1% in 2022).

Main limitations: Bacterial strains from sub-clinically infected carrier horses may be under-represented in this study, given the nature of data collection via positive laboratory diagnoses.

Conclusions: The rapid change in genomic population structure observed among *S. equi* isolates recovered from UK horses between 2016 and 2022 cannot be explained by transmission via sub-clinical long-term carriers. Instead, it suggests that transmission from acutely infected or recently recovered horses may be a far more significant contributor to strangles endemicity than previously thought.

Ethical animal research: Approved by Royal Veterinary College's Clinical Research Ethical Review Board (URN 2020 1973–2).

Informed consent: Not stated.

Competing interests: A. Waller is CSO at Intervacc.

Funding: The Horse Trust.

20 | Pinning the tail of the evolution of *Streptococcus equi* in populations of horses and donkeys

H. Wilson[1], A.J. van Tonder[2], C. Ruis[2,3], N. Lefrancq[2,4], A. McGlennon[5], C. Bustos[6,7], A. Léon[8,9], S. Frosth[10], J. Dong[11], A.M. Blanchard[12], M. Holden[13], A. Waller[14] and J. Parkhill[2]

[1]*PHG Foundation, linked exempt charity of University of Cambridge, Cambridge, UK;* [2]*Department of Veterinary Medicine, University of Cambridge, Cambridge, UK;* [3]*Victor Phillip Dahdaleh Heart & Lung Research Institute, University of Cambridge, Cambridge, UK;* [4]*Department of Genetics, University of Cambridge, UK* [5]*EIDS, Department of Veterinary Medicine, University of Cambridge;* [6]*Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Cátedra de Enfermedades Infecciosas, Argentina;* [7]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina;* [8]*LABÉO, Research Department, St Contest, Caen, France;* [9]*Normandie Univ, UNICAEN, INSERM, DYNAMICURE UMR 1311, Caen, France;* [10]*Swedish University of Agricultural Sciences, Sweden;* [11]*Department of Veterinary Medical Science, Shandong Vocational Animal Science and Veterinary College, Weifang, China;* [12]*School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK;* [13]*Infection Group, School of Medicine, University of St Andrews, North Haugh, St Andrews, United Kingdom;* [14]*Intervacc AB, Stockholm, Sweden*

Email: hjw58@cam.ac.uk

Background: *Streptococcus equi* subsp. *equi* continues to evolve and disseminate globally causing disease and considerable economic losses. Largescale genomic analysis of *S. equi* and its ancestor *S. zooepidemicus* can be utilised to identify evolutionary events leading to the successful expansion of *S. equi*.

Study design: Genomic analysis.

Methods: 1201 *S. equi* sequences and 66 sequences representative of the diversity of *S. zooepidemicus* were used for genetic analysis.

Results: A novel *S. equi* lineage was identified, comprising isolates from donkeys in Chinese farms, which diverged nearly 300 years ago, after the emergence of *S. equi* from *S. zooepidemicus*, but before the global sweep identified by Harris et al. [1]. Ancestral state reconstruction demonstrated that *slaA*, *seeL* and *seeM* were acquired by the global *S. equi* after the divergence of the basal donkey lineage. We identified the equibactin locus in both *S. equi* populations, but not *S. zooepidemicus*, reinforcing its role as a key *S. equi* virulence mechanism. Evidence of a further population sweep beginning in the early 2000s was detected in the UK. This clade now accounts for more than 80% of identified UK cases since 2016. Several sub-lineages demonstrated increased fitness, within which the acquisition of a new, fifth prophage containing additional toxin genes was identified.

Main limitations: The global SARS-CoV2 pandemic meant that genomic data outside of the UK was not available after 2020.

Conclusions: Acquisition of the equibactin locus was a major determinant in *S. equi* becoming an equid-exclusive pathogen, but that other virulence factors were fixed by the population sweep at the beginning of the 20th century. Evidence of a secondary population sweep in the UK and acquisition of further advantageous genes implies that *S. equi* is continuing to adapt and therefore continued investigations are required to determine further risks to the equine industry.

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Not applicable.

Competing interests: A. Waller is CSO at Intervacc AB.

Funding: Petplan Charitable Trust grant: S19-741-780.

Reference:

[1] Harris, SR, Robinson, C, Steward, KF, Webb, SK, Paillot, R, Parkhill, J, Holden, MTG, Waller, AS. Genome specialization and decay of the strangles pathogen, *Streptococcus equi*, is driven by persistent infection. Genomic Res. 2015;25:1360–1371. 10.1101/gr.189803.115.

21 | Analysis of the distribution of functional groups of genes in Argentinian *Streptococcus equi* subsp. *equi*

C.P. Bustos[1,2], G. Retamar[1], H. Wilson[3], S. Frosth[4], L. Frykberg[4], B. Guss[4], A. Muñoz[1], A. Waller[4,5] and M. Mesplet[1]

[1]*Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Cátedra de Enfermedades Infecciosas, Argentina;* [2]*Consejo Nacional de Investigaciones Cientificas y Tecnicas, Argentina;* [3]*Cambridge University, UK;* [4]*Swedish University of Agricultural Sciences, Sweden and* [5]*Intervacc AB, Sweden*

Email: carlabustos@fvet.uba.ar

Background: *Streptococcus equi* subsp. *equi* is one of the major horse pathogens affecting the equine industry. Although molecular tools and genomics are useful to track epidemiological relationships of *S. equi*, the analysis of its metabolism and virulence is essential to understand its behaviour.

Objectives: To analyse the distribution of functional groups of genes in Argentinian *S. equi* genomes.

Study design: Molecular analysis of *S. equi*.

Methods: A total of 44 Argentinian strains of *S. equi* isolated from healthy horses and horses with acute Strangles were included in the study. All the strains were previously sequenced by Illumina technology and annotated by the RAST Server (Rapid Annotations using Subsystem Technology). The distribution of functional groups of genes were analysed among the *S. equi* genomes comparing them according to their origin and epidemiological relationships.

Results: Although *S. equi* genomes were similar, the variability was observed among the genes associated with protein metabolism and carbohydrates. The comparison of the distribution of the functional genes showed that some strains isolated from the same outbreak presented some differences even if they encoded the same *seM*-type or were closely related according to cgMLST. On the other hand, a similar number of genes in all categories was found among the strains obtained from healthy horses.

Main limitation: Pangenome analysis was not performed.

Conclusions: Argentinian strains of *S. equi* presented genetic differences related to metabolism even when they had closely related core genome sequences.

Ethical animal research: Not required: analysis of microorganisms
Informed consent: Not stated.
Competing interests: A.S. Waller is employed by Intervacc AB
Funding: Universidad de Buenos Aires and Agencia Nacional de Promocion de la Investigación, el Desarrollo Tecnológico y la Innovacion, Argentina.

22 | Core genome multilocus sequence typing schemes for epidemiological investigation of *Taylorella equigenitalis* and *Taylorella asinigenitalis*

S. Kozak[1], D. Merda[2], M.-F. Breuil[1], S. Petry[1] and F. Duquesne[1]
[1]*ANSES, Normandy Laboratory for Animal Health, Physiopathology and Epidemiology of Equine Diseases Unit, Goustranville, France and* [2]*SPAAD unit, Paris Est University, Anses, Maisons-Alfort, France*
Email: sofia.kozak.ext@anses.fr

Background: The *Taylorella* genus is composed of two species:(i) *Taylorella equigenitalis*, mostly found in horses and responsible for contagious equine metritis and (ii) *Taylorella asinigenitalis*, mainly found in donkeys in which the pathogenicity remains unclear. As of today, monitoring of the *Taylorella* genus is performed using MultiLocus Sequence Typing (MLST; https://pubmlst.org/organisms/taylorella-spp/)[1] and despite being suited for phylogenic studies, it is only based on <0.5% of the genome which limits its use for finer epidemiological investigations.
Objectives: To develop genome-wide typing methods (core genome MLST) for the monitoring of the *Taylorella* genus
Study design: Genome analysis of microorganisms.
Methods: Genomic data of 246 *T. equigenitalis* and 44 *T. asinigenitalis* (18 countries, 1977–2022) were used to detect all common genes and build the typing schemes, which were subsequently evaluated using 124 and 24 additional genomes of *T. equigenitalis* and *T. asinigenitalis*. The average number of alleles per locus was calculated and allelic distances were represented using minimum-spanning trees. Epidemiologically related strains were used to define clonal group thresholds.
Results: While perfect congruence was obtained between MLST and cgMLST for *T. asinigenitalis*, comparison of the two methods showed that cgMLST performed better to discriminate closely related genotypes of *T. equigenitalis*. The results showed a high genetic stability of both species over the years.
Main limitations: Limited number of epidemiologically related *T. equigenitalis* strains and low number of *T. asinigenitalis* strains.
Conclusions: This work allowed us to gain insights into the genome-wide relatedness of 370 *T. equigenitalis* and 68 *T. asinigenitalis* which fully represents this genus diversity to date, and to propose highly robust typing methods for future investigations.
Ethical animal research: Not required: retrospective analysis of microorganisms
Informed consent: Not applicable
Competing interests: None declared
Funding: The French Horse and Riding Institute, IFCE (http://www.ifce.fr) and the Fonds Eperon (https://www.fondseperon.com/).
Reference:
[1] Duquesne F, Hébert L, Breuil MF, Matsuda M, Laugier C, Petry S. Development of a single multi-locus sequence typing scheme for *Taylorella equigenitalis* and *Taylorella asinigenitalis*. Vet. Microbiol. 2013;167(3–4):609–618.

Bacteriology 6: Clinical

Michel D'Ornano Wednesday 11.00–13.00

23 | *Mycoplasma equirhinis*, a disregarded player in equine respiratory disorders?

M. Martineau[1,2,3], É. Kokabi[1,2], C. Ambroset[3], M. Jaÿ[3], F. Tardy[3,4] and A. Leon[1,2]
[1]*LABÉO, Research Department, 14 280 Saint Contest, France;* [2]*Normandie Université, UNICAEN, UNIROUEN, DYNAMICURE, UMR 1311, Inserm,14 000 Caen, France;* [3]*Université de Lyon, UMR Mycoplasmoses animales, VetAgro Sup, Anses, laboratoire de Lyon and* [4]*Mycoplasmology, Bacteriology and Antibioresistance unit, Anses, laboratoire de Ploufragan-Plouzané-Niort*
Email: matthieu.martineau@laboratoire-labeo.fr

Background: Respiratory disorders are associated with poor performance and economic losses in the equine industry.[1] *Mycoplasma* (*M.*) spp. have been regularly reported in up to 15% of respiratory specimens but their clinical contribution remains unclear.
Objectives: Characterisation of *Mycoplasma* species and evaluation of their role in equine respiratory disorders.
Study design: retrospective descriptive
Methods: In total, 1948 respiratory samples were collected and analysed from 1764 horses in France over the 2020–2022 period.[2] Mycoplasmas were detected by PCR post enrichment and culture. Detection results were interpreted using clinical scoring, risk factor analysis as well as testing for association with other potential etiological agents The genomes of several *M. equirhinis* isolates were sequenced and compared.
Results: The prevalence of mycoplasmas was refined to 16.1%, with a predominance of *M. equirhinis* species (85.3%). The *M. equirhinis* prevalence remained steady whatever the clinical score. Nonetheless, it increased significantly in the presence of *Streptococcus equi* subsp. *zooepidemicus* or EHV-5 virus. In bronchoalveolar lavages, the detection of *M. equirhinis* was associated with neutrophil-mediated inflammation, suggesting it could participate in an inappropriate immune response as observed in equine asthma. *M. equirhinis* genomes were shown to be highly homogeneous, with a few mobile genetic elements, such as an *M. arginini*-like ICE and a *M. arthritidis*-like prophage. The repertoire of genes putatively associated with virulence was limited to cytoadherence and immune escape, like its closest phylogenetic neighbour, *M. hominis*.

Main limitation: Lack of a quantitative method to detect *M. equirhinis* directly from clinical specimens.

Conclusions: *M. equirhinis* is not a primary pathogen but could contribute to clinical signs in association with other opportunistic pathogens. It could play a part in the equine respiratory disease complex essentially through dysregulation of the host immune response, as is well known for other mycoplasma species in other respiratory complexes.

Ethical animal research: Not required: retrospective case series and analysis of microorganisms.

Informed consent: Not stated.

Competing interests: None declared.

Funding: IFCE (Institut Français du Cheval et de l'Equitation), Fonds Eperon and GIS Centaure.

References:

[1] Wood JLN, Newton JR, Chanter N, Mumford JA. Association between respiratory disease and bacterial and viral infections in British racehorses. J Clin Microbiol 2005;43(1):120–126. DOI:10.1128/JCM.43.1.120-126.2005.

[2] Martineau M, Kokabi E, Taiebi A, Lefebvre S, Pradier S, Jaÿ M, Tardy F, Leon A. Epidemiology and pathogenicity of *M. equirhinis* in equine respiratory disorders. Vet Microbiol. 2023;287:109926. DOI:10.1016/j.vetmic.2023.109926.

24 | Involvement of *Pseudomonas aeruginosa* in human and animal health in Normandy: Resistance, adaptation factors, and biofilm

S. Le Hello[1,2], M. Pottier[1,3], L. Rodrigues[1,2], F. Gravey[1,2], S. Petry[4] and A. Leon-Seck[1,3]

[1]*Normandie Université, UNICAEN, UNIROUEN, DYNAMICURE, UMR 1311, Inserm,14 000 Caen, France;* [2]*CHU Caen, Department of infectious agents, bacteriology, F-14000 Caen, France;* [3]*LABÉO, Research Department, Caen, France and* [4]*ANSES, Normandy Laboratory for Animal Health, Physiopathology and Epidemiology of Equine Diseases Unit, Goustranville, France*

Email: lehello-s@chu-caen.fr

Background: *Pseudomonas aeruginosa* is one of the leading causes of healthcare-associated infections in humans. This bacterium is less represented in veterinary medicine, despite causing difficult-to-treat infections due to its capacity to acquire antimicrobial resistance, produce biofilms and persist in the environment.

Objectives: Determine the susceptibility profiles of a large panel of *P. aeruginosa* strains to antipseudomonal antibiotics and didecyldimethylammonium chloride (DDAC), widely used as a disinfectant.

Study design: Genomic surveillance.

Methods: A phenotypical and genomic characterisation carried out on 180 strains isolated in hospitals and 168 strains from animals, particularly horses. Susceptibility profiles to antipseudomonal antibiotics and DDAC were established using disk diffusion and broth microdilution respectively. A genomic study was performed on 121 strains (77 human and 44 animal) to determine their resistome and putative clonal relatedness.

Results: 28 Sequence Types (ST) were found among strains of animal origin and 18 for strains of hospital origin. ST strains considered among the top ten high-risk clones of *P. aeruginosa* for human health were found in hospitals and animals. While veterinary medicine has a very few therapeutic options to control *P. aeruginosa* infections, resistance rates to critical antibiotics (carbapenems) in humans were found for both populations. In addition, an important representation of a decreased susceptibility phenotype to DDAC was observed and was more represented in the hospital setting (62.5%) than in patients (28.2%) and animals (11.3%). Biofilm formation did not appear to be directly involved in decreased susceptibility to DDAC but was a persistent structure of the bacteria in the environment.

Main limitations: Knowledge of the mechanisms underlying this decreased susceptibility to DDAC is limited: preliminary observations suggest overexpression of the efflux resistance-nodulation-division pump.

Conclusion: All these observations reinforce the need to study the different reservoirs of resistance in *P. aeruginosa*, using a "One health" approach.

Ethical animal research: Not required: analysis of microorganisms

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Conseil Régional de Normandie, Ministère de l'Agriculture et de la Souveraineté Alimentaire

25 | Seasonal variations in the antibody response to putative agents of mucoid placentitis

A.E. Page[1], E.N. Adam[1], M. Johnson[1] and R. Ruby[2]

[1]*Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546 USA and* [2]*University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY 40511, USA*

Email: a.page@uky.edu

Background: Equine mucoid placentitis, often referred to as nocardioform placentitis, is a distinctive disease that is characterised by the development of mucoid material between the endometrium and chorioallantois. The resulting placental insufficiency can result in abortion or small, weak foals. To date, multiple different bacterial species have been associated with disease, with *Crossiella equi* and *Amycolatopsis* spp. the most common in central Kentucky. Previous analysis of archived samples showed significant increases in serum antibodies against *C. equi* during the summer months.

Objectives: To observe serial changes in antibodies using a recombinant protein-based enzyme-linked immunosorbent assay (ELISA).

Study design: Prospective, observational study.

Methods: Sera from 76 Thoroughbred broodmares on 8 central Kentucky farms with a history of mucoid placentitis were sampled every 4 weeks from April 2021 through April 2023. Sera were analysed

using a custom-designed recombinant protein ELISA for total IgG antibodies. Placentae from these mares were submitted to the University of Kentucky's Veterinary Diagnostic Laboratory for gross examination with or without histologic, microbiologic, and molecular analysis. One-way, repeated measures analysis of variance was utilised to determine significant changes in population antibody levels.

Results: Significant increases in antibody concentrations occurred between late-May and late-August in both years, when compared to antibody concentrations in January. Five broodmares with mucoid placentitis during the study demonstrated a variable antibody response prior to parturition.

Main limitations: Lack of an experimental model for mucoid placentitis prevents complete validation of this ELISA and sensitivity/specificity calculations. Additionally, the use of recombinant proteins could fail to capture additional antibodies directed against other mucoid placentitis-associated organisms.

Conclusions: Antibodies against the two main putative agents of mucoid placentitis increase during the summer, suggestive of exposure during this time of year. Further examination of epidemiologic and environmental factors is warranted to better understand this disease and the variable antibody response in affected mares.

Ethical animal research: Approved by University of Kentucky Institutional Animal Care and Use Committee.

Informed consent: Owner or farm manager consent was obtained.

Competing interests: None declared.

Funding: Grayson-Jockey Club Research Foundation and the Mary K. Oxley Foundation.

26 | Hitching a ride: Is the movement of horses driving transmission of *Streptococcus equi* amongst the UK horse population?

A. A. McGlennon[1,2], A. S. Waller[3], J. R. Newton[2] and K. L. Verheyen[1]
[1]*Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, AL9 7TA, UK;* [2]*EIDS, Department of Veterinary Medicine, University of Cambridge, CB3 0ES, UK and* [3]*Intervacc, Hägersten, Sweden*
Email: aam94@cam.ac.uk

Background: Strangles, caused by the bacterium *Streptococcus equi* (*S. equi*), remains endemic in the UK with ~300 laboratory diagnoses annually. We hypothesised that whole genome sequences (WGS) of UK *S. equi* isolates could be utilised to identify and better understand transmission events.

Objectives: To model transmission of UK *S. equi* strains by combining epidemiological and WGS data.

Study design: Retrospective cross-sectional genomic transmission analysis.

Methods: A dated phylogenetic tree of 447 *S. equi* WGS recovered from UK horses between 2016 and 2022 was reconstructed. Transmission analysis using Transphylo was conducted, informed by specific input priors, including an estimated time between infection to onward transmission of 42 ± 10 days. Inference was performed in triplicate for 1 000 000 Markov chain Monte Carlo iterations, and a posterior probability ≥0.5 indicated a plausible transmission event. Clinical history and geographical location data were also used where these were available.

Results: Sixteen plausible transmission pairs were identified, approximately two-thirds of which were between horses from different regions ($n = 10$, 62.5%, 95% CI 35%–85%), the rest within the same region ($n = 6$, 37.5%, 95% CI 15%–65%). Two horses were detected as transmitting to two separate recipients each. Onward transmission was found within a six-month period among nine sampled horses across all four UK countries, with plausible transmission events identified between five horses.

Main limitations: There was a low sampling proportion among these data relative to overall cases in the UK, providing a snapshot of broader, but unsampled transmission events.

Conclusion: Transmission of genetically related strains between diverse UK regions suggests that the movement of horses across the UK facilitates *S. equi* transmission. Our data emphasise the need for improved equine biosecurity and health practices and highlight that a real-time sampling-to-sequence pipeline could inform interventions and minimise the further spread of infection.

Ethical animal research: Approved by the Royal Veterinary College's Clinical Research Ethical Review Board (URN 2020 1973-2).

Informed consent Explicit owner informed consent not stated.

Competing interests: A. Waller is CSO at Intervacc.

Funding: The Horse Trust.

27 | Insight into the biosecurity and isolation practices' knowledge related to strangles prevention in the UK equestrian community

L. Henry[1], E. Taylor[2], H. Whitelegg[3], R. Paillot[1] and S. Mooney[1]
[1]*Faculty of Science & Engineering, School of Agriculture, Animal & Environmental Sciences, Anglia Ruskin University (ARU Writtle), Lordship Road, Writtle, Chelmsford, CM1 3RR, UK;* [2]*The Horse Trust, Speen Princes Risborough, Buckinghamshire, UK and* [3]*Redwings Horse Sanctuary, Norwich, UK*
Email: romain.paillot@aru.ac.uk

Background: Strangles remains one of the most frequently reported equine infectious diseases, with more than 200 strangles cases reported in the UK alone in 2023. While Strangles vaccines are available, the implementation of biosecurity measures, diagnostic screening and carrier identification often remain the primary approach to prevent new outbreaks.

Objectives: To obtain insights about the biosecurity/isolation practices currently in place in the UK equestrian community to prevent or mitigate the occurrence of Strangles.

Study design: Surveys of horse owners

Methods: Two surveys were posted on over 350 equine-related Facebook groups and BHS-approved riding centres (December 2023 to January 2024). The first survey targeted horse owners

(152 respondents) and the second focused on yard owners/workers (131 respondents).

Results: The horse owner survey reported that only 30.9% of respondents were aware of existing isolation policies for new horse arriving on the yard (36.2% for existing horse developing contagious diseases). This was significantly different from yard owners/workers answers ($p < 0.001$), with 71.0% of respondents confirming the presence of specific procedures for new arrival (77.9% for horse developing illness). Reported isolation duration greatly varied (1 week or less, 19.4%; 1–2 weeks, 49.6%; >2 weeks, 29.5%) for new arrivals (no significant difference between respondent categories; $p = 0.7$). While hygiene/biosecurity procedures were relatively well identified by the respondents (even when no policies were reported), 48.7% of horse owners reported a complete absence of facility disinfection (22.9% for yard owners/works; $p < 0.001$) and the presence of a biosecurity plan had limited influence on the yard choice made by horse owners (56.0%). Irrespective of the respondent category, the lack of facility/area and space constraint remained the main reason mentioned for a policy absence (52.1%: $p = 0.5$). Welfare concerns for isolated horses was mentioned by 5.1% of respondents. While most respondents have a reasonable knowledge of biosecurity measures, the lack of space/facilities remains the primary limiting factor. These results are currently used to focus future Strangles Awareness Week campaigns in order to help equestrians overcoming these barriers.

Main limitations: Self-selection bias in survey respondents.

Conclusions: The proportion of horse owners that are unaware of biosecurity measures/requirements or their benefit/need in relation to strangles remains high.

Ethical animal research: Approved by Writtle University College Ethical committee (ref 1927, approved on the 18DEC23).

Informed consent: Completion of the survey was taken as consent.

Competing interests: None declared.

Funding: None.

28 | Description of four forms of equine epizootic lymphangitis

U. Wernery, F. Graiban Al Mheiri, and S. Joseph
Central Veterinary Research Laboratory, Dubai, United Arab Emirates
Email: cvrl@cvrl.ae

Background: Equine epizootic lymphangitis (EEL) is a pyogranulomatous fungal disease that affects not only horses, mules, and donkeys in Asia, South America and Africa but also other animal species including wildlife and laboratory animals [1]. EEL is caused by the fungus *Histoplasma capsulatum* var. *farciminosum* (HCF) and is the most prevalent disease in Ethiopian horses after African horse sickness with a prevalence of up to 20% in some low-lying humid areas. EEL is a WOAH-listed disease that possesses economic and public health importance. Working equids make up the majority of the Ethiopian equid population which are used for transport, as cart and riding animals, and in the agricultural sector with a total equid population of 11.3 million. No satisfactory treatment is known and severely infected horses are abandoned by their owners in remote areas to die.

Objectives: To investigate the different forms of EEL in the disease epicentre in Bishoftu (Debre Zeit), south of Addis Ababa, and its surroundings.

Study design: Descriptive clinical report.

Methods: During several visits to the equine veterinary hospital of the College of Veterinary Medicine and Agriculture in collaboration with the Society for the Protection of Animals Abroad (SPANA), and The Donkey Sanctuary in Bishoftu and rural areas, the pathological alterations of different forms of EEL were recorded and presented by several images.

Results: The clinical conditions of EEL can be divided into four different forms: cutaneous, ocular, respiratory, and asymptomatic.

Main limitation: In Ethiopia, EEL is an important health and welfare issue that should be addressed by the Ethiopian government.

Conclusion: EEL is a devastating disease of equids and presents itself in four different forms. A vaccine should be developed in the future from the isolated HCF strains to reduce the disease impact on the equine population and to improve the livelihood of Ethiopian families.

Ethical animal research: Not required: descriptive clinical report.

Informed consent: Not stated.

Competing interests: None declared.

Funding: None.

Reference:

[1] A Mheiri FG, Wernery, U. Equine Epizootic lymphangitis: a synopsis. J. Trop. Dis. 2023;11(4), 1 000 396. 10.35241/2329-891X.23.11.396.

29 | Exposure of naïve strangles free horses to carriers of *S. equi*

M. Riihimäki[1], S. Frosth[2], A. Waller[3], S. Hanche-Olsen [4], I. Falk[1] and J. Pringle[1]
[1]*Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2]*Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [3]*Intervacc AB, Hägersten, Sweden;* [4]*Department of Companion Animal Clinical Sciences, Norwegian University of Life Sciences, As, Norway*
Email: miia.riihimaki@slu.se

Background: Recovered carriers of *Streptococcus equi* subsp *equi* (*S. equi*) may retain live bacteria and can have a key role in disease transmission. The majority of clinically healthy carriers remain PCR positive yet culture negative for *S. equi* months after clinical disease. Unfortunately, their infectious capacity is unknown.

Objectives: To identify the likelihood that qPCR positive, bacterial culture negative, *S. equi* carriers can spread *S. equi* to naïve horses and thus determine the role of carriers in the transmission of this disease.

Study design: In vivo experiments.

Methods: *S. equi* naïve ponies (2 naïve ponies per carrier) were co-housed with carriers ($n = 10$). Clinical exams were performed daily, and sampling from upper airways was performed weekly from day 7 until an individual horse had three consecutive negative samples for *S. equi* by qPCR or culture. Weekly sampling of naïve animals included blood samples for serology, as well as nasal—and endoscopic guttural pouch lavage. The samples were analysed with qPCR and bacterial culture and whole genome sequencing when culture positive.

Results: Mild abnormal clinical signs with transient fever (>38.5°C) up to 39.1°C were observed in 50% of naïve ponies. All naïve ponies were culture negative in all samplings, but eight had qPCR positive samples for *S. equi*.

Main limitations: Risk of environmental DNA contamination from dead *S. equi* bacteria and thus false positive qPCR results. Risk of false negative bacterial culture results due to low numbers of bacteria and potentially attenuated strains.

Conclusions: Immunologically naïve horses co-housed with solely PCR positive silent carriers of *S. equi* developed mild transient fever but did not develop classical clinical signs of strangles. The lack of disease in naïve ponies may be due to a low infection dose and/or the persistence and shedding of attenuated *S. equi* strains.

Ethical animal research: Approved by the Swedish Ethical Committee on Animal Experiments (diary nr 5.8.18-20 527/2020).

Informed consent: Horse owners provided written informed consent.

Competing interests: None declared.

Funding: Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, 2019–02127.

30 | The prevalence of *S. equi* carriers in the Netherlands, and implications for carrier-susceptible contacts at competitions

R. Houben[1], E. Broens[2], M. Broekhuizen-Stins[2], M.M. Sloet van Oldruitenborgh-Oosterbaan[1], K. van Maanen[3] and H. Heesterbeek[4]

[1]*Utrecht University, Faculty of Veterinary Medicine, Equine Sciences, Yalelaan 114, Utrecht, The Netherlands;* [2]*Utrecht University, Faculty of Veterinary Medicine, Department of Biomolecular Health Sciences Infectious Diseases & Immunology, Utrecht, The Netherlands;* [3]*GD Animal Health, Deventer 7400 AA, The Netherlands and* [4]*Utrecht University, Faculty of Veterinary Medicine, Population Health, Utrecht, The Netherlands.*

Email: r.m.a.c.houben@uu.nl

Background: *Streptococcus equi (S. equi)* carriers are thought to be important drivers for strangles outbreaks. Limited data are available on the prevalence of carriers in European horse husbandry settings, and no data are available on how frequently carriers contact susceptible horses.

Objectives: To estimate the prevalence of *S. equi* carriers among apparently healthy horses and ponies in the Netherlands, and to estimate the opportunities for contact of carriers with susceptible horses at Dutch competitive events.

Study design: Cross-sectional survey and simulations.

Methods: PCR analyses of three repeated nasopharyngeal lavage at weekly intervals and Bayesian true prevalence estimation. To estimate the annual number of carrier-susceptible contacts at competitions, simulations drew estimates from the Bayesian true prevalence posterior distribution, assigned carrier status to horses in a real-world network based on Dutch sports and racing records, assigned non-susceptible status to a proportion of horses in the network informed by published seroprevalence surveys, and counted the number of direct contacts, defined as presence at the same location on the same day, between carrier and susceptible horses for an entire year.

Results: A full set of three lavages was available for 166 horses on 86 premises. The estimated true prevalence was 3.8% (95% Credible Interval 1.2%–7.7%). The median annual number of carrier-susceptible contacts in the simulation runs was 1.0×10^6 (IQR 7.3×10^5–1.4×10^6).

Main limitations: Our target of 200 participants in the cross-sectional survey was not reached. Seropositivity is an imperfect proxy for resistance to infection for *S. equi*, therefore the simulations may have overestimated the number of susceptible horses.

Conclusions: Our carrier prevalence estimate is similar to a recent report from the UK. A large number of carrier-susceptible contacts at competitions means that even if the probability of transmission per contact in these settings is small, it may still be of epidemiological importance.

Ethical animal research: Approved by Utrecht University's Institutional Animal Welfare Body (IVD; protocol number 5204-2-05 and the Ethical Review Board of Utrecht University's Geography Department (DGK S-23016) determined that ethical review for use of personal data was not required.

Informed consent Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: Partially funded by Utrecht University's ‘‘Jublieumfonds.’’

Bacteriology 7: Diagnostics 1

Lexington Wednesday 15.00–16.00

31 | New approaches for evaluation of microbiological quality in equine clinics using Fourier transform infrared spectroscopy

E. Kokabi[1,2], J. Leprou[1,2], N. Menard[3], P.-H. Pitel[1,3] and A. Leon-Seck[1,2,3]

[1]*LABÉO, Research Department, Saint-Contest, 14 000 Caen, France;* [2]*Normandie Université, UNICAEN, UNIROUEN, INSERM, DYNAMICURE UMR 1311, 14 000 Caen, France and* [3]*LABÉO, Animal Health Department, Saint-Contest, 14 000 Caen, France*

Email: elena.kokabi@laboratoire-labeo.fr

Background: Veterinary clinics are confronted with nosocomial infections caused by pathogens that persist in the absence of complete eradication or that adapt too quickly to their environment,[1] but with less epidemiological surveillance than in the human health sector.

Objectives: Inspired by the protocols used in hospitals, we developed a process to evaluate the microbiological quality of veterinary structures.
Study design: Surveillance protocol development.
Methods: Hospital surveillance consists of (1) an exchange with the responsible to target risk zones, (2) sampling and microbiological analysis, and (3) results' discussion and optimisation of initial cleaning protocol if necessary. Literature and methodology reviews were conducted for four matrices: air, surfaces, water, and materials (e.g., endoscopes); an equine clinic was used to compare the sampling methods (air sampling *vs*. sedimentation boxes, or wipes *vs*. agar dishes for surfaces). Contamination indicator organisms (ESKAPE) and their antimicrobial resistance profiles were targeted.[2] A new rapid typing technology using Fourier transform infrared spectroscopy[1] was used to determine strain clonality.
Results: Two strains of *Staphylococcus* (*pseudintermedius* and *xylosus*) with an antibiotic-resistant phenotype to methicillin were isolated by air analysis. Eight strains of *Enterobacter hormaechei* were isolated by surface sampling. Six of these strains carried extended-spectrum beta-lactamases and were found in different zones. IR-Biotyper analysis demonstrated that the neonatal stable and operating room strains belonged to the same clone.
Main limitations: We have focused our investigation only on bacteria, fungi, and yeasts' detection but viruses should be included.
Conclusion: Our process represents a biosecurity approach to reduce the introduction of harmful organisms, control environmental pathogens, prevent the transmission of infectious diseases, and improve human and animal safety.
Key Manufacturer:
1 IR-Biotyper, Bruker https://www.bruker.com/en/products-and-solutions/microbiology-and-diagnostics/microbial-strain-typing.html.
Ethical animal research: Not applicable.
Informed consent: Not applicable.
Competing interests: None declared.
Funding: None.
References:
[1] Stull JW, Weese JS. Hospital-associated infections in small animal practice. Vet Clin North Am Small Anim Pract. 2015;45(2):217–233. DOI: 10.1016/j.cvsm.2014.11.009.
[2] Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front Microbiol. 2019;10:539. DOI: 10.3389/fmicb.2019.00539.

32 | Multiplex technology for improved diagnostics and treatment efficacy evaluation for Lyme disease

B. Wagner
Department of Population Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
Email: bw73@cornell.edu

Background: Lyme disease is a tick-borne disease caused by the spirochete *Borrelia burgdorferi* in the USA. Pathogen detection is inconsistent and not the method of choice. Antibodies against *B. burgdorferi* are used to support the diagnosis of Lyme disease in people, dogs and horses. *Borrelia* spirochetes cause persistent infection and clinical signs in dogs and horses appear typically months after infection. This makes Lyme disease challenging to diagnose and often difficult to treat.
Objectives: To evaluate a sensitive, quantitative assay that advances Lyme diagnostics in dogs and horses and provides a tool for measurement of treatment success.
Study design: A fluorescent bead-based Lyme Multiplex assay was developed using recombinant *B. burgdorferi* antigens, including outer surface protein A (OspA), OspC, OspF, and the peptide C6. **Methods:** The quantitative assay was validated for horses and dogs using large numbers of patient samples and compared to Western blot results. In addition, longitudinal antibody profiles were evaluated in dogs and horses after experimental infection and with or without antibiotic treatment.
Results: Antibodies against *B. burgdorferi* can be detected as early as 2 weeks post exposure to infected ticks. OspC and OspF-specific serum antibody profiles can distinguish early and chronic infection, respectively. After treatment, declining antibodies support the decrease in antigenic load and thereby the removal of *B. burgdorferi* from the mammalian host. By 3 months post treatment initiation, successful treatment is supported by a decrease in antibody quantities of 40% or more. By 6 months, Lyme Multiplex test results are negative again. In contrast, treatment failure and *B. burgdorferi* persistence is indicated by steady or rising antibodies.
Main limitations: *B. burgdorferi* antibodies are indirect indicators of treatment success. Poor sensitivity of direct pathogen detection.
Conclusions: Sensitive, quantitative assays fine-tune Lyme disease diagnostics and enable an objective treatment efficacy evaluation in dogs and horses.
Ethical animal research: Not required: excess material from clinical samples was used.
Informed consent: Not stated.
Competing interests: B. Wagner holds a patent on the assay technology described in this abstract.
Funding: Not applicable.

33 | Detection of *Streptococcus equi* subspecies *equi* in water buckets

A. Rydén[1], J. Pringle[1], Y. Blom[1], I. Hansson[2] and M. Riihimäki[1]
[1]*Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden and* [2]*Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden*
Email: anneli.ryden@slu.se

Background: Strangles, caused by the bacteria *Streptococcus equi* subspecies *equi* (*S. equi*), is the most reported infectious disease in horses worldwide. The spread occurs by direct horse-to-horse contact and indirectly through environments [1].

Objective: This study aimed to investigate whether bacteria of *S. equi* and/or its DNA could be detected in experimental contaminated or potentially contaminated water.

Study design: in vitro experiments.

Methods: The study comprised three parts. Initially, water-filled buckets were experimentally contaminated with an overnight culture of viable *S. equi* (2.1–2.5 $\times$ 10^8 CFU/mL) previously archived from clinical cases. Subsequently, water was obtained from confirmed strangles outbreaks three to six weeks after the horses had recovered. Finally, water samples were collected from two different horse groups, each consisting of one silent carrier and two naive horses. Samples were taken regularly from contaminated buckets and buckets in stables and paddocks. All samples were analysed by bacteriological analyses and qPCR.

Results: In the experimentally contaminated water buckets, *S. equi* survived up to 19 days in the summer and at least 73 days in winter conditions. However, *S. equi* could not be detected in the samples from water buckets in stables or paddocks via culture or qPCR. Notably, a commensal bacterium in horses, *Streptococcus equi* subspecies *zooepidemicus*, was detected in samples from one paddock, demonstrating the method's sensitivity for *Streptococcus* spp.

Main limitations: Detection relied on conventional methods, which may have limited sensitivity or specificity, potentially affecting detection of low bacterial concentrations in water samples.

Conclusions: The results indicate that environmental factors affect the survival of *S. equi* and the concentration of *S. equi* in water from a natural outbreak of strangles is most likely lower compared to the concentration of *S. equi* in the experimentally contaminated water.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, Formas (221-2013-606 and 2019-02127).

Reference:

[1] Taylor SD, Wilson WD. Streptococcus equi subsp. equi (Strangles) Infection. Infectious Respiratory Diseases 2006;5(3):211–217.

34 | Assessment of serum microagglutination test based on flagellar antigens for differentiating between *S.* Abortusequi and *Salmonella* Typhimurium infections

Y. Kinoshita, H. Niwa and T. Ueno

Equine Research Institute, Japan Racing Association, Tochigi, Japan.

Email: kinoshita@equinst.go.jp

Background: *Salmonella* Abortusequi (SA) and *S.* Typhimurium (ST) belong to the O4 serogroup. SA can cause abortion in horses, whereas ST is known to cause enteritis. The O-antigen factors these serotypes have in common make them difficult to differentiate by the O-antigen-based serum agglutination test.

Objectives: To evaluate whether a flagellar-antigen-based serum microagglutination test could differentiate between infections with the two serotypes.

Study design: Assay assessment.

Methods: Equine sera from three groups were used: (1) experimental SA infection of 7 horses, with 4 intrauterine and 3 nasogastric tube inoculations; (2) experimental ST infection by nasogastric tube inoculation of 8 horses; and (3) 16 mares that aborted because of SA infection, with 9–13 serum samples collected from each horse at intervals of several weeks. Serum samples were preabsorbed with ST antigen and subjected to microagglutination testing with SA flagellar antigen. Reverse assays were performed on the antigens used for absorption and agglutination. A negative result was defined as the absence of agglutination at a serum dilution of 1/20 after the addition of flagellar antigen. **Results:** (1) All horses, except for one inoculated by nasogastric tube, showed agglutination positivity for SA antigen at 1–2 weeks after SA inoculation, and no agglutination reaction to ST antigen was observed in any sample. (2) After ST inoculation, all horses tested positive in agglutination testing for ST antigen but negative for SA antigen. (3) All horses were agglutination positive for SA antigen after abortion, whereas all but one sample was negative for ST antigen; the serum titres of the sample were 1:320 for SA antigen and 1:80 for ST antigen.

Main limitations: Small sample size and no sera from clinical ST infections.

Conclusions: The flagellar-antigen-based serum microagglutination test is potentially useful for differentiating between *S.* Abortusequi and *S.* Typhimurium infections.

Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute of the Japan Racing Association.

Informed consent: Informed consent was obtained from the owners of the farms with equine abortion outbreaks.

Competing interests: None declared.

Funding: Japan Racing Association.

Bacteriology 8: Diagnostics 2

Lexington Wednesday 16.30–17.30

35 | Multi-centred field evaluation of a *Salmonella* spp. point-of-care PCR assay using equine faeces and environmental samples

N. Pusterla[1], P. Naranatt[2], H. Swadia[2], L. Winfield[3], A. Hartwig[3], S. Barnum[1] and E. Mendonsa[2]

[1]*Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA;* [2]*Fluxergy, Irvine, California, USA and* [3]*Steinbeck and Peninsula Equine Clinics, Salinas, California, USA*

Email: npusterla@ucdavis.edu

Background: Several studies have evaluated the use of qPCR for the detection of *Salmonella* spp. in faecal and environmental samples.

The advantages of qPCR are its increased sensitivity, rapid turn-around-time, and cost-effectiveness. However, samples for *Salmonella* spp. testing still require shipment to an external veterinary diagnostic laboratory, delaying time to result availability. The introduction of microfluidic card technology has opened the field for rapid point-of-care (POC) molecular assays, including faecal and environmental *Salmonella* spp. testing.

Objectives: to evaluate a novel POC PCR assay for the detection of *Salmonella* spp. in faeces and environmental samples.

Study design: Prospective study.

Methods: The study was performed at two veterinary hospitals. A total of 143 faecal samples and 132 environmental samples were collected for POC PCR *Salmonella* spp. testing as well as qPCR testing. Each sample was inoculated into selenite broth and incubated for 18–24 h. For the POC PCR assay, 14 µL of selenite broth were mixed with 126 µL of PCR reaction mix and pipetted into a microfluidic test card targeting the invA and ttrC gene of *Salmonella enterica*. For qPCR analysis, 200 µL of the selenite broth were processed for DNA purification and *Salmonella* spp. testing targeting the invA gene.

Results: A total of 15/275 (5.4%) tests gave an indeterminate result for the POC PCR *Salmonella* spp. assay likely due to faecal inhibition. The remaining tests gave either a negative (225 samples) or a positive result (35 samples). The POC PCR assay showed strong agreement of 92.4% with the gold standard of qPCR for the detection of *Salmonella* spp. in all study samples. The overall agreement between the POC PCR *Salmonella* spp. assay and qPCR assay was 88.1% for faeces and 97.0% for environmental samples.

Main limitations: The study protocol did not further pursue indeterminate results and it would have been interesting to determine the outcome of retesting such samples. Another limitation of the study was the inability to culture each faecal and environmental sample.

Conclusions: The POC PCR assay was able to reliably detect *Salmonella* spp. in faeces and environmental samples when compared to the gold standard of qPCR. Further, *Salmonella* spp. results were available in less than 24 hours, including sample incubation and enrichment, and PCR analysis.

Ethical animal research: Not required: excess material from clinical samples was used.

Informed consent: Owners gave consent for their animals' inclusion

Competing interests: P. Naranatt, H. Swadia and E. Mendonsa work for Fluxergy.

Funding: Fluxergy provided the test kits.

36 | *Salmonella enterica* detection methods in equine faeces: A systematic review

E.C. Herring[1], H.W. Aceto[2], P.S. Morley[3], A.M. O'Connor[4], N. Pusterla[5], S.C. Rankin[6] and B.A. Burgess[1]

[1]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA; [2]Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; [3]Veterinary Education, Research, and Outreach (VERO) Program, Texas A&M University/West Texas A&M University, Canyon, TX; [4]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI; [5]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA; [6]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

Email: brandy.burgess@uga.edu

Background: The natural history of equine salmonellosis and limitations of commonly used detection methods hamper identification of truly negative horses and its management in facilities. While enriched culture and polymerase chain reaction (PCR) are frequently used for its diagnosis, understanding of their accuracy remains incomplete. As such, objective information about test reliability is critical for improved infection control.

Objectives: To identify, appraise, and synthesise available information on diagnostic sensitivity and specificity of enriched culture and PCR for detection of *Salmonella* in equine faecal samples; and to identify factors that drive heterogeneity in test performance.

Study design: Systematic review

Methods: A literature search was conducted in PubMed, CAB Abstracts, Web of Science, Agricola, and PubAg. References were subjected to title/abstract screening, then full-text screening, by two independent reviewers. Data on study design, population characteristics, diagnostic test protocols and performance were extracted, and assessed for risk of bias. Results were synthesised using descriptive statistics.

Results: Of 1091 studies identified, 81 full texts were screened, and 21 articles were selected for inclusion. Preliminary results suggest that on average, PCR is more sensitive (mean: 86.7%; range: 60.0%–100%) than culture (mean: 25.2%; 0%–86.7%) on a per sample basis, while culture is more specific (mean: 100%; 100%–100%) than PCR (mean: 88.6%; 71.0%–100%). Testing methods used were variable and incomplete reporting of methods and results was common.

Main limitations: Review was limited to published literature written in English; study heterogeneity and incomplete reporting precluded the ability to perform a meta-analysis.

Conclusions: The lack of standardised methods to detect *Salmonella* in equine faecal samples results in highly variable estimates of test performance. Improvements in study design and reporting, as well as consensus among experts and institutions regarding optimal *Salmonella* detection methods, would aid in improving the diagnostic landscape for equine salmonellosis.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: None.

37 | Review of the use of PCR for diagnosis of contagious equine metritis: The case of non-reproducible *Klebsiella* positive samples in France in 2024

A. Carpentier[1], L. Le Franc[2], A. Taiebi[2], M. Duchatellier[2], K. Klewer[1], P. Pourquier[1], G. Fortier[3,4] and A. Leon-Seck[3,5]
[1]*Innovative Diagnostics—IDvet, 310 rue louis pasteur, 34 790 Grabels, France;* [2]*IDEQUI—3 rue Nelson Mandela, 14 280 Saint-Contest, France;* [3]*LABÉO, Research Department, 1 route de Rosel, 14 280 Saint-Contest, France;* [4]*Normandie University, UNICAEN, BIOTARGEN UR7450, 14 000 Caen, France and* [5]*Normandie University, UNICAEN, UNIROUEN, DYNAMICURE, UMR 1311, Inserm,14 000 Caen, France*
Email: alix.carpentier@innovative-diagnostics.com

Background: Contagious equine metritis (CEM), caused by *Taylorella equigenitalis*, is a notifiable disease in many countries, responsible for reproductive disorders, making diagnosis essential before each reproduction season for mares and stallions. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are also often identified in equine metritis, obliging laboratories to perform analysis for these 3 pathogens systematically. Bacteriology is the gold standard diagnostic method, and since 2024, PCR use has been expanded in several studbooks as in the HBLB Codes of Practice.

Objectives: to evaluate the performance of a freeze-dried quadruplex qPCR kit[1] for detection of the 3 pathogens with a direct lysis protocol.

Study design: assay validation

Methods: Samples from the 2024 reproductive season were tested with the new kit and results were compared to those obtained with PCR and bacteriology usually used at LABÉO. Limit of detection and exclusivity were measured for each pathogen. Subsequently, 292 DNA extracts from fresh AMIES-charcoal swabs were tested randomly among samples received at LABÉO.

Results: LD_{PCR} was determined at 6.25, 75, and 6.25 gene copies/PCR for *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa* respectively. For all three pathogens, measured sensitivity was 100% ($n = 89$; CI_{95}: 95.9%–100%), with good exclusivity. Of 292 samples taken from LABÉO, none was positive for *T. equigenitalis* and 1 sample was positive for *P. aeruginosa* with both PCR methods. For *K. pneumoniae*, 178 samples were positive with the PCR usually used at LABÉO, while only 21 were positive on the new PCR and 4 were confirmed in bacteriology.

Main limitations: Number of field positive samples for *T. equigenitalis*.

Conclusions: The new PCR kit gives rapid results for three pathogens simultaneously and better correlation with bacteriology results regarding *K. pneumoniae*, which simplifies the workflow in laboratories while increasing the results' accuracy.

Key manufacturer:1 Quadruplex qPCR kit, IDvet: www.innovative-diagnostics.com.

Ethical animal research: Not required: excess material from clinical samples was used.

Informed consent: Not stated

Competing interests: None declared.

Funding: None.

38 | Development and validation of a specific recombinant based ELISA for detection of anti-borrelia antibodies in equine serum

A. Carpentier*[1], O. Mercier[1], S. Roche[1], K. Klewer[1], P.-H. Pitel[2], G. Fortier[2,3] and Philippe Pourquier[1]
[1]*Innovative Diagnostics - IDvet, 310 rue louis pasteur, 34 790 Grabels, France;* [2]*LABEO Frank Duncombe—1 route de Rosel, 14 280 Saint-Contest, France and* [3]*Normandie University, UNICAEN, BIOTARGEN UR7450, 14 000 Caen, France*
Email: alix.carpentier@innovative-diagnostics.com

Background: Equine borreliosis is a bacterial infection caused by *Borrelia burgdorferi* and transmitted by ticks of the family Ixodidae. This zoonotic disease poses a risk not only to equines but also other animals and humans, as horses can serve as reservoir hosts, transmitting infected ticks to people. Manifesting vague clinical signs such as lameness, fever and performance decline, equine borreliosis presents a challenge for diagnosis. Due to low concentrations of the bacteria in bloodstream, direct detection using PCR or culture is reliable only in tissue samples. Therefore, serology is the method of choice for effective diagnosis of this disease. Western-blot is a gold-standard but it is not adapted to rapid routine screening and is used as a confirmation test. An ELISA is needed for first intention screening.

Objectives: This study describes development and validation of the ID Screen® Borreliosis Double Antigen Multi-species ELISA[1], based on highly conserved proteins from *Borrelia burgdorferi*, allowing detection of antibodies in horse sera.

Study design: Assay evaluation.

Methods: Specificity was evaluated with 249 horse sera from Iceland, where circulation of ticks and *Borrelia* are very low. Sensitivity was measured by comparison with Western-blot (Anti-borrelia Euroline Horse IgG, EUROIMMUN), using 27 horse sera provided by LABEO.

Results: Measured specificity on Icelandic horses was 100% (CI95% 98.5%–100%, $n = 248$). Of 27 Western-blot positive sera, 26 were detected, giving a sensitivity of 96% (CI95%: 81.3%–99.3%, $n = 27$).

Main limitations: Western-blot availability was limited, so the choice was to test only positive sera.

Conclusions: This ELISA shows excellent specificity and high analytical sensitivity. It is a reliable tool for anti-Borrelia antibody detection, to be taken into account alongside clinical signs and differential diagnosis, for example in the case of piro-like syndrome.

Key manufacturer: 1 https://www.innovative-diagnostics.com/produit/id-screen-borreliosis-double-antigen-multi-species/.

Ethical animal research: The authors attest that ethics committee oversight is not required in their institute.

Informed consent: Owners of Icelandic horses gave consent for research in general. Positive sera were purchased from LABEO.

Competing interests: AC, OM, SR, KK and PP are employees of IDvet.

Funding: IDvet.

Bacteriology 9: Antimicrobials 2

Michel D'Ornano Thursday 11.00–12.00

39 | Does antibiotic treatment change the microbial resistome of mares?

E. Doster[1], L.J. Pinnell[1], S. Tamm[1], G.O. Veneklassen[2], M.M. Murphy[1,3], C.A. Wolfe[1], K.N. Beavers[4], H.R. Duddy[1], M.G. Papich[5], J.M. Looman[2], V. Smalley[2], S. Pruett[2], A. Veneklassen[2], J.L. Pipkin[3] and P.S. Morley[1]

[1]VERO Program, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, Canyon, TX, USA; [2]Timber Creek Veterinary Hospital, Canyon, TX, USA; [3]College of Agriculture and Natural Sciences, West Texas A&M University, Canyon, TX, USA; [4]College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA and [5]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Email: pmorley@tamu.edu

Background: Antimicrobial resistance (AMR) presents a global challenge. Veterinarians and caretakers require access to antimicrobial drugs (AMDs) to treat bacterial infections in horses, but it is crucial to prioritise AMD use practices that minimise potential impact of AMR on horses and the public.

Objectives: To assess the effects of common antimicrobial treatment regimens on microbial communities (microbiome) and AMR genes (resistome) in three biological niches: faeces, nasal passage, and vagina.

Study design: Randomised clinical trial.

Methods: Forty healthy mares used as embryo transfer recipients were randomly assigned to 5 groups: Group 1—gentamicin sulfate IM and penicillin G procaine IM; Group 2—oxytetracycline IV; Group 3—ceftiofur sodium IM; Group 4—sulfadiazine-trimethoprime PO; Group 5—untreated controls. Antibiotic treatments were administered q24hrs for 4 days. Samples were collected on Days 0, 1, 5, 6, 14, 15, 21, and 22 for microbiome and resistome analysis using 16S rRNA gene sequencing and target-enriched shotgun sequencing, respectively. Statistical analyses included Pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg correction for alpha diversity, and ANCOM-BC and pairwise PERMANOVA with Benjamini-Hochberg correction for beta diversity.

Results: All antibiotic treatments significantly impacted the microbiome and resistome compared to untreated controls. Effects were most pronounced immediately post-treatment, with some persisting for 16 days. Impacts varied across biological niches.

Main limitations: Microbiome and resistome of horses with bacterial infections may differ from healthy horses and may responding differently to AMD exposures. Comingling of horses by group may also affect microbiome and resistome composition.

Conclusions: Common AMD treatments were associated with changes in the microbiome and resistome, with varying magnitude and duration of effects. Further research should investigate whether these changes influence clinical response to subsequent treatments.

Ethical animal research: Approved by the West Texas A&M University Animal Care and Use Committee (protocol #2022.09.003).

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: Grayson Jockey Club Research Foundation and Texas A&M University.

40 | Gut microbiome and resistome development in foals

M.M. Murphy[1,2], L.J. Pinnell[1], E. Doster[1], C. Wolfe[1], E. Morah[3], K. Shields[2,3], J. Looman[3], G.O. Veneklasen[3], J.L. Pipkin[2] and P.S. Morley[1]

[1]VERO Program, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, Canyon, TX, USA; [2]Department of Agricultural Sciences, West Texas A&M University, Canyon, TX, USA and [3]Timber Creek Veterinary Hospital, Canyon, TX, USA

Email: pmorley@tamu.edu

Background: Gut microbiome development in early-life is crucial for health and disease patterns in various species, yet limited data exist on healthy foals' microbiome, especially regarding establishment of core antimicrobial resistance genes' (ARGs) populations.

Objectives: Characterise early life development of hindgut bacterial communities (microbiomes) and all ARGs (the resistomes) in foals, comparing them to their dams.

Study design: Prospective longitudinal study.

Methods: Fourteen healthy foal and maternal recipient (dam) pairs ($n = 28$) were enrolled. All foals were produced through embryo transfer and were housed in the same facility throughout the study. Faecal samples were collected per rectum from dams at 300 days of gestation, and from foals and dams post-birth, and on days 2, 7, 14, 21, 28, 60, 90, and 120. Microbiome and resistome were analysed using 16S rRNA gene sequencing and target-enriched shotgun sequencing, respectively. Statistical analyses included pairwise Wilcoxon rank-sum tests for alpha diversity and ANCOM-BC and pairwise PERMANOVA for beta diversity. Ordination and hierarchical clustering were used to examine similarities in population structures.

Results: Hierarchical clustering identified unique microbiome structures for samples collected 0–28 days compared to other samples, with richness and diversity increasing until around day 90. Foals' hindgut communities stabilised between 90 and 120 days when they more closely resembled dams' microbiomes. Conversely, resistome richness decreased significantly until day 60, stabilizing thereafter. Dams' microbiomes and resistomes remained stable, though ARG diversity was lower and more varied than in foals.

Main limitations: Study duration was limited but sampling and sequencing was more extensive than previously published studies. Future research should explore microbial communities in other niches and compare foals with gastrointestinal disease or receiving antimicrobial treatment.

Conclusions: Early-life gut microbiome of foals undergoes significant changes, akin to other species. These findings provide a basis for other investigations of factors affecting health and disease in horses.
Ethical animal research: Approved by the West Texas A&M University's (WTAMU) Institutional Animal Care and Use Committee (Approval number 2020.02.004).
Informed consent Owners gave consent for their animals' inclusion.
Competing interests: None declared.
Funding: Texas A&M University.

41 | Antimicrobial resistance of *Klebsiella pneumoniae* isolates from Thoroughbred mare reproductive samples

A. Kawarizadeh[1,2], L. Hardefeldt[1,4], K. Mitchell[3], K. Bailey[1,4], M. Marenda[2], C. Chicken[3], A. Blishen[3], R. Bushell[2] and J. Gilkerson[1,4]
[1]*The Centre for Equine Infectious Disease, Melbourne Veterinary School, Faculty of Science, University of Melbourne, Parkville, VIC 3010, Australia;* [2]*Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, University of Melbourne, Parkville, VIC 3010, Australia;* [3]*Scone Equine Hospital, Scone, NSW 2337, Australia and* [4]*National Centre for Antimicrobial Stewardship, Peter Doherty Institute for Infection and Immunity, Melbourne, Australia*
Email: a.kawarizadeh@unimelb.edu.au

Background: *Klebsiella pneumoniae*, a Gram-negative bacterium, has been isolated from various pathological conditions affecting the equine reproductive system. Although *K. pneumoniae* constitutes a relatively small proportion of bacteria isolated from the equine reproductive tract, their propensity to acquire and disseminate antimicrobial resistance (AMR) genes highlights the importance of their study.
Objectives: To characterise AMR patterns and profiles of *K. pneumoniae* isolates from the reproductive tract of mares.
Study design: Retrospective case series.
Methods: *K. pneumoniae* isolates from reproductive samples collected from Thoroughbred mares in Australia were used to study antimicrobial resistance patterns and profiles of isolates and 22 191 reproductive samples were cultured between 2020 and 2022. Colony morphology, biochemical and negative motility tests were used to identify *K. pneumoniae* isolates. Antimicrobial susceptibility testing was performed.[1,2] The results were interpreted according to Clinical and Laboratory Standards Institute guidelines.
Results: *K. pneumoniae* was isolated from 0.45% (99/22191) of the reproductive tract samples collected. The frequency of resistance to the high importance antimicrobials, ceftiofur and enrofloxacin was 49% and 47%, respectively. Multidrug resistance (resistance to 3 or more classes of antimicrobials) was detected in 52% of isolates. The 99 isolates exhibited 19 different AMR patterns.
Main limitations: Further research is needed to understand the emergence and transmission dynamics of *K. pneumoniae* in equine populations. Isolates were only from Thoroughbreds and one geographical region.
Conclusions: This study revealed resistance to high importance antimicrobials in nearly half the *K. pneumoniae* isolates from mare reproductive tract samples with a significant proportion exhibiting multidrug resistant profile.
Key manufacturers: 1 1 Vet Equine EQUIN1F Sensititre™ plates, https://www.thermofisher.com/; 2 Sensititre™ ARIS™ 2X machine https://www.thermofisher.com/.
Ethical animal research: Not required: analysis of microorganisms.
Informed consent: Not applicable.
Competing interests: None declared.
Funding: Centre for Equine Infectious Disease.

42 | Antimicrobial resistance and genetic diversity of *Klebsiella pneumoniae* from different clinical sources in horses

F. Gravey[1,2], C. Sévin [3], S. Castagnet[1,4], N. Foucher[3], K. Maillard [4,5], J. Tapprest [3], A. Léon [1,4], B. Langlois [1], S. Le Hello [1,2] and S. Petry [3]
[1]*Université de Caen Normandie, Université de Rouen Normandie, INSERM, DYNAMICURE UMR1311, CHU Caen, Department of Infectious Agents, Bacteriology, F-14000 Caen, France;* [2]*CHU Caen, Department of infectious agents, Bacteriology, F-14000 Caen, France;* [3]*ANSES, Normandy Laboratory for Animal Health, Physiopathology and Epidemiology of Equine Diseases Unit, Goustranville, France and* [4]*LABÉO, Research Department, Caen, France*
Current address for Dr Maillard: *Département du Calvados, 5 place Félix Eboué, Caen, France*
Email: sandrine.petry@anses.fr

Background: *Klebsiella pneumoniae* is a major cause of infections and reproductive disorders among horses, ranked in recent French studies as the sixth most frequently isolated bacterial pathogen in equine clinical samples. Multidrug Resistant (MDR) *K. pneumoniae* strains are considered a major global concern by the World Health Organization, including the veterinary medicine.
Objectives: To (i) characterise by a genomic approach 119 equine *K. pneumoniae* strains responsible for various manifestations of infection, (ii) describe the main antibiotic resistance profiles and acquired resistance genes, and (iii) describe the proportion of virulence genes.
Study design: *In vitro* analysis of microorganisms.
Methods: 119 equine *K. pneumoniae* strains collected between 1996 and 2020, from necropsies, suspected bacterial infections (mainly genital) and contagious equine metritis analyses were studied. Antimicrobial susceptibility profiles were determined using 35 molecules by disk diffusion method. After Illumina sequencing, *in silico* analyses were performed including species identification, MLST, cgMLST, genetic determinants identification: O-antigen, K-antigen (compared with multiplex K125 PCR), virulence genes, antibiotic resistance genes, plasmid types.
Results: A wide genomic population diversity was observed and highlighted 39% MDR and 9% hypervirulence including 5% MDR-

hypervirulent strains. An increase in resistance to 29 antibiotics was observed for necropsy-associated strain isolations during the period 2008–2020 compared with 1996–2007.

Main limitations: Retrospective study including two distinct but complementary sources of equine *K. pneumoniae* strains.

Conclusions: These findings emphasise the importance of improving the surveillance of *K. pneumoniae* in equine diagnostic tests to detect high-risk MDR-hypervirulent *K. pneumoniae* strains, which are currently not detected by the simple K1, K2 and K5 serotype approach.[1]

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The French Horse and Riding Institute, IFCE (http://www.ifce.fr) and GIS CENTAURE Recherche Equine, and internal research fund ANSES and DYNAMICURE UMR1311.

Reference:

[1] Gravey F, Sévin C, Castagnet S, Foucher N, Maillard K, Tapprest J, Léon A, Langlois B, Le Hello S, Petry S. Antimicrobial resistance and genetic diversity of *Klebsiella pneumoniae* strains from different clinical sources in horses. Front Microbiol. 2024;14:1334555. doi: 10.3389/fmicb.2023.1334555.

Bacteriology 10: Diagnostics 3

Michel D'Ornano Thursday 12.00–13.00

43 | Prevalence of antibodies to *Streptococcus equi* on horse farms in Australia

S. Dennis, K. Jeffers, J. Allen, C. El Hage, L. Hardefeldt, K. Bailey and J. Gilkerson

Centre for Equine Infectious Disease, Melbourne Veterinary School, The University of Melbourne, Victoria, Australia

Email: jrgilk@unimelb.ed.au

Background: Strangles is an endemic disease in all countries with large horse populations, however, little is known about the prevalence of exposure in Australian horses.

Objectives: This study describes the prevalence of horses with antibodies to *Streptococcus equi* on different horse properties, to improve our understanding of the risk of silent carriage.

Study design: Surveillance study

Methods: Blood samples were collected from 923 horses from 36 farms across Queensland, NSW and Victoria to determine the proportion of horses with antibodies to *Streptococcus equi*.All serum samples were tested using a dual antigen iELISA.[1] Horses and ponies of different breeds were sampled for this study.

Results: The seroprevalence on each property ranged from 0% (8 properties) to 50% (11/22 horses). The seroprevalence of samples tested from horse properties differed between states: Queensland 9% ($n = 40/427$), Victoria 16% ($n = 56/357$) and NSW 32% ($n = 44/139$). Overall, the seroprevalence in all samples tested to date was 15% ($n = 140/923$).

Main limitations: These are not a representative sample of all horses in these states, and results should be interpreted with care.

Conclusions: Seropositive horses with no previous vaccination history have most likely been previously infected. A proportion of infected horses will remain silent carriers of *S. equi*, thus an improved understanding of the seroprevalence of *S. equi* is informative as to the likelihood of introducing a carrier into a susceptible population and will influence the decision to undertake prospective screening programmes.

Ethical animal research: University of Melbourne animal ethics approval 2024-20 886-52 629-7.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: Agrifutures Australia.

Reference:

[1] Robinson C, Steward KF, Potts N, Barker C, Hammond T-A, Pierce K, Gunnarsson E, Svansson V, Slater J, Newton JR, Waller AS. Combining two serological assays optimises sensitivity and specificity for the identification of *Streptococcus equi* subsp. *equi* exposure. Vet J 2013;197:188–191.

44 | Development and application of an iELISA for the detection of antibody against *Salmonella* Abortusequi

K. Guo[1]*, Z.N. Zhang[1]*, Y. Yang[1]*, W.G. Zhang[1], J.H. Wang[1], S.J. Li[1], X.Y. Chu[1], W. Guo[1], D.Q. Liu[1], Y.X. Wang[1], Z. Hu[1] and X.J. Wang[1,2]

[1]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China and* [2]*Institute of Western Agriculture, the Chinese Academy of Agricultural Sciences, Changji, China*

*These authors contributed equallly.

Email: huzher@126.com

Background: Equine abortus salmonellosis is a bacterial disease that causes high abortion rates in susceptible equids and therefore significant economic losses. Although the tube agglutination test (TAT) is a commonly used serological test for *S.* Abortusequi, it is not highly specific or sensitive, and the development of more sensitive, specific and rapid assays is therefore urgently required.

Objectives: To establish an iELISA, which could be applied for detecting antibody against *S.* Abortusequi.

Study design: Assay development and validation.

Methods: An iELISA was developed for the specific detection of flagellum protein (FljB) antibodies against *S.* Abortusequi. Negative sera from horses sampled in a surveillance programme ($n = 1030$) were used to establish the baseline for a negative population, and reference antisera positive against other viruses or bacteria were used to test the cross reactivity of the technique. The performance of the iELISA

was evaluated against that of the standard TAT, and was tested using field serum samples from clinical cases.

Results: The iELISA was 8–16 times more sensitive than TAT. ROC analysis showed that the iELISA was accurate, with an area under the curve (AUC) = 0.9943 (95% CI, 0.9815–1.000). The diagnostic sensitivity of the iELISA was 98.9% (95% CI, 93.84%–100.00%), which was higher than that of TAT (DSe 38.6; 95% CI, 29.14%–49.08%). The diagnostic specificity of the iELISA was 100.0% (95% CI, 95.82%–100.0%). When the 508 clinical samples were tested, the FljB iELISA had a positive detection rate of 51.38% (261/508, 95% CI, 51.24%–51.51%), which was higher than that of TAT (44/508).

Main limitations: The collection of serum samples does not cover the whole of China.

Conclusion: The iELISA developed in this study is rapid, sensitive, specific and repeatable, and is likely to be a suitable test for large-scale serological surveys for the detection and control of *S*. Abortusequi infection.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences.

Informed consent: Not stated.

Competing interests: None declared.

Funding: Nature Science Foundation of Heilongjiang Province, China (TD2022C006; LH2022C109), and the National Key Research and Development Program of China (No. 2021YFD1800500).

45 | Development and application of a competitive ELISA for the detection of antibodies against *Salmonella* Abortusequi in equids

K. Guo[1], W. Guo[1], D.Q. Liu[1], W.G. Zhang[1], Y. Yang[1], Z.N. Zhang[1], S.J. Li[1], J.H. Wang[1], X.Y. Chu[1], Y.X. Wang[1], Z. Hu[1]** and X.J. Wang[1,2,]**

[1]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China and* [2]*Institute of Western Agriculture, the Chinese Academy of Agricultural Sciences, Changji, China*

Email: huzher@126.com

Background: The high abortion rate associated with *Salmonella* Abortusequi infection in equids has re-emerged over the past ten years and has caused serious economic losses to China. Our previous studies showed that the flagellin FljB gene could distinguish *S*. Abortusequi from most *Salmonella* serotypes. No specific cELISA serological method capable of the identification of *S*. Abortusequi has been published to date.

Objectives: To develop a specific serological diagnostic method for *S*. Abortusequi.

Study design: Assay development and validation

Methods: The flagellin antigen was used to develop a cELISA that could be used in horse and donkey serum samples using a monoclonal antibody (Mab) specific for FljB. A cELISA was established using the purified MAb coating the plate, and incubation of the mixture of HRP-conjugated FljB with the undiluted serum sample. The performance of the cELISA assay and the tube agglutination test (TAT) assay was compared with respect to sensitivity and specificity, by testing a panel containing 660 *S*. Abortusequi positive and 515 negative serum samples, all of which had been characterized by Western blotting. ROC analyses were performed to determine the cut-off value and estimate the detection specificity and sensitivity.

Results: ROC analysis showed that the AUC values of cELISA (AUC = 0.9941; 95% CI, 0.9898–0.9984) were higher than that of TAT (AUC = 0.7705; 95% CI, 0.7437–0.7972). A cut-off value of 39.5% was selected with Sp and Se values of 100 (95% CI, 99.26–100.00) and 97.58 (95% CI, 96.10–98.50), respectively.

Main limitations: The sensitivity is lower than that of Western blot, and there are individual false negative results.

Conclusion: The cELISA has excellent futures compared with TAT, such as shortened detection time, no need to pre-treat sera, and easy interpretation of the results. It is thus more suitable for disease surveillance.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (approval no. 200812-01 and 211 116-05).

Informed consent: Not stated.

Competing interests: None declared.

Funding: The Nature Science Foundation of Heilongjiang Province, China (TD2022C006; LH2022C109), and the National Key Research and Development Program of China (No. 2021YFD1800500).

46 | Strangles outbreaks management with a new double antigen ELISA

A. Carpentier*[1], O. Mercier[1], R. Bonjour[1], K. Klewer[1], P. Pourquier[1], G. Fortier[2,3] and A. Leon-Seck[2,4]

[1]*Innovative Diagnostics—IDvet, 310 rue louis pasteur, 34 790 Grabels, France;* [2]*LABÉO, Research Department, -1 route de Rosel, 14 280 Saint-Contest, France;* [3]*Normandie University, UNICAEN, BIOTARGEN UR7450, 14 000 Caen, France and* [4]*Normandie University, UNICAEN, UNIROUEN, DYNAMICURE, UMR 1311, Inserm,14 000 Caen, France.*

Email: alix.carpentier@innovative-diagnostics.com

Background: Strangles is an equine bacterial infection caused by *Streptococcus equi* subs. *equi*. It affects the upper respiratory tract of horses and is characterised by abscessation of the lymph nodes. Due to its rapid spread, the presence of asymptomatic carriers and its ability to cause considerable economic damage, strangles represents a major health concern for equids. Serological tools are useful to confirm an outbreak after PCR testing, to assist in sanitary management, and to assess the risk of haemorrhagic purpura.

Objectives: To evaluate the performance of a new double antigen ELISA[1] for strangles.

Study design: Assay validation.

Methods: Positive, negative, and asymptomatic horses' samples collected between 2014 and 2023 were tested and correlated to PCR

and bacteriology results when available. Samples tested were from 5 origins (i) a first outbreak in Normandy included 22 horses, symptomatic or not that were sampled during clinical signs and two weeks later, (ii) a second outbreak in Lyon where 24 horses were sampled, (iii) 464 sera from a longitudinal study performed in France by LABÉO. For specificity assessment, 159 horses were sampled from Iceland where strangles has never been observed. For exclusivity evaluation, 8 horses from Southern France with respiratory symptoms associated with *S. equi* subsp. *zooepidemicus* positive PCR were sampled.

Results: Measured specificity of the test was 98.5% ($n = 159$, $CI_{95\%}$: 95.2%–99.5%). Individual detection rate among infected herds was 77% ($n = 74$, $CI_{95\%}$: 66.2%–85.1%). The ELISA was negative on samples from horses infected with *S. equi* subsp. *zooepidemicus*, meaning good exclusivity.

Main limitations: Since all sera were obtained from field infections, the exact date of infection was unknown.

Conclusions: Data indicate that the new IDvet ELISA test offers reliable diagnostic performance and is useful for sanitary management of strangles.

Key manufacturer: 1 Double antigen ELISA for strangles, IDvet, https://www.innovative-diagnostics.com.

Ethical animal research: The authors attest that ethics committee oversight is not required in their institute.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: AC, OM, RB, KK and PP are employees of IDVet.

Source(s) of funding: IDvet.

Parasitology 1: Cestodes

Lexington Tuesday 11.00–12.00

47 | Anoplocephalidae infections in horses in Italy

F. Buono[1], D. Piantedosi[1], E. Castaldo[1], S. Scarcelli[1], C. Roncoroni[2], F. Veronesi[3] and V. Veneziano[1]

[1]*Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy;* [2]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Roma, Italy and* [3]*Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia, Italy*

Email: francesco.buono@unina.it

Background: Equine tapeworms *Anoplocephala perfoliata*, *Anoplocephala magna* and *Paranoplocephala mamillana* have a worldwide distribution [1]. Although horses with a low infection rate do not show clinical signs, *A. perfoliata* can cause hyperemia, mucosal thickening, diphteric membranes and necrotic ulcers at the site of attachment [2].

Objectives: To evaluate the prevalence of *Anoplocephala* spp. in horses in Italy from 2014 to 2023.

Study design: Cross-sectional.

Methods: Individual coprological examinations were performed on 12 056 horses. Sex, age, body condition score (BCS), access to pasture, living area and sampling period were recorded. Analysis was performed using the Proudman technique with a Sheather's sugar solution.

Results: An overall prevalence of *Anoplocephala* spp. eggs were found in 452/12056 (3.7%) animals of which 256/452 were females (56.6%), followed by intact males (140/452–31.0%) and geldings (56/452, 12.4%). Further analysis showed 173/452 (38.3%) aged between 1 and 4 years, followed by <1 year (121/452–26.8%), between 5 and 15 years (120/452–26.5%), and >15 years (38/452–8.4%), 424/452 (93.8%) showed a BCS = 3, 402/452 horses (88.9%) had access to pasture. Moreover 141/452 (31.2%) were recorded in autumn, followed by 129/452 (28.5%) in winter, 111/452 (24.6%) in spring, and 71/452 (15.7%) in summer. Sex, access to pasture, age group, and living area were significantly associated with the infection ($p < 0.05$).

Main limitations: Difficulty in diagnosis of *Anoplocephala* due to discontinuous egg excretion.

Conclusion: The results of this large-scale study showed a low prevalence of *Anoplocephala* in Italian horses. It is crucial to identify positive animals (> 20 tapeworms) to perform cestodicide treatments to prevent clinical disease.

Ethical animal research: Research ethics committee oversight not required: retrospective analysis of clinical data.

Informed consent: Not stated.

Competing interests: None declared.

Funding: Partially funded by grants from the Ministry of Health of the Italian Republic (IZSLT 11/15 RC, IZSLT 9/18 RC) and research program regarding the "Horse Endoparasite and Anthelmintic Resistance (AHR) Control Program Guide" years 2019–2023.

References

[1] Nielsen MK. Equine tapeworm infections: Disease, diagnosis and control Equine Vet Educ. 2016; 28(7):388–395.

[2] Hedberg-Alm Y, Penell J, Riihimäki M, Osterman-Lind E, Nielsen MK, Tydén E. Parasite Occurrence and Parasite Management in Swedish Horses Presenting with Gastrointestinal Disease-A Case–Control Study. Animals 2020;10(4):638.

48 | Equine anticestodal treatment failure

M.K. Nielsen, C. Finnerty, M. Smith and N. Ripley

University of Kentucky, Veterinary Science, Gluck Equine Research Center, Lexington, Kentucky 40 546, USA

Email: martin.nielsen@uky.edu

Background: Over the past decades, reports of equine anthelmintic resistance have been restricted to strongylid, ascarid, and oxyurid parasites. However, veterinarians in the Central Kentucky area have been reporting concerns of anticestodal treatment failure in recent years.

Objectives: The objective was to investigate treatment failure claims for products containing praziquantel or pyrantel.

Study design: Longitudinal.

Methods: Six yearling and two mare cohorts present in different locations were monitored for the presence of anoplocephalid eggs in their

faecal samples pre and post administration of anticestodal products. The cohorts were treated with either pyrantel pamoate (13.2 mg base/kg) or praziquantel (1, 1.5, or 2.5 mg/kg depending on the product administered). Tapeworm faecal egg counts were determined at the day of treatment and again 14 days post administration. All horses testing positive for anoplocephalid egg counts pre- or post-treatment were included in the evaluation. Faecal egg counts were determined with either the OvaTector or Mini-FLOTAC techniques. Treatment efficacy was estimated using a Bayesian hierarchical model analysis of faecal egg count data with determination of model-estimated mean faecal egg count reductions (FECRs) and 90% Credible Intervals.

Results: One pyrantel and three different praziquantel products were evaluated and neither effectively eliminated anoplocephalid infection. In total, 24 yearlings were egg count positive before praziquantel treatment, and 27 were positive at 2 weeks post treatment. Similarly, 9 and 7 mares were positive pre and post praziquantel administration, respectively. Fourteen yearlings were positive both pre and post pyrantel pamoate administration. Pyrantel efficacy estimates ranged from 47 to 57% across the tested groups, whereas praziquantel efficacy ranged from 24% to 41%.

Main limitations: A standard testing procedure for determining anticestodal efficacy has not been developed, and commonly used faecal egg counting techniques suffer from low diagnostic sensitivities for tapeworm detection.

Conclusions: These data suggest complete anticestodal treatment failure.

Ethical animal research: Not required: clinical surveillance

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: None.

49 | Gastro-intestinal helminth infections in German horses with a focus on large strongyles, *Anoplocephala* spp. and cyathostomins using coproscopic, serological and molecular methods

J.S. Schmidt[1], J. Krücken[1], E. Bousquet[2], J. Bartz[3], C.J. Austin[4], K.L. Lightbody[4], J.B. Matthews[4], M.K. Nielsen[5] and G. von Samson-Himmelstjerna[1]

[1]*Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Robert-von- Ostertag-Str. 7, 14 163 Berlin, Germany;* [2]*Virbac, Virbac, 13e rue LID BP 27, Carros cedex 06511, France;* [3]*Virbac Tierarzneimittel GmbH, Rögen 20, 23 843 Bad Oldesloe, Germany;* [4]*Austin Davis Biologics Ltd, Unit 1 Denfield Lodge, Lower Street, Great Addington, Northants, NN14 4BL, UK and* [5]*M.H. Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA*

Email: jennifer.s.schmidt@fu-berlin.de

Background: Recent but regional studies in Germany reported high frequencies of infections with *Anoplocephala* spp. and *Strongylus* spp., which are both clinically relevant.

Objectives: Obtain new prevalence data for the most relevant equine gastro-intestinal helminths in several federal states of Germany.

Study design: Cross-sectional.

Methods: The study was conducted in six different German federal states with 970 horses from 96 stables. Faecal ($n = 970$), saliva ($n = 969$) and blood ($n = 957$) samples were analysed. Risk factors for the different infections were identified based on analysis of questionnaire data regarding, for example, husbandry, pasture management and deworming. The egg counts per gram faeces (EPG) were determined through the Mini-FLOTAC method for the respective worm eggs. Worm egg isolation, followed by DNA isolation was performed on farm level. To determine the prevalence of *Strongylus vulgar*is, a species-specific real-time PCR was performed. A high-resolution melting curve analysis was conducted for *Strongylus edentatus*, *Strongylus equinus* and *Strongylus asini*. For *S. vulgaris*, serological data were obtained[1] and antibodies against *Anoplocephala* spp. in saliva were determined[1].

Results: A prevalence at horse level of 47.4% (95% confidence interval (CI) 44.3%–49.8%) was found for the gastrointestinal strongyles with farm level prevalence of 95.8%. The prevalence at stable level was 10.4% for the large strongyles determined by real-time PCR, however only *S. vulgaris* and *S. edentatus* were found. Antibodies for *S. vulgaris* were detected in 23.3% of the horses and antibodies against *Anoplocephala* spp. in 19.4% (95% CI 17.3–21.7%) of the horses with a farm level prevalence of 38.5%.

Main limitations: Voluntary instead of random participation could have had an influence on the statistical presentation of the results. Sampling at different times of the year and geographical regions could have had an influence on the results, as egg excretion shows seasonality.

Conclusion: Frequent occurrence of large strongyle-DNA positive samples and very high seroprevalence of *Strongylus vulgaris* were encountered. The observed *Anoplocephala* spp. seroprevalence was much higher than the coproscopic results suggest. Strong regional differences were evident for the prevalence of strongyles and tapeworms.

Key manufacturer: 1 Equisal® Tapeworm test and Small Redworm Test are manufactured and sold by Austin Davis Biologics. https://www.austindavis.co.uk/.

Ethical animal research: Approved by Landesamt für Arbeitsschutz, Verbraucherschutz und Gesundheit Brandenburg, approval number Reg 2347-A-3-1-2021; Landesdirektion Sachsen, approval number Reg 25-5131/541/9; Regierung von Oberbayern, approval number Reg 55.2-2532.Vet_03-22-14; Landesamt für Natur, Umwelt und Vervraucherschutz Nordrhein-Westfalen, LANUV, approval number Reg 81-02.04.40.2022.VG015; Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, approval number Reg 33.19–42 502–04-22-00103; and Minesterium für Energiewende, Klimaschutz, Umwelt und Natur Schleswig Holstein, approval number Reg V244-45729/2022.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: JB is employed by Virbac Tierarzneimittel GmbH and EB by Virbac France. CJA reports an affiliation to Austin Davis Biologics and KLL and JBM are employed by Austin Davis Biologics. GvS-H has worked repeatedly as a consultant for different veterinary pharmaceutical and diagnostic companies. Other authors declare no competing interests.

Funding: The Freie Universität Berlin, Virbac Tierarztneimittel GmbH and Virbac France.

50 | Hide and caecum: looking for antimicrobials in the equine tapeworm *Anoplocephala perfoliata*

H.M. Northcote[1], B. Wititkornkul[2], P.M. Brophy[1], R.E. Wonfor[1] and R.M. Morphew[1]

[1]*Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Wales, SY23 3EE, UK;* [2]*Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, 50200, Thailand*

Email: hmn2@aber.ac.uk

Background: Gastrointestinal helminths have been observed to significantly change the host intestinal microbiome. One hypothesised mechanism for these changes is antimicrobial proteins/peptides (AMPs) released through secretory products (ESP), including extracellular vesicles (EVs). *Anoplocephala perfoliata* (*Aper*) has often been neglected in molecular research, however, recent generation of -omic datasets allows for greater investigation of host–parasite-microbiome relationships.

Objectives: To identify the potential antimicrobial nature of *Aper* EVs (*Aper*EV) using multi-omics and antimicrobial analysis.

Study design: In vitro experiments.

Methods: A comparative bioinformatic approach was utilised to identify potential AMPs within Aper EVs, Aper EV-free ESP (*Aper*ESP) and somatic proteome (*Aper*S). Peptides from each Aper fraction were extracted by acidic methanol treatment and identified via LC–MSMS and MASCOT. MS/MS Ion Search (MS/MS). *Aper* secretome antimicrobial activity was assessed following incubation with *Escherichia coli* and *Bacillus megaterium* and the optical density (OD) was monitored over 24 hours.

Results: In total, 34 potential AMPs were identified in the *Aper* transcript compared to 130, and 13 from a rumen fluke and a liver fluke respectively. Notably, 6, 8, and 4 were localised to the EVs. Additionally, 4 and 25 potential AMP IDs were resolved in the *Aper*S and *Aper*ESP, respectively. Furthermore, MS/MS identified 613 unique peptides from 246 proteins across each *Aper* fraction (<0.05). OD assays of *Aper* secretomes demonstrated no antimicrobial activity.

Main limitations: Limitations of this study involve limited molecular databases for *Aper* (i.e., genome) and a lack of antimicrobial analysis against physiologically relevant bacteria.

Conclusions: *Anoplocephala perfoliata* may have the ability to impact the surrounding microbial environment through AMPs. The initial lack of antimicrobial activity could suggest potential AMPs may not possess antimicrobial activity similar to helminth defence proteins. Greater investigation into the *Anoplocephala perfoliata* peptidome may provide a novel source of bioactive AMPs. Furthermore, further work to investigate activity against host-relevant bacteria and *in vitro* caecal models is required.

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Eleanor and David James scholarship, Aberystwyth University and grant funding (Welsh Government: Establishing Cutting Edge Veterinary Research Laboratories for Wales; European Development Regional Fund Sêr Cymru programme Grant—80 761-AU185) for the acquisition of the Ultimate 3000 RSL Nano with ES082 EASY-Spray Source.

Parasitology 2: Protozoans and Tick borne parasites

Lexington Tuesday 12.00–13.00

51 | Seroprevalence of intracellular tick-borne pathogens in horses in Austria

J. Pikalo[1], I. Stimac[1], A. Homann[1], S. Wilfinger[1], O. Rother[2], H.-P. Fuehrer[1], J.M. Cavalleri[2] and A. Joachim[1]

[1]*Institute of Parasitology, Department of Biological Sciences and Pathobiology, Center for Pathobiology, University of Veterinary Medicine, Vienna, Austria and* [2]*Clinical Center for Horses, Clinical Department for Small Animals and Horses, University of Veterinary Medicine, Vienna, Austria*

Email: jutta.pikalo@vetmeduni.ac.at

Background: Equine granulocytic anaplasmosis (EGA) and equine piroplasmosis (EP) are caused by tick-borne pathogens. The pathogens are the intracellular bacterium *Anaplasma phagocytophilum* and the intracellular protozoa *Babesia caballi* and *Theileria equi*. While EGA is considered endemic in Central Europe, EP is a sporadic disease in Switzerland, Austria, and Germany. It must be regarded as underdiagnosed, however, as horses persistently infected with *T. equi* are also repeatedly detected in Central Europe.[1]

Objectives: To fill the data gap on the current (sero)prevalence of these pathogens in Austria.

Study design: Cross-sectional.

Methods: Horses were convenience sampled by veterinarians throughout Austria after public announcement of the study Throughout Austria 350 horses were sampled. Commercial (c)ELISA tests[1,2] were used.

Results: Antibodies against *A. phagocytophilum* were detected in 192 horses (54.8%). In addition, 20 samples (5.7%) yielded questionable results. Of the 19 horses (4.99%) that tested positive for antibodies against piroplasms, ten (2.62%) had antibodies against *B. caballi* and six (1.57%) against *T. equi*. Three horses (0.79%) had antibodies against both piroplasms. Twelve horses showed antibodies against *A. phagocytophilum* and at least one equine piroplasmosis pathogen.

Main limitations: The subpopulation sampled so far is small and not evenly distributed over Austria according to the total horse population of the country.

Conclusions: The high rates suggest that these pathogens are apparently not only sporadically present in the horse population in Austria,

but regularly, and that recurrent/regular transmission and clinical cases occur.

Key manufacturers: 1 Euroimmun, Lübeck, Germany, https://www.euroimmun.com/; 2 VMRD, Pullman, USA, https://www.vmrd.com/.

Ethical animal research: Conducted in compliance with the Ethics Committee (EC) from the VetMedUni Vienna (GZ: #2022-0.343.848).

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: None.

Reference

[1] Joachim, A, Cavalleri, JV, Berger, S. Equine anaplasmosis and equine piroplasmosis in Germany, Austria, and Switzerland—previously anecdotal, now relevant? Schweiz Arch Tierheilkd 2022;164(1):35–50. 10.17236/sat00335/.

52 | Field detection of equid trypanosomiasis in a low resource setting with remote lab diagnostic support

A.G. Raftery[1], S. Jallow[2], K. Garcia[3], A. MacLeod[1], P. Capewell[1] and D.G.M. Sutton[1]

[1]School of Biodiversity, One Health and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bearsden Road, Glasgow, UK; [2]Gambia Horse and Donkey Trust, Sambel Kunda, Gambia and [3]Faculty of Veterinary Medicine, Veterinary Medical Sciences, University of Calgary, 3330 Hospital Drive NW, TRW2D09, Canada

Email: a.raftery1@research.gla.ac.uk

Background: Equid trypanosomiasis is widespread in lower income countries where access to sensitive diagnostic tools is limited. The condition has high morbidity and mortality but overuse of trypanocidals may result in resistance.

Objectives: In equids with naturally occurring trypanosomiasis (P), is a diagnostic algorithm based upon clinical examination plus packed cell volume (PCV) and microscopic evaluation of buffy coat (I) equivalent to PCR for *Trypanosoma* sp. on FTA cards (C) for diagnosis of trypanosomiasis (O)?

Study design: Cross sectional, observational, single gate design in Gambia.

Methods: All equids in selected villages were examined. For each equid a history, clinical examination and blood sample (PCV, buffy coat evaluation, FTA card for *Trypanosoma* sp. & Piroplasm PCR) were taken. The clinical diagnostic algorithm was: 2/4: (BCS≤1.5, anaemia, pyrexia, dull demeanour) and/ or parasitaemia on microscopy. Animals were treated according to algorithm results. Recent trypanocidal treatment led to exclusion.

Results: From 210 equids examined, 28% (58/210) were positive for ≥1 *Trypanosoma* sp. by PCR. Twenty-five percent (53/210) reached algorithm criteria for trypanocidal treatment. The clinical algorithm was compared to PCR, and sensitivity (26/58; 45%), specificity (125/152; 82%), positive predictive value (26/53; 49%) and negative predictive value (125/157; 80%) were calculated. Likelihood ratio was 2.5. Piroplasm status was not associated with specific clinical signs (103/210; 50%).

Main limitations: No follow up, PCR is an imperfect gold standard.

Conclusions: The algorithm was inferior to PCR but useful for identifying uninfected equids in a high challenge region. The algorithm identified that subclinical equine trypanosomiasis was common (32/58; 55%) and an uncharacterised disease was resulting in unnecessary trypanocidal use (27/53; 51%). This algorithm can aid in resource allocation in similar endemic regions. Further investigation is needed to determine if subclinical equine trypanosomiasis would become clinical with sequential use of the algorithm and to identify the uncharacterised disease.

Ethical animal research: Ethical approval for the study was provided by the research ethics and welfare committee, School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow and the Department of Livestock Services, Gambia.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: AGR is funded by the Vet Fund (University of Glasgow).

53 | Development of a comparative titration method to assess the sensitivity and specificity of antigens for the dourine complement fixation test

L. Hébert

ANSES, Laboratory for Animal Health Normandy site, Physiopathology and Epidemiology of Equine Diseases Unit (PhEED), 1180 route de l'Église 14 430 Goustranville, France

Email: laurent.hebert@anses.fr

Background: Dourine is an equine parasitic disease caused by *Trypanosoma equiperdum*. Thanks to eradication policies, dourine is currently present in only a few regions of the world. The test method recommended by the World Organization for Animal Health (WOAH) to control dourine during international movement of equids is the complement fixation test (CFT). The CFT specificity and sensitivity is based on antigens constituted by whole cell lysates of *T. equiperdum*. Due to the lack of biological reference material, the means of controlling the performance of these antigens are insufficient and generally only based on control sera from immunised horses.

Objectives: To develop procedures and reagents to improve the specificity and sensitivity of antigens produced for dourine CFT.

Study design: Assay development.

Methods: Using a positive serum from an experimentally infected horse and a serum from a horse likely to induce false-positive results, we developed a comparative titration method and evaluated the performance of different dourine CFT antigen batches. Antigen production and CFTs were performed according to the recommendations of the WOAH Terrestrial Manual with minor modifications and the comparative titration according to the method developed here.

Results: The comparative titration method using experimentally infected and false-positive sera has revealed that some antigen

batches lack specificity or sensitivity that were undetectable using only sera from immunised horses.

Main limitations: The number of positive sera from horses naturally infected with *T. equiperdum* is limited by the difficult access to field samples from endemic areas.

Conclusions: Validation and titration of a dourine CFT antigen batch using serum from immunised horses is not sufficient to guarantee the sensitivity and specificity of dourine CFT. Here, the comparative titration method improved the validation process of antigen batches, and thus the quality of sanitary control of dourine during international equine movements.

Ethical animal research: Approved by the Ethics Committee of Comité d'éthique en experimentation animale Val de Loire (DGRI agreement APAFIS#2015010908456425, the Ethics Committee ComEth Anses/ENVA/UPEC (protocol code 20/12/12–24, and ANSES/ENVA/UPEC's Animal Ethics Committee under DGRI agreement APAFIS#2019100111108827 ## 22 234.

Informed consent: Informed consent was given for inclusion of the privately owned horse.

Competing interests: None declared.

Funding: The European Commission (https://ec.europa.eu/info/index_en, accessed on 23 April 2024) through DG SANTÉ funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness, and ANSES internal research fund.

54 | Clinical and diagnostic features of equine protozoal myeloencephalitis cases with *Sarcocystis neurona* rtPCR-positive cerebrospinal fluid

M. Valdes Soto[1], J. Morrow[2], A. Graves[2] and S. DeNotta*[1]
[1]*University of Florida College of Veterinary Medicine, Gainesville, FL, USA;* [2]*Equine Diagnostic Solutions, LLC, Lexington, Kentucky, USA*
*Corresponding author email: s.denotta@ufl.edu
Email: valdessoto.m@ufl.edu

Background: Equine protozoal myeloencephalitis (EPM) is a common cause of neurologic disease in horses in the United States. Antemortem diagnosis of EPM relies on detection of antibodies against *Sarcocystis neurona* in serum and cerebrospinal fluid (CSF). The diagnostic utility of a recently described CSF rtPCR assay for EPM diagnosis remains to be elucidated.

Objectives: To describe the clinical and diagnostic features of horses with EPM and *S. neurona* rtPCR-positive CSF.

Study design: Retrospective medical record review with prospective survey-based study.

Methods: Medical records of horses evaluated for EPM via SnSAG2/4/3 ELISA serum: CSF titre ratios and *S. neurona* rtPCR between 2021 and 2023 were reviewed. Owners provided case follow-up information via telephone interview or online survey.

Results: Thirty-eight cases were identified, four of which were positive on CSF *S. neurona* rtPCR. All four rtPCR-positive horses presented with typical EPM clinical signs (asymmetrical ataxia and muscle atrophy). Two rtPCR-positive horses had serum / CSF titre ratios >100, and would have been deemed negative for EPM by serologic ratios alone. Two rtPCR-positive horses were retested after treatment, with one becoming negative and one remaining positive. In all four rtPCR-positive horses, rtPCR CT values were near positive cut-off values in all four positive horses, suggesting low concentrations of sample DNA. Confirmatory rtPCR testing in two additional laboratories failed to confirm positive rtPCR status.

Main limitations: Retrospective study. Low overall case numbers precluded statistical comparison between groups. Definitive EPM diagnoses via necropsy could only be performed in horses who died or were euthanised.

Conclusions: In this cohort, rtPCR testing identified cases of EPM that would have been diagnostically missed using the standard SnSAG2/4/3 ELISA serum: CSF titre ratios. DNA concentrations in positive cases were low, resulting in high CT values and potentially contributing to low assay sensitivity.

Ethical animal research: Authors declare no IACUC or other approval was needed in their institute.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: Jennifer Morrow and Amy Graves work at Equine Diagnostic Solutions (EDS), the laboratory at which study samples were processed. The *S. neurona* rtPCR in cerebrospinal fluids test is commercially available at EDS. No other authors declare a conflict of interest.

Funding: None.

Parasitology 3: Strongylids

Lexington Wednesday 11.00–13.00

55 | Phytochemicals' effect on cyathostomins' ecological composition

J. Malsa[1], A. Chereau[2], F. Guégnard[2], D. Serreau[2], A. Gesbert[3], F. Reigner[3], N. Mach[1], G. Fleurance[4,5], A.R. Williams[6#] and G. Sallé[2#]
[1]*INRAE, ENVT, Université de Toulouse, UMR 1225 Interactions Hôtes-Agents Pathogènes, Toulouse, France;* [2]*INRAE, Université de Tours, UMR 1282 Infectiologie et Santé Publique, Nouzilly, France;* [3]*INRAE, UEPAO, Nouzilly, France;* [4]*IFCE, Pôle développement, innovation et recherche, Saint-Genès-Champanelle, France;* [5]*INRAE, Université Clermont Auvergne, VetAgro Sup, UMR 1213 Herbivores, Saint-Genès-Champanelle, France and* [6]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark*
Email: joshua.malsa@inrae.fr

Background: The unregulated use of anthelminthic treatments has led to a rise in drug-resistant parasite isolates, necessitating the identification of new control strategies such as bioactive forages rich in secondary metabolites (phytochemicals). However, their use in the field raises questions about their effect on parasite ecological composition.

Objectives: To investigate the potential structural variations inside the cyathostomins community during *in vitro* exposure to pyrantel, carvacrol and cinnamaldehyde, and species with reduced sensitivity to these compounds.

Study design: *In vitro* experiments.

Methods: A larval development assay was performed in the presence of increasing concentrations of the phytochemicals carvacrol and cinnamaldehyde, before identifying living L3 larvae by metabarcoding. The assay was performed on strongyle eggs collected from faecal samples (same isolate) from experimental Welsh ponies from the INRAE experimental farm, purified and dropped in six replicates plates at 5000 eggs per wells. After 48 h incubation, each phytochemical was added to the L1/L2 larvae in a five concentrations' gradient and incubated for six days. The DNA from living L3 larvae was extracted, sequenced and analysed with R software.

Results: The alpha diversity decreased significantly from the third concentration of cinnamaldehyde (0.7 ± 0.15, mean ± SD); and for the highest concentrations of pyrantel (0.88 ± 0.19) and carvacrol (1.08 ± 0.17) compared to the control (≥ 1.33 ± 0.12, $P \leq 0.003$). Nevertheless, absolute abundances of *Coronocyclus labiatus*, *Cylicocyclus (Cyc.) ashworthi* and *Cyc. leptostomus* increased significantly at the highest concentration of pyrantel ($P \leq 0.02$). Similar results were observed for *Cyc. nassatus* ($P = 0.03$) and *Cyc. ashworthi* ($P = 0.01$) for carvacrol and cinnamaldehyde respectively.

Main limitations: These *in vitro* results are only an approximation of what would happen *in vivo*.

Conclusions: Two phytochemicals selectively reduced the development of cyathostomins species *in vitro*. In addition, they appear to promote the development of *Cyc. nassatus* and *Cyc. ashworthi*. The narrow spectrum of these two phytochemicals may reshape cyathostomins communities in the field, resulting in the selection of species less sensitive to pyrantel.

Ethical animal research: Approved by the French Ministry of Research under protocol number APAFIS #35631–202202281021526.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Institut Français du Cheval et de l'Equitation (IFCE), and the Institut Carnot France Futur Elevage (F2E).

56 | Understanding the relationship: Cyathostomin infections and host gut microbiota interactions in warmblood mares and foals in Germany

L.G. Klass[1,2], V.H.J. Diaz[3], S.K. Forslund[3], M. Fulde[4], L. Peachey[5], S. Andreotti[6], J. Krücken[1,2] and G. von Samson-Himmelstjerna[1,2]

[1]*Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Robert-von-Ostertag-Str. 7, 14 163 Berlin, Germany;* [2]*Veterinary Centre for Resistance Research, Freie Universität Berlin, Robert-von-Ostertag-Str. 8, 14 163 Berlin, Germany;* [3]*Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Robert-Rössle-Str. 10, 13 125 Berlin, Germany;* [4]*Institute of Microbiology and Epizootics, Freie Universität Berlin, Robert-von-Ostertag-Str. 7, 14 163 Berlin, Germany;* [5]*Bristol Veterinary School, Faculty of Health Sciences, University of Bristol, Langford House, Langford, BS40 5DU, Bristol, UK and* [6]*Bioinformatics Solutions Center, Freie Universität Berlin, Takustr. 9, 14 195 Berlin, Germany*

Email: grace.klass@fu-berlin.de

Background: Cyathostomins, a group of 50 morphologically recognised species, are the most prevalent gut parasites in horses. Larval cyathostominosis can be life-threatening, yet species involvement and pathogenesis are poorly understood. Studies have suggested links to host gut microbiota but current data is lacking.

Objectives: Obtain new data of individual cyathostomin species' prevalence and gut microbiota changes in connection to helminth infections in mares and foals in Germany.

Study design: Longitudinal cohort.

Methods: Individual fresh faecal samples ($N = 550$) and general husbandry data were collected from naturally exposed warmblood mares and foals ($N = 50$) in Germany in 2022. Egg counts per gram faeces (Epg) using Mini-FLOTAC, followed by egg isolation and cytochrome-c-oxidase-subunit-I-amplicon sequencing of individual strongyle-type egg positive samples were used to differentiate cyathostomin species. Host gut microbiota was analysed using full-length 16S sequencing of fresh faecal samples.

Results: Overall, 19.2% of foal samples and 8.5% of mare samples were strongyle-type-egg positive (Epg > 5). Co-infection with *Parascaris* spp. was common in foals (44.6%). Sequencing-based taxonomic differentiation showed *Cylicostephanus longibursatus* (28%), *Cylicostephanus minutus* OTUII (21%) and *Cyathostomum pateratum* (19%) were most common in mares, while *Coronocyclus coronatus* (28%) dominated in foals and *Cya. paternatum* was rare (0.5%). *Triodontophorus brevicauda* (3%) was only detected in foals. Large strongyles were not detected. Microbiota data showed good taxonomic resolution, with 96% of ASVs assigned to family-level and 50% genus-assigned. *Firmicutes*, *Bacteriodetes* and *Verrucomicrobiota* were the dominant phyla. Beta-diversity differed between mares and foals, but not sexes. Horses shedding helminth eggs did not show significant microbiota differences compared to non-shedding animals.

Main limitations: Low cyathostomin prevalence limited the power of findings. Recruitment of participants, instead of random sampling, could have biased results.

Conclusion: Helminth prevalence was lower than expected, likely due to dry weather and management practices. Cyathostomin species and gut microbiota composition differ significantly between mares and foals, but not between helminth egg-shedding and non-shedding individuals.

Ethical animal research: Not required: analysis of microorganisms.

Informed content: All animal owners gave consent for the data collection.

Competing interests: GvS-H has worked repeatedly as a consultant for different veterinary pharmaceutical and diagnostic companies.

Funding: German Research Foundation (Research Training Group 2046 "Parasite Infections: From Experimental Models to Natural Systems", Project C5-3, DFG Grant No. 251133687).

57 | First report of triple anthelmintic resistance in strongylids on a French Thoroughbred stud farm

A. Merlin[1,2], N. Larcher[1,2] and J-C. Vallé-Casuso[1,2]

[1]*ANSES, Laboratory for Animal Health in Normandy, Physiopathology and Epidemiology of Equine Diseases Unit, 14 430 Goustranville, France and* [2]*Mixed Technological Unit "Equine health and welfare—Organisation and traceability of the equine industry" (UMT SABOT), Goustranville, France*

Email: aurelie.merlin@anses.fr

Background: The frequent and indiscriminate use of anthelmintics (AH) has resulted in the development of AH resistance in strongylids against the licenced classes.

Objectives: To assess the anthelmintic resistance in strongylid nematodes against commonly used anthelmintic drugs in France.

Study design: Field surveillance.

Methods: The efficacy of fenbendazole[1], pyrantel[2] and ivermectin[3] was assessed in Thoroughbred yearlings kept on a French racehorse stud farm from March to December 2023. Faecal egg count reduction tests were conducted in three different groups of yearlings (a group of 6 males, a group of 13 females and a group of 8 females and 3 males) following the new World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines. The efficacy of fenbendazole was tested in two groups once during the monitoring period, the efficacy of ivermectin in 3 groups twice and the efficacy of pyrantel in one group once. For each faecal egg count reduction test, the 90% confidence interval (CI) of the percentage faecal egg count reduction was calculated using the hybrid Frequentist/Bayesian analysis method.

Results: The resistance in strongylids was observed to fenbendazole (upper 90% CI = [32.2%–36.4%] < expected efficacy of 99%), pyrantel (upper 90% CI = 96.6% < expected efficacy of 98%), and ivermectin (upper 90% CI = [88.3–94.8] < expected efficacy of 99.9%) in all the groups in which these drugs were tested. The number of animals in each group was sufficient to reach ≥80% power for the resistance test.

Main limitations: One farm and a small sample size.

Conclusions: This study provides the first evidence of triple resistance in strongylids to all three licenced classes of AH in France, and more specifically to three of the four molecules available.

Key manufacturers: 1 Panacur® (Intervet, Angers Technopole, Beaucouze, France): https://www.msd-sante-animale.fr/produits/panacur-pate-2/); 2 Eqvalan® (Boehringer Ingelheim Animal Health, Lyon, France): https://www.boehringer-ingelheim.com/fr/sante-animale/nos-produits/eqvalan-pate; 3 Strongid® (Zoetis, Malakoff, France): https://www2.zoetis.fr/

Ethical animal research: Not required: descriptive clinical report.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: French Agency for Food, Environmental and Occupational Health & Safety (Anses).

58 | Anthelmintic treatments and strongylid community: alterations in the species composition and parasite community structure in domestic horses

T.A. Kuzmina[1,2], A. Königová[1], L. Burcakova[1,3], V. Kharchenko[2] and M. Varadi[1]

[1]*Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Kosice 04001, Slovakia;* [2]*I. I. Schmalhausen Institute of Zoology NAS of Ukraine, B. Khmelnytsky street, 15, Kyiv, Ukraine and* [3]*University of Veterinary Medicine and Pharmacy in Kosice, Komenskeho 73, Kosice, 04181, Slovakia*

Email: takuzmina@gmail.com; taniak@izan.kiev.ua

Background: Anthelminthic drugs are widely used in horses.

Objectives: We estimated the alterations in the species composition and structure of strongylid communities in domestic horses influenced by use of anthelmintics.

Study design: Longitudinal field surveillance.

Methods: Fifty-five horses from five horse farms in Ukraine and Slovakia with different anthelmintic treatment patterns were enrolled in the study. Strongylid nematodes (93 749 specimens) were collected after horse deworming with macrocyclic lactones (ML). Comparative data collected from 22 horses in 2004 (9119 specimens) were included in the strongylid community analysis.

Results: Twenty-seven strongylid species were collected – 4 species of large strongylids and 23 of cyathostomins. In a farm with rare anthelmintic treatments, 26 species were found (4 large strongylids, 22 cyathostomins); in two farms with deworming >2–3 times/year, 9 or 12 cyathostomin species were found. In farms with 2 treatments/year, 19 or 21 species (two species of *Triodontophorus* spp. and cyathostomins) were detected. Regular horse treatment with MLs leads to a decrease in species diversity; *Strongylus* spp. and rare cyathostomin species disappeared first. The most prevalent and abundant species (10–12) survived massive use of anthelmintics and formed the strongylid community in regularly dewormed horses. Dominance of the most prevalent species (*Cylicocyclus nassatus*, *Cylicostephanus longibursatus*, *Cyathostomum catinatus*) dramatically increased after regular use of MLs; *C. nassatus* became a "super-dominant species" in the strongylid community (the Berger-Parker dominance index = 74.4). General structure of the strongylid community gradually transformed from multimodal (with dominant, subdominant, background, and rare species) to a bimodal (core–satellite mode).

Main limitations: Limited to five farms.

Conclusions: Regular anthelmintic treatments led to a decrease in species diversity, and an increase in dominance, and transformed strongylid community structure.

Ethical animal research: Not required: analysis of microorganisms.
Informed consent: Not stated.
Competing interests: None declared.
Funding: EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia, project No. 09I03-03-V01-00015.

59 | Circulating microRNAs associated with *Strongylus vulgaris* infection

K. Toft[1], M.L. Honoré[1], M.K. Nielsen[2]; L.N. Nielsen[1], P. Nejsum[3], B Fromm[4], S.M. Thamsborg[1], S. Cirera[5] and T.H. Pihl[1]
[1]*Department of Veterinary Clinical Sciences, University of Copenhagen, Taastrup 2620, Denmark;* [2]*Veterinary Science, University of Kentucky, Lexington, Kentucky, USA;* [3]*Department of Clinical Medicine, University of Aarhus Faculty of Health, Aarhus, Denmark;* [4]*UiT The Arctic University of Norway, Tromso, Norway and* [5]*Faculty of Health and Medical Sciences, University of Copenhagen, Kobenhavn 2200, Denmark*
Email: katrine.t.nielsen@live.dk

Background: The migratory lifecycle of *Strongylus vulgaris* poses a significant threat to horses. Current diagnostic methods are ineffective in detecting migrating life stages, necessitating new biomarkers for infection.
Objective: To investigate parasite- and horse-derived microRNAs (miRNAs) associated with *S. vulgaris* infection.
Study design: Exploratory study.
Methods: Blood samples were collected from naturally infected ($n = 27$) and uninfected ($n = 28$) horses. Arterial tissue samples were collected from the Cranial Mesenteric Artery (CMA) and Aorta of ten infected horses. Small-RNA sequencing was performed on a subset of plasma samples to identify miRNAs of interest. Selected horse and parasite-derived miRNAs were evaluated by qPCR in plasma from a larger horse cohort and horse-derived miRNAs were additionally evaluated in arterial tissues. Differential expression analysis was conducted for both sequencing and qPCR data. Diagnostic potential was assessed using Receiver Operator Characteristics (ROC) curve analysis.
Results: Small-RNA sequencing identified 462 horse-derived and 135 parasite-derived miRNAs in the plasma samples. In the sequencing data, 81 horse-derived miRNAs showed differential abundance when comparing infection groups, while parasite-derived miRNA showed no difference. qPCR validation resulted in 7 differentially abundant horse-derived miRNAs in plasma, while none of the selected parasite-derived miRNAs were detectable in plasma using qPCR. ROC analysis indicated that the miRNA Eca-Mir-140-3p had the best diagnostic potential, especially when adjusted for horse age (AUC: 0.78 and 0.96, respectively). Eight miRNAs displayed differential abundance in CMA tissue compared to aortic tissue, and again Eca-Mir-140-3p showed significant differential abundance.
Main limitations: The two infection groups compared in this study were located on different continents and managed differently, which can be confounding factors. The differentially abundant miRNAs should be further characterised and evaluated for associations with non-parasitic factors.
Conclusions: Changes in horse-derived miRNAs might be associated with *S. vulgaris* infection, warranting further investigation. Parasite-derived miRNAs were not detectable in plasma using qPCR.
Ethical animal research: Approved by the Institutional Animal Care and Use Committee at the University of Kentucky (protocol no. 2012-1046); the Danish animal experimental council (approval no. 2018-15-0201-01480 and 2022-15-0201-01210) and by the ethical board of the University of Copenhagen Large Animal Teaching Hospital; and the committee for animal experimentation in Lund, Sweden (approval no. 5.8.18–02993/2022).
Informed consent: Owner consent was obtained for all included patients.
Competing interests: None declared.
Funding: Independent Research Fund Denmark (case number 0136-00101B), the Danish Horse Levy Foundation, University of Copenhagen's Graduate School, the Denmark America Foundation, the Hartmann Foundation, the William Demant Foundation and the Sveland Foundation. Materials for sample collection and processing were received from E-vet, Scanvet and Eickemeyer.

60 | Do simple surveillance data inform the anthelmintic resistance debate? UK horse fecal worm egg count results (2012–2023)

F. Whitlock and J.R. Newton
Equine Infectious Disease Surveillance (EIDS), Department of Veterinary Medicine, University of Cambridge, Cambridge, UK.
Email: fw371@cam.ac.uk

Background: Anthelmintic resistance threatens effective equine parasite control globally. Surveillance data should help inform and monitor progress with anthelmintic resistance mitigation strategies. Understanding laboratory faecal worm egg count testing (FWECT) methods and positivity thresholds (FWECT-thresholds) are necessary for accurate clinical decision-making and inferring from surveillance data. Since 2005, quarterly data summaries from FWECT performed by a network of UK veterinary diagnostic laboratories have been compiled in surveillance reports.
Objectives: Assess strongyle FWECT methods and positivity thresholds used by UK laboratories. Investigate factors associated with quarterly laboratory FWECT positivity rates for the period 2012–2023.
Study design: Laboratory survey and analysis of quarterly laboratory surveillance report submissions.
Methods: A FWECT methods and thresholds survey was issued to 28 surveillance reporting laboratories. Percentages of strongyle FWEC tests reported positive (PTP) each quarter by individual laboratories between 2012 and 2023 were analysed using multiple mixed-effects linear regression analysis, including laboratory-level random-effects and fixed-effects variables for ordered categories of FWECT-thresholds, year quarters and groups of three consecutive years.

Results: Responses were returned from 13/28 laboratories and demonstrated variable FWECT methods and strongyle positivity thresholds, with samples commonly but not exclusively reported positive at >50 and >200 eggs per gram (epg). Regression modelling of 892 quarterly laboratory surveillance report submissions of PTP confirmed significantly decreased PTP with increasing FWECT-thresholds relative to baseline (>50 epg): −16% with >200 epg ($P = 0.04$) and −47% with >300 epg ($P = 0.002$). Overall, controlling for between-laboratory variation, quarter and FWECT-thresholds, there remained evidence for a significant gradient in increasing PTP over the study period relative to baseline (2012–2014): +4.1% 2015–2017 ($P = 0.007$), +4.5% 2018–2020 ($P = 0.004$) and +8.3% 2021–2023.

Main limitations: Limited survey responses, with some laboratories' FWECT-thresholds unspecified.

Conclusions: Controlling for laboratories, year quarters and FWECT-thresholds, there was strong residual evidence from FWECT summary data for increasing strongyle burdens in UK horses between 2012 and 2023.

Ethical animal research: Not required: review of laboratory data.

Informed consent: Not stated.

Competing interests: None declared.

Funding: Department for Environment, Food and Rural Affairs (Defra), British Equine Veterinary Association, Horserace Betting Levy Board, Thoroughbred Breeders' Association, Racehorse Owners Association and International Thoroughbred Breeders' Federation.

61 | Farm size and biosecurity measures associated with *Strongylus vulgaris* infection in horses

Y. Hedberg Alm, E. Tydén, F. Martin, J. Lernå and P. Halvarsson
Department of Animal Biosciences, Parasitology Unit, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden
Email: ylva.hedberg.alm@slu.se

Background: Selective anthelmintic treatment, advocated due to evolving anthelmintic resistance, has been associated with an increase in *Strongylus vulgaris* prevalence. Reverting to routine interval anthelmintic treatments is not viable and therefore, identifying other management factors correlated with *S. vulgaris* infection is vital.

Objectives: To investigate possible risk factors associated with the presence of *S. vulgaris* infection in residing horses on Swedish horse establishments.

Study design: Internet-based questionnaire survey.

Methods: A questionnaire, created using the internet-based survey platform Netigate, was distributed to owners of equine establishments throughout Sweden via established equine platforms and social media channels. The survey was available for response from 21 May until 1 September 2022. Questions were closed ended with branching logic paths.

Results: Four factors were significantly associated with *S. vulgaris* infection, with an increased odds of infection seen in livery yards (OR 1.67, 95% CI 1.18–2.36, $p = 0.004$) and premises with more than ten residing horses (OR 2.42, 95% CI 1.64–3.56, $p < 0.001$). A lower odds of infection was seen in establishments using quarantine routines (OR 0.69, 95% CI 0.50–0.96, $p = 0.03$) and anthelmintic treatment of new horses prior to arrival at the premise (OR 0.37, 95% CI 0.18–0.74, $p = 0.005$).

Main limitations: In this study, due to the presence of *S. vulgaris* infection being based on *S. vulgaris* diagnostics performed at the farm level, any association between faecal diagnostic use and risk of infection could not be investigated.

Conclusions: Although the use of diagnostics for *S. vulgaris* can keep infection rates low, large farms or livery yards with many different horse owners, and those with low use of biosecurity measures applied to new horses arriving at the premises, are associated with a higher risk of infection.

Ethical animal research: The authors attest that ethics committee oversight is not required in their institute.

Informed consent: Survey participation is taken as consent.

Competing interests: None declared.

Funding: Swedish and Norwegian Foundation for Equine Research, grant number H-15-47-097.

62 | Gastrointestinal strongyles and ascarids: A nationwide risk factor study

C. Carta[1], P. Zeinoun[1], C. Tamponi[1], L. Cavallo[1], A. Varcasia[1], A. Scala[1] and M.K. Nielsen[2]
[1]*Laboratory of Parasitology, Veterinary Teaching Hospital, Department of Veterinary Medicine, University of Sassari, Sassari, Italy and* [2]*Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA*
Email: c.carta1@studenti.uniss.it

Background: High levels of anthelmintic resistance in equine strongylid and ascarid parasites prompts implementation of diagnostic surveillance and a better understanding of factors affecting parasite infection dynamics.

Objectives: To analyse strongylid and ascarid test results determined over a 10-year period and identify factors associated with faecal egg count (FEC) magnitude in equine establishments across Italy.

Study design: Field survey.

Methods: Samples collected by veterinarians across Italy were sent to our laboratory along with completed questionnaires for analysis and data cataloguing. Between 2015 and 2024, 25 028 equine faecal samples were collected and analysed from 1427 equine operations located across Italy. FECs were determined with a McMaster method. Farm and horse data were recorded. Mixed linear models were developed to analyse factors associated with strongylid and ascarid egg shedding.

Results: Overall, strongylid and ascarid prevalence was 45.7% and 6.4%, respectively. Horses in central Italy had significantly higher strongylid FECs compared to both southern and northern regions ($p = 0.02$ and $p < 0.001$). Female horses showed higher strongylid FECs than males ($p = 0.004$). Moreover, the study highlighted the

impact of housing conditions on infestation levels, with pastured horses demonstrating higher strongylid FECs than those kept in paddocks or stables ($p < 0.001$). Horses recently treated with macrocyclic lactones had lower FECs than those treated with pyrantel ($p = 0.0454$). Stabled horses had significantly lower ascarid FECs than those in paddocks ($p = 0.008$), and younger horses (aged 1–4 years) showed higher ascarid FECs than older ones ($p = 0.01$).

Main limitations: Some data entries had missing information, and the number of horses included and samples submitted varied between years.

Conclusions: These findings emphasise the need for targeted parasite management strategies that consider geographic factors, sex variations, housing conditions, and age demographics among equine populations in Italy.

Ethical animal research: Not required: retrospective analysis of clinical samples and data.

Informed consent: Not stated.

Competing interests: None declared.

Funding: Partially funded by "Fondo di Ateneo per la ricerca 2019" of Prof. Antonio Scala, of the University of Sassari, Italy.

Parasitology 4: Piroplasmosis

Lexington Thursday 09.00–10.30

63 | Rapid screening testing for equine piroplasmosis using a fluorescence flow cytometry haematology analyser

A. Ochi[1], Y. Toya[2], M. Sengoku[2], S. Tsuchiya[2], D.Kishi and, T. Ueno[1]
[1]*Equine Research Institute, Japan Racing Association, Tochigi, Japan and* [2]*Sysmex Corporation, Hyogo, Japan*
Email: aki_ochi@equinst.go.jp

Background: Equine piroplasmosis (EP) is a protozoal disease of equids that is caused by *Theileria equi* and *Babesia caballi*. Rapid and accurate methods that are suitable for detecting these parasites in the field are crucial for controlling and preventing EP. Diagnosis is conventionally performed by microscopic, molecular, and serological methods. These methods are time-consuming, so faster testing methods are needed to manage EP effectively. We have reported an *in vitro* study showing that the automated fluorescence flow cytometry haematology analyser[1] detects EP-infected red blood cells (RBCs) in approximately 1 min (under review).

Objectives: To evaluate the performance of the automated haematology analyser *in vivo*.

Study design: *In vivo* experiments and field testing.

Methods: XN-31 was evaluated by using blood samples from experimentally EP-infected horses. Two horses were inoculated with *T. equi* (USDA strain; 4.6×10^9 infected RBCs/head) or *B. caballi* (USDA strain; 1.6×10^9 infected RBCs/head). Horses were observed daily for clinical signs. Blood samples were collected daily for 10 days following inoculation and examined by using haematology analyser and PCR. Next, to assess the performance of the haematology analyser in the field, we examined 120 competition horses on site during the 2020 Tokyo Olympic Games.

Results: The experimentally EP-infected horses were positive by PCR at 1 or 2 days post-infection (dpi) and by the haematology analyser at 2 or 3 dpi. In the field testing during the Olympic Games, two acute EP cases were suspected using the haematology analyser and then confirmed by PCR. These horses had been negative for EP upon pre-export testing. In addition, the monitoring of infected horses using the haematology analyser detected a decrease in parasitaemia.

Main limitations: the haematology analyser is a diagnostic instrument for human malaria and was modified to detect EP-infected RBCs. Only two horses were infected experimentally.

Conclusion: Given that the fluorescence flow cytometry haematology analyser's results were confirmed by PCR and available in 1 min, it shows promise as a method to screen for EP.

Key manufacturer: 1 Fluorescence flow cytometry haematology analyser XN-31, Sysmex, Japan. https://www.sysmex.co.uk/products/diagnostics/haematology/xn-31-malaria-diagnostics/.

Acknowledgments: Part of this study was performed at the 2020 Tokyo Olympic Games. We thank Dr. Takashi Yamanaka, a director of biosecurity at the 2020 Tokyo Olympic Games, for his help and scientific comments.

Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute of the Japan Racing Association.

Informed consent: The owners, the representatives of the national teams, and the Japan Olympic committee gave informed consent.

Competing interests: Yuji Toya, Mikako Sengoku and Seiichito Tsuchiya are employees of Sysmex which produces the XN-31 haematology analyser.

Funding: Japan Racing Association.

64 | The seroprevalence of equine piroplasmosis in Argentine horses and comparative evaluation of diagnostic assays for its detection

B. Ahedor[1], T. Sivakumar[1], N.M. Mumbi Ngigi[1], M. Sol Colombo[2], G.J. Polledo[2], M.L. Giorgi[2], L. Hébert[3], N. Yokoyama[1] and T. Becú[2]
[1]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan;* [2]*Clinica Equina SRL, Alem 1698—Capitan Sarmiento, Buenos Aires, Argentina and* [3]*ANSES, Normandy Laboratory for Animal Health, Physiopathology and Epidemiology of Equine Diseases Unit, 14 430 Goustranville, France*
Email: tbecu@clinicaequina.com.ar

Background: Equine piroplasmosis (EP) is a disease caused by *Theileria equi* and *Babesia caballi* in horses. The availability of epidemiological data is important for devising EP control strategies.

Objectives: To assess the seroprevalence of *T. equi* and *B. caballi* in Argentine horses using different sero-diagnostic assays.

Study design: Cross-sectional.

Methods: This study used sera obtained from horses in El Chaco ($n = 149$) and Buenos Aires ($n = 414$) during clinical examination, pre-export testing, or routine breeding procedures. Horses from El Chaco were tested for *T. equi* and *B. caballi* antibodies using the indirect immunofluorescence antibody test (IFAT), competitive ELISA (cELISA), and complement fixation test (CFT), while horses from Buenos Aires were screened using IFAT and cELISA.

Results: In El Chaco, *T. equi* and *B. caballi* were detected using IFAT in 97 (65.1%) and 3 (2%), cELISA in 105 (70.5%) and 3 (2%), and CFT in 63 (42.3%) and 0 (0.0%) horses, respectively. In Buenos Aires, IFAT detected *T. equi* in 15 (3.6%) and *B. caballi* in 17 (4.1%) horses, while cELISA found them in 10 (2.4%) and 2 (0.5%) horses, respectively.

Main limitations: This study did not include all provinces. Past and ongoing infections could not be differentiated.

Conclusions: The higher EP-positive rate in El Chaco compared with Buenos Aires underscores the importance of regulating horse movement between provinces to avoid disease outbreaks. Lower positive rates by CFT, compared to those by IFAT and cELISA, highlight its unsuitability for EP diagnosis. In both provinces, the rates of *T. equi* from IFAT and cELISA were comparable, but IFAT had a higher *B. caballi*-positive rate than cELISA in Buenos Aires. This indicates the importance of using IFAT to detect *B. caballi* in Argentina. Our findings suggest that EP control strategies in Argentina should consider its differential distribution among regions, determined through active surveillance with suitable diagnostic assays.

Ethical animal research: Not required: excess material from clinical samples was used.

Informed consent: Not stated.

Competing interests: None declared.

Funding: The European Commission (https://ec.europa.eu/info/index_en, accessed on 19 April 2024) through DG SANTÉ funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness, internal research fund from ANSES, and the Japan Society for the Promotion of Science (KAKENHI 22KK0095).

65 | Correlation between clinical and laboratory parameters and outcome in horses with piroplasmosis

H. Badenhorst[1] and E.C. Schliewert[1,2]

[1]*Department of Companion Animal Clinical Studies, Faculty of Veterinary Medicine, University of Pretoria, South Africa and* [2]*School of Biodiversity, One Health and Veterinary Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow, UK*

Email: tine.schliewert@web.de

Background: Equine piroplasmosis, an intraerythrocytic protozoal disease of equids, causes major losses in endemic countries. Infection with either *Theileria equi*, *Babesia caballi* or both parasites causes haemolysis. Clinical signs of acute disease include pyrexia, icterus, and petechia. There is little published information about the clinical signs and prognosis in horses with acute piroplasmosis.

Objective: To describe the clinical and laboratory findings in horses which presented with equine piroplasmosis, and to investigate the correlation between the clinical and laboratory findings and survival.

Study design: Retrospective case series.

Methods: Clinical and laboratory findings and treatment of horses presented to the Onderstepoort Veterinary Academic Hospital, South Africa from 01/2013 to 06/2020 were analysed and correlation with outcome determined using Pearson's correlation coefficient.

Results: Fifty-two horses with acute piroplasmosis confirmed on blood smear (45/52) and/or PCR (13/52) were included. On admission, mean temperature was 38.7°C (SD ± 1.1°C), mean heart rate, 61 beats/minute (SD ±14), and median respiratory rate, 27 breaths/minute (IQR 18–39). Mucous membranes were pale pink in 24 horses and icteric in 28 horses. Haematocrit ranged from 9% to 37% (median 20.5%; IQR 15–27.5). Treatment included antiprotozoal drugs (51/52 horses [49/52 imidocarb, 1/52 diminazene aceturate, 1/52 both]), anticholinergic drugs (42/52 [24/52 hyoscine butylbromide, 14/52 glycopyrrolate, 4/52 both]), corticosteroids (32/52), intravenous fluid therapy (34/52) and blood transfusion (20/52). Duration of hospitalisation ranged from 2 to 22 days (median, 5; IQR, 4–7). Three horses did not survive to discharge. No correlation between any clinical or laboratory variables and duration of hospitalisation or survival to discharge was found.

Main limitations: Small number of cases, retrospective study.

Conclusion: Tachycardia, tachypnea, pyrexia and pronounced anaemia are common in acute piroplasmosis. Regardless of clinical signs, most horses survive if appropriate therapy is implemented.

Ethical animal research: University of Pretoria Research and Animal Ethics approval number REC 41–20.

Informed consent: Not stated.

Competing interests: None declared.

Funding: None.

66 | Diagnostic performance of a rapid immunochromatographic test for the simultaneous detection of antibodies to *Theileria equi* and *Babesia caballi* in horses and donkeys

F. Jongejan[1,6*], C. Du[2*], E. Papadopoulos[3], V. Blanda[4], S. Di Bella[4], V. Cannella[4], A. Guercio[4], D. Vicari[4], S. Tirosh-Levy[5], A. Steinman[5], G. Baneth[5], S. van Keulen[6], I. Hulsebos[6], L. Berger[6] and X. Wang[2]

[1]*Vectors and Vector-Borne Diseases Research Programme, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, Republic of South Africa;* [2]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China;* [3]*Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, University Campus, Thessaloniki, Greece;* [4]*Istituto Zooprofilattico Sperimentale della Sicilia "A.Mirri", Palermo, Sicily, Italy;* [5]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, 7 610 001 Rehovot, Israel and* [6]*TBD International BV,*

BioScience Center, Wagenin- gen University & Research, Runderweg 6, 8219 PK Lelystad, The Netherlands
*These authors contributed equally to this work.
Email: ducheng@caas.cn

Background: Equine piroplasmosis is caused by two tick-borne protozoan parasites, *Theileria equi* and *Babesia caballi*, which are clinically relevant in susceptible horses, donkeys, and mules. Moreover, equine piroplasmosis significantly constrains international horse trade and equestrian events. Rapidly diagnosing both parasites in carrier animals is essential to implement effective control measures.
Objectives: In this study, we developed a card to detect antibodies against *T. equi* and *B. caballi* based on two colloidal gold immunochromatographic strips.
Study design: A test for the simultaneous detection of antibodies to *T. equi* and *B. caballi* was evaluated using samples from horses and donkeys collected in Greece, Israel, and Italy.
Methods: Blood samples were collected from 255 horses and donkeys. The panel consisted of 129 horses sampled at four locations in northern Greece, 105 donkeys sampled at four locations in Sicily, and 21 horses sampled at two locations in Israel. The immunochromographic test and the cELISA were performed according to the manufacturer's instructions, and the results were subjected to statistical analysis to determine the sensitivity and specificity of both tests.
Results: The immunochromatographic test provided a result within 15 min detecting both pathogens simultaneously. The overall coincidence rate between the rapid test and the cELISA for detecting antibodies against *T. equi* was 93% and 92.9% for *B. caballi*. The agreement for *T. equi* detection using both tests was high in Greece (93.8%) and Italy (95.2%) and moderate in Israel (76.2%).
Main limitations: The immunochromographic test can also be helpful for the differential diagnosis of clinical cases, since seropositivity may rule out equine piroplasmosis since it does not indicate current or active infection.
Conclusions: The rapid immunochromographic test detected *T. equi* and *B. caballi* simultaneously in the field, potentially replacing the laborious cELISA and is thus recommended for import/export purposes.
Ethical animal research: Approved by the Internal Research Review Committee of the Koret School of Veterinary Medicine Veterinary Teaching Hospital (KSVM-VTH/23_2014).
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: None declared.
Funding: None.

67 | Equine neonatal piroplasmosis: Challenges in clinical-diagnostic and therapeutic work up

I. Imposimato[1], C. Castagnetti[1], F. Freccero[1,2], A. Lanci[1], V. Facile[1], L. Urbani[1], F.M. Dini[1], R. Galuppi[1], A. Balboni[1] and J. Mariella[1]

[1]*Università degli Studi Bologna, Dipartimento di Scienze Mediche Veterinaire, Ozzano dell'Emilia, Emilia-Romagne, Italy and*
[2]*University of Helsinki, Faculty of Veterinary Medicine, Helsinki, Finland*
Email: ilaria.imposimato2@unibo.it

Background: Data available about clinical-diagnostic and therapeutic management of neonatal equine piroplasmosis (EP) are limited. Vertical transmission of EP is underestimated, mainly in endemic areas.[1]
Objectives: To describe the clinical-diagnostic and therapeutic workup of foals with hyperacute EP infection in an endemic area in northern Italy.
Study design: Retrospective case series.
Methods: Three foals (0–30 days) were referred at the Equine Perinatology Unit (University of Bologna, Italy) with jaundice and pyrexia. Anaemia, hyperbilirubinemia and uraemia were revealed by haematochemistry; liver abnormalities and decreased gastrointestinal (GI) motility by ultrasound. Since EP was suspected, foals with their dams were tested by 18S rRNA PCR assays[2] and the amplicons sequenced. Four genetically different piroplasms were detected. Two foals were infected by *Babesia caballi;* one of the dams was negative, the other one was coinfected with a piroplasm similar to the *Theileria haneyi*-like species reported in China and *B. caballi*. One foal was infected by *T. equi* and its dam by a genetically different *Theileria* sp. Foals were treated with NSAIDs, 2 with oxytetracycline 8 mg/kg q12h IV diluted in NaCl 0.9% and 1 with doxycycline 10 mg/kg q12h PO. Clinical condition deteriorated in all foals, requiring intensive care and blood transfusions. Based on clinical judgment. Imidocarb dipropionate (ID) was administered intramuscularly 2.2–4 mg/kg, two to four administration q24/72 h. All cases manifested gastrointestinal side effects and responded to IV scopolamine 0.3 mg/kg and fluid therapy.
Results: Foals were discharged following a normal clinical examination after 8, 10 and 50 days of hospitalisation.
Main limitations: Low case number.
Conclusions: For a successful outcome neonatal EP should be promptly managed with careful attention. Laboratory tests detecting all equine piroplasms should be used because different species of *Theileria* were detected for the first-time in Italy.
Ethical animal research: Not required: retrospective case series.
Informed consent: Owners gave consent for their animals' inclusion
Competing interests: None declared.
Funding: None.

References

[1] Bartolome del Pino LE, Meana A, Zii M, Cersini A. Evidence of transplacental transmission of equine piroplasms *Theileria equi* and *Babesia caballi* in an Italian breed mare. Folio Parasitologica 2023; 70: 005. DOI:10.14411/fp.2023.005
[2] Alhassan A, Pumindonming W, Okamura M, Hirata H, Battsetseg B, Fujisaki K, Yokoama N, Igarashi I. Development of a single-round and multiplex PCR method for the simultaneous detection of *Babesia caballi* and *Babesia equi* in horse blood Veterinary Parasitology 2005;129:43–49. doi: 10.1016/j.vetpar.2004.12.018.

68 | Development of a real-time quantitative PCR based on a TaqMan-MGB probe for the rapid detection of *T. haneyi*

B. Zhou[1*], K. Chen[1*], Y. Chen[1], C. Du[1**] and X. Wang[1,2**]
[1]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China and* [2]*Institute of Western Agriculture, the Chinese Academy of Agricultural Sciences, Changji, China*
Email: ducheng@caas.cn

Background: Equine piroplasmosis (EP) is a tick-borne disease caused by three apicomplexan protozoan parasites, *Theileria equi (T. equi)*, *Babesia caballi (B. caballi)* and *Theileria haneyi (T. haneyi)*. This disease is considered to be reportable by the World Organization for Animal Health (WOAH).

Objectives: To develop a new real-time quantitative PCR (qPCR) to detect *T. haneyi*.

Study design: Assay development and validation.

Methods: A TaqMan MGB probe was used in the development of the qPCR assay. The chr1sco (chromosome 1 single-copy open reading frame (ORF)) gene, which has no detectable orthologs in *T. equi* or *B. caballi* was selected and a qPCR method was developed based on the chr1sco gene. First a plasmid containing the chr1sco gene was constructed and used to establish the standard curves. This qPCR was further validated by comparison with an optimized nested PCR (nPCR) assay in the analysis of clinical samples.

Results: The novel qPCR technique demonstrated high specificity for detecting additional frequent equine infectious pathogens and sensitivity for detecting diluted standard plasmids. The agreement between nPCR assay and the newly established qPCR assay for *T. haneyi* was 85.42%.

Main limitations: The *T. haneyi* specific qPCR produced false-negative results, because the chr1sco gene of *T. haneyi* is a single-copy, which mean that samples with low pathogenic load might show high Ct values.

Conclusions: The novel *T. haneyi* specific qPCR showed high sensitivity and specificity by comparison with an optimised nPCR assay and thus could contribute to the accurate diagnosis of *T. haneyi* infections in horses.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (SYXK [Hei] 2020-009).

Informed consent: Not stated.

Competing interests: None declared.

Funding: The National Key Research and Development Program of China (grant number 2022YFD1800200) and the Natural Science Foundation of Heilongjiang Province (Team Program, grant number TD2022C006).

Virology 1: Influenza

Michel D'Ornano Tuesday 09.00–10.30

69 | Detection of equine influenza virus genes in the air around infected horses

M. Nemoto, Y. Kambayashi, H. Bannai, T. Yamanaka and K. Tsujimura
Equine Research Institute, Japan Racing Association, Tochigi, Japan
Email: nemoto_manabu@equinst.go.jp

Background: Equine influenza virus (EIV) can spread quickly among horses by airborne infection. However, to our best knowledge, there are no reports of EIV recovery from the air around infected horses.

Objectives: To evaluate whether EIV can be recovered from the air in the stalls of infected horses by using an air sampler.

Study design: Experimental challenge study.

Methods: Two horses without antibodies were experimentally infected with A/equine/Ibaraki/1/2007. Horses were kept in individual stalls during the study. Air was collected daily in the stalls by using an air sampler[1] for 13 days post-infection (dpi). Nasopharyngeal swabs were also collected from the horses daily. Viral genes in the air and nasopharyngeal swabs were detected by real-time reverse transcription – polymerase chain reaction. Virus titration was conducted by using 10- or 11-day-old embryonated chicken eggs.

Results: Viral genes were detected in air samples and nasopharyngeal swabs from both horses. The copy numbers of viral genes peaked at 2 or 3 dpi in air samples and at 1 dpi in swabs. Although live viruses were isolated in swabs from the two horses at 1–6 or 7 dpi, no live viruses were isolated from air samples. The average copy numbers in swabs were higher than those in air samples at 1–10 dpi, but the copy numbers were reversed at 11–13 dpi. This discrepancy suggests that the air sampler had collected viral genes derived from environmental surfaces in the stalls, rather than those immediately shed by the horses.

Main limitation: Infectious virus could not be isolated from the air by the air sampler.

Conclusions: This study confirms that horses infected with EIV release virus into the air. Air sampling may be useful to detect infected horses without the need for invasive sampling.

Key manufacturer: 1 AerosolSense Sampler, Thermo Fisher Scientific. https://www.fishersci.com/shop/products/aerosolsense-airborne-pathogen-detection-solution/2900AA.

Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute with accession number 22–23.

Informed consent: Not applicable.

Competing interests: The AerosolSense Sampler was lent by Thermo Fisher Scientific. The authors declare no other conflicts of interest.

Funding: Japan Racing Association.

70 | New generation EIV virus like particle (VLP) vaccine

S. Reedy[1], M.M. O'Kennedy[2,5], A. Khan[1], E. Olajide[1], A. Cullinane[3], J. Daly[4], C. Abolnik[5] and T.M. Chambers[1]
[1]*University of Kentucky, Department of Veterinary Science, USA;* [2]*CSIR Advanced Chemicals & Life Sciences, South Africa;* [3]*Irish Equine Centre, Kildare, Ireland;* [4]*University of Nottingham, UK and* [5]*Department of Production Animal Studies, University of Pretoria, South Africa*
Email: sereed0@uky.edu

Background: Conventional equine influenza (EI) vaccines are widely used, but a need exists for a platform that facilitates prompt manufacturing of a highly immunogenic, antigenically matched, updated vaccine product. Virus-like particles (VLPs) are protein shells that resemble virions but lack the viral genome and are non-infectious. Plant-produced VLPs have been used successfully against other viruses including human and avian influenzas.

Objective: To conduct a pilot safety/dose response study of a plant produced bivalent VLP vaccine[1] expressing the hemagglutinin proteins of Florida clade (FC) 1 (A/equine/Tipperary/1/2019) and 2 (A/equine/Wexford/2014) viruses in 1:1 ratio.

Study design: The dose response study was conducted using four antigen levels (0, 250, 500, 1000 HAU/dose component), with three EIV seronegative weanling horses per group.

Methods: All horses were co-pastured. Vaccines were administered on Days 0 and 28. Horses were observed daily and systemic and site reactions were recorded on day (D) 0, 1, 2, 3, and 7 each time. Sera were collected weekly for hemagglutination inhibition test using FC1 and FC2 viruses. All horses were challenged with 5×10^7 EID_{50} of aerosolised[2] FC1 virus (A/equine/KY/14) on D56. Horses were observed daily for clinical signs. Nasopharyngeal swabs were collected to quantify viral RNA using qPCR and infectious virus by titration in embryonated hens' eggs.

Results: Vaccine adverse reactions were unremarkable. All vaccinated groups seroconverted. Post-challenge, all vaccinated horses had minor clinical scores and were negative for viral replication. Control horses did not seroconvert until post challenge, had significant clinical scores and detectable virus replication. Vaccinated horses had sporadic positive qPCR results D2–6, but were otherwise negative, whereas control horses were positive from D2 to 7.

Main limitations: Small group sizes. Further testing would be needed for licensure and marketing.

Conclusion: The VLP vaccines were safe and effective in this natural host influenza challenge model.

Key manufacturers: 1 The commercial adjuvant Montanide (Seppic, France; https://www.seppic.com/en/montanide-gel)was used in the production of this vaccine; 2 Flexineb E2 mask (Flexineb® North America, USA; https://www.flexineb.us/flexineb-nebulizer).

Ethical animal research: Approved by the IACUC (Chambers Protocol # 2022–4028, approved March 2022) at the University of Kentucky. R&D license agreements were in place to use the pEAQ-Hypertrans expression vector from Leaf Expression Systems (LES, UK) and the *N. benthaminan* glycosylation mutant ΔXT/FT (University of Natural Resources and Life Sciences, BOKU, Vienna) plants. The vaccine was produced with approval from CSIR Research Ethics Committee approval reference number 311/2020 and South Africa Department of Agriculture, Land Reform and Rural Development (DALRRD) Section 20 reference number 12/11/1/1/12 (1470).

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Grayson-Jockey Club Research Foundation.

71 | Antigenic and molecular characterisation of viruses responsible for outbreaks of equine influenza (2021–2024)

M. Garvey, L. Dayot, R. Gallagher, G. Lukaseviciute and A. Cullinane*
Virology Unit, The Irish Equine Centre, Johnstown, Naas, County Kildare, Ireland
Email: mgarvey@irishequinecentre.ie
*Corresponding author: ACullinane@irishequinecentre.ie

Background: Genetic evolution and antigenic drift of equine influenza (EI) virus in addition to vaccine efficacy is continually monitored by the World Organization for Animal Health (WOAH) reference laboratories and the Expert Surveillance Panel (ESP).

Objectives: Genetic and antigenic analysis of representative EI viruses (2021–2024).

Study design: Cross-sectional.

Methods: A pan-reactive influenza type A real-time RT-PCR was used for confirmatory diagnosis and sequencing of the haemagglutinin (HA) and neuraminidase (NA) genes performed when Ct < 30. Phylogenetic analyses were inferred using maximum likelihood. Virus isolation of representative EI viruses was carried out in embryonated eggs, for antigenic characterisation by haemagglutination inhibition (HI) using specific ferret antisera and virus neutralisation with equine antisera.

Results: Since 2021, fourteen outbreaks (2021:7, 2022:4, 2023:2, and 2024:1) of EI were confirmed on 13 premises in Ireland. Disease affected two mixed, one Thoroughbred (TB) and 10 non-TB premises with mixed ($n = 10$), vaccinated ($n = 1$), unvaccinated ($n = 1$) and unknown ($n = 1$) vaccination status. Sequencing of viruses (2021:5, 2022:3, 2023:1) from eight premises identified them as belonging to clade 1 of the H3N8 Florida sub-lineage (FC1) and closely related to viruses recently circulating in Europe and North America. Alignment of the predicted amino acid sequence of HA showed there are between 14 and 17 amino acid differences between these FC1 strains and the FC1 WOAH recommended vaccine strain A/eq/South Africa/4/03 of which 12 have been fixed for many years. Antigenic characterisation of three representative viruses indicated a close relatedness to A/eq/South Africa/4/03. Experiments with sera from vaccinated sport horses indicated that antibodies against the current vaccine strains neutralise recently circulating FC1 strains.

Main limitations: Some viruses could not be isolated and/or sequenced due to low viral load.

Conclusions: These experiments provided evidence to support the concept that the current WOAH recommendations for influenza vaccine composition do not require modification and are fit for purpose.

Acknowledgements: The staff in the Virology Unit at the Irish Equine Centre for diagnostic testing of samples.

Ethical animal research: Not required: retrospective clinical description and analysis of microorganisms

Informed consent: Not stated.

Competing Interests None declared.

Funding: Department of Agriculture, Food and the Marine, Ireland.

72 | Whole genome sequencing of equine influenza virus to enhance surveillance in the United Kingdom

J. Lyall[1], F. Whitlock[1], E. Drinkwater[1], A. Rash[2], L. Walker-Panse[1], D. Elton[2], R. Newton[1] and N.A. Bryant[1]

[1]*Department of Veterinary Medicine, University of Cambridge, Cambridge, UK and* [2]*Animal Health Trust, Newmarket, Suffolk, UK*

Email: jel1000@cam.ac.uk

Background: The control of H3N8 equine influenza virus (EIV) by vaccination depends on the antigenic similarity of vaccine strains to currently circulating viruses. Vaccine failure can result in increased spread, morbidity, cancellation of equine sporting events and associated economic loss to the industry. An industry funded EIV surveillance programme continues to operate in the UK.

Objectives: To monitor the genetic and antigenic changes occurring in EIV circulating in the UK to identify evidence of antigenic drift or shift potentially causing vaccine breakdown in the field.

Study design: Prospective molecular surveillance.

Methods: UK equine veterinary practices collect and submit respiratory samples for laboratory testing from horses with suspected EIV. Samples were tested for EIV by qRT-PCR, with positive samples analysed by whole genome sequencing. Phylogenetic analyses were completed for all eight viral segments to identify nucleotide changes and signs of genetic reassortment. Amino acid sequences were compared to current WOAH-recommended vaccine strains. HI assays provided information on continued vaccine effectiveness to novel viruses.

Results: Whole genome sequences were obtained from 88 viral swab samples representative of the period 1963 to 2024. All viruses circulating in the UK since 2018 were classified as H3N8 Florida Clade 1 (FC1). Although no reassortments for any segments were seen amongst these, a gradual accumulation of amino acid changes in all FC1 segments continues to be observed up to 2024.

Main limitations: EIV surveillance relies on owners reporting clinical signs and getting their horses tested and positive samples being submitted for sequencing, meaning only a proportion of outbreaks contribute to surveillance. A lack of coordinated European wide surveillance reduces the effectiveness of individual countries' efforts in tracking emerging EIV variants.

Conclusions: The ongoing accumulation of amino acid changes in all FC1 viral segments reinforces the importance of continued surveillance of EIV to inform vaccine strain selection.

Ethical animal research: Not required: excess material from clinical samples was used.

Informed consent: Not stated.

Competing interests: None declared.

Funding: Horserace Betting Levy Board.

73 | Evaluation of a triplex assay for detecting equine influenza virus and equine herpesvirus types 1 and 4 by using a microfluidic-chip-based mobile real-time PCR device for point-of-care testing

K. Tsujimura, T. Yamanaka, Y. Kinoshita, M. Nemoto, H. Bannai, Y. Kambayashi, N. Kawanishi and M. Ohta

Equine Research Institute, Japan Racing Association

Email: kotsuji@equinst.go.jp

Background: Point-of-care testing (POCT) methods for equine respiratory viruses include immunochromatography for equine influenza virus (EIV) and loop-mediated isothermal amplification for equine herpesvirus types 1 and 4 (EHV-1 and -4). However, highly sensitive real-time PCR methods are difficult to implement in clinical practice.

Objective: To evaluate the specificity and sensitivity of a triplex assay for EIV and EHV-1 and -4 by using a microfluidic-chip-based mobile real-time PCR device[1] (PicoGene, GoFoton, Inc.).

Study design: Assay evaluation.

Methods: Published primers and probes were used for the assay. Three MGB probes were labelled at the 3′-end with NED (EIV), FAM (EHV-1). or Cy5 (EHV-4). Detection of viral genes was automatically determined when the fluorescence signal exceeded a threshold. Assay specificity was tested by analysing isolated strains of EIV, EHV-1, EHV-4 and 10 non-target equine respiratory pathogens. Detection of 10 or 1 copies of each positive control nucleic acid was performed five times to determine the assay sensitivity. Detection until post-infection-day 14 of the viral genes in archived nasal samples collected from horses experimentally inoculated with EIV, EHV-1, or EHV-4 was compared between our assay and each individual real-time PCR assay performed on a conventional instrument.

Results: The assay discriminated between EIV, EHV-1, and EHV-4 and did not detect other pathogens. Positive controls for all target viruses were 100% detectable at 10 copies. Detection rates of the one copy of the positive controls were, for EIV, 40%; for EHV-1, 0%; and for EHV-4, 100%. Detection of viral genes in experimental samples was consistent between the triplex assays on the mobile device and the individual assays on the conventional instrument.

Main limitation: Field samples were not examined.

Conclusion: The mobile real-time PCR device is highly sensitive in POCT for EIV, EHV-1, and EHV-4 in clinical practice.

Key manufacturer: 1 Microfluidic-chip-based mobile real-time PCR device from PicoGene, GoFoton, Inc., https://www.gofoton.com/

Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute (approval numbers 17–21 and 20–20).
Informed consent: Not applicable.
Competing interests: None declared.
Funding: Japan Racing Association.

74 | Combining genomic and epidemiological data to track the spread of equine influenza

L. Mojsiejczuk[1], F. Whitlock[1,2], H. Chen[1], C. Magill[1], E. Aranday-Cortes[1], J. Bone[1], N. Bryant[2], J.R. Newton[2], T. Chambers[3], S. Reedy[3], M. Nemoto[4], T. Yamanaka[4], J. Hughes[1] and P.R Murcia[1]
[1]*Centre for Virus Research, Institute of Infection & Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK;* [2]*Department of Veterinary Medicine, University of Cambridge, Cambridge, UK;* [3]*Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, Kentucky, USA and* [4]*Equine Research Institute, Japan Racing Association, Shimotsuke, Tochigi, Japan*
Email: laura.mojsiejczuk@glasgow.ac.uk

Background: Equine Influenza virus (EIV) is the aetiological agent of a major respiratory disease in horses, is highly contagious and associated with significant economic losses to the horse industry. In late 2018, an EIV of the Florida Clade 1 (FC1) lineage was introduced from North America to Europe, triggering one of the largest epizootics recorded in the UK, and replacing the former endemic FC2 lineage.
Objectives: To characterise the evolutionary dynamics during a nationwide equine influenza (EI) epizootic and analyse international spread patterns of EIV.
Study design: Molecular epidemiology and phylogeographic study.
Methods: EIV complete genomes for over half of the UK outbreaks were sequenced and analysed with existing genomic and ecological information in a multi-step phylodynamic approach.
Results: The findings reveal a single EIV introduction from North America to Europe in mid-October 2018, indicating that the virus circulated for ~8 weeks before outbreaks in France in mid-December raised the alert. At least eight independent introductions from Europe to the UK were identified, with only three clusters spreading and persisting for several months. At the sub-national scale, the East, West-Midlands, and North-West England were the main viral sources during the epizootic. The number of affected premises in a region and the number of horses in the premises area were key predictors of subsequent within-country viral spread. Phylogeographic analysis evidenced a source-sink model for intercontinental EIV migration, with a source population evolving in North America and directly or indirectly seeding lineages into sink populations in other continents.
Main limitations: Limited availability of complete genome sequences for both UK and international outbreaks, potentially limiting the extent of our analyses.
Conclusions: The results suggest that the interplay between virus evolution, host immunity, population structure and spatial ecology shape epizootics, providing new information to design effective measures to control EI.
Ethical animal research: Not required; retrospective analysis of surveillance data.
Informed consent: Not stated.
Competing interests: None declared.
Funding: BBSRC grant BB/V002821/1.

Virology 2: African horse sickness and other viruses

Lexington Tuesday 09.00–10.30

75 | African horse sickness causes disseminated intravascular coagulopathy

E.-C. Schliewert[1,2], E.H Hooijberg[1], C. Potgieter[3] and A. Goddard[1]
[1]*Department of Companion Animal Clinical Studies, Faculty of Veterinary Medicine, University of Pretoria, South Africa;* [2]*School of Biodiversity, One Health and Veterinary Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow, UK and* [3]*Human Metabolomics, Faculty of Natural and Agricultural Sciences, North-West University, Potchefstroom, South Africa*
Email: tine.schliewert@web.de

Background: African Horse Sickness (AHS), a vector-borne disease endemic to sub-Saharan Africa, is caused by African Horse Sickness Virus (AHVS). In naïve horses infections have high morbidity and mortality rates. The pathogenesis of AHS is poorly understood, but endothelial cell damage and loss of endothelial cell barrier function results in oedema, effusion and haemorrhage. Virus or viral antigen is found in microvascular endothelial cells.
Objective: To evaluate haemostatic changes in horses with experimental AHSV infection.
Study design: Prospective, longitudinal, experimental study.
Methods: Four horses negative for AHSV antibodies were infected with AHSV. Blood was obtained before inoculation, then q24h until euthanasia. Haemostasis tests included thromboelastography (TEG), fibrinogen, prothrombin time, activated partial thromboplastin time, D-dimers, antithrombin-III, coagulation factors II, VIII, VIII, X, and XII and thrombocyte concentration. Tissue samples (lung, heart, spleen) were examined microscopically. Due to the small numbers, changes are described based on visual inspection.
Results: All horses were humanely euthanised due to severe clinical signs typical of AHS. TEG analysis was consistent with development of disseminated intravascular coagulation (DIC) in all horses at the time of euthanasia. Severe coagulopathy was also observed in classical coagulation testing: an increase in prothrombin time, activated partial thromboplastin time, D-dimer concentrations and decreases in the activity of coagulation factors II, VII, X, and XII at the time of euthanasia. Fibrinogen, plasminogen activity and plasminogen inhibitor activity remained unchanged. All horses developed severe thrombocytopenia. Histopathology revealed no evidence of microthrombi in either tissue.

Main limitation: Small number of cases.
Conclusion: Horses infected with AHSV develop consumptive coagulopathy with haemorrhagic tendencies consistent with DIC. The main reason for this is likely the vascular damage caused by viral replication within the endothelial cells. The vascular changes together with the severe DIC likely result in multiorgan dysfunction resulting in multiorgan failure and contribute to the high mortality of AHS.
Ethical animal research: University of Pretoria Animal and Research Ethics Committee Approval REC 0195-19.
Informed consent: Not applicable.
Competing interests: None declared.
Funding: None.

76 | Safety and immunogenicity of a sarcoid vaccine in horses

O. Jacob[1], B. Hause[2], K. Peters-Smith[2], E.N. Adam[3], A.E. Page[1], C. Floyd[1], C. Tucker[1], D. Wang[4] and F. Li[4]
[1]University of Kentucky, Maxwell H Gluck Equine Research Center, 1400 Nicholasville Road, Lexington, Kentucky, USA; [2]Cambridge Technologies, Worthington, Minnesota, USA; [3]University of Kentucky, Veterinary Science, Lexington, Kentucky, USA and [4]University of Kentucky, College of Agriculture Food and Environment, Veterinary Science, Lexington, Kentucky, USA
Email: ocja224@uky.edu

Background: Equine sarcoids are the most common skin tumour of horses. They are caused largely by bovine papillomavirus genotype 1 or 2. Sarcoids can interfere with the horses' health, comfort, and use. They are difficult to treat and can result in significant economic challenges. There is no vaccine available to protect equines from sarcoids in the USA.
Objective: To determine the safety and protective antibody response in horses immunised with a recombinant baculovirus vector vaccine expressing the L1 protein of bovine papillomavirus.
Study design: Vaccine trial in horses.
Methods: Baculovirus expressing L1 protein culture fluids were inactivated and formulated with CA50, a proprietary adjuvant. A group of 10 clinically healthy, sarcoid-free horses was immunised intramuscularly with 1 mL of vaccine at 3-week intervals. A control group of 5 clinically normal horses was immunised with adjuvant plus culture medium. Physiological parameters were monitored daily. Serum samples were collected from all animals pre-vaccination and at intervals post-vaccination. The vaccine trial ended at 12 weeks after the second immunisation.
Results: All horses appeared normal clinically throughout the study with no clinically relevant adverse reactions to vaccination observed. A GFP-expressing pseudo-virus based virus-neutralising antibody assay detected a robust neutralising antibody response in all immunised horses following two doses. Pre-vaccination serum from the vaccination group and sera from the control group had no detectable virus-neutralising antibodies. A total of 6 out of 10 vaccinated horses had neutralising antibody titres ≥1280.
Main limitations: Group size was limited and a challenge study was not conducted.
Conclusions: Immunisation with bovine papillomavirus L1 antigen produced in baculovirus stimulated neutralising antibody titres in all horses. Neutralising antibodies are an established correlate of protection for papillomaviruses. As such we anticipate this vaccine may protect horses from sarcoids.
Ethical animal research: Reviewed at University of Kentucky under IACUC protocol.
Informed consent: Not applicable.
Competing interests: Dr B. Hause and Dr K. Peters-Smith are employed by the commercial laboratory that developed the vaccine. Other authors have declared no competing interests.
Funding: William Robert Mills Endowment; Cambridge Technologies.

77 | Safety and immunogenicity of African horse sickness Disabled Infectious Single Animal (DISA)-DIVA vaccines in horses

D. Craig[1], S. Joseph[2], R.G.P. van Gennip[3], P.A. van Rijn[3], C. Hebel[2] and U. Wernery[2]
[1]Emirates Racing Authority, Dubai, United Arab Emirates; [2]Central Veterinary Research Laboratory (CVRL), Dubai, United Arab Emirates; [3]Wageningen Bioveterinary Research (WBVR), Lelystad, the Netherlands
Email: david.craig@emiratesracing.com

Background: African Horse Sickness (AHS) is a severe disease of equids (WOAH-notifiable disease, category A) caused by nine serotypes of AHS-virus (AHSV) showing limited cross-neutralisation. A safe, efficacious, broadly protective DIVA-vaccine for AHS is a prerequisite to control AHS and to improve the safety of international equine trade. Promising AHS Disabled Infectious Single Animal (DISA) DIVA vaccines for all nine serotypes, shortly named DISA1to DISA9, became available for *in vivo* studies. A DISA-DIVA cocktail vaccine (DISA1-9) was successfully studied in the artificial IFNAR −/− mouse model.
Objective: To evaluate safety and immunogenicity of novel vaccines.
Study design: Vaccine trial.
Methods: Ten horses were intramuscularly immunised twice at a 4-week interval with DISA1-9 containing equal amounts of each DISA vaccine. Horses were immunised again after one year. Horses were intensively monitored post immunisation for clinical signs, adverse effects and for viremia by PCR-testing, while antibodies were monitored throughout the experiment.
Results: Horses did not show any adverse clinical signs after immunisations and remained PCR-negative. ELISA-positivity was observed in four horses as early as two weeks post prime-immunisation. One week after booster immunisation, all horses showed maximal blocking which declined very slowly but remained ELISA-positive (mean blocking of 63%) after one year. Blocking was maximal again after annual booster immunisation. Neutralising antibody titres varied between individual horses and serotypes, but were positive for all serotypes for all horses except one at eight weeks post boost immunisation.

Antibody titres also declined but then increased after annual booster immunisation.

Conclusions: DISA1 to DISA9 in cocktail vaccine DISA1-9 are completely safe and induce neutralizing antibodies against all nine AHSV serotypes suggesting broad protection. Hence, DISA1-9 should now be studied for protection by vaccination-challenge trials in horses.

Ethical animal research: Approved by the Ethical Committee of the Ministry of Climate Change and Environment (MOCCAE) in Dubai, UAE.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: Central Veterinary Research Laboratory, Dubai.

78 | Equine immune response after vaccination with a cocktail AHS vaccine DISA1-9 in a field trial: a real-world challenge

C. Hebel[1], D. Craig[2], S. Joseph[1], R.G.P. van Gennip[3], P.A. van Rijn[3] and U. Wernery[1]

[1]*Central Veterinary Research Laboratory (CVRL), Dubai, United Arab Emirates;* [2]*Emirates Racing Authority, Dubai, United Arab Emirates and* [3]*Wageningen Bioveterinary Research (WBVR), Lelystad, the Netherlands*

Email: christiana@cvrl.ae

Background: African Horse Sickness (AHS) is a highly contagious and deadly disease threatening equids (WOAH-notifiable disease, category A). Current challenges include nine serotypes with limited cross-protection. The development of nine DISA (Disabled Infectious Single Animal) vaccines, DISA1 to DISA9, offering serotype specific protection and broad protection with a cocktail vaccine (DISA1-9) will pave the way for potential effective control of AHS and safer horse trade.

Objective: Assess the safety and immune response of DISA1-9 in horses in a field trial in Kenya.

Study design: Field trial.

Methods: 28 horses (group 1) which had not received a prior AHS vaccine and were negative by ELISA (<50%), received two intramuscular doses of a DISA1-9 four weeks apart. They were monitored for side effects and illness after vaccination, as well as antibody development. A further 143 horses (group 2) which had received an inactivated AHS vaccine in previous years and were positive in ELISA, were injected with one intramuscular DISA 1–9 dose.

Results: No side effects were reported after vaccination in both groups. Group 1 horses developed antibodies within four weeks, which increased by 79% after the booster. Group 2 also responded with an increase of antibodies against the virus compared with pre-DISA 1–9 vaccination levels (12%). One horse in group 2, despite having high antibodies from the inactivated AHS vaccine, as well as a booster of DISA1-9 vaccine, was infected with AHSV-Serotype 9, developed AHS fever, but survived.

Main limitations: No virus neutralisation tests (VNT) were performed.

Conclusions: The field trial with AHS DISA1-9 vaccine yielded promising results. The vaccine demonstrated safety and induced an immune response with increased antibodies in both previously vaccinated and unvaccinated horses, suggesting broad protection. Revaccination of previously vaccinated horses induced increased seroconversion. However, an important consideration is the seasonal presence of AHS-transmitting vectors. with an increased pressure during certain periods.

Ethical animal research: Approved the Ethical Committee under the Ministry of Climate Change and Environment (MOCCAE) in Dubai, UAE and the Ethical Committee of the Supervision of the District Organization of Laikipia/ Meru County Kenya.

Informed consent: Owners gave consent for their animals' inclusion

Competing interests: None declared.

Funding: None.

79 | Genomic variation and receptor specificity of the equine adenoviruses

C.A. Hartley, P.K. Vaz, A.B. Mekonnen, J.R. Gilkerson and J.M. Devlin

Centre for Equine Infectious Disease and Asia Pacific Centre for Animal Health, Melbourne Veterinary School, The University of Melbourne, Victoria 3010, Australia

Email: carolah@unimelb.edu.au

Background: Two equine adenovirus species within the *Mastadenovirus* genus are known. Each show different tropisms *in vivo*, where equine adenovirus-1 (EAdV1) is associated with respiratory infections and equine adenovirus-2 (EAdV2) with gastrointestinal infections. Equine adenovirus-1 is sporadically detected in surveys of horses with respiratory disease. Experimental infection of horses with EAdV1 shows respiratory disease severity is influenced by age and immune status.

Objectives: To better understand genomic variation and species specificity of EAdV1 and EAdV2.

Study design: In vitro experiments.

Methods: To determine the full genome sequences of a panel of EAdV1 isolates to compare whole genome and gene specific heterogeneity. The *in vitro* host species specificity and receptor specificity of EAdV1 and EAdV2 strains were also compared using a panel of equine and non-equine cell types *in vitro*. The full genome sequence of 8 EAdV1 isolates was determined using the Illumina NovaSeq 600 platform. Competition infection assays compared receptor specificity of EAdV1 and EAdV2.

Results: The full genomes of 8 EAdV1 isolates showed 98.5%–100% nucleotide pairwise identity, with the most heterogeneity in the hexon gene sequence (88.5%–100%), while the receptor-binding fibre protein had a more stable gene sequence (99.1%–100%). The nucleotide pairwise identity to EAdV2 hexon gene ranged from 65.7% to 67%. The gene encoding the fibre protein was very different between species with EAdV1 almost twice the size of EAdV2 (2508 and 1422 nucleotides respectively). Competition

assays revealed EAdV1 and EAdV2 use separate receptors for virus entry.

Main limitations: Limited EAdV2 isolates to characterise.

Conclusion: The EAdV1 genome is reasonably stable across the 8 isolates characterised, with higher levels of variation in the hexon gene. Viruses such as these, with DNA genomes that can be easily manipulated and with viral species showing distinct tropisms, may have potential as vaccine vectors in equids and other species.

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Special Virology Fund; Centre for Equine Infectious Diseases; Asia Pacific Centre for Animal Health.

80 | Clinical study of potential factors influencing the histological score of pulmonary fibrosis in horses with interstitial fibrosing lung disease

I. Desjardins[1], E. Lauteri[1], C. Normand[2,3], A. Tortereau[1], E. Hue[2,3] and S. Pronost[2,3]

[1]*VetAgro sup, Université de Lyon, 69 280 Marcy l'Etoile, France;* [2]*LABÉO, 14 280 Saint-Contest, France and* [3]*Normandie Université, UNICAEN, BIOTARGEN, 14280 Saint-Contest, France*

Email: isabelle.desjardins@vetagro.sup.fr

Background: Pulmonary fibrosis (PF) is a tissue repair mechanism that can be abnormally dysregulated by ageing, predisposing environmental factors and microbes. It is poorly documented in horses. An association between PF disorders and γ-herpesvirus infection has been established in several species.

Objectives: This study aims to establish a histological fibrosis score (HFS) system on lung histological sections and to assess the potential relationship with age, EHV-5 viral load in lung tissue and clinical outcome.

Study design: Retrospective clinical study.

Methods: Clinical information from horses presented for respiratory distress and interstitial lung diseases (ILD) was recorded. Antemortem transthoracic lung biopsies or post-mortem pulmonary samples from horses with severe generalised interstitial radiographic patterns, and previous histological identification of replacement of alveoli by fibrosis and collagen were selected. The severity of PF was assessed blindly using a previously described histologic scoring (/8) [1]. EHV-5 qPCR (Ct) was performed on lung tissue. Shapiro–Wilk, Fisher and Spearman correlation tests were used to analyse the data.

Results: Twenty-five cases were included. Median age was 14-yo (4–29-yo). EHV-5 was detected in 12 of 25 horses (48%). HFS ranged from 1 to 6/8 (median 4/8) and was not different between EHV-5-or negative groups ($p = 0.7$). Eighteen out of 25 horses (72%) and 12/25 (50%) with PF survived to discharge and 3 months, respectively, with no statistical difference between EHV-5 positive and negative horses ($p = 0.7$). HFS was moderately negatively correlated to short-term survival ($r = -0.55$, $p = 0.005$) but not survival at 3 months ($r = -0.37$, $p = 0.07$). HFS was not statistically correlated to age ($r = -0.15$, $p = 0.4$), nor Ct ($r = -0.07$, $p = 0.7$).

Main limitations: Small number of cases, retrospective nature of the analysis.

Conclusions: EHV- 5 is a common cause of PF in horses. Age, EHV-5 viral load, and severity of PF do not seem to influence survival rate of equines affected by fibrosing ILD.

Ethical animal research: Approved by VetAgro sup Ethic Committee (n°2040).

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: None.

Reference:

[1] Hübner RH, Gitter W, El Mokhtari NE, Mathiak M, Both M, Bolte H, Freitag-Wolf S, Bewig B. Standardized quantification of pulmonary fibrosis in histological samples. Biotechniques. 2008;44 (4):507–511. doi 10.2144/000112729.

Virology 3: Coronavirus, Rotavirus and Arteritis virus

Michel D'Ornano Tuesday 15.00–17.30

81 | Equine coronavirus infection and replication in equine intestinal enteroids

Y. Kambayashi [1], T. Suzuki [2], M. Nemoto [1], A. Ochi [1], D. Kishi [1], T. Ueno[1], H. Bannai [1], N. Kawanishi [1] and K. Tsujimura [1]

[1]*Equine Research Institute, Japan Racing Association, 1400–4 Shiba, Shimotsuke, Tochigi, Japan;* [2]*Division of Pathology and Pathophysiology, Hokkaido Research Station, National Institute of Animal Health, 4, Hitsujigaoka, Toyohira, Sapporo, Hokkaido, Japan*

Email: y_kambayashi@equinst.go.jp

Background: Equine coronavirus (ECoV) is a member of the Betacoronavirus genus. It primarily infects the intestinal tract and causes fever and diarrhoea in horses. Intestinal enteroids, which are derived from intestinal stem cells in crypts and recapitulate the three-dimensional cell structure *in vivo*, are a novel *in vitro* tool for studying the infectious mechanism of enteric pathogens in humans and animals.

Objectives: To investigate the ability of equine intestinal enteroids to sustain ECoV infection.

Study design: Experimental inoculation study.

Methods: Three-dimensional equine intestinal enteroids were generated from stem cells in crypts of the duodenum, jejunum, and ileum harvested from a 3-year-old Thoroughbred and subjected to several passages. The presence of cell types was confirmed by immunohistochemistry (IHC). The dissociated intestinal enteroids were grown as a monolayer on a 48-well plate and inoculated with ECoV. Culture media was collected at 1, 6, 12, 24, 48, and 72 h post-inoculation (hpi). Quantitative reverse transcription—polymerase chain reaction (RT-qPCR) was performed to detect ECoV replication. Intestinal

enteroids monolayers on a 24-well plate were inoculated with ECoV and fixed at 72 hpi for electron microscopy (EM).

Results: IHC analysis revealed that the generated equine intestinal enteroids contained various cell types expressed in the equine intestinal epithelium *in vivo*. RT-qPCR showed that the viral gene copy number had increased by more than 1000-fold at 72 hpi compared to 2 hpi in enteroids derived from any intestinal tissues. Virus particles were observed in the cytoplasm and on the cell membrane by EM. These results indicate that ECoV replicated substantially in equine intestinal enteroids.

Main limitation: Intestinal enteroids were established from only one horse.

Conclusion: Equine intestinal enteroids generated from the duodenum, jejunum, and ileum supported ECoV replication and may be a valuable tool for *in vitro* studies of ECoV.

Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute of the Japan Racing Association with accession numbers 21-6.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Japan Racing Association.

82 | Seasonal trends of equine coronavirus in Sweden based on samples analysed at Swedish Veterinary Agency 2019–April 2024

J. Lindahl[1], B. Lakic[1], J. Johansson Wensman[1,2], M. Malmberg[2] and G. Gröndahl[2]

[1]Department of Animal Health and Antibiotic Strategies, Swedish Veterinary Agency, Uppsala, Sweden and [2]Swedish University of Agricultural Sciences, Uppsala, Sweden

Email: johanna.lindahl@sva.se

Background: Equine coronavirus (ECoV) is an emerging pathogen in the horse population, causing fever and gastrointestinal disease, sometimes fatal. Since it was first described in 2017 in Sweden, it has grown to become the most common infectious disease diagnosed in horses by the Swedish Veterinary Agency (SVA).

Objectives: This study aimed to investigate the seasonal trend of equine coronavirus in Sweden

Study design: Retrospective review of clinical records.

Methods: All data on equine coronavirus (ECoV) analyses performed at the SVA during 2019–April 2024 was retrieved from the data base and analysed for seasonal trends.

Results: In total 2689 samples had been sent to SVA for analyses of ECoV by PCR, and of these, 509 (18.93%, 95% confidence interval (CI) 17.46%–20.46%) samples were positive. Except for the year 2019 when only 110 samples were analysed in total, there were 466–696 samples submitted each year between 2020 and 2023, and 147 in the first 4 months of 2024. There were significant differences between years, with 2020 having the highest proportion of positive samples (24.89%, 95% CI 21.02%–29.08%), compared to the years with lowest proportion positive; 2023 (12.67%, 95%CI 10.19%–15.51%) and the first 4 month of 2024 (12.93%, 95% CI 7.96–19.45). Positive samples were found every month of the year, with July–September having the lowest number of positives (3.03%–6.45%) and January–March having the highest (22.99%–27.17%). Most common were faecal samples, but there was 1 blood sample and 11 nasal swabs submitted, all of which were negative. The faecal samples included faeces (2469 samples, 18.75% positive), rectal swabs (148 samples, 22.30% positive) and 11 pooled faecal samples (18.18% positive).

Main limitations: The data are based on the samples submitted to SVA, and may not reflect the actual number of cases.

Conclusions: While ECoV seems to occur in a seasonal pattern, positive cases can be found all year round in Sweden.

Ethical animal research: Not required: descriptive clinical report.

Informed consent: Not stated.

Competing interests: None declared.

Funding: Formas.

83 | Drug library screening for putative antiviral compounds' discovery against equine arteritis virus

A.-L. Pocheron[1], M. Antonijevic[1,2], G. Bouet[1], D. Froger[1], F. Lecouturier[1], A. Madeline[1], H. Munier-Lehmann[3], S. Pronost[4,5], P.-O. Vidalain[6,7], P. Dallemagne[2] and J.-C. Valle-Casuso[1]

[1]ANSES, Animal Health Laboratory—Normandy site, Equine Diseases Physiopathology and Epidemiology team, Goustranville, France; [2]NU, UNICAEN, CERMN (Centre d'Etudes et de Recherche sur le Médicament de Normandie), Caen, France; [3]Institut Pasteur, Unité de Chimie et Biocatalyse, CNRS UMR 3523, 75 015, Paris, France; [4]LABÉO, Pôle d'analyses et de recherche de Normandie, Saint-Contest, France; [5]NU, UNICAEN, BIOTARGEN (Biologie, Génétique et Thérapies Ostéoarticulaires et Respiratoires), Saint- Contest, France; [6]Equipe Chimie et Biologie, Modélisation et Immunologie pour la Thérapie (CBMIT), Université Paris Descartes, CNRS UMR 8601, 75 006, Paris, France and [7]Centre International de Recherche en Infectiologie, Univ Lyon, Inserm U1111, Université Claude Bernard Lyon 1, CNRS UMR5308, ENS de Lyon, F-69007, Lyon, France

Email: anne-lise.pocheron@anses.fr

Background: Equine arteritis virus (EAV) induces extensive vascular endothelium damage that can lead to abortion, neonatal death, reduced fertility, or loss of performance. Of the two vaccines currently on market, only the inactivated one is ANMV-approved (French Agency for Veterinary Medicinal Products) in Europe. Since 2023, vaccine supply delays or unavailability have been source of great concern in the equine industry. Since no curative treatment for EAV is currently marketed, finding other solutions to limit the viral spread such as antiviral molecules are needed. Our previous work showed that Ribavirin impairs the EAV-replication *in vitro* in equine dermal cells (EDc) [1] which made us confident of finding other anti-EAV molecules with a lower cytotoxic and higher anti-EAV profile.

Objective: To identify candidate compounds through drug repositioning and library screening which is quicker and cost-saving compared to *de novo* drug discovery.

Study design: In vitro experiments.

Methods: Three libraries were screened using a high-throughput *in cellulo* phenotypic-based protocol on their cytotoxicity and cytoprotective capabilities in EDc.

Results: Out of more than 3000 molecules, 92 were identified as hit ones, and for 80 of them we confirmed their capability to decrease the EAV production *in vitro*. Most of the molecules already on market (with known mechanisms of action) seem to target ionic channel blockade and lipid metabolism modulation; both pathways are already known to impact viral replication of other *Nidovirales* virus such as SARS-CoV-2.

Main limitation: Results need to be confirmed.

Conclusion: These new molecules, showing antiviral capacities or expanding their antiviral spectrum, have been included in a translational global health research project to look for their antiviral capacity against other animal or human viruses (SARS-CoV-2, FCoV). Through the study of equine infectious disease, this project will increase the number of antiviral molecules that could be of use in future pandemics.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Supported by the Normandy region and the European Union with the European Regional Development Fund (FEDER).

Reference:

[1] Valle-Casuso J-C, Gaudaire D, Martin-Faivre L, Madeline A, Dallemagne P, Pronost S, Munier-Lehmann H, Zientara S, Vidalain P-O, Hans A. Replication of equine arteritis virus is efficiently suppressed by purine and pyrimidine biosynthesis inhibitors. Sci Rep. 2020;10(1):10100. doi: 10.1038/s41598-020-66944-4

84 | Identification of equine arteritis virus receptors

C.J. Thieulent[1], S. Sarkar[2], M. Carossino[1] and U.B.R. Balasuriya[1]

[1]*Louisiana Animal Disease Diagnostic Laboratory and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA;* [2]*Department of Genetics, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

Email: thieulentcome@gmail.com

Background: Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory, systemic, and reproductive disease of equids with worldwide distribution. EAV causes significant economic losses to the horse industry due to abortion storms, neonatal mortality, respiratory disease, and the establishment of long-term persistent infection (LTPI) in the reproductive tract of infected stallions. Equine CXCL16 (EqCXCL16S) was recently identified as a cell entry receptor for EAV *in vitro*. However, EAV has a broad host-cell tropism and infects various cell lines that do not express EqCXCL16S. Thus, investigation of other cell proteins as potential additional cellular receptor(s) or accessory molecules warrants further investigation.

Objectives: To identify the additional host cell protein(s) involved in EAV infection.

Study design: *In vitro* experiment.

Methods: Peripheral blood mononuclear cells (PBMCs) collected from healthy University-owned horses and seven equine and non-equine cell lines were used in this study. Virus overlay protein-binding assay in combination with Far-Western blot and LC–MS/MS analysis were performed to identify EAV-binding protein(s). Virological and classic cell culture methods (e.g., cell transfection, immunofluorescence, plaque assays) were used to confirm the role of putative EAV attachment factors.

Results: A 57 kDa protein expressed in two EAV-susceptible cell lines, equine endothelial cells (EECs) and equine dermal cells (E. Derm), was identified as a possible EAV-binding protein. This unidentified protein, present in the membrane fraction of the cells, was subsequently identified as vimentin. Screening a wide range of cells from different mammalian species determined that only those expressing vimentin are susceptible to EAV infection. Pre-treatment of EECs with an anti-vimentin antibody induces a significant reduction in viral input.

Main limitations: All experiments were conducted *in vitro*, which may not reflect the *in vivo* conditions.

Conclusions: Our data demonstrated the importance of vimentin in EAV infection *in vitro*. The identification of other EAV (co)-receptors is under investigation.

Ethical animal research: Approved by the institute's IACUC.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The NIH-USDA NIFA R01 Research Grant Program Dual Purpose with Dual Benefit: Research in Biomedicine and Agriculture using Agriculturally Important Domestic Animal Species grant number 2019-67016-29 102 (award number AWD-47990-1) from the USDA National Institute of Food and Agriculture to UBRB.

85 | Follow-up of the monitoring of equine rotavirus A infection in foals with diarrhoea in Argentina

F. Alamos[1,2], C. Olguin Perglione[1,2], M.S. Tordoya[1,2], A.E. Brettschneider[1,2], V. Parreño[1,2], M. Barrandeguy[3], M. Carossino[4,5] and M.A. Vissani[1,2,5]

[1]*Instituto de Virología, CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina;* [2]*Instituto de Virología e Innovaciones Tecnológicas, UEDD, CONICET, Buenos Aires, Argentina;* [3]*Plataforma de Salud Animal, INIA La Estanzuela, Colonia, Uruguay;* [4]*Louisiana Animal Disease Diagnostic Laboratory and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana, USA and* [5]*Instituto de Investigación en Veterinaria, Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador, Buenos Aires, Argentina*

Email: alamos.florencia@inta.gob.ar

Background: Equine rotavirus A (ERVA) is the major cause of diarrhoea in foals. Based on the VP7 and VP4 outer capsid proteins, ERVA are classified into G- and P-genotypes respectively, with G3P[12] and

G14P[12] the most predominant worldwide. While ERVA G3P [12] was the predominant genotype in Argentina, the detection of G14P[12] has steadily increased following its first detection in 2000. The prevalence of both genotypes has shown cyclic alternations from that time.

Objectives: To determine the ERVA G-types circulating in Argentina during 2021–2023.

Study design: Analytical time series study.

Methods: A total of 105 faecal samples corresponding to 28 foal diarrhoea outbreaks, were analysed and characterised by G3/G14 multiplex genotyping qPCR.

Results: Equine ERVA was detected in 25% (26/105) of the samples, corresponding to 32% (9/28) of the reported outbreaks. Overall, 96% (25/26) of ERVA strains were identified as G3 and 4% (1/26) as G14. Based on annual frequencies, a predominance of G3 strains was observed during the current period reaching 100% in 2021–2022 and 93% in 2023.

Main limitations: The number of samples analysed was limited.

Conclusions: The detection rate of ERVA presented during this 3-year study period is similar to the one in previous reports. Yet, unlike previous years, these results showed a higher prevalence of G3. When analysing these results along with the ones in previous periods, a 4-year cyclical alternation of prevalence between the variants can be observed (G14 in 2016–2019, G3 in 2020–2023). As vaccination in pregnant mares is a common practice in our country and the vaccine includes only the G3 strain, the alternation between ERVA variants observed can be attributed to the pressure exerted by vaccination; outbreaks of G14 occurring because the strain is not contained in the vaccine, possibly exerting a natural immune pressure to allow a new cycle of G3.

Ethical animal research: Not required: retrospective analysis of clinical data

Informed consent: Not stated.

Competing interests: None declared.

Funding: INTA and INTA-HARAS Agreement.

86 | Isolation and identification of a G3P[12] genotype donkey-derived rotavirus

Z. Zhang[1], J. Chang[1,2], Z. Hu[1*] and X. Wang[1,2*]

[1]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China and* [2]*Institute of Western Agriculture, the Chinese Academy of Agricultural Sciences, Changji, China*

Email: wangxiaojun@caas.cn

Background: Rotavirus has been confirmed as a major cause of non-bacterial diarrhoea in newborn donkeys under three months old, with a high risk of mortality. Donkeys are susceptible to Group A Rotavirus (Do.RVA), particularly the G3P[12] and G14P [12] genotypes, which are most prevalent and pathogenic for equine animals. Outbreaks have led to significant economic losses for breeders.

Objectives: Controlling this disease is a crucial task in the donkey industry, necessitating the isolation of rotavirus for further research.

Study design: Analysis of microorganisms.

Methods: Using intestinal tissues from foals with clinical signs and positive RT-qPCR results, the study aimed to isolate and identify donkey-derived rotavirus using *in vitro* viral culture techniques. Tissues were homogenised, and the supernatant was treated with trypsin before inoculation onto rhesus monkey kidney cells (Marc-145). After 3–4 days, the viral culture fluid was collected and passaged until typical cytopathic effects (CPE) were observed. The virus was confirmed by ultracentrifugation and electron microscopy. The VP4, VP6, and VP7 gene segments were amplified by RT-PCR, sequenced, and genotyped.

Results: By the sixth passage, typical CPE was observed, with early cell rounding and clumping, followed by unclear cell boundaries, darkening of the cytoplasm, and increased granularity. Electron microscopy revealed numerous spherical viral particles approximately 70 nm in diameter. Genotyping showed the rotavirus to be Group A G3P [12]. The virus titre, calculated by the Karber method, ranged from $10^{-5.125}$ to $10^{-5.375}$ $TCID_{50}$/mL.

Main limitations: The study is preliminary, and further detailed research on the viral strains is needed.

Conclusion: The isolation and identification of donkey-derived rotavirus provide a significant foundation and reference for pathogenicity studies, vaccine development, and antibody production.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences.

Informed consent: Not required: analysis of microorganisms.

Competing interests: None declared.

Funding: None.

87 | Novel equine rotavirus B infection (2022–2023)

A. Graves[1], J. Morrow[1] and P. Morresey[2]

[1]*Equine Diagnostic Solutions, LLC, 1501 Bull Lea Rd. Suite 104, Lexington, KY 40511, USA and* [2]*Rood and Riddle Equine Hospital, PO Box 12 070, Lexington, KY 40580, USA*

Email: pmorresey@roodandriddle.com

Background: During the spring of 2021 a highly contagious infectious diarrhoea syndrome appeared in central Kentucky. This was subsequently determined to be a novel rotavirus B of ruminant origin. A diagnostic PCR test became available mid-2021 allowing routine deployment, starting in 2022.

Objectives: To determine demographic and presentation parameters of the affected foal population, and the influence of any co-infecting agents.

Study design: Retrospective analysis of referral hospital patient records providing survey data.
Methods: Medical records for the years 2022 and 2023 consisting of admissions to an equine referral hospital of foals up to 7 days of age with overt or suspected diarrhoea were included. Only records with complete information were included. Age at onset of clinical signs, gender, objective presenting clinical information, and faecal PCR diagnostic results were included.
Results: Overt diarrhoea was present in the majority of patients admitted for diarrhoea signs that met inclusion criteria. Age at admission or onset of signs, gender, admission presence of hypothermia, hyperthermia, abnormal haematocrit, leukopenia, leucocytosis, electrolyte derangements, signs of abdominal discomfort, and molecular confirmation of co-infecting agent toxin production (*Clostridium perfringens* and *Clostridioides difficile*) were not associated with non-survival. Where mortality occurred orthopaedic sepsis, gastrointestinal compromise not directly related to viral infection, or financial restrictions prompted euthanasia. Of interest the majority of non-survivors had absent gastrointestinal co-infecting agents.
Main limitations: Incompleteness of patient demographic information, geographically restricted case population.
Conclusions: The novel rotavirus B infection of a naïve neonatal population had profound clinical effects on affected foals. Characteristic signs and restricted age range allowed accurate presumptive diagnosis and appropriate patient triage. Clostridial toxin production is commonplace in these cases and should be addressed in therapeutic plans. Survival with treatment is good.
Ethical animal research: Not required: retrospective analysis of clinical data.
Informed consent: Not stated.
Competing interests: Financial and management interest in Equine Diagnostic Solutions.
Funding: None.

88 | Identification and genetic characterisation of equine arteritis virus strains isolated in France in 2021

D. Froger[1], A. Madeline[1], M. Delerue[2], F. Lecouturier[1] and J.-C. Valle Casuso[1]
[1]*ANSES Laboratory for Animal Health in Normandy, Goustranville, France and* [2]*French horse and riding institute (IFCE), 61 310 Gouffern-en-Auge, France*
Email: delphine.froger@anses.fr

Background: The causative agent of equine viral arteritis (EVA) is a virus belonging to the *Equarterivirinae* family in the order *Nidovirales*. The virus is transmitted either by the respiratory or venereal routes. Even depending on the viral load and the virulence of the equine arteritis virus (EAV) strain, EVA is mostly a sub- clinical disease. EAV infection can be responsible for fulminating pneumonia, enteritis, or pneumo-enteritis and death in young foals, and EAV infection can lead to abortion in pregnant mares. EVA outbreaks have major economic consequences for the equine industry since EAV can persist in the reproductive tracts of infected stallions, and can be thus excreted in their semen. In 2021, 6 new outbreaks of EVA happened in 5 different departments in France.
Objective: Isolated EAV strains' phylogenetic classification.
Study design: in vitro analysis.
Methods: Viral RNAs were extracted from semen collected from 6 EAV shedding stallions for molecular amplification of EAV genome targeted portion and phylogenetic analysis of obtained sequences. A 518-nucleotide EAV genome portion was amplified and sequenced. A phylogenetic tree was then built using the Maximum Likelihood method and MEGA X software.
Results: All genome targets were amplified successfully. Six new EAV sequences were classified in their respective phylogenetic groups: 4 isolated strains clustered to the European 1 subgroup and 2 to the European 2 subgroup.
Conclusions: Molecular characterisation of the isolated EAV strains will improve our surveillance program by classifying strains circulating in France, and can also identify new emergent strains, as happened in France in 2007. Furthermore, phylogenetic analysis is a useful tool to identify any epidemiological links between the outbreaks, and extremely helpful in highlighting the potential links between genetic markers and strain virulence.
Ethical animal research: Not required: analysis of microorganisms.
Informed consent: Not stated.
Competing interests: None declared.
Funding: None.

Virology 4: Other viruses

Lexington Wednesday 09.00–10.30

89 | Evaluating humoral immunity against Hendra virus in foals

K.J. Carey[1], I. Smith[2], J. Barr[3], S. Caruso[3], G.G. Au[3], C.A. Hartley[1], K.E. Bailey[1], W. Perriam[4], K. McIntosh[5], C. Broder[6] and J.R. Gilkerson[1]
[1]*Centre for Equine Infectious Disease, Melbourne Veterinary School, The University of Melbourne, Parkville VIC, Australia;* [2]*CSIRO Health and Biosecurity, Black Mountain Laboratories, Black Mountain ACT, Australia;* [3]*CSIRO Australian Centre for Disease Preparedness (ACDP), East Geelong VIC, Australia;* [4]*Gundy Veterinary Services, Scone NSW, Australia;* [5]*Northern Rivers Equine, Kyabram VIC, Australia and* [6]*Department of Microbiology and Immunology, Uniformed Services University, Bethesda MD, USA*
Email: careyk@student.unimelb.edu.au

Background: Hendra virus (HeV) is a zoonotic infection that causes fatal disease in horses and humans. The Equivac® HeV vaccine[1]

induces neutralising antibodies in adult horses, however the foal response to vaccination is largely undescribed.

Objectives: To investigate neutralising HeV antibodies in foals in response to HeV vaccination.

Study design: Field study.

Methods: Serum samples were collected on two horse farms, one with HeV vaccinated broodmares and the second with unvaccinated broodmares. Mares and their foals were sampled and vaccinated following current protocols. All samples were tested using a Luminex® microsphere immunoassay (MIA). A subset of samples was tested with a HeV virus neutralisation test (VNT) and a Spearman's rank correlation test was used to determine an MIA protective threshold (MIA-PT) that correlated to the minimum protective neutralising titre of 32 (VNT-PT).

Results: A strong positive correlation between the VNT titres and MIA results (correlation coefficient = 0.94) was observed and the known VNT-PT correlated to a median fluorescent intensity (MFI) of approximately 4000 (MIA-PT). Foals of vaccinated mares acquired protective levels of HeV maternally derived antibody (MDA), although passive antibodies waned to below MIA-PT levels in most foals from 3 to 6 months of age. Foals of vaccinated mares had a suboptimal response to vaccination. Foals from unvaccinated mares responded to vaccination, but not to the same level as previously unvaccinated mares.

Main limitations: The role of cellular immunity was not considered in this study.

Conclusions: The Luminex® MIA correlates well with neutralising HeV antibodies and provides a high throughput screening option without the need for PC4 facilities. Vaccinated mares transfer protective levels of MDA to their foals, however MDA waning leaves foals susceptible from 3 months old. Most foals had poor response to initial vaccination, suggesting that foals from both vaccinated and unvaccinated mares may be susceptible to infection at the recommended time of vaccination, but may not respond adequately.

Key manufacturers: 1 Equivac® HeV vaccine for horses manufactured by Zoetis©. https://www.zoetis.com.au/all-products/portal-site/equivac-hev.aspx.

Ethical animal research: Approved by the University of Melbourne Ethics committee under the Ethics Application number 24871.

Informed consent: Vaccination and sampling is part of normal management at farm one. An owner consent form was signed by the owner of farm two.

Competing interests: None declared.

Funding: Centre for Equine Infectious Disease, The University of Melbourne.

90 | Equine dermatitis outbreak associated with parapoxvirus

J. Virtanen[1,2], K.Hautala[1], M. Utriainen[2], L. Dutra[2], K. Eskola[3,4], N. Airas[1], R. Uusitalo[1,2,5], E. Ahvenainen[2], T. Smura[2], T. Sironen[1,2], O. Vapalahti[1,2], R. Kant[1,2], A.-M.K. Virtala[1] and P.M. Kinnunen[1]

[1]Department of Veterinary Biosciences, Faculty of Veterinary Medicine, P.O. Box 66, 00014 University of Helsinki, Finland; [2]Department of Virology, Faculty of Medicine, University of Helsinki, Finland; [3]Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland; [4]Animal Health and Welfare Department, Finnish Food Authority and [5]Department of Geosciences and Geography, Faculty of Science, University of Helsinki, Finland

Email: katja.hautala@helsinki.fi

Background: Parapoxviruses (PPV) cause skin and mucous membrane lesions in several species and are often zoonotic. Equine parapoxvirus (EqPPV) was reported in a severely sick horse in Finland in 2016. In 2021–2022, an infectious pastern dermatitis outbreak occurred in horses around Finland.

Objectives: To identify the cause of the outbreak, develop diagnostics and describe the epidemiology as well as clinical signs of the infection.

Study design: Clinical report and epidemiological survey.

Methods: Skin samples from 26 affected horses representing 11 stables were subjected to bacteriological, histological and virological analysis. Epidemiological survey data were collected among case and control Finnish horse farms by online survey was analysed to describe the outbreak.

Results: EqPPV was identified as a probable cause of the epidemic and co-infections with potentially pathogenic and zoonotic bacteria were observed. Nearly complete virus genome with little sequence variation was sequenced and annotated from several samples. Histopathologically, suppurative and ulcerative dermatitis was diagnosed. Having racehorses at the farm was a risk factor for the infection. Skin symptoms consistent with PPV infection were reported in humans in case farms more often than in control farms.

Main limitations: Samples from the humans taking care of sick horses and presenting typical skin lesions for PPV infection were not obtained. Therefore, the zoonotic nature of EqPPV could not be confirmed.

Conclusions: Pastern dermatitis spread broadly among racehorses in Finland during winter of 2021–2022. EqPPV had been described in one horse earlier but was not known to cause epidemics, making it unfamiliar to the racing community and veterinarians. Reports of skin lesions in humans and the zoonotic nature of other PPVs makes this disease a potential zoonosis. This work offers help in early recognition, diagnostics and disease control, if pastern dermatitis associated with EqPPV occurs again.

Ethical animal research: The authors confirmed that ethics committee oversight for the epidemiological survey is not required in their institute. Skin samples were collected for clinical purposes.

Informed consent: Participants gave consent.

Competing interests: None declared.

Funding: The Sakari Alhopuron rahasto; Erkki Rajakosken rahasto; Eläinlääketieteen Tutimuksen Tukisäätiö; Suomen Eläinlääketieteen Säätiö); and Niemi-säätiö

Reference:

Virtanen J, Hautala K, Utriainen M, Dutra L, Eskola K, Airas N, Uusitalo R, Ahvenainen E, Smura T, Sironen T, Vapalahti O, Kant R,

Virtala A-MK, Kinnunen PM. Equine dermatitis outbreak associated with parapoxvirus. Journal of General Virology 2023;104(12):001940. https://doi.org/10.1099/jgv.0.001940

91 | Venezuelan equine encephalitis virus (VEEV) indirect ELISA development

M. Chevrier[1], T. Helle[1], M. Dumarest[1], Y. Boudjadi[1], E. Bréard[1], M. Turpaud[1], M.A. Vissani[2], R. Lenin Vinueza[3], L. Mathews-Martin[1], J. Vanhomwegen[4], S. Zientara[1], C. Beck[1], S. Martin-Latil[1], P. Desprès[4], G. Gonzalez[1] and C.V. Migné[1*]

[1]*Anses, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR 1161 Virologie, Laboratoire de Santé Animale, Maison-Alfort, France;* [2]*Instituto de Virología e Innovaciones Tecnológicas (IVIT), Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina; Cátedra de Enfermedades Infecciosas, Escuela de Veterinaria, Universidad del Salvador, Buenos Aires, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) scientific career, Buenos Aires, Argentina;* [3]*Escuela de Medicina Veterinaria, Universidad San Francisco de Quito, Quito, Ecuador and* [4]*Interactions Moléculaires Flavivirus-Hôtes, Department of Virology, Institut Pasteur, Paris, France*

Email: manon.chevrier@vet-alfort.fr

Background: Venezuelan equine encephalitis virus (VEEV) is a re-emerging zoonotic pathogen for which equines are the most susceptible among mammals, with a potentially high mortality rate (19 to 83%). The virus, transmitted between vertebrates and mosquitoes, is responsible for unpredictable sporadic epizootic outbreaks in equids and humans in Central and South America. To date, VEEV is not circulating in Europe. However, in the context of global change, intensification of international trade and the presence of potential competent vectors (mosquitoes of the genus *Aedes* spp. and *Culex* spp), its risk of emergence in Europe is not negligible. Due to its specificity and sensitivity, the gold standard to detect VEEV remains the Virus Neutralisation Test (VNT) performed at the European Union Reference Laboratory (EURL). However, it is time-consuming and requires skilled personnel working in biosafety level 3 laboratories.

Objectives: To anticipate the emergence of VEEV in Europe, the European Commission mandated the EURL for equine diseases to develop an easy-to-implement and fast serological detection method to diagnose VEEV equine infection.

Study design: Assay development

Methods: We developed *in-house* indirect enzyme-linked immunosorbent assay (ELISA) using the E2 glycoprotein of VEEV. The performance of the assay was evaluated using 469 samples collected in Ecuador and Argentina from horses either non-infected, or naturally infected with VEEV and/or EEEV or EEEV/WEEV vaccinated and compared with VNT.

Results: The VEEV ELISA offered satisfactory performance with sensitivity and specificity of 89.2% and 98.6% respectively.

Conclusions: The VEEV ELISA is an easy-to implement alternative diagnostic method to virus neutralisation test. It can be used in sero-epidemiological studies to estimate the accurate distribution of the virus across the Americas and to assess the introduction of the virus in new areas such as Europe.

Ethical animal research: Not stated.

Informed consent: Not stated.

Competing interests: None declared.

Funding: European Union Laboratory for equine diseases.

92 | Different regions of the 3′ end of WNV genome modulate its virulence and transmission in vertebrate hosts and mosquitoes

L. Fiacre[1,2], A. Nougairède[3], C. Migné[1], M. Bayet[2], M. Cochin[3], M. Dumarest[1], T. Helle[1], A. Exbrayat[4], N. Pagès[2], D. Vitour[1], J.P. Richardson[1], A.-B. Failloux[5], M. Vazeille[5], E. Albina[4], S. Lecollinet[#2] and G. Gonzalez[#1]

[1]*ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR Virologie, Laboratoire de Santé Animale, F-94700 Maisons-Alfort, France;* [2]*UMR ASTRE, CIRAD, Petit-Bourg, Guadeloupe;* [3]*Unité Des Virus Emergents (UVE), Aix-Marseille Université, IRD 190, INSERM 1207, Marseille, France;* [4]*ASTRE, CIRAD, INRAe, Université de Montpellier, Montpellier, France and* [5]*Institut Pasteur, Université Paris Cité, Arboviruses and Insects Vectors, Paris, France*

#Contributed equally.

Email: gaelle.gonzalez@anses.fr

Background: West Nile virus (WNV) is a neurotropic mosquito-borne virus belonging to the *orthoflavivirus* genus. WNV's amplification cycle involves birds as amplifying hosts and mosquitoes, mainly of the genus *Culex*, as vectors. Subsequent to WNV emergence in New York in 1999, intense and fast WNV spread occurred in the Americas. WNV induces outbreaks of varying intensity in humans, horses and birds. Beyond environmental variables, the importance of WNV epizootics or epidemics could be explained by differences in strain virulence and transmission.

Objectives: To characterise the genetic determinants of WNV pathogenesis and transmission in vertebrates and arthropods.

Study design: *In vitro* and *in vivo* experiments.

Methods: Several chimeras were generated between two WNV strains differing in their levels of virulence either by classical (infectious clone) or by recently developed (ISA—Infectious Subgenomic Amplicons) reverse genetic methods. The replicative capacity of the chimeras was assessed *in vitro* in mammalian Vero, avian CCL141 and C6/36 mosquito cells.

Results: Infection of Vero cells suggested a role for NS1, NS3 and NS4A proteins in WNV replication 24 and 48 h post-infection, whereas NS5 and the 3'UTR region were found to modulate viral fitness in C6/36. *In vivo* analysis after infection of BALB/c mice, 1-day old chickens as well as *Culex pipiens* mosquitoes allowed deeper

observations; specifically, different genomic regions were shown to modulate virulence in mammals and birds and vector competence in mosquitoes.

Conclusions: Precise mapping of WNV virulence determinants will allow more rapid assessment of emerging strains' epidemic potential, as well as the development of effective preventive or control solutions.

Ethical animal research: Biosecurity measures for the biocontainment of parental and chimeric viruses generated in this study have been reviewed and validated by the French Ministry of Higher Education, Research and Innovation under the DUO10537. In vivo experiments were approved by the Bioethics Committee CODA-IPS (agreement number: LA1230174) and the joint Anses-UPEC-Alfort Veterinary School ethics committee (permit number: 15/02/11-13.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: CIRAD—ANSES PhD fundings.

93 | New 2D/3D *in vitro* models of West Nile virus-infected equine brain cells for antiviral discovery

M. Cochet[1§], N. Berry[1§], V. Chaillot[1], T. La Rosa[2], F. Piumi[1], K. Gorna[1], G. Gonzalez[1], A. Danckaert[3], N. Aulner[3], O. Blanchet[4], S. Zientara[1], F.X. Donadeu[5], H. Munier-Lehmann[6], J. Richardson[1], A. Benchoua[7], B. Pain[2] and M. Coulpier[1*]

[1]*INRAE, Anses, Ecole Nationale Vétérinaire d'Alfort, UMR VIROLOGIE, Laboratoire de Santé Animale, 94 700 Maisons-Alfort, France;* [2]*INRAE, USC1361, SBRI, Lyon, France;* [3]*Institut Pasteur Paris, UTechS Photonics Bioimaging/C2RT, Université Paris Cité, 75 015 Paris, France;* [4]*Centre de Ressources Biologiques, BB-0033-00038, CHU Angers, 49 933 Angers, France;* [5]*Division of Translational Bioscience, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK;* [6]*Institut Pasteur, Université Paris Cité, CNRS UMR3523, PF-CCB, 75015 Paris, France and* [7]*CECS, I-STEM, AFM, 91100 Evry, France*

Email: muriel.coulpier@vet-alfort.fr

Background: Outbreaks of West Nile virus (WNV) occur periodically, affecting equine populations. Whereas vaccines are commercialised, specific antiviral treatments do not exist so far.

Objectives: To provide the veterinary community with new *in vitro* models of infection, specific to the horse brain, and to identify chemical compounds that block WNV replication.

Study design: In vitro.

Methods: We developed relevant 2D and 3D cellular models representative of the equine brain, determined their susceptibility to WNV and evaluated the efficiency and toxicity of antiviral compounds. Equine induced pluripotent stem cells (eiPSC) were induced into the neural lineage and either differentiated to equine neural progenitors (eNPC, 2D model) or used to generate cerebral organoids (eCO, 3D model). Equine NPC and eCO were infected with WNV (lineage 1/2) and treated with 41 and 3 chemical compounds, respectively, whose efficiency and toxicity was assessed.

Results: eNPCs and eCO derived from eiPSC were both permissive to WNV. Using eNPC in a microplate assay, one nucleoside analog, 2'C-methylcytidine, blocked WNV infection, whereas other compounds, some known to display anti-viral activity in human cell lines, were either toxic or non-active in eNPC. An unexpected proviral effect of statins was revealed, whereas statins are antiviral in human cells, including in human NPC. Using eCO, the efficiency of 2'C-methylcytidine was confirmed and showed an absence of antiviral activity of ribavirine and arbidol.

Main limitations: eNPC are not the main target of WNV in equine brain.

Conclusions: The results emphasise the importance of using tissue- and species-relevant cellular models for assessing antiviral activity of compounds and identify a potential lead for future drug development. It also provides a valuable discovery platform for the veterinary community that can be applied to diverse neurotropic equine viruses.

Ethical animal research: Approved by the "Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale" of Henri Mondor Hospital, France. Authorisation and declaration numbers at the Research Ministry are AC-2017-2993 (CHU Angers) and DC-2019-3771 (UMR Virology). A rabbit immunisation protocol used for antibody production complied with EU legislation (authorisation 12/04/11-6 accorded by the ANSES/ENVA/UPEC ethical committee).

Informed consent: Human foetus was obtained after legal abortion with written informed consent from the patient.

Competing interests: None declared.

Funding: The French National Research Institute for Agriculture, Food and Environment (INRAE), the Ecole Nationale Vétérinaire d'Alfort (EnvA), the Fonds Eperons, the Institut Français du Cheval et de l'Equitation (IFCE), the DIM1Health (Ile de France, NB), the Biotechnology and Biological Sciences Research Council (UK). The UtechS PBI, part of the France–BioImaging infrastructure network (ANR-10-INSB-04; Investments for the Future) received funding from the Région Île-de-France (DIM1Health) and the Institut Pasteur.

94 | Development of an *in vitro* model to study equine rhinitis A virus pathogenesis

G. Chen[1,2], C. Hartley[1,2], J. Gilkerson[1,2] and A. Diaz-Méndez[1,2]

[1]*Asia-Pacific Centre for Animal Health (APCAH), Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Victoria, Australia and* [2]*Centre for Equine Infectious Disease (CEID), Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Victoria, Australia*

Email: guihuac@student.unimelb.edu.au

Background: Equine Rhinitis A Virus (ERAV) is classified with foot-and-mouth disease virus (FMDV) in the *Aphthovirus* genus of the family *Picornaviridae* and causes febrile respiratory disease, viremia, and

urinary shedding in horses. The pathogenesis of ERAV within the respiratory and urinary tracts is not well-understood.

Objectives: To explore ERAV's pathogenesis across various cell infection models, including equine foetal lung cells (EFLs), equine tracheal bronchial epithelial cells (ETBECs) and rabbit kidney cells (RK13).

Study design: In vitro experiments.

Methods: The pathogenic effects of ERAV on EFLs, ETBECs and RK13s were examined by assessing cytopathic effect and viral replication. ERAV infected cell cultures were studied using Haematoxylin and Eosin staining, transmission electron microscopy and immunofluorescence to evaluate morphology and viral presence. $TCID_{50}$ and qPCR were used to quantify viral replication.

Results: Post-ERAV infection, RK13 cells displayed significant cytopathic effects with complete lysis by 2 dpi, while EFLs retained monolayer morphology up to 7 dpi, even with high viral titres. One-step growth curve of ERAV in RK13 and EFL cells indicated a lag phase until 4 hpi, log phase up to 12 hpi, and stationary phase beyond 20 hpi. ETBECs maintained microvilli integrity post-ERAV infection at 24 hpi.

Main limitations: While this *in vitro* study does not fully emulate *in vivo* conditions, it establishes some groundwork for understanding the cellular pathogenesis of ERAV.

Conclusion: The findings of this research enhance our understanding of ERAV kinetics and its pathogenesis across various equine primary cell lines and RK13 cells. Future studies should focus on cellular responses to ERAV, including gene regulation and cytokine production.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Centre for Equine Infectious Disease and Faculty Research Start-up Funds from the University of Melbourne. Guihua Chen was supported by the Melbourne Research Scholarship and the Merlie Ivy Merchant Research Scholarship.

Virology 5: Epidemiology

Michel D'Ornano Wednesday 15.00–17.30

95 | What influences field veterinary practitioners' decision to protect themselves from zoonotic infections? A qualitative study

N.V. Jasper and B.A. Burgess

Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

Email: nichelle.jasper@uga.edu

Background: While veterinarians are educated on zoonotic risk and prevention, they often do not take recommended precautions while practicing.[1] This may be exacerbated for equine and livestock field practitioners where variable working conditions present challenges. Limited access to clean water, sanitation facilities, and PPE may prevent implementation of effective prevention strategies and impact decision-making.

Objectives: To identify factors influencing field practitioner decision-making relative to implementation of transmission risk reduction strategies.

Study design: Qualitative cross-sectional study.

Methods: Virtual focus groups were conducted via Zoom. Currently practicing AVMA-member equine and livestock field practitioners, who self-identified, were screened with an online survey including demographics, perceptions of zoonotic risk, and typical on-farm infection control practices. Virtual focus groups were conducted via Zoom, transcripts were generated, checked for accuracy, and thematic analysis was conducted using NVivo.

Results: A pilot study revealed that veterinarians engage in situational risk assessment influenced by knowledge of prevalent diseases, local climate and environment and availability of PPE. Importantly, these decisions often occurred after initial animal interaction. In the study reported here, participants agreed about the importance of zoonotic disease exposure risk, but differed in their perceived level of risk, decision-making, and prevention strategies in scenario-based discussion.

Main limitations: Response bias due to participants' fear of being judged; selection bias which may limit generalisability of results due to participant self-selection.

Conclusions: The results of this study demonstrate that while field service practitioners have a general awareness of their risk of exposure to zoonotic disease, the perceived risk level is inconsistent across practitioners. This suggests that without employing 'standard' infection control practices, veterinarians may incur more zoonotic diseases unnecessarily.

Ethical animal research: Study approved by the Institutional Review Board.

Informed consent: Participants gave consent.

Competing interests: None declared.

Funding: None.

Reference:

[1] Wright JG, Jung S, Holman RC, Marano NN, McQuiston JH. Infection control practices and zoonotic disease risks among veterinarians in the United States. J Am Vet Med Assoc 2008;232:1863–1872.

96 | Isolation of *Histoplasma capsulatum* var. *farciminosum* from Ethiopian horses with chronic equine epizootic lymphangitis

F. Al Mheiri[1,2], M. Joseph[1], J. Kinne[1], S. Joseph[1], P.C.Y. Woo[3,4], S.V. Jose[1], J.-M.V. Cavalleri[2] and U. Wernery[1]

[1]*Central Veterinary Research Laboratory, Dubai, United Arab Emirates;* [2]*University of Veterinary Medicine Vienna, Vienna, Austria;* [3]*Doctoral Program in Translational Medicine and Department of Life Sciences,*

National Chung Hsing University, Taichung 402, Taiwan and [4]The iEGG and Animal Biotechnology Research Center, National Chung Hsing University, Taichung 402, Taiwan
Email: fgraiban@cvrl.ae

Background: *Histoplasma capsulatum* var. *farciminosu*m (HCF) is a fungal pathogen recognised as the cause of equine epizootic lymphangitis (EEL) in equids, leading to severe health consequences for the animals and great losses that cause a devastating economic impact on their owners.

Objectives: To isolate and identify HCF from 15 horses showing clinical signs of EEL in Ethiopia. Forty-six specimens were collected from 15 EEL-infected Ethiopian horses to isolate HCF. The isolated HCF strains were identified by their characteristic growth pattern, colony morphology, staining, and PCR. Two the American Type Culture Collection (ATCC) strains served as controls. Additionally, tissue samples were collected for histopathological analyses.

Study design: Clinical case series.

Methods: Microbiological analyses were carried out by culturing 46 specimens on different agars incubated at 26°C and 37°C for 45 days. Histopathological analyses were performed on tissue samples fixed in 10% formalin, dehydrated, embedded in paraffin wax sliced, and stained with Haematoxilin & Eosin and Gomori methenamine silver.

Results: Twenty fungal and 18 bacterial species were isolated from the 46 samples and identified. Twelve unidentified fungal strains by conventional biochemical tests were identified by PCR, and 6 of the fungal strains were confirmed as HCF. One of the 4 histopathologic samples of the infected skin had high numbers of yeast organisms (HCF) located both intracellularly and extracellularly of the macrophages.

Main limitations: The poor isolation success rate has several reasons but is mainly caused by contamination and overgrowth of the specimens by different fungal and bacterial species.

Conclusions: HCF was identified in some horses with equine epizootic lymphangitis. Future research should concentrate on establishing more selective culture media for Histoplasma.

Ethical animal research: Not required: clinical case series.

Informed consent: Not stated.

Competing interests: None declared.

Funding: None.

97 | Five degrees of separation: The contact network of horses at competitions in the Netherlands

R. Houben[1], K. van Maanen[2], M.M. Sloet van Oldruitenborgh-Oosterbaan[1] and H. Heesterbeek[1]
[1]Utrecht University, Faculty of Veterinary Medicine, Equine Sciences, Utrecht, The Netherlands and [2]GD Animal Health, Deventer 7400 AA, The Netherlands
Email: r.m.a.c.houben@uu.nl

Background: Dynamics of infectious diseases are influenced by population contact structure. Limited data is available on horse contact networks.

Objectives: To describe the contact network of horses participating in sports or racing competitions in the Netherlands, and to compare static and dynamic representations.

Study design: Network census.

Methods: Participation records from the Royal Dutch Equestrian Sports Organisation and Dutch racing records for 2022 were made available upon request. Four networks were analysed: sport horses and racehorses, with horses as nodes and presence at the same event as edges; and sports locations and racing locations, with locations as nodes and travel of horses from one event to the next as directed edges. Annual static and temporal network metrics were calculated.

Results: The sport horse network was the largest network, with 41 018 nodes, its diameter (highest number of links in the shortest path between any two nodes) was five, and the network had "small world" properties, a topology that is favourable for spreading of infectious disease. All static annual networks were fully (strongly) connected. The connectedness of the networks was robust to targeted node removal, except for the racing locations network. The temporal "reach" distribution of nodes suggested that static representations of the networks overestimated the network connectedness.

Main limitations: Lack of information on contacts on the horses' home premises.

Conclusions: The Dutch equestrian competition network is highly connected. Since 4/5 Dutch premises house at least one horse that participates in competitions at least occasionally, this connectedness affects most if not all Dutch horses. Targeting high-risk horses or locations for preventive measures may not be equally effective in all networks.

Ethical animal research: The Ethical Review Board of Utrecht University's Geography Department (DGK S-23016) ruled that ethical review of use of personal data was not required for the study protocol.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: None.

98 | Investigation of the role of exercise in the detection of selected respiratory pathogens from nose wipes collected from healthy horses

N. Pusterla[1], C. Wright[2], S. Barnum[1], K. Lawton[1], J. Blea[3], B. Farwell[2] and B. Craig[2]
[1]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA; [2]Merck Animal Health, Madison, NJ 07940, USA and [3]School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA
Email: npusterla@ucdavis.edu

Background: The detection of subclinical shedders of respiratory pathogens represents a diagnostic challenge as such horses generally shed either intermittently or for a short time period. As exercise is known to increase airway clearance by removing viscous secretions

from the respiratory airways, it is appealing to consider testing healthy horses following regular exercise in an attempt to detect respiratory pathogens.

Objectives: To determine the frequency and quantitation of selected respiratory pathogens from nose wipes collected pre- and post-exercise in different populations of performance horses.

Study design: Prospective study.

Methods: One hundred and twenty-four horses of different disciplines (racing, jumper/hunter, and dressage horses) with an age ranging from 2 to 20 years (median 4 years) were enrolled in the study. There were 52 females and 72 males (geldings/stallions). Each horse had one disposable 4 × 6-inch saline cloth wipe taken from the nose area prior to and immediately after routine exercise. The wipes were processed for nucleic acid extraction and tested by qPCR for selected common (equine herpesvirus (EHV)-1, EHV-4, *Streptococcus equi* ss *equi* (*S. equi*)) and commensal respiratory (EHV-2, EHV-5, *S. equi* ss *zooepidemicus* (*S. zooepidemicus*)) pathogens. All results were quantitated and reported as number of target genes per million cells.

Results: The frequency of pathogen detection increased for all selected respiratory viruses and bacteria from pre- to post-exercise (EHV-1 7.2/10.5%; EHV-2 47.6/61.3%; EHV-4 0/1.6%; EHV-5 58.1/69.3%; *S. equi* 0/1.6%; *S. zooepidemicus* 52.4/69.3%). EHV-1 (17 horses) and EHV-4 (2 horses) were found exclusively in racing horses, and *S. equi* in one racing and one jumper/hunter horse. EHV-2 and EHV-5 were detected with greater frequency in racing horses when compared to jumper/hunter and dressage horses. *S. zooepidemicus* was detected with similar frequency among horses from the different disciplines. Absolute quantitation of the respiratory pathogens in pre- and post-exercise samples showed no statistical differences (Mann–Whitney *U* test, $p < 0.05$).

Main limitations: Study limitations related to the overrepresentation of young racing horses as study subjects.

Conclusions: The data showed that exercise increased the shedding of selected common and commensal respiratory pathogens without significantly altering absolute quantitation. Nose wipes collected post-exercise may be a suitable and less invasive sample type to document silent shedding of selected respiratory pathogens in healthy performance horses.

Ethical animal research: Not required: clinical surveillance with non-invasive sampling.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: UC Davis.

99 | Emergence of equid herpesvirus 1 myeloencephalopathy in Argentina

F. Alamos[1,2], M.S. Tordoya[1,2], C. Gabaglio[1,2], C. Olguin Perglione[1,2], M. Barrandeguy[3] and M.A. Vissani[1,2,4]

[1]Instituto de Virología CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), Dr. Nicolás Repetto y De Los Reseros s/n°, Hurlingham B1686LQF, Buenos Aires, Argentina; [2]Instituto de Virología e Innovaciones Tecnológicas (IVIT), UEDD, CONICET, Buenos Aires, Argentina; [3]Plataforma de Salud Animal, INIA La Estanzuela, Colonia, Uruguay and [4]Instituto de Investigación en Veterinaria, Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador, Buenos Aires, Argentina

Email: alamos.florencia@inta.gob.ar

Background: Myeloencephalopathy due to EHV-1 (EHM) is an emerging disease worldwide. In Argentina, variants A2254 and G2254 of EHV-1 have been associated with abortion, but their neurological manifestation had not been registered until 2021.

Objectives: To communicate the virological findings corresponding to one outbreak plus three additional cases of EHM in 2021 and 2022.

Study design: Descriptive study.

Methods: Premises A and C: 40 and 5 training high-performance polo horses, respectively. Premise B: 15 horses in a polo breeding farm. Premise D: 6 training Thoroughbred horses. Samples obtained were nasopharyngeal swabs (NS; n:95), central nervous system (CNS; n:2), cerebrospinal fluid (CSF; n:2), lung (n:1) and unclotted blood (n:87) of 14 animals showing clinical signs and of 34 cohabiting animals. Viral isolation in cell culture, PCR, and discriminative (A2254G polymorphism) real-time PCR was performed to detect and characterise EHV1.

Results: EHV-1 was detected in 20 NS, 10 unclotted blood samples, 2 CNS, 2 CSF and 1 lung from 12 affected animals, and in 4 NS and 1 unclotted blood samples from 5 cohabiting animals. In premise A, 11 horses showed neurological and/or respiratory signs [morbidity: 28% (11/40); mortality: 5% (2/40)]. In premises B, C and D, only one horse was affected in each; horses in B and D died, and the horse in C survived without sequelae. Variant G2254 was responsible for the outbreak of EHM in premise A, while A2254 was detected in the other three premises. No new clinical cases or viral excretion were registered after 18 days of quarantine in any of the premises.

Main limitations: Lack of samples from premise B.

Conclusions: An association of EHV-1 A2254 variant with abortions and G2254 with neurological disease is not exclusive. The emergence of EHM in Argentina severely affected the equine industry, which might be of future concern.

Ethical animal research: Not required: field outbreak management

Informed consent: Not stated.

Competing interests: No competing interests have been declared.

Funding: INTA and INTA-HARAS Agreement.

100 | Antibody testing to detect viral exposure in contact horses during an equine herpes myeloencephalopathy outbreak

G.A. Perkins[1], B. Wagner[2], A. Rollins[2], H. Sfraga[1], E. Pearson[1] and M. Cercone[1]

[1]Departments of Clinical Sciences and [2]Population Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Email: gap7@cornell.edu

Background: Two experimental equine herpesvirus-1 (EHV-1) challenge studies showed that stable, moderately high serum anti-EHV-1

antibodies paired with rapidly increasing nasal mucosal antibodies (mucAbs) prevent EHV-1 infection, viral shedding and cell-associated viremia.

Objective: To investigate serum and mucAbs concentrations in in-contact horses during a naturally occurring EHV-1 incident. We hypothesised that EHV-1 antibody testing can confirm EHV-1 exposure in non-clinical horses during an outbreak.

Study design: Descriptive longitudinal case series.

Methods: Two horses with neurological signs from one farm were admitted to an equine hospital. EHV-1 was confirmed by PCR. Five concurrently hospitalised in-contact horses, 4 of which were vaccinated against EHV- 1/4 within 7-months were studied. In-contact horses had temperatures measured, along with serum and nasal swab samples taken for EHV-1 PCR and antibody quantification between 1 and 29 days of potential EHV-1 exposure.

Results: None of the in-contact horses developed fever or clinical signs of EHV-1. PCR results were negative. Only mild seroconversion was observed. MucAbs were initially low and increased rapidly in four in-contact horses who were considered exposed to EHV-1 yet neither infected nor infectious. One horse without an increase in mucAbs was not exposed.

Main limitations: Small number of horses.

Conclusions: Repeatedly measuring serum and mucAbs provide information on EHV-1 exposure and, together with clinical monitoring and PCR, could enable improved management of EHM outbreaks and release from quarantine. Serum antibodies obtained at the same time provide information on pre-existing EHV-1 immunity.

Ethical animal research: Not required: samples were obtained as part of EHV-1 outbreak management.

Informed consent: Horse owners gave verbal consent for inclusion.

Competing Interest: B. Wagner is Director of Serology and Immunology Lab at the Animal Health Diagnostic Center, Cornell University where the EHV-1 Risk Assessment Assay is offered. Dr Wagner has a patent submitted as the inventor of the test; however, she does not receive compensation from the sale of this test.

Funding: Harry M. Zweig Memorial Fund for Equine Research at Cornell University.

101 | Retrospective review of herpes virus positive cases at the Donkey Sanctuary, UK from 2023 to 2024

C. Goding, C. Platts, S.L. Norris, E. McGoldrick and J. Cook
The Donkey Sanctuary, Devon, UK
Email: curtis.goding@thedonkeysanctuary.org.uk

Background: Equine herpesviruses (EHV) are among the most diagnosed infectious diseases of equids. The type and clinical presentation of herpesvirus infection in donkeys can differ to that of horses. There is especially limited donkey-specific literature regarding gamma herpes viruses.

Objectives: The aim of this study was to retrospectively review positive herpesvirus cases in equids across 10 sanctuary sites in the south of England, examine the type of viruses identified and associated clinical manifestations.

Study design: Retrospective case review.

Methods: Identification of herpesvirus positive q-PCR results over a 15-month period from January 2023 to March 2024 through laboratory records. All descriptive statistical analysis was performed using R v4.2.1 and RStudio v2023.06.1.

Results: A total of 21 positive test results from 20 individual cases were examined. The population consisted of 19 donkeys and 1 pony, with ages ranging from 7 to 31. Sample types included nasopharyngeal, nasal, and ocular swabs, eye tissues and cerebrospinal fluid (CSF). EHV-2 was identified most frequently (12/21), including once in CSF, followed by EHV-8, formerly known as asinine herpesvirus-3, (8/21) and then EHV-5 (1/21). Overall, 43% (9/21) of positive cases were associated with outbreaks of disease. Serous nasal discharge was the most reported presenting sign across all positive cases (9/21), found also in 88% (7/8) of EHV-8 cases. Ultimately 70% of all positive cases resolved, 30% were euthanised, and of those 83% (5/6) had concurrent or chronic illness.

Main limitations: Due to the study design, the dataset available for analysis is limited. Consequently, only descriptive statistics are included.

Conclusion: Herpesvirus positive cases in donkeys and ponies at The Donkey Sanctuary, UK are often associated with outbreaks and a variety of clinical manifestations, from mild respiratory signs to severe neurological, respiratory, and ophthalmic disease. Screening for EHV-1 and EHV-4 in acutely unwell donkeys may overlook virus types associated with severe and infectious disease.

Ethical animal research: Approved by The Donkey Sanctuary Research and Ethics Review Committee (RERC).

Informed consent: Not stated.

Competing interests: None declared.

Funding: None.

102 | Control of the respiratory form of equine rhinopneumonitis among yearlings at a breeding and training farm by updating their vaccination program

H. Bannai*[1], Y. Kambayashi[1], K. Kume[2], N. Takebe[2], Y. Endo[2], N. Kawanishi[1], M. Nemoto[1] and K. Tsujimura[1]
[1]*Equine Research Institute, Japan Racing Association and* [2]*Hidaka Training and Research Farm, Japan Racing Association*
*Corresponding author: Hiroshi Bannai
Email: bannai@equinst.go.jp

Background: At the Japan Racing Association's Hidaka Training and Research Farm, the respiratory form of equine rhinopneumonitis was endemic among yearlings soon after their introduction from July to September, thus delaying training schedules. To date, vaccination started in late September or October—only after all yearlings purchased at the sales had been introduced; those introduced earlier were kept with naïve immune status before vaccination.

Objective: To evaluate the efficacy of a new vaccination program starting earlier.
Study design: Descriptive study.
Methods: Yearlings in 2021–2022 and 2022–2023 were allocated to three groups according to their date of farm introduction. Each group received live equine rhinopneumonitis vaccine[1] at the earliest time after introduction, from early August to late September, with a second dose 2 months later. Sera collected monthly from August to April were subjected to enzyme-linked immunosorbent assay for equid alphaherpesvirus 1 (EHV-1) and 4 (EHV-4) and virus-neutralisation testing. The numbers of pyretic horses (≥38.5°C) with EHV-1/4 infection and the whole-population infection rate in each month were compared with those in previous years (from 2018–2019 to 2020–2021).
Results: Each group had significant antibody responses after the first and second vaccinations. The number of pyretic horses with EHV-1/4 infection was 8 in 2021–2022 and 5 in 2022–2023—fewer than in previous years (range, 14–18). Although infection rates in the previous years peaked from August to September (range, 6.84‰–10.94‰), those in the same period under the new vaccination program were much lower, at 1.22‰–1.67‰ in 2021–2022 and 2.55‰–3.76‰ in 2022–2023.
Main limitation: No statistical analysis was available because of the small number of years.
Conclusion: Endemic equine rhinopneumonitis among yearlings from summer to fall was substantially controlled by initiating vaccination at the earliest timepoint after introduction.
Key manufacturer: 1 Equi N Tect ERP, Nisseiken, Japan)
Ethical animal research: Approved by the Ethics and Research Promotion Committee of the Equine Research Institute (approval number 2018-3263-07).
Informed consent: Not applicable.
Competing interests: The authors declare no conflicts of interest.
Funding: Japan Racing Association.

Virology 6: Diagnostics

Michel D'Ornano Thursday 09.00–10.30

103 | Stream of revelation: detection of equine herpesvirus-1 (EHV-1) in urine during myeloencephalopathy outbreaks

A. Velloso Alvarez[1], E. Jose-Cunilleras[2,3], A. Dorrego-Rodriguez[4], I. Santiago-Llorente[5], M. de la Cuesta-Torrado[1], L. Troya-Portillo[2,3], B. Rivera[4], V. Vitale[1], L. de Juan[4,6] and F. Cruz-Lopez[4].
[1]*University Cardenal Herrera CEU, Valencia, Spain.* [2]*Unitat Equina, Fundació Hospital Clínic Veterinari, Cerdañola del Valles, Spain.* [3]*Department of Animal Medicine and Surgery, Universitat Autònoma de Barcelona, Cerdañola del Valles, Spain.* [4]*VISAVET Health Surveillance Centre, Universidad Complutense, Madrid, Spain.* [5]*Hospital Clínico Veterinario Complutense, Universidad Complutense, Madrid, Spain.* [6]*Animal Health Department, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain*
Email: fatimacr@ucm.es

Background: The detection of EHV-1 by real-time PCR in nasal swabs (NS), whole blood, brain, and spinal cord samples has been extensively described. However, there remains a notable gap in understanding the excretion of the virus in urine, the patterns of DNA detection, and the potential role of urine in viral dissemination during an outbreak.
Objectives: To ascertain the presence of EHV-1 DNA in urine during natural infection and to compare the DNA detection patterns of EHV-1 in urine, buffy coat (BC), and NS.
Study design: Prospective clinical study.
Methods: During the hospitalisation of twenty-one horses involved in two EHV-1 myeloencephalopathy outbreaks in Spain in 2021 and 2023, urine, whole blood, and NS samples were collected at various intervals. Viral DNA load was compared in BC-urine samples from 2021 and NS-urine samples from 2023 by quantitative, real-time PCR.
Results: EHV-1 was detected in a total of eighteen hospitalised horses during the 2021 and 2023 outbreaks, and EHV-1 positivity was confirmed in urine samples from 11 horses across both outbreaks. Comparative analysis revealed that, in contrast to BC samples, the presence of viral DNA persisted in urine for a longer duration (up to 22 days from first fever vs. 13 days) and exhibited significantly higher concentrations in urine samples. However, in comparison to NS, the detection of EHV-1 in urine showed a lower duration and significantly lower DNA concentrations.
Main limitations: These include a restricted sample size and variations in sampling times and protocols between BC and NS samples across the two distinct natural infection outbreak scenarios.
Conclusions: Detection of EHV-1 in urine from naturally infected horses suggests that non-invasively collected urine could serve as a valuable complement to blood and NS samples in the management of infected horses during an outbreak.
Ethical animal research: Not required: performed on archived material collected previously during clinical procedures.
Informed consent: Not stated.
Competing interests: None declared.
Funding: None.

104 | Evaluating non-invasive sampling techniques for the molecular surveillance of Equid herpesvirus (EHV) in naturally shedding yearling horses

A. Khan[1], E. Olajide[1], M. Friedrich[2], A. Holt[2] and L.S. Goehring[1]
[1]*Department of Veterinary Science, Martin-Gatton College of Agriculture, Food and the Environment, University of Kentucky and* [2]*Lincoln Memorial University, Tennessee, USA*
Email: akh242@uky.edu

Background: Equid alphaherpesvirus 1 (EHV-1) is a highly contagious respiratory tract pathogen of horses, and initial infection may be

followed by myeloencephalopathy or abortion. Surveillance and early detection have focused on PCR assays using nasal swabs which are not tolerated well.

Objectives: To assess non-invasive sampling techniques as surveillance tools in naturally infected horses with Equid gammaherpesvirus 2 (EHV-2) as a surrogate.

Study design: Indoor, *in vivo* experimental control study using horses.

Methods: Horses were individually housed for 10-h periods on 2 consecutive days. Sampling involved nasal swabs, nostril wipes (2.54 cm^2 area), environmental swabs (6.45 cm^2 area); droplet catching devices (4.5 cm^2 area) for indirect transmission detection, and air sampling. The latter was done via 2 strategies: combined air samples collected going from horse to horse (200 L/horse), and a collective air sample collected at a stationary central point for 6 h (18 m^3 air). Initial screening was done through quantitative PCR followed by absolute quantitation via digital PCR.

Results: Nine horses on day 1, and 11 horses on day 2 were EHV-2 positive with 100% positivity of nostril wipes, 81.8% of environmental surface, and 90.9% of droplet catching devices. Nostril wipes mean DNA copies detection per cm^2 sampled concentration (D1:3.1×10^5 and D2:5.6×10^5) was significantly ($P < 0.05$) alike nasal swabs (D1:3.5×10^5 and D2:3.6×10^5) followed by environmental swabs (D1: 5.1×10^4 and D2: 3.5×10^4) and droplet catchers (D1: 3.7×10^3 and D2: 3.3×10^5) respectively. Overall, 100% of the air samples collected were positive on both qPCR and dPCR. In individual air samples a mean concentration of 9.0×10^3 (Day1) and 1.1×10^4 (Day2) and in collective air samples 1.1×10^3 (Day1) and 1.2×10^3 (Day2) DNA copies detected per m^3 air sampled.

Conclusion: Environmental samples look promising in replacing direct contact sampling. Environmental and air sampling could become efficient surveillance tools at equestrian events; however, it needs threshold calculations for minimum detection levels.

Ethical animal research: This study was approved by University of Kentucky Institutional Animal Care and Use Committee (2023-4251).

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The International Equestrian Federation (FEI), Lausanne, Switzerland.

105 | Cerebral organoids: why shouldn't horses benefit from these models? Application to equine herpesviruses' (EHVs) studies

B. Pain[1], T. LaRosa[1], C. Poinsignon[1], C. Fortier[2,3], N. Aurine[1], C. Normand[2,3], E. Hue[2,3] and S. Pronost[2,3]

[1]*University of Lyon, Université Lyon 1, INSERM, INRAE, SBRI, Stem Cell and Brain Research Institute, U1208, USC1361, Bron, France;* [2]*LABÉO, 14 280 Saint Contest, France and* [3]*Normandie Université, UNICAEN, BIOTARGEN, 14000 Caen, France*

Email: bertrand.pain@inserm.fr

Background: The study of viruses is inseparable from their host, and the relevance of *in vitro* models is dependent on their ability to mimic an aspect of the *in vivo* environment. Technological, ethical, and legislative developments have led to the emergence of organoid models in many species. The study of equine herpesviruses could in turn benefit from this new approach.

Objectives: To validate a cerebral organoid model derived from equine reprogrammed pluripotent stem cells (ePSCs) to obtain a new relevant *in vitro* 3D model to study host-pathogen interactions, in particular neurotropic viruses.

Study design: Development of new 3D models and proof of concepts.

Methods: Equine cerebral organoids (eCOs) were generated from ePSCs in neural induction medium and grown individually in wells. At D50, the eCOs were infected with one of the three EHV-1 strains (A2254, G2254, and C2254) respectively or with a reference EHV-4 strain at 10000 or 1000 pfu. After 5 days, nucleic acid extraction from organoids and their culture supernatants was performed and viral load of EHV-1 or EHV-4 was determined as previously described.[1]

Results: After 5 days post-contact with 10 000 pfu/ml, all organoids are positive for EHV-1 and the viral load increased by more than 3 Log. An increase of the viral load in the culture supernatant was also measured. The same observations were made after infection with 1000 pfu/ml. Organoids appear to be also permissive for EHV-4 even if the strain seems to replicate less than the 3 EHV-1 strains.

Main limitations: Lack of specific antibodies for immunocytochemistry of eCOs.

Conclusions: These results show for the first time the capacity of the eCOs model to replicate three different strains of EHV-1 and one EHV-4 strain, opening the way for further studies on cell-virus interactions in a neural environment.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Eperon HVE4 IRCP Fund N49-2019, CRB-ANIM-ANR-11-INBS-0003 to BP.

Reference:

[1]: Thieulent CJ, Hue ES, Fortier CI, Dallemagne P, Zientara S, Munier-Lehmann H, Hans A, Fortier GD, Pitel P-H, Vidalain P-O, et al. Screening and Evaluation of Antiviral Compounds against Equid Alpha-Herpesviruses Using an Impedance-Based Cellular Assay. Virology 2019;526:105–116.

106 | Molecular characterisation of varicellovirus equid alpha1 viruses responsible for disease outbreaks (2023–2024)

M. Garvey, L. Dayot, R. Gallagher, G. Lukaseviciute and A. Cullinane*

Virology Unit, The Irish Equine Centre, Johnstown, Naas, County Kildare, Ireland

Email: mgarvey@irishequinecentre.ie

*Corresponding author: ACullinane@irishequinecentre.ie

Background: Surveillance of varicellovirus equid alpha1 (EHV-1) viruses is within our remit as a World Organisation for Animal Health reference laboratory.

Objectives: To genetically classify EHV-1 viruses and assess the prevalence of the ORF30 DNApol D752/N752/H752 genotypes and the Valencia marker ORF11 A713G.

Study design: Cross-sectional.

Methods: Multi-locus sequence typing (MLST) of seven open reading frames (ORFs) was carried out directly from clinical samples as described previously [1]. One virus was isolated in rabbit kidney (RK-13) cells prior to whole genome sequencing (WGS).

Results: Eighteen EHV-1 outbreaks (neurological = 1, multiple abortion = 2, single abortions = 15) were confirmed during the study period. Viruses were characterised by MLST as belonging to six U_L clades: 1, 5, 7, 8, 9 and 10. The most prominent circulating clade was 7. Both the virus from the case of neurological disease and the virus responsible for multiple abortions ($n = 5$) on a single premises over a five month period, belonged to clade 7. The latter was confirmed by WGS. Viruses from the same outbreak had identical MLST profiles and on one farm this was also true for viruses isolated in different years. However, the clade 7 virus associated with the neurological case differed in its genetic profile from a virus (clade 1) associated with an abortion on the same premises nine months earlier. Four of 16 outbreaks analysed to date were associated with the ORF30 D752 genotype, all occurred in 2024 and none were associated with neurological disease. Neither the ORF30 H752 nor the ORF11 A713G genotypes were detected.

Main limitations: Viruses associated with respiratory disease outbreaks were not characterised due to insufficient DNA.

Conclusions: All viruses characterised belonged to previously recognised U_L clades. Clade 7 continues to predominate in Ireland. This study corroborates other findings that the ORF30 D752 genotype is not a reliable predictor of neurological disease.

Key manufacturer: 1 Equi N Tect ERP, Nisseiken, Japan, https://www.jp-nisseiken.co.jp.

Acknowledgements: The staff in the Virology Unit at the Irish Equine Centre for diagnostic testing of samples.

Ethical animal research: Not required: excess material from clinical samples was used.

Informed consent: Not stated.

Competing Interests: None declared.

Funding: The laboratory work was funded by the Department of Agriculture, Food and the Marine, Ireland.

Reference:

[1] Garvey M, Lyons R, Hector RD, Walsh C, Arkins S, Cullinane A. Molecular Characterisation of Equine Herpesvirus 1 Isolates from Cases of Abortion, Respiratory and Neurological Disease in Ireland between 1990 and 2017. Pathogens. 2019;8(1):7. doi: 10.3390/pathogens8010007.

107 | Evaluation of a $PtCl_4$ and PMAxx™-assisted PCR to evaluate capsid integrity of *Varicellovirus equidalpha4* (EHV-4)

C. Normand[1,2], C. Fortier[1,2], A. Guenoux[1,2], C. Lupo[3], P.-H. Pitel[1], E. Hue[1,2,†] and S. Pronost[1,2,†]

[1]*LABÉO, 14 280 Saint-Contest, France;* [2]*Normandie Université, UNICAEN, BIOTARGEN, 14280 Saint-Contest, France and* [3]*RESPE-French Network for Surveillance of Equine Diseases, 14 280 Saint Contest, France*

[†]These authors contributed equally.

Email: camille.normand@laboratoire-labeo.fr

Background: *Varicellovirus equidalpha4* (EHV-4) is an endemic horse pathogen worldwide. EHV-4 is responsible for many respiratory outbreaks and can sporadically induce abortion. Sero-epidemiological investigations show that more than 70% of tested animals were positive. During outbreaks, EHV-4 can be excreted by silent shedders as a source of environmental contamination. Currently, PCR methods are able to detect EHV-4 but are unable to differentiate between infectious and degraded virus. This could be done by cell culture, but it is time-consuming.

Objectives: Develop a new PCR technology for rapidly differentiating between infectious and degraded viruses.

Study design: *In vitro* experiments.

Methods: A PCR test was developed to amplify a long fragment of 892 pb allowing amplification of EHV-4 but also EHV-1, EHV-8 and EHV-9. EHV-4-spiked PBS was heat-inactivated at 2 temperatures then pre-treated with platinum (IV) chloride ($PtCl_4$) or a propidium monoazide (PMAxx™) which can permeate viruses with damaged capsids. These compounds will intercalate into nucleic acids and inhibit PCR amplification in degraded viruses. Different concentrations of $PtCl_4$ and PMAxx™ were tested. Results were expressed by calculating the ΔCt value between a treated sample and the untreated native virus.

Results: After amplification in the presence of PMAxx™ for the inactivated virus at 95°C, a ΔCt value of 11.02 ± 1.85 was observed compared to the untreated, native virus. Better results were observed with the use of PMAxx™ compared with using $PtCl_4$. In addition, this technology was evaluated to assess a disinfection process by heat treatment at 56°C.

Main limitations: Toxicity of $PtCl_4$. Currently, not performed in field conditions.

Conclusions: The proof of concept was established after a 95°C heat treatment of a viral sample. PMAxx™-assisted PCR could be a new way to evaluate the infectivity of environmental samples contaminated by equine herpesviruses.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Eperon HVE4 IRCP Fund N49-2019, the IFCE (Institut Français du Cheval et de l'Equitation) grant number Rech-CS-2020-2023-023-HVE4_IRCP. CENTAURE European project co-financed by the Conseil Régional de Normandie, European Union within the framework of the ERDF-SSE operational programme 2014-2020.

108 | ImpedanCELL: A core facility to screen and evaluate antiviral compounds against equine viruses and a new approach to sero-neutralisation

E.S. Hue[1,2,3], C. Normand[1,3], C.J. Thieulent[1,3], G. Sutton[1,3], F. Carnet[1,3], C. Fortier[1,2,3], E. Brotin[3,4,5], C. Denoyelle[3,4,5] and S. Pronost*[1,2,3]

[1]LABÉO, Saint-Contest, France; [2]Normandie Université, UNICAEN, BIOTARGEN, Saint-Contest, France; [3]Normandie Université, UNICAEN, ImpedanCELL, Saint-Contest, France; [4]Normandie Université, UNICAEN, ANTICIPE—Unité de recherche interdisciplinaire pour la prévention et le traitement des cancers, Caen, France and [5]UNICANCER/CRLC—Centre Régional de Lutte contre le Cancer François Baclesse, Caen, France

Email: erika.hue@laboratoire-labeo.fr and *Corresponding author: stephane.pronost@laboratoire-labeo.fr

Background: ImpedanCELL (labelled IBiSA, national recognition by the major research institutions in France) is an innovative and original core facility for the study of real-time high throughput cellular activity using impedance measurement (xCELLigence technology) and real-time cellular imaging. It is located in the equine research department of LABÉO, Saint-Contest, for infectious disease applications (biosafety level 2) and in Comprehensive Cancer Center F. Baclesse, Caen, for all non- infectious applications. The core facility is equipped with cutting-edge technologies based on xCELLigence Real-Time Cell Analysis (RTCA) and live-cell imaging devices (Incucyte S3) both allowing the study of proliferation, cell death, adhesion, migration and invasion in various domains including virology, bacteriology, immunology, toxicology, oncology.

Objectives: To illustrate different applications of the ImpedanCELL platform for (1) the screening and identification of antiviral compounds against equine viruses and (2) development of real-time neutralisation test, a new serological approach.

Study design: *In vitro* experiments.

Methods: A high-throughput screening of 2891 compounds was performed against *Varicellovirus equidalpha1* (EHV-1) [1] and 42 compounds against EHV-4.[2] Efficacy of the identified compounds was confirmed by qPCR and microscopy observation. The technology has also been tested to measure antibody levels quantitatively (RTNA).

Results: Six compounds were identified for their antiviral potency on different cell lines (E. Derm, RK13 and EEK cells) against alpha-EHV including EHV-1 variants (ORF30 2254 A/G/C) and EHV-4. A strong correlation was observed between RTNA and seroneutralisation test for EHV-1 and equine influenza virus (EIV).

Main limitations: The number of samples tested for RTNA.

Conclusions: This work has shown that impedance measurement is very useful for screening antiviral compounds and quantified serological responses for different equine viruses (alpha-EHV, EIV). The core facility is open to collaborations and services for both academic and industrial partners and offers theoretical and practical courses.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: CENTAURE European project co-financed by the Conseil Régional de Normandie, European Union within the framework of the ERDF-SSE operational programme 2014–2020

References:

[1] Thieulent C, Hue ES, Sutton G, Fortier C, Dallemagne P, Zientara S, Munier-Lehmann H, Hans A, Paillot R, Vidalain P-O, Pronost S. Identification of antiviral compounds against equid herpesvirus-1 using real-time cell assay screening: Efficacy of decitabine and valganciclovir alone or in combination. Antiviral Res. 2020;183:104931. doi:10.1016/j.antiviral.2020.104931.

[2] Normand *et al*., *Viruses*, 2024 (viruses-2 961 355, *in press*).

Virology 7: Antiviral Immunology 1

Michel D'Ornano Thursday 15.00–16.00

109 | Equine herpesvirus type 1 specific T cells in the upper respiratory tract

C.M. Holmes and B. Wagner

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Email: cmh335@cornell.edu

Background: Equine herpesvirus type 1 (EHV-1) is a respiratory pathogen of horses, with yearly outbreaks impacting the equine industry. During EHV-1 infection, horses develop a systemic cytotoxic $CD8^+$ T cell (CTL) response and IFN-γ producing $CD8^+$ T cells following viral exposure. Virus specific T cell responses at the primary entry site of EHV-1 infection, the upper respiratory tract (URT), have not yet been explored.

Objectives: To compare peripheral and mucosal URT T cells that contribute to the mucosal immune response to EHV-1.

Study design: In vitro experiments.

Methods: T cells were collected from healthy adult horses with ($n = 5$) or without ($n = 5$) immunity to EHV-1 and assessed for their functional capacity. Cells were cultured *in vitro* and stimulated with phorbol 12-myristate 13- acetate and ionomycin (PMA/iono) or infected with the Ab4 strain of EHV-1. Cells were stained for IFN-γ, LFA-1, CD4, and CD8, and analysed by flow cytometry.

Results: Following PMA/iono stimulation, both peripheral (43.4 ± 14.5%) and mucosal lymphocytes (30.5 ± 18.1%) had a population of $CD8^+$ IFN-γ^+ cells, with a significant increase compared to cells kept in medium (peripheral: 0.2 ± 0.2%, mucosal: 1.8 ± 1.6%) ($p < 0.0001$). A higher percentage of peripheral compared to mucosal cells were activated by PMA/iono ($p = 0.017$). In response to EHV-1, horses with immunity had higher percentages of peripheral $CD8^+$ IFN-γ^+ cells (13.5 ± 8.5%) compared to horses without immunity (2.7 ± 2.2%) ($p = 0.0016$). In contrast, mucosal cells did not respond to *in vitro* EHV-1 infection in either group.

Main limitations: The low cell recovery from mucosal samples of healthy horses can make analysis of T cell responses to EHV-1 challenging.

Conclusions: Healthy horses have functional mucosal T cells which can be activated upon PMA/iono stimulation. In contrast to peripheral T cells, EHV-1 specific mucosal T cells were not detected in the nasal cavity of healthy horses. This points towards the rarity of EHV-1 specific mucosal T cells.

Ethical animal research: Approved by the Institutional Animal Care and Use Committee at Cornell University (protocol #2011-0011).

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The American Quarter Horse Foundation and The Harry M. Zweig Memorial Fund for Equine Research.

110 | Use of an equine neurological model of EHV-1 to identify host immune factors that contribute to the development of equine herpesvirus myeloencephalopathy

G. Soboll Hussey[1*], K. Giessler[1,2], L. Goehring[3], S. Jacob[1], A. Davis[1], M. Esser[1], Y. Lee[1,4], L. Zarski[1] and P.S. Weber[5]

[1]Dept. of Pathobiology & Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA; [2]Bavarian Health and Food Safety Authority, Oberschleissheim, Germany; [3]MH Gluck Equine Research Center, College of Agriculture, Food & Environment, University of Kentucky, Lexington, KY, USA; [4]In Vivo Animal Core, Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI, USA and [5] Dept of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA

Email: husseygi@msu.edu

Background: Equine Herpesvirus 1 (EHV-1) causes respiratory disease, abortions, and myeloencephalitis (EHM) in ~10% of infected horses worldwide. In contrast, in mares >20 years, EHM incidence increases to 70%.

Objectives: To undertake EHV-1 infection of old mares to induce EHM and identify host specific factors contributing to EHM.

Study design: In vivo experiments

Methods: Old mares (>17 years) and young horses (2 years) were infected with EHV-1 and studied for 21 days post infection. Nasal viral shedding and viremia were assessed by qPCR. Cytokine/chemokine responses were evaluated in nasal secretions and cerebrospinal fluid by Luminex and in blood by qRT-PCR. EHV-1 specific IgG sub-isotype responses were measured by ELISA.

Results: All young horses developed respiratory disease and a biphasic fever, but only 1/9 young horses exhibited ataxia. In contrast, respiratory disease was absent in old horses, but 7/10 old horses developed severe EHM. Old horses presented significantly decreased nasal viral shedding but higher viremia that coincided with a single fever peak. Consistent with the clinical signs, non-EHM horses showed an early upregulation of IFN-alpha (nasal secretions), IRF7/IRF9, IL-1beta, CXCL10, Tbet and IFN gamma (blood). In contrast, IFN-alpha in nasal secretions of EHM horses was low and induction of cellular immunity in the blood was delayed. Finally, EHM horses showed significantly higher IL-10 levels in nasal secretions, PBMCs and CSF and higher serum IgG3/5 antibodies.

Main limitations: In our neurological model, the "at risk profile for EHM" might be biased towards the profile of old mares. However, when comparing our findings with results from 4 yearling horses with EHM from previous studies, we find that their immunophenotype mirrors the "at risk phenotype".

Conclusions: It is suggested that a regulatory or TH-2 profile correlates with an increased risk for EHM and future vaccine development for protection against EHM must target shifting this "at risk" immunophenotype.

Ethical animal research: Approved by Michigan State University's Institutional Animal Care and Use Committee, under protocol "PROTO201800015".

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Grayson Jockey Club Research Foundation.

111 | Mucosal antibodies against equine herpesvirus type-1 and their role in preventing infection

B. Wagner, C. Holmes, N. Eady, C. Schnabel and S. Babasyan

Department of Population Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Email: bw73@cornell.edu

Background: Equine herpesvirus type-1 (EHV-1) infects through the epithelium of the upper respiratory tract (URT). Previous research has shown that mucosal antibodies (mucAbs) correlate with protection.

Objectives: We hypothesised that mucAbs neutralise EHV-1 at the URT and prevent infection of respiratory epithelial cells. Our objective was to provide evidence that mucAbs are neutralising EHV-1 and to characterise the mucAb isotypes most efficient in preventing infection.

Study design: In vivo experiments.

Methods: Nasal wash and swab samples from EHV-1 immune and non-immune research horses were used to analyse neutralising nasal mucAbs ($n = 8$ per group) and viral replication (($n = 4$ per group) at the URT after experimental EHV-1 challenge *in vivo*. Horses were experimentally infected with 10^7 PFU EHV-1 Ab4. Nasal swab and wash samples were collected pre- and post-infection. Swabs were used for viral detection. Wash samples were used for mucAb quantification and to purify IgA and IgG isotypes. Mucosal isotypes were tested in neutralisation assays.

Results: Contrary to non-immune horses, immune horses had no fever, clinical signs, viral shedding or viremia after EHV-1 infection. Immune horses had pre-existing mucAbs which rapidly increased after infection. Mucosal IgG1 and IgG4/7 neutralised EHV-1 *in vitro*, while IgA did not. In non-immune horses, virus was fully replicating, while mucAb

development was delayed. Virus isolation, EHV-1 PCR, and RNA sequencing indicated a significantly reduced EHV-1 load at the URT of immune horses, and viral gene expression profiles were incomplete.

Main limitations: Classical neutralisation assays are not sensitive enough to identify mucAbs. This study required purification of mucosal antibodies from larger nasal wash sample volumes.

Conclusions: Mucosal IgG1 and IgG4/7 can effectively neutralise EHV-1 and are instrumental in preventing EHV-1 replication at the URT. In immune horses with mucAbs, viral replication is compromised and EHV-1 cannot progress to the viremic stage.

Ethical animal research: Approved by the Institutional Animal Care and Use Committee at Cornell University (protocol #2011-0011).

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Harry M. Zweig Memorial Fund for Equine Research at Cornell University, and the Agriculture and Food Research Initiative competitive grant no 2015-67015-23 091 supported by the US Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA).

112 | Modulation of equid herpesvirus-1 replication dynamics *in vitro* using CRISPR/Cas9-assisted genome editing

R.T. Hassanien[1], C.J. Thieulent[2], M. Carossino[2], G. Li[3] and U.B.R. Balasuriya[2]

[1]*Virology Department, Animal Health Research Institute, Agriculture Research Center (ARC), Dokki, Giza 12 618, Egypt;* [2]*Louisiana Animal Disease Diagnostic Laboratory and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA and* [3]*Department of Veterinary Diagnostics and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA*

Email: thieulentcome@gmail.com

Background: Equid alphaherpesvirus-1 (EHV-1) is one of the most common and widespread equine viruses that pose a serious threat to the global equine industry. This virus causes respiratory disease, ocular disease, abortions, neonatal mortalities, and neurological disorders. EHV-1 genes are co-ordinately expressed and temporally regulated in immediate-early, early, and late genes during lytic infection. During the past decade, genome editing technologies have rapidly evolved in biological research, and the CRISPR/Cas9 system has emerged as one of the most powerful genome-editing tools. Lately, various studies have used this system to impair herpesvirus replication *in vitro*, such as HSV-1, HSV-2 HCMV, and EBV.

Objectives: To assess the ability of the CRISPR/Cas9 system to suppress EHV- 1 lytic infection by specifically targeting essential viral genes.

Study design: *In vitro* experiment.

Methods: A novel approach was employed using single guide RNA (sgRNAs) to target three essential (ORF30, ORF31, and ORF7) and one non-essential (ORF74) EHV-1 genes. The efficacy of these sgRNAs in inhibiting viral replication was evaluated *in vitro* using plaque assay and qPCR. Next-generation sequencing (NGS) was utilised to detect EHV-1 editing sites containing insertions, deletions, or mutations (indels).

Results: The findings revealed that sgRNAs targeting essential lytic genes effectively reduced EHV-1 replication, while those targeting ORF74 (non-essential gene) had a minimal impact. Notably, the sgRNAs targeting ORF30 demonstrated the most potent effect in suppressing EHV-1 replication. NGS analysis identified variants with deletions in the specific cleavage site of selective sgRNAs. Moreover, we observed a synergistic effect between the double combination of sgRNAs targeting ORF30 and ORF7, which significantly reduced viral replication to a greater extent than the use of a single sgRNA.

Main limitations: While CRISPR/Cas9 system is efficient *in vitro*, its' *in vivo* application is limited.

Conclusions: These data demonstrate that the CRISPR/Cas9 system can be used to inhibit EHV- 1 replication *in vitro*. Its application will be evaluated against other equine DNA and RNA viruses.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: USAID (Higher Education Initiative, Graduate Scholarships for Profession [GSP]), Postdoctoral Research Program for R.T.H., as a Visiting Research Scholar in Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA and USAID GSP and self-generated research funds (PG008671) from U.B.R.B. LADDL and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

Virology 8: Antiviral Immunology 2

Michel D'Ornano 16.30–17.30

113 | Use of transcriptomic analysis of peripheral blood mononuclear cells collected from horses during equine herpesvirus-1 (EHV-1) and 4 (EHV-4) infection and horses with and without EHV-1 myeloencephalitis (EHM)

G. Soboll Hussey, G. Berríos-Vázquez and L. Zarskil

Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI 48824, USA

Email: husseygi@msu.edu

Background: Equine herpesvirus 1 (EHV-1) causes respiratory disease, abortions, and myeloencephalitis (EHM) in ~10% of infected horses worldwide. In contrast, EHV-4 is a major cause of respiratory disease but does not cause EHM.

Objectives: To compare the viral and host specific transcriptome in horses infected with EHV-1 and EHV-4 to uncover differences in the pathogenesis between EHV-1 and EHV-4 and horses with and without EHM.

Study design: In vivo experiments.

Methods: PBMCs were collected from horses infected with EHV-1, (with and without EHM), and EHV-4 and viral and host transcriptomic profiles were compared. PBMCs were collected from horses pre-infection, and during the febrile phase following infection with EHV-1 and EHV-4. RNA was extracted and illumina sequencing and library preparations were performed. Differentially expressed host genes (DEGs) between EHV-1 and EHV-4 infected horses (and horses with and without EHM) were identified and functional pathway analysis and *in silico* cell sorting were completed. Additionally, viral gene counts were determined and compared.

Results: When comparing EHV-4 with EHV-1 infected horses, a total of 618 DEGs were found. Interestingly, viral gene expression indicated establishment of viremia following infection with EHV-1 and EHV-4, but EHV-4 gene expression was significantly lower. Functional pathway analyses showed significant upregulation of genes involved in cell proliferation, cellular immunity, inflammation, and viral defence in EHV-4 infected horses compared to EHV-1 infected horses. Finally, in horses exhibiting EHM, a dysregulation of T-cell activation was identified and overall responses skewed towards a TH-2 phenotype.

Main limitations: Transcriptomic analysis was limited to bulk PBMC from each horse.

Conclusions: It is suggested that infection with EHV-4 leads to increased activation of cellular immunity and decreased viremia compared to infection with EHV-1. Moreover, the transcriptomic profile of horses exhibiting EHM following infection with EHV-1 is characterised by a dysregulation of T-cell activation and a regulatory/TH-2 immunophenotype.

Ethical animal research: Approved by Michigan State Animal Care and Use Committee.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: USDA/NIFA.

114 | A screening study identified decitabine as an inhibitor of *Varicellovirus equidalpha4* enhancing the innate antiviral response

C. Normand[1,2], C. Thieulent[1,2], C. Fortier[1,2,3], Gabrielle Sutton[1,2], C. Senamaud-Beaufort[4], Laurent Jourdren[4], Corinne Blugeon[4], Pierre-Olivier Vidalain[5], Stéphane Pronost[1,2,3†] and Erika Hue[1,2,3†]

[1]LABÉO, 14 280 Saint-Contest, France; [2]Normandie Université, UNICAEN, BIOTARGEN, 14280 Saint-Contest, France; [3]Normandie Université, UNICAEN, ImpedanCELL, 14 280 Saint-Contest, France; [4]GenomiqueENS, Institut de Biologie de l'ENS (IBENS), Département de biologie, École normale supérieure, CNRS, INSERM, Université PSL, 75005 Paris, France and [5]CIRI, Centre International de Recherche en Infectiologie, Team Viral Infection, Metabolism and Immunity, Univ Lyon, Institut National de la Santé et de la Recherche Médicale (Inserm), U1111, Centre National de la Recherche Scientifique (CNRS), UMR5308, Ecole Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, 69 007 Lyon, France

†These authors had contributed equally.

Email: camille.normand@laboratoire-labeo.fr

Background: *Varicellovirus equidalpha4* (EHV-4) is a frequent respiratory pathogen of the horse. EHV-4 sporadically induces abortion or neonatal death and although not clearly demonstrated its involvement in neurological forms is strongly suspected. Despite preventive treatments using vaccines against EHV-1/EHV-4, the resurgence of alpha-EHV infection still constitutes an important threat to the horse industry.

Objectives: Testing the efficacy of different compounds on EHV-4 and studying the mode of action of the most effective one.

Study design: Screening and transcriptomic approach.

Methods: A screening of 42 antiviral compounds was performed *in vitro* on E. Derm cells infected with EHV-4405/76 reference strain (VR2230). Formation of cytopathic effects was monitored by Real-Time Cell Analysis (xCELLigence and video-microscopy) and the viral load was quantified by qPCR.

Results: Potential antiviral activities were confirmed for 8 molecules from the 42 compounds (idoxuridine, vidarabine, pritelivir, cidofovir, valganciclovir, ganciclovir, aphidicolin, and decitabine). Decitabine is the most potent compound against EHV-4 *in vitro* with an EC_{50} value of 1.16 ± 0.31 μM and 0.28 ± 0.05 μM measured by xCELLigence and by qPCR, respectively. A transcriptomic analysis revealed an increase in expression of various genes involved in the interferon response.

Main limitations: Study performed on one cell line with one reference strain.

Conclusions: This work confirms the effect of ganciclovir against EHV-4 *in vitro*. The study was unable to demonstrate *in vitro* the antiviral activity of acyclovir against EHV-4, a molecule frequently used in the field by veterinary practitioners against equine herpesviruses. The compound with the best efficacy in inhibiting EHV-4 replication *in vitro* is decitabine. Transcriptomic analysis of infected cells treated with decitabine revealed activation of the innate antiviral response by stimulation of the interferon pathway.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Eperon HVE4 IRCP Fund N49-2019, the IFCE (Institut Français du Cheval et de l'Equitation) grant number Rech-CS-2020-2023-023-HVE4_IRCP. CENTAURE European project co-financed by the Conseil Régional de Normandie, European Union within the framework of the ERDF-SSE operational programme 2014–2020.

115 | Intramuscular EHV vaccination results in systemic and mucosal antibodies

B. Wagner, C. Schnabel and A. Rollins

Department of Population Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Email: bw73@cornell.edu

Background: Equine herpesvirus type-1 (EHV-1) infects through the epithelium of the upper respiratory tract (URT). Mucosal antibodies (mucAbs) against EHV-1 were shown previously to correlate with protection from disease. EHV vaccination is typically performed intramuscularly (i.m.). Transfer of systemic vaccine induced antibodies to the URT has not been shown.

Objectives: The hypotheses were (i) intramuscular vaccination will result in both systemic and mucAbs, and (ii) frequent vaccination will not increase antibodies beyond certain concentrations. The objective was to provide information on systemic and mucAb responses after frequent vaccination of horses with prior EHV vaccination and/or infection history.

Study design: Descriptive longitudinal vaccination study.

Methods: Fourteen Icelandic research horses were used, 5–13 years-old, ten mares, five geldings. All horses had existing EHV-1 antibodies prior to this vaccination study and thus resemble adult client horses with an EHV vaccination history. Horses were vaccinated[1] i.m. on days 0, 22 and 2, 3, 6 and 8 months. Serum and nasal swab samples were collected at different times post-vaccination and used for antibody detection in a new sensitive EHV-1 Risk Evaluation assay. EHV-1 specific antibody responses were compared pre- and post-vaccination by Friedman tests with Dunn's post-tests.

Results: EHV-1 specific serum antibodies plateaued after the second and subsequent vaccinations. MucAbs significantly increased beyond pre-vaccination levels after the fourth vaccination and consisted mostly of IgG4/7.

Main limitations: Small horse numbers.

Conclusions: Intramuscular vaccination resulted in increasing mucAbs at the URT which likely can neutralise EHV, thereby preventing disease. Frequent vaccination increased mucAbs while serum antibodies were less affected.

Key manufacturer: 1 Calvenza EHV® https://bi-animalhealth.com/equine/vaccines/calvenza.

Ethical animal research: Approved by the Institutional Animal Care and Use Committee at Cornell University (protocol #2011–0011).

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Harry M. Zweig Memorial Fund for Equine Research at Cornell University.

116 | Early mucosal immune responses to equine herpesvirus type 1 infection

C.M. Holmes, S. Babasyan and B. Wagner

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Email: cmh335@cornell.edu

Background: Equine herpesvirus type 1 (EHV-1) is a highly prevalent, respiratory pathogen in horses, which infects through the upper respiratory tract (URT). EHV-1 first replicates locally at the URT before cell-associated viremia is established, which can result in severe clinical outcomes, like abortion and/or equine herpesvirus myeloencephalopathy. The URT consists of a heterogenous population of immune and structural cells which contribute to the mucosal barrier. Thus, the early mucosal immune response is essential in limiting infection or mediating protection against EHV-1.

Objectives: Here the nasal transcriptome was analysed during early infection to identify mucosal immune pathways contributing to immunity against EHV-1.

Study design: in vivo experiment.

Methods: Immune ($n = 4$) and non-immune horses ($n = 4$) were experimentally infected with EHV-1. Clinical signs, virus shedding, viremia and mucosal immunity were evaluated. RNA sequencing was performed on nasopharyngeal swabs. Bead-based assays were used to measure protein concentrations in the same samples for confirmation of the top RNAseq results during early infection.

Results: Immune horses had a rapid upregulation of innate immune genes, including the homeostatic regulator, antileukoproteinase (SLPI, $p = 0.045$, immune: 829 ± 730, non-immune: 108 ± 39 reads). Meanwhile interferon stimulated genes (ISGs), including IFN-induced protein with tetratricopeptide repeats 2 (IFIT2, $p < 0.0001$, immune: 1586 ± 491, non-immune: 6067 ± 2617 reads), were upregulated in non-immune horses. Protein concentrations of SLPI and IFN-α were upregulated during early infection in concert with gene expression.

Main limitations: Small sample number. Samples were collected longitudinally during infection, limiting cell collection to low invasive and thus lowered cell yield.

Conclusions: Immune horses upregulated mucosal SLPI RNA and protein secretion immediately after EHV-1 infection, while non-immune horses lacked early SLPI expression and responded with type I IFN pathway upregulation. This suggested a role of early mucosal SLPI secretion in protection from EHV-1 infection and the need for type IFN production for locally controlling the virus in non-immune horses.

Ethical animal research: Approved by the Institutional Animal Care and Use Committee at Cornell University (protocol #2011-0011).

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Harry M. Zweig Memorial Fund for Equine Research.

Virology 9: Hepatitis

Michel D'Ornano Friday 09.00–10.30

117 | Chronic hepatitis in horses with equine hepacivirus infection

M. Jager[1], D. Luethy[2], T.J. Divers[3], G.R. Van de Walle[4] and J.E. Tomlinson[2]

[1]*Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA;*

[2]Department of Clinical Studies New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA; [3]Department of Clinical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA and [4]Baker Institute for Animal Health, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Email: joytom@vet.upenn.edu

Background: Equine hepacivirus (EqHV) is closely related to hepatitis C virus (HCV), which causes persistent infection and chronic hepatitis in people. EqHV causes subclinical hepatitis during acute resolving infection, however, there is limited information on hepatitis associated with chronic infection.

Objectives: To report 26 clinical cases of chronic hepatitis in horses infected with EqHV.

Study design: Case series.

Methods: Horses presented with the following inclusion criteria: (1) chronic hepatitis, defined as at least one month duration of elevated serum liver biomarkers and/or elevated serum liver biomarkers with findings of chronicity on liver histopathology, such as fibrosis; (2) serum or liver EqHV RT-qPCR positive; and (3) liver histopathology performed. Liver biopsies were independently reviewed and scored by 17 individual features.

Results: Twenty-six horses met inclusion criteria. Two horses had acute resolving infections and bacterial cholangiohepatitis. Eight horses died within 6 months and persistent infection could not be verified. Sixteen horses had persistent hepaciviral infection of ≥6 months follow-up. These 16 horses had a median age of 16 years old (range, 6–20) years old, comprised 6 mares and 10 geldings, and all were light breeds. Median duration of documented hepatitis was 18 (5–120) months with median duration of documented EqHV viremia of 12 (6.6–42) months. Predominant histopathologic findings were lymphocytic inflammation and nodules, bridging and dissecting fibrosis, and individual hepatocyte necrosis.

Main limitations: The definitive cause of hepatitis in these horses cannot be determined.

Conclusions: The similarities between these cases and HCV suggest it is likely that EqHV causes chronic hepatitis and liver failure in horses.

Ethical animal research: If samples were requested for research purposes, procedures were covered under Cornell University Institutional Animal Care and Use Committee oversight (IACUC #2014−-0024).

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: JET received speaker honoraria for presentations including information presented in this manuscript.

Funding: National Institutes of Health, National Institute of Allergy and Infectious Diseases K08AI141767; National Institutes of Health, NIH Office of the Director T32ODO011000; U.S. Department of Agriculture, National Institute of Food and Agriculture 2022-67 015-36 343

118 | Equine hepacivirus and equine parvovirus-hepatitis in hospitalised horses in Austria

D. Lale[1,2], E.E. Dirks[1,3], I. Preining[1], A. Gömer[4], E. Steinmann[4], J.-M.V. Cavalleri[1] and A.S. Ramsauer[1,2]

[1]University Equine Hospital, Clinical Unit of Equine Internal Medicine, University of Veterinary Medicine Vienna, Vienna, Austria; [2]Institute of Virology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland; [3]Unit for Hospital Hygiene, Infection Prevention and Control, Department of Infectious Diseases, Robert Koch Institute, 13 353 Berlin, Germany and [4]Department of Molecular and Medical Virology, Faculty of Medicine, Ruhr-University Bochum, Bochum, Germany

Email: dilara.lale@uzh.ch

Background: Equine hepacivirus (EqHV) and equine parvovirus-hepatitis (EqPV-H) are associated with subclinical to clinically relevant hepatitis in horses. Limited data exists on infection and viral shedding of these equine liver viruses.

Objectives: To study the frequency of EqHV and EqPV-H occurrence, possible viral shedding routes, and co-infection in hospitalised horses.

Study design: Cross-sectional study.

Methods: Serum, faecal, nasal, and buccal swab samples of 116 hospitalised horses at the University Equine Hospital of the University of Veterinary Medicine Vienna between 02/2021–03/2022 were collected. Luciferase immunoprecipitation system assay (LIPS) was used to detect the presence of anti-EqHV and anti-EqPV-H antibodies in the serum. Equine hepaci virus RNA was detected by RT-qPCR and qPCR was used for EqPV-H DNA detection.

Results: Seroprevalence of EqHV and EqPV-H was 35.3% (41/116) and 10.3% (12/116). Equine hepacivirus RNA was detected in 4.3% (5/116) of the hospitalised horses and 12.9% (15/116) tested positive for EqPV-H DNA. Two horses were co-infected with EqHV and EqPV-H and seroconversion was detected for both viruses. One co-infected horse's nasal and buccal sample tested positive for EqHV RNA. Equine parvovirus-hepatitis DNA was detected in both nasal and faecal samples of one horse, as well as in a nasal sample of another horse, both revealing EqPV-H viremia.

Main limitations: Further infection studies are needed to assess the infectious potential of the viral material shed from the nasal, buccal, and faecal samples.

Conclusions: In the hospitalised horse population studied, both subclinical co-infection and single infection of EqHV and EqPV-H were identified. There might be a risk of horizontal transmission since nucleic acids of both viruses were detected in nasal samples and EqPV-H DNA in one faecal sample. To prevent iatrogenic transmission, screening of donor horses for these hepatic viruses before using equid-derived products is advisable.

Ethical animal research: Approved by the ethics committee of the University of Veterinary Medicine Vienna (study reference number ETK-124/08/2020).

Informed consent: Owner-informed consent was obtained.

Competing interests: None declared.

Funding: The Foundation PRO Pferd, Switzerland (PR 2020-11).

119 | Detection of equine hepacivirus RNA and equine parvovirus-hepatitis DNA in *Stomoxys calcitrans* in eastern Austria

V. Frisch[1], S. Ramsauer[1,2], I. Preining[1], M.S. Unterköfler[4], T. Borysova[1], M. Haller[1], J. Wigger[3], M. Lyrakis[3], H.-P. Fuehrer[4] and J.-M.V. Cavalleri[1]

[1]*Equine Internal Medicine, Clinical Centre for Equine Health and Research, Department for Small Animals and Horses, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria;* [2]*Institute of Virology, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 266a, 8057 Zurich, Switzerland;* [3]*Platform for Bioinformatics and Biostatistics, Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria and* [4]*Institute of Parasitology, Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria*

Email: frivicky@hotmail.com

Background: Equine hepatic viruses are detected worldwide in equids with a global prevalence of equine hepacivirus (EqHV) ranging up to 16.1% and equine parvovirus-hepatitis (EqPV-H) up to 37% in horses. Recently, an equid hepatitis B virus (EqHBV) was detected in donkeys and zebras with a prevalence of up to 3.2%. Iatrogenic transmission via blood products was demonstrated for EqHV and EqPV-H, while vertical transmission of EqHV was observed. To date, transmission via vectors has not been detected or investigated for all three viruses.

Objectives: To investigate the detection of EqHV RNA, EqPV-H and EqHBV DNA in *Stomoxys calcitrans*.

Study design: Descriptive epidemiological investigation.

Methods: In 2021 and 2022 from July to October, 802 *S. calcitrans* were caught with CO_2 baited BG sentinel traps in two horse stables near Vienna, and at the University of Veterinary Medicine, Vienna every other week. A maximum of five flies´ heads and thoraxes including legs and wings were pooled and analysed for the presence of EqHV RNA by RT-qPCR and EqPV-H and EqHBV DNA by qPCR. The minimum infection rate (MIR) and the infection rate via maximum likelihood estimation (IR-MLE) were calculated.

Results: Between 2021 and 2022 for EqHV the MIR ranged up to 3.87% and the IR-MLE up to 3.93%. For EqPV-H, the MIR ranged up to 1.1% and the IR-MLE up to 1.11%. Highest values for both viruses were detected in autumn 2021 and 2022. EqHBV could not be detected.

Main limitations: The amounts of viral DNA/RNA were close to the limit of detection for EqHV and EqPV-H. No final conclusion of the infectious potential can be drawn.

Conclusions: EqHV RNA and EqPV-H DNA could be detected in the head and thorax region of *S. calcitrans*, a role as mechanical vectors for these viruses has to be evaluated further.

Ethical animal research: Not required.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Stiftung Pro Pferd.

120 | Preliminary data on biomolecular investigation of equid hepatitis B virus in Italian horses, donkeys and mules

G. Pacchiarotti[1], R. Nardini[1], A. Cersini[1], G. Brocherel[1], G. Bruni[1], E. Sezzi[1], G. Terracciano[1], N. Cavaliere[2], S. Costarelli[3], C. De Martinis[4], C. Di Pancrazio[5], F. Gobbo[6], M.L. Mandola[7], G. Purpari[8], A. Ruiu[9], M. Tonni[10] and M.T. Scicluna[1]

[1]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", via Appia Nuova, 1411 Rome, Italy;* [2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, via Manfredonia, n. 20, Foggia, Italy;* [3]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Via G.Salvemini, 1, Perugia, Italy;* [4]*Istituto Zooprofilattico Sperimentale del Mezzogiorno Via Salute, 2—Portici (Naples), Italy;* [5]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale," Campo Boario Teramo, Italy;* [6]*Istituto Zooprofilattico Sperimentale delle Venezie Viale dell'Università 10, Legnaro (Padua), Italy;* [7]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, Turin, Italy;* [8]*Istituto Zooprofilattico Sperimentale della Sicilia Via Gino Marinuzzi 3—Palermo, Italy;* [9]*Istituto Zooprofilattico Sperimentale della Sardegna, Via Duca degli Abruzzi n. 8 Sassari, Italy and* [10]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" Via Bianchi, 9, Brescia, Italy*

Email: roberto.nardini@izslt.it

Background: Equid hepatitis B virus (EqHBV) is a recently discovered virus (Family: *Hepadnaviridae*) affecting equids and causing hepatic disease.[1] To date only donkeys and zebras seem to be susceptible, while investigations in horses did not detect virus circulation.[1] For Italy, data are limited.[1]

Objectives: Assessing the bio-molecular EqHBV national prevalence in a potentially susceptible horse production category and in donkeys and mules of two Central Italian regions.

Study design: Cross sectional.

Methods: Horse serum samples belonging to the Work/Meat category were collected at national level, between 2019 and 2022, with sampling criteria set to detect an unknown prevalence with 95% confidence level and 5% standard error. Donkeys and mules sera were collected during surveillance activities for equine infectious anaemia, between November 2023 and March 2024, regardless of their production category. Horses, donkeys and mules serum samples were analysed using a specific Real-Time PCR.[1]

Results: Up to March 2024, no EqHBV PCR positive samples were detected among the 394 horse serum samples analysed. Instead, among the 185 donkey and 68 mule samples, only five donkey samples were PCR positive. Preliminary EqHBV positivity in donkey sera is 2.7%.

Main limitations: For mules and donkeys, a convenience sampling was performed and no sequencing confirmation is available at present.

Conclusions: This study confirms that EqHBV affects donkeys, but not horses or mules. The prevalence obtained in donkeys (2.7%) highlights that the infection is present in the Central Regions of Italy. A broader sampling at national level would be beneficial to better define

prevalence levels.[1] Upon completion, results of the phylogenetic analysis will be made available.

Ethical animal research: The samples were collected for the implementation of the 'National plan for the surveillance and control of infectious anaemia in equidae" (Ministerial Decree 2 February 2016).

Informed consent: Not stated.

Competing interests: None declared.

Funding: Italian Ministry of Health RC IZSLT1018.

Reference:

[1] Rasche A, et al. A hepatitis B virus causes chronic infections in equids worldwide. Proceedings of the National Academy of Sciences 2021;118:13 e2013982118. https://doi.org/10.1073/pnas.2013982118.

121 | Prevalence of equine hepatitis associated viruses in the Austrian equine population

O. Rother[1], J. Pikalo[2], A. Gömer[3], I. Preinig[1], I. Stimach[2], E. Steinmann[3], A. Joachim[2] and J.-M.V. Cavalleri[1]

[1]*Clinical Centre for Equine Health and Research, Clinical Department for Small Animals and Horses, University of Veterinary Medicine Vienna, Vienna, Austria;* [2]*Institute of Parasitology, Centre of Pathobiology, Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria and* [3]*Department of Molecular and Medical Virology, Ruhr-University Bochum, Bochum, Germany*

Email: orietta.rother@vetmeduni.ac.at

Background: Equine hepatitis associated viruses include equine parvovirus (EqPV-H), equine hepacivirus (EqHV) and equine hepatitis B virus (EqHBV). In Austria, prevalence of EqPV-H and EqHV have only been partially analysed. In the surrounding area of Vienna (eastern Austria) in 2017, EqPV-H had a DNA-prevalence of 8.9% and seroprevalence of 30.1%, while EqHV had RNA-prevalence of 4.15% and seroprevalence of 45.9%. Infection rates of EqHBV for horses remain unknown, despite *in vitro* models suggesting the potential for infection of horses.

Objectives: To determine the prevalence of EqPV-H, EqHV and EqHBV in the Austrian equine population.

Study design: National cross-sectional study.

Methods: Convenience sampling of 255 non-systemically ill appearing horses located in six out of nine federal states of Austria (Lower Austria, Burgenland, Styria, Salzburg, Tyrol, Carinthia) was performed between May and September 2023. Serum samples ($n = 255$) were analysed for the presence of EqPV-H-, EqHBV-DNA and EqHV-RNA by quantitative PCR. Antibodies directed against EqPV-H and EqHV using a luciferase immunoprecipitation system were investigated in 131 sera.

Results: EqPV-H-DNA- prevalence was 3.1% ($n = 8/255$) and seroprevalence was 25.9% ($n = 34/131$). EqHV RNA prevalence was 1.2% ($n = 3/255$) and seroprevalence was 54.2% ($n = 71/131$). Five of the horses that had antibodies against EqPV-H were also DNA positive. Two horses with antibodies against EqHV were also RNA positive. Thirty horses (22.9%) had antibodies against EqPV-H and EqHV. But EqHBV-DNA could not be detected in any sample.

Main limitations: Sampled population is rather small and not evenly distributed over Austria. Seroprevalence was calculated from a subset of sampled horses. Seroprevalence of EqHBV was not investigated.

Conclusions: DNA-/RNA-prevalence of EqPV-H/EqHV is lower and seroprevalence is comparable to previous results in eastern Austria. No infection with EqHBV could be detected. Local differences and risk factors for infection need to be further investigated.

Ethical animal research: Approved by the Ethics Committee of the University of Veterinary Medicine Vienna and the Austrian Federal Ministry of Education, Science and Research (GZ: 2022–0.343.848).

Informed consent: Owner informed consent was obtained.

Competing interests: None declared.

Funding: Internal funding supplemented with contributions by individual donors to the University of Veterinary Medicine Vienna.

122 | First report of biomolecular prevalence of equine parvovirus hepatitis in Italian horses

G. Pacchiarotti[1], R. Nardini[1], A. Cersini[1], G. Brocherel[1], G. Bruni[1], E. Sezzi[1], G. Terracciano[1], N. Cavaliere[2], S. Costarelli[3], C. De Martinis[4], C. Di Pancrazio[5], F. Gobbo[6], M.L. Mandola[7], G. Purpari[8], A. Ruiu[9], M. Tonni[10] and M.T. Scicluna[1]

[1]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", via Appia Nuova, 1411 Rome, Italy;* [2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, via Manfredonia, n. 20, Foggia, Italy;* [3]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Via G.Salvemini, 1, Perugia, Italy;* [4]*Istituto Zooprofilattico Sperimentale del Mezzogiorno Via Salute, 2—Portici (Naples), Italy;* [5]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale," Campo Boario Teramo, Italy;* [6]*Istituto Zooprofilattico Sperimentale delle Venezie Viale dell'Università 10, Legnaro (Padua), Italy;* [7]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, Turin, Italy;* [8]*Istituto Zooprofilattico Sperimentale della Sicilia Via Gino Marinuzzi 3—Palermo, Italy;* [9]*Istituto Zooprofilattico Sperimentale della Sardegna, Via Duca degli Abruzzi n. 8 Sassari, Italy and* [10]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" Via Bianchi, 9, Brescia, Italy*

Email: roberto.nardini@izslt.it

Background: Equine serum hepatitis (Theiler's disease) is a potentially fatal disease of horses, significantly associated with equine biological products' administration. In 2018, a novel equine parvovirus hepatitis virus (EqPV-H) was described and associated with Theiler's disease [1]. EqPV-H is an ssDNA virus (genus *Copiparvovirus*, family *Parvoviridae*), causing sub-clinical hepatitis to severe fatal disease in horses. The presence of EqPV-H has been verified worldwide, but data for Italy are limited.[2]

Objectives: Assessing EqPV-H country prevalence in the Italian horse population.

Study design: Cross-sectional.

Methods: The sampling was designed to detect at national level, an unknown prevalence level with 95% confidence level and 5% standard error and was stratified into four categories to better represent the Italian horse population structure: equestrian, competition, work/meat and reproduction. Serum samples used were those collected during surveillance for equine infectious anaemia and were analysed by a specific Real-Time PCR [3]. Prevalence were calculated with 5% Confidence Interval (CI). Differences among categories were evaluated by Fisher's Exact Test.

Results: In 2023, 1581 samples were analysed, with an overall EqPV-H prevalence of 3.41% (CI:0.95–5.88). Prevalence within categories were: 3.55% (CI:0–8.49) for equestrian, 5.32% (CI:0.38–10.25) for competition, 1.0% (CI:0–5.9) for work/meat and 3.83% (CI:0–8.78) for reproduction. A statistical difference was detected between work/meat and all the other categories: equestrian ($p = 0.0172$), competition ($p = 0.0004$) and reproduction ($p = 0.01$).

Main limitations: No sequencing confirmation is available at present.

Conclusions: This study suggests that, although subclinical, EqPV-H is present in Italy. These data are coherent with that previously reported in Italy.[2] Upon completion, results of the phylogenetic analysis will be made available.

Ethical animal research: The samples were collected for the implementation of the 'National plan for the surveillance and control of infectious anaemia in equidae'' (Ministerial Decree 2 February 2016).

Informed consent: Not stated.

Competing interests: None declared.

Funding: Italian Ministry of Health RC LT1018.

References:

[1] Divers TJ, Tennant BC, Kumar A, McDonough S, Cullen J, et al. New Parvovirus associate with serum hepatitis in horses after inoculation of common biological product. Emerg Infect Dis. 2018;24 (2):303–310. doi: 10.3201/eid2402.171031.

[2] Diakoudi G, et al. Atti di Congresso SIDILV, XXI Congresso Nazionale—Identificazione molecolare di Parvovirus-Hepatitis equino in italia, Abstract 151, pg. 75.

[3] Altan, E, Li Y, Sabino-Santos Jr, G, Sawaswong V, Barnum S, Pusterla N, Deng X, Delwart E. Viruses in horses with neurologic and respiratory diseases. https://doi.org/10.3390/v11100942.

Virology 10: Surveillance

Michel D'Ornano Friday 11.00–12.00

123 | Extensive survey of equine infectious diseases in Argentina: equine infectious anaemia, glanders, surra, and dourine

L. Hébert[1], M. Sol Colombo[2], G. Polledo[2], M. Giorgi[2], T. Sivakumar[3], J.C. Vallé-Casuso[1], N. Yokoyama[3] and T. Becù[2]

[1]ANSES, Normandy Laboratory for Animal Health, Physiopathology and Epidemiology of Equine Diseases Unit, 14 430 Goustranville, France; [2]Laboratorio de Diagnóstico, Clinica Equina SRL, Alem 1698—Capitan Sarmiento, Buenos Aires, Argentina and [3]National Research Center for Protozoan Diseases, WOAH Reference Laboratory for Equine Piroplasmosis, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan

Email: laurent.hebert@anses.fr

Background: Argentina is a vast country with different equine populations including Thoroughbreds, polo ponies and cross-breeds. These populations can live in a variety of ecosystems, and do not all have the same access to veterinary care. The presence of numerous equine diseases has already been described in Argentina, but their impact on the various equine populations remains poorly described.

Objectives: Evaluate the seropositivity levels of three equine populations: Thoroughbreds, polo ponies and cross-breed working animals, for five internationally regulated diseases: equine infectious anaemia (EIA), glanders, surra and dourine.

Study design: Cross sectional.

Methods: This study was carried out on serum samples collected during breeding and exportation controls or during clinical procedures from 200 Thoroughbred horses and 200 polo ponies from Buenos Aires Province and on 149 cross-breed animals from Chaco province (Northern Argentina) collected between 2022 and 2023. The horses' serological status was determined using: agar-gel immunodiffusion (AGID) for EIA, Complement fixation test (CFT) for glanders and dourine, ELISA and card agglutination Trypanosomiasis test (CATT/*Trypanosoma evansi*) for surra.

Results: For the Thoroughbreds and polo ponies, seropositivity was 0% for EIA, 0% for glanders, 0.5% for surra (ELISA) and 0% for dourine. For horses in Chaco province, the seropositivity was 78.5% for EIA, 0.7% for glanders, 21.5% for surra (ELISA), 32.2% for surra (CATT/*T.evansi*), 0% for dourine.

Main limitations: No direct detection of pathogenic agents. Concordance between the surra ELISA and CATT/*T evansi* is poor.

Conclusions: This study shows the high-health-status of Thoroughbred and polo ponies in Argentina but the high exposure of horses in Chaco province to at least EIA and surra. These observations demonstrate the ability of compartmentalisation to maintain healthy equine populations in a country where diseases are circulating in given geographical areas.

Ethical animal research: Not required: retrospective analysis of surveillance data.

Informed consent: Not stated.

Competing interests: None declared.

Funding: The European Commission (https://ec.europa.eu/info/index_en) through DG SANTÉ funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness, ANSES and Clinica Equina SRL.

124 | Equine viral diseases in Argentina during the last six years and their impact on the equine industry

V. Vera[1,2], M.S. Tordoya[1,2], F. Alamos[1,2], C. Olguin[1,2], C. Gabaglio[1,2], M. Barrandeguy[3] and M.A. Vissani[1,2,4]

[1]*Instituto de Virología, CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina;* [2]*Instituto de Virología e Innovaciones Tecnológicas, UEDD, CONICET, Buenos Aires, Argentina;* [3]*Plataforma de Salud Animal, INIA La Estanzuela, Colonia, Uruguay and* [4]*Instituto de Investigación en Veterinaria, Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador, Buenos Aires, Argentina*

Email: vera.valentina@inta.gob.ar

Background: Occurrence of viral diseases is a permanent threat to the renowned horse industry in Argentina.

Objectives: To communicate the virological findings registered from 2018 to 2023.

Study design: Descriptive study.

Methods: Virus detection was performed by conventional, nested or real time PCR, and antibody detection, by sero-neutralisation and IgM ELISA for equine arteritis virus (EAV) and West Nile virus (WNV), respectively. Samples: 300 abortions from 175 premises; 216 nasopharyngeal swabs (NS) from 52 respiratory outbreaks; 201 stools from 56 diarrhoea outbreaks, and different types of samples from 84 neurological cases. Also, 744 NS and 406 semen samples for equine influenza (EI) pre-export and EAV pre-import certification respectively were tested. Regarding serology requests 21 140 sera for EAV and 2872 for WNV antibody detection were received.

Results: Equid herpesvirus 1 (EHV-1) was detected in 7% (12/175) of the abortion cases. Fourteen percent (7/52) of the respiratory outbreaks were positive for EHV-4, while 21% (11/52) were EI positive, related to the 2018 outbreak in Argentina. Rotavirus A was detected in 40% (22/56) of the diarrhoea outbreaks. EHV-1 was the cause of 4% (4/84) of the neurological cases in 2021–2022, while Western equine encephalomyelitis virus was detected in 21% (18/84), related to the recent reemergence of this disease. EI was not detected in NS from horses in pre-export quarantine and EAV was not detected in imported semen. EAV serology showed 1% (172/21140) of horses were seropositive, corresponding to horses infected in the last registered outbreak (2010) or imported as vaccinated. No WNV IgM seropositive horses were detected.

Main limitations: Limited number of samples.

Conclusions: These results address the importance of viral disease surveillance and diagnosis to take well-grounded measures for prevention and control, and to certificate the sanitary condition of Argentinian horses.

Ethical animal research: Not required: review of laboratory records.

Informed consent: Not stated.

Competing interests: None declared.

Funding: INTA and INTA-HARAS Agreement.

125 | Laboratory network surveillance of equine diseases

C. Lupo[1], C. Fortier[2,3], E. Kokabi[2,4], A. Léon[2,4], S. Pronost[2,3], A. Couroucé[1,3,5], P. Tritz[6], C. Marcillaud-Pitel[1], S. Zientara[7], P.-H. Pitel[2] and J.-L. Cadoré[8]

[1]*RESPE- French Network for Surveillance of Equine Diseases, 14 280 Saint Contest, France;* [2]*LABÉO, Research Department, 14 280 Saint Contest, France;* [3]*Normandie University, UNICAEN, BIOTARGEN UR7450, 14 000 Caen, France;* [4]*Normandie University, UNICAEN, UNIROUEN, DYNAMICURE, UMR 1311, Inserm,14 000 Caen, France;* [5]*ONIRIS, CISCO-ONIRIS, Route de Gachet, 44 307 Nantes Cedex, France;* [6]*RESPE Sentinel Veterinarian, Veterinary clinic, 57 380 Faulquemont, France;* [7]*UMR VIROLOGIE, Laboratoire de Santé Animale, INRAE, Anses, Ecole Nationale Vétérinaire d'Alfort, 94 700, Maisons-Alfort, France and* [8]*Université de Lyon, VetAgro Sup, Campus vétérinaire de Lyon, Département des animaux de compagnie de loisir et de sport, 69 280 Marcy L'Etoile, France*

Email: epidemiologiste@respe.net

Background: The French Network for Surveillance of Equine Diseases (RESPE) is an online veterinary reporting and information system created in 1999 for early detection of equine syndromes and non-notifiable diseases in France. It aims to provide an early warning to all the equine industry stakeholders to implement immediate and collective management measures when necessary. To increase its surveillance coverage, the RESPE is developing a laboratory-based surveillance component, that centralises laboratory analysis results from a network of sentinel laboratories. Diagnostic laboratory results from different laboratories must be comparable and reliable to ensure effective epidemiological indicators for surveillance use. However, equine influenza, strangles, and equine herpesvirus-1 and -4 (EHV-1 and EHV-4) infections which pose significant risks to the equine industry, are non-notifiable diseases. Thus, diagnostic laboratory methods are not standardised, and each laboratory can develop its own test.

Objectives: To compare the PCR diagnostic laboratory results for equine influenza, strangles, EHV-1 and EHV-4 infections obtained by different laboratories.

Study design: In vitro studies.

Methods: Interlaboratory comparisons (ILC) were performed to assess the capacity of participating laboratories in diagnosing the targeted diseases using their PCR methods. ILCs were organised in 2022 and 2023. These consisted of sending panels of samples of known status, i.e. containing or not containing targeted pathogen DNA or RNA. The results obtained by the laboratories using their own method on the blinded samples were compared with the results obtained by LABÉO, RESPE's support laboratory.

Results: Six laboratories participated in the ILCs. The percentage of participating laboratories that correctly identified the samples was 80% for equine influenza and EHV-1, and 100% for strangles and EHV-4.

Main limitations: Samples were artificially contaminated and none were at the detection limit.

Conclusions: These results highlighted the importance of ensuring the reliability and comparability of the laboratory diagnostic methods for effective surveillance of equine infectious diseases.

Ethical animal research: Not required: quality improvement activity.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: French Institute of horse and riding (IFCE), Conseil Départemental du Calvados, Fonds EPERON, French Ministry in charge of Agriculture, Conseil Départemental de la Manche, National Horseracing Federation (FNCH).

126 | Sindbis virus (SINV) in the Netherlands: evidence for local circulation in wild birds and horses

K. Streng[1]*, C.M. Holicki[2]*, J.C. Hesson[3,4], H. Graham[5], F. Chandler[2], L. Krol[6], R. Blom[7], E. Münger[2], C.J.M. Koenraadt[7], M. Schrama[6], Å. Lundkvist[3], M.P.G. Koopmans[2], H. van der Jeugd[8], W.H.M. van der Poel[1,5]* and R.S. Sikkema[2,8]*

[1]Quantitative Veterinary Epidemiology, Wageningen University & Research, Wageningen, the Netherlands; [2]Viroscience, Erasmus Medical Center, Rotterdam, the Netherlands; [3]Department of Medical Biochemistry and Microbiology, Zoonosis Science Center, Uppsala University, Sweden; [4]Biologisk Myggkontroll, Nedre Dalälvens Utvecklings AB, Gysinge, Sweden; [5]Wageningen Bioveterinary Research, Lelystad, the Netherlands; [6]Institute of Environmental Sciences, Leiden University, Leiden, the Netherlands; [7]Laboratory of Entomology, Plant Sciences Group, Wageningen University, Wageningen, the Netherlands and [8]Dutch Centre for Avian Migration and Demography, NIOO—KNAW, Wageningen, the Netherlands

* These authors contributed equally to this article.

Email: heather.graham@wur.nl

Background: Sindbis virus (SINV) is maintained in an enzootic transmission cycle between birds and mosquitoes (mainly *Culex* spp.). Horses and humans are considered dead-end hosts. Infections in non-symptomatic horses have been described through the detection of neutralising antibodies, while neurological signs in horses have also been reported.

Objectives: To determine SINV circulation in the Netherlands.

Study design: Cross-sectional.

Methods: Throughout the Netherlands, mosquitoes, wild birds, and horses were sampled and screened for SINV RNA and/or neutralising antibodies, between 2020 and 2022. Mosquitoes ($n = 12\,884$) and birds ($n = 10\,983$) were tested for SINV RNA by real-time reverse transcription (RT) PCR. Horses ($n = 371$) and a subset of wild bird sera ($n = 110$) were tested for SINV neutralising antibodies by a Plaque Reduction Neutralisation Test (PRNT).

Results: We report the first evidence for local circulation of SINV in the Netherlands. Serological evidence of SINV infections was detected in three horses without international travel history between January 2021 and December 2022, of which one horse showed neurological symptoms at the time of sampling. Twelve seropositive (12/110) birds were detected in a 16 km radius around the three seropositive horses and a RT-PCR-positive bird sampled in October 2022. Based on the sampling time and ringing history, six out of 12 birds were considered residents.

Main limitations: Serological investigation of birds was performed only for birds sampled in areas where the seropositive horses and the RT-PCR positive bird were detected.

Conclusions: This study describes for the first time the circulation of SINV in the Netherlands and the second time SINV antibodies have been detected in horses in Europe. This highlights the importance of surveillance for SINV as well as the need for increased awareness among veterinarians and health practitioners.

Ethical animal research: Horses in this study were sampled under license no. AVD40100202114384) of the Dutch Central Authority for Scientific procedures on Animals (CCD). Sampled birds were ringed and sampling was performed under ethical permit AVD801002015342 and AVD80100202114410 issued to NIOO-KNAW.

Informed consent: Owners gave consent for their animals' inclusion

Competing interests: None declared.

Funding: The Dutch Research Council (NWA.1160.1S.210), European Union's Horizon 2020 research and innovation programme under (Grant No. 874735, VEO and the DURABLE project, co-funded by Health Emergency Preparedness and Response (HERA) and the European-Union's EU4Health programme (Grant No. 101102733).

Virology 11: Equine infectious anaemia

Michel D'Ornano Friday 12.00–13.00

127 | A new non-serological diagnostics approach for equine infectious anaemia virus

J.-C. Valle Casuso[1], F. Lecouturier[1], N. Wiernasz[1], A. Madeline[1], D. Froger[1] and S. Zientara

[1]ANSES Laboratory for Animal Health in Normandy, Goustranville, France and [2]UMR Virology, Maisons-Alfort, France

Email: jose-carlos.valle-casuso@anses.fr

Background: Equine infectious anaemia virus (EIAV) is a significant worldwide threat to the equine population. Current control measures rely only on serological diagnosis. Still, there is a critical gap for this disease diagnosis because, during the viraemic phase, when infected horses are most infectious, they do not present a detectable level of anti-EIAV antibodies. To address this, other diagnostic approaches were tried

Objective: To develop an early and universal EIAV detection tool.

Study design: Molecular analysis.

Methods: This study used a next-generation sequencing approach to characterise the EIAV strains that infected the horses, from which our extensive collection of positive EIAV samples were obtained.

Results: After aligning the genetic data obtained by characterising more than 70 EIAV strains, partially conserved regions suitable for PCR amplification were identified. The designed RT-qPCR and qPCR tests successfully amplified these regions and identified the presence of the EIAV genome integrated into the horse genome. We identified more than 85% of the serological positive samples from only the 655 samples with the previously published qPCR. Performing a multiplex qPCR using the best two pairs of primers and probes increased the detection ratio to 95% of the serological positive samples. In addition to those results, some preliminary tests performed on PBMC isolated from blood from a positive EIAV horse also confirmed the possibility of detecting virus integrated into the horse genome.

Conclusions: The innovative NGS viral characterisation approach contributes to developing a universal PCR-based assay for early EIAV detection. These advances will be a valuable tool to enhance EIAV surveillance and management programs for positive EIAV animals and maybe limit the euthanasia measures used in some countries.

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Not stated.

Competing interests: None declared.

Funding: Chaire d'Excellence Region Normandie.

128 | Development of an antigen capture ELISA for quantifying the different strains of equine infectious anaemia virus

B. Zhou[1], C. Du[1*] and X. Wang[1,2*]

[1]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China and* [2]*Institute of Western Agriculture, the Chinese Academy of Agricultural Sciences, Changji, China*

*Corresponding author: Prof. Xiaojun Wang, email: wangxiaojun@caas.cn

*Co-corresponding author: Cheng Du; email: ducheng@caas.cn

Email: zhoubingqian9901@163.com

Background: Equine infectious anaemia Virus (EIAV), a member of the Lentivirus genus, poses a significant threat to the equine industry. Known for its high mutation rate, EIAV exhibits strain diversity discernible through genomic sequencing. The genetic heterogeneity within the gag gene, encoding a key structural protein, leads to variability in the translated p26 proteins, affecting their antigenic characteristics. However, most current ELISA methods, which target the p26 protein, are not inclusive of all viral strains due to this diversity.

Objectives: To develop a polyclonal antibody-based antigen capture ELISA (AC-ELISA) for broad-spectrum detection of diverse EIAV strains.

Study design: Assay development.

Methods: The AC-ELISA was refined by incorporating a murine monoclonal antibody and a rabbit polyclonal antibody, both specific to the p26 protein, to enhance its detection capabilities for EIAV strains. One p26-specific monoclonal antibody was developed in mice and the other polyclonal antibody was developed in rabbit. The mAb 1G11 was coated in microtiter plates as the capture antibody, the polyclonal antibody was used as tracing antibody and the anti-rabbit HRP conjugate as the detection system.

Results: The newly developed p26 AC-ELISA demonstrated equivalent sensitivity to detecting purified p26 protein, highlighting its high sensitivity for EIAV. When rigorously compared with the standard reverse transcriptase (RT) assay, the AC-ELISA proved its sensitivity, accuracy, and reliability, emerging as a robust alternative for EIAV quantification. Sample analysis using this refined assay confirmed its effectiveness in quantifying both American and Chinese EIAV strains in cell lysates and culture media.

Main limitations: This AC-ELISA includes a multi-step detection process, which results in a relatively extended duration for analysis.

Conclusions: The results underscore the assay's potential as a valuable diagnostic tool for the equine industry and for research into EIAV epidemiology and pathogenesis.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (SYXK [Hei] 2020–009).

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The Nature Science Foundation of Heilongjiang Province, China (TD2022C006).

129 | Development and evaluation of a real-time quantitative PCR for the detection of equine infectious anaemia virus

S. Li[1*], K. Guo[1*], X. Wang[1*], Y. Lin[1], J. Wang[1], Y. Wang[1], C. Du[1**], Z. Hu[1,2] and X. Wang[1,2,3]

[1]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agriculture Sciences, Harbin, China;* [2]*WOAH Reference Laboratory for Equine Infectious Anemia, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences, Harbin, China and* [3]*Institute of Western Agriculture, the Chinese Academy of Agricultural Sciences, Changji, China*

Email: huzher@126.com

Background: Equine infectious anaemia (EIA) has a worldwide distribution and causes severe economic losses to the equine industry. The EIA virus (EIAV) genomes' sequences from different countries are highly diverse, which poses a great challenge for pathogen identification with PCR. Phylogenetic analysis showed that although gag is the most conserved structural gene, it still has great genome variability. Currently, most existing PCR methods are designed based on the gag gene sequence and therefore do not cover all the viral strains, especially Asian EIAV strains.

Objectives: To establish an updated real-time quantitative PCR, which could be applied for detecting more EIAV strains.

Study design: Assay development.

Methods: A tat-gag based real-time quantitative PCR (TG-qPCR) for the detection of EIAV was developed by targeting the fragment between the tat and gag genes, which was relatively conserved in all the known EIAV strains. The performance of the TG-qPCR was evaluated against that of the standard qPCR (recommended by WOAH) by testing viral RNA extracted from viral supernatants of $EIAV_{DLV2\text{-}6}$ and $EIAV_{UK3}$, proviral DNA from peripheral blood mononuclear cells of artificially immunised horses, and virus nucleic acid from EIAV positive serum samples.

Results: The TG-qPCR assay had high specificity, sensitivity, and reproducibility. The detection limit of the TG-qPCR assay was 1 copy/reaction for both viral RNA and proviral DNA based on the Poisson distribution. Compared to the qPCR, the TG-qPCR has better inclusivity and can detect not only Asian EIAV strains but also almost all the representative EIAV strains from other continents.

Main limitations: Viral nucleic acid enrichment is needed since the amount of virus in blood is relatively low.

Conclusion: The TG-qPCR assay could serve as an effective tool for the early diagnosis of clinical EIA disease.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences.

Informed consent: Not stated.

Competing interests: None declared.

Funding: The National Key R&D Program of China (2021YFD1800500), and the National Key Research and Development Program of China (2022YFD1800200).

130 | Development and evaluation of a test strip for the rapid detection of antibody against equine infectious anaemia virus

Z. Zhang[1*], K. Guo[1*], X. Chu[1,3], M. Liu[4], C. Du[1,3**], Z. Hu[1,3**] and X. Wang[1,2,3**]

[1]State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China; [2]Institute of Western Agriculture, the Chinese Academy of Agricultural Sciences, Changji, China; [3]WOAH Reference Laboratory for Equine Infectious Anemia, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China and [4]Shenzhen Lvshiyuan Biotechnology Co., Shenzhen, China

*Zenan Zhang and Kui Guo contributed equally to this work.

Email: 905404134@qq.com

Background: Equine infectious anaemia (EIA) is a contagious disease of horses caused by the equine infectious anaemia virus (EIAV). Surviving horses become lifelong carriers because of the integration of the viral genome into that of the host, and these horses can produce and transmit the virus to other animals. This increases the difficulty of imposing practical control measures to prevent epidemics of this disease.

Objectives: To develop a rapid and simple method for detecting the disease in order to control the spread of EIA.

Study design: Assay development and validation.

Methods: A colloidal gold immunochromatographic (GICG) test strip was designed and developed to detect antibodies against EIAV based on the double-antigen sandwich. Both the p26 and gp45 proteins were used as the capture antigens.The sensitivity of the test was compared with those of AGID, Western blotting, and two commercial ELISA kits. The specificity, stability, and reproducibility of the test were assessed. The performance of the test strip was further evaluated using 31 serum samples from experimental horses immunised with the attenuated EIAV vaccine, as well as with 1186 clinical serum samples.

Results: The sensitivity of the test strip was higher than commercially available ELISA tests and AGID. The strip has good specificity and stability. When testing clinical serum samples ($n = 31 + 1014$), the test strip surprisingly provided greater sensitivity and a higher number of "true positive" results than other techniques.

Main limitations: It is difficult to quantify the antibodies in serum samples. Furthermore, it may lead to a risk of false positive results.

Conclusion: The GICG test strip has demonstrated great potential in field trials as a simple and effective tool for the detection of antibodies against EIAV.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The National Key R&D Program of China (2021YFD1800500), the National Key Research and Development Program of China (2022YFD1800200), and the Natural Science Foundation of Heilongjiang Province of China (TD2022C006).

Accepted: 14 August 2024
DOI: 10.1111/evj.14215

Equine Veterinary Journal WILEY

ABSTRACT

Poster Presentations

131 | Case of larval cyathostominosis in a young stallion in Croatia

N. Konstantinović[1] and A.M. Kovač[2]
[1]*Department of Parasitology and Parasitic Disease with Clinic, Faculty of Veterinary Medicine, University of Zagreb, Croatia and* [2]*Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, Croatia*
Email: nkonstantinovic@vef.hr

Background: Small strongyles (subfamily *Cyathostominae*) are the most common parasites of horses today. A three-year-old stallion was purchased and placed in a livery yard that had no parasitological monitoring in place. A month after arrival, the first clinical episode occurred, the signs including skin changes, poor performance, periodical lethargy, and occasional diarrhoea. After deworming, adults and larvae of small strongyles were found. If moxidectin was withheld, the clinical signs would reappear.
Objectives: Investigate the case of a young stallion that had symptoms consistent with the occurrence of larval cyathostominosis.
Study design: Case study.
Methods: The specimens found in faeces were microscopically examined. FEC testing was carried out throughout the year using the FLOTAC method. Following clinical episodes, haematology and biochemistry analyses were done. Assessments of body condition score and bodyweight were carried out. The diet was balanced with good quality hay ad libitum, pelleted alfalfa, wheat bran, sugar beet pulp, rolled oats, and complete feed mixtures with the addition of brewer's yeast, methionine, biotin, and linseed oil.
Results: The results of FEC were mostly negative, except three times when strongyle-type eggs appeared. Their count ranged from one to eight eggs per gram of faeces. The blood work was unremarkable. The stallion kept showing clinical episodes when moxidectin dewormer was not given every 3 months. As he reached the age of 4 years, clinical episodes gradually resolved.
Main limitations: Small sample size.
Conclusions: Larval cyathostominosis is often overlooked due to negative FEC results, vague clinical symptoms, and unremarkable blood results. This is the first documented case in Croatia, where FEC monitoring is still rarely done consequently increasing the possibility of the development of parasitic disease and antiparasitic resistance in equines.
Ethical animal research: Not required: descriptive clinical report.
Informed consent: The owner gave informed consent for his animals' inclusion.

Competing interests: None declared.
Funding: None.

132 | Use of a microfluidic immunofluorescence assay kit to detect equine influenza antigen

N. Kawanishi[1], Y. Kinoshita[1], Y. Kambayashi[1], H. Bannai[1], K. Tsujimura[1], T. Yamanaka[1], A. Cullinane[2] and M. Nemoto[1]
[1]*Equine Research Institute, Japan Racing Association, Shimotsuke, Tochigi, Japan and* [2]*Virology Unit, Irish Equine Centre, Naas, Kildare, Ireland*
Email: nanako_kawanishi@equinst.go.jp

Background: Equine influenza virus (EIV) infection is one of the most important respiratory diseases in the equine industry. Rapid diagnosis, facilitated by point-of-care testing, is essential to control disease outbreaks.
Objectives: To elucidate the usefulness of a microfluidic immunofluorescence assay kit (LumiraDx SARS-CoV-2 and Flu A/B Test[1]) for equine influenza diagnosis.
Study design: Experimental assay comparison.
Methods: A total of 11 EIV strains and 16 non-EIV pathogens, including equine coronavirus (ECoV) were tested to elucidate the specificity of the microfluidic immunofluorescence assay kit. To validate the clinical usefulness, nasopharyngeal swab samples of three horses experimentally infected with EIV were tested with the microfluidic immunofluorescence assay kit, two rapid antigen detection kits, and real-time reverse transcription-polymerase chain reaction (RT-PCR). The rapid antigen detection kits were Quick Chaser Flu A, B (QC; Mizuho Medy; based on immunochromatography) and Quick Chaser Auto Flu A, B (QCA; Mizuho Medy; based on silver amplification immunochromatography).
Results: The microfluidic immunofluorescence assay kit detected the 11 EIV strains but not ECoV or the other non-EIV pathogens, suggesting that it had high specificity for EIV antigens. With RT-PCR as a reference assay, the microfluidic immunofluorescence assay kit showed a sensitivity of 60.7% for evaluating specimens from experimentally infected horses. The kit had higher sensitivity than QC (53.6%) and the same sensitivity as QCA (60.7%).
Main limitation: No clinical specimens of natural occurring EI were used.
Conclusion: The microfluidic immunofluorescence assay kit could be a useful diagnostic tool for detecting EIV in the field.[1]

Key manufacturer: 1 Microfluidic Immunofluorescence Assay Kit, LumiraDx SARS-CoV-2 and Flu A/B Test, LumiraDx UK Ltd.

Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute with accession numbers 18–22 and 21–28.

Competing interests: The microfluidic immunofluorescence assay instrument was supplied by Shionogi & Co., Ltd. and LumiraDx Ltd. to the Equine Research Institute. None of the authors had any other conflicts of interest.

Funding: Japan Racing Association.

Reference:

[1] Kawanishi N, Kinoshita Y, Kambayashi Y, Bannai H, Tsujimura K, Yamanaka T, Cullinane A, Nemoto M. Performance of a microfluidic immunofluorescence assay kit for equine influenza virus antigen detection. J. Equine Vet. Sci. 2023;131:104956.

133 | Investigation of the frequency and selected prevalence factors of EHV-4 viremia in horses with acute onset of fever and respiratory signs

N. Pusterla[1], S. Barnum[1], K. Lawton[1], K. James[2] and B. Craig[3]

[1]*Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA;* [2]*Department of Obstetrics, Gynaecology and Reproductive Biology, Massachusetts General Hospital, Boston, MA 02114, USA and* [3]*Merck Animal Health, Madison, NJ 07940, USA*

Email: npusterla@ucdavis.edu

Background: EHV-4 is an endemic virus of young horses associated with upper airway infection. In comparison to EHV-1, EHV-4 has seldom been associated with complications such as abortion and myeloencephalopathy, due to the low affinity of this virus to infect mononuclear cells and to induce viremia.

Objectives: To document the frequency of EHV-4 viraemia in horses presented for acute onset of fever and respiratory disease and to determine if EHV-4 viraemia was associated with demographic parameters and clinical signs.

Study design: Retrospective study.

Methods: Samples consisting of nasal swabs and whole blood collected from horses with acute onset of fever and respiratory disease between January 2020 and December 2023 were used in this study. Case selection included all equids with EHV-4 qPCR-positive nasal secretions. Controls consisted of every case submitted before and after each EHV-4 qPCR-positive case. Purified nucleic acid from blood samples collected from EHV-4 qPCR-positive horses and control cases was tested for EHV-4 by qPCR. Selected demographic and clinical prevalence factors were compared between nasal secretion EHV-4 qPCR-positive and -negative horses. Further, the prevalence factors were also compared between horses with EHV-4 qPCR-positive nasal secretions with and without EHV-4 viraemia.

Results: Over the study period, 183 EHV-4 qPCR-positive horses and 376 EHV-4 qPCR-negative horses were selected. In general, EHV-4 qPCR-positive horses were younger, and displayed a lower rate of anorexia and a higher rate of nasal discharge compared to the EHV-4 qPCR-negative horses. A total of 25/183 (13.7%) EHV-4 qPCR-positive horses tested qPCR-positive for EHV-4 in blood, while none of the blood samples from control horses tested qPCR-positive for EHV-4. When both EHV-4 qPCR-positive horses with and without viraemia were compared, only age and limb oedema were determined to be significant.

Main limitations: Study limitations related to the retrospective nature of the samples submitted to a single laboratory.

Conclusions: The data support the observation that EHV-4 viraemia is rarely detected in EHV-4 infected horses, which explains the low level of reported complications, such as abortion and myeloencephalopathy.

Ethical animal research: Not required: retrospective case series.

Informed consent: Not stated.

Competing interests: None declared.

Funding: Equine Disease Infectious Disease Research Laboratory, School of Veterinary Medicine, University of California, Davis, Davis, California, USA.

134 | Molecular characterisation of *Histoplasma capsulatum* sensu *lato* from Ethiopian horses reveals two distinct phylogenetic clades

P.C.Y. Woo[1,2,3], F. Al Mheiri[4], J. Cavalleri[5], S. Joseph[4], J.Y.M. Tang[3], M. Joseph[4], C.-C. Tsang[6], S.K.P. Lau[3] and U. Wernery[4]

[1]*Doctoral Program in Translational Medicine and Department of Life Sciences, National Chung Hsing University, Taichung 402, Taiwan;* [2]*The iEGG and Animal Biotechnology Research Center, National Chung Hsing University, Taichung 402, Taiwan;* [3]*Department of Microbiology, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong;* [4]*Central Veterinary Research Laboratory, Dubai, United Arab Emirates;* [5]*University of Veterinary Medicine Vienna, Vienna, Austria and* [6]*School of Medical and Health Sciences, Tung Wah College, Homantin, Hong Kong*

Email: pcywoo@hku.hk

Background: Equine epizootic lymphangitis (EEL) is a highly prevalent and contagious systemic infectious disease affecting horses in many parts of Ethiopia caused by *Histoplasma capsulatum* sensu *lato*.

Objectives: To identify 12 suspected isolates of *H. capsulatum* sensu *lato* or yeasts unidentified by conventional biochemical tests isolated from Ethiopian horses with EEL and to perform molecular typing on six *H. capsulatum* sensu *lato* isolates.

Study design: In vitro analysis.

Methods: The 12 suspected isolates of *H. capsulatum* sensu *lato* or yeasts were characterised by ITS sequencing. Molecular typing was performed by multi-locus sequence analysis (MLSA). Internal Transcribed Spacer rRNA sequencing was performed by PCR amplification and sequencing the ITS region of the fungal isolates. MLSA was achieved by sequencing four housekeeping gene loci and phylogenetic analysis of the concatenated nucleotide sequences.

Results: Six of the 12 isolates were identified as members of *H. capsulatum* sensu *lato* and the other six were *Candida krusei* ($n = 3$), *Trichosporon asahii* ($n = 1$), *Geotrichum silicola* ($n = 1$) and *Moeszio-myces aphidus* ($n = 1$) respectively. MLSA and phylogenetic analysis of the concatenated nucleotide sequences of the four distinct gene loci [*arf* (462 bases), *H-anti* (410 bases), *ole1* (338 bases) and *tub1* (272 bases)] for the six *H. capsulatum* sensu *lato* isolates showed that three isolates and reference strain ATCC 58332 were identical and belonged to the Eurasian clade within LAm A (*H. suramericanum*), and those of the other three and reference strain ATCC 28798 were identical and belonged to the African clade.

Main limitations: Limited number of fungal isolates.

Conclusions: Two distinct phylogenetic clades of *Histoplasma capsula-tum* sensu *lato* were circulating in Ethiopian horses with EEL. Advanced molecular technologies and bioinformatics tools are crucial for accurate identification and typing of pathogens as well as discov-ery of novel microorganisms in veterinary microbiology.

Ethical animal research: Approved by the Ethics Committee of the Central Veterinary Research Laboratory.

Informed consent: Not applicable.

Competing interests: P.C.Y. Woo has provided scientific advisory/ laboratory services for Gilead Sciences, Incorporated; International Health Management Associates, Incorporated; Merck & Corporation, Incorporated; Micrología Molecular S.L. and Pfizer, Incorporated. The other authors report no conflict of interest.

Funding: Partly supported by the Higher Education Sprout Project by the Ministry of Education (MOE-112-S-023-A) in Taiwan.

135 | Equine Psittacosis and the emergence of *Chlamydia psittaci* as an endemic cause of equine reproductive loss and foal illness in Southeastern Australia

C. El-Hage[1]*, A. Legione[1], J. Devlin[1], K. Hughes[2], C. Jenkins[3] and J. Gilkerson[1]

[1]*Asia Pacific Centre for Animal Health, The Melbourne Veterinary School, The University of Melbourne, Parkville, VIC 3010, Australia;* [2]*School of Agricultural, Environmental and Veterinary Sciences, Faculty of Science and Health, Charles Sturt University, Wagga Wagga, NSW 2650, Australia and* [3]*Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW 2568, Australia*

Email: cmeh@unimelb.edu.au

Background: Prior to 2014, Equine Psittacosis (EP) disease due to *C psittaci* was rarely reported as a cause of reproductive loss in horses. Since then, there have been sporadic reports from SE Australia includ-ing epizootics and zoonotic spread.

Objectives: To summarise reported equine reproductive loss due to *C psittaci* in Australia including nature and occurrence of disease and geographic location of each case.

Study design: Retrospective case series.

Methods: Case of EP were reviewed from the records of state and federal veterinary authorities and from published reports between 2018 and 2022 (inclusive) in Australia where *C.psittaci* was deemed the likely cause of reproductive loss based on PCR-positive results in tissues, and exclusion of other likely pathogens, including EHV-1 and 4.

Results: A total of 31 cases of *C. psittaci* associated equine reproduc-tive loss were reported in New South Wales (NSW) and Victoria (VIC). There were 21 confirmed cases from 11 properties in NSW and 10 cases from 6 properties in VIC. These were the only two Australian states or territories where cases were reported and were restricted to their eastern regions. Where multiple *C.psittaci*-associated equine reproductive loss events occurred at a single location, all cases occurred within three weeks, or the following year.

Main limitations: This was a retrospective summary of disease reports, relying on submission of tissues to laboratories for analysis. Comprehensive case information is limited and EP may likely be underrepresented.

Conclusions: The emergence of EP as a cause of equine reproductive loss is now well established and endemic in SE Australia. It is vital that equine practitioners, and associated personnel, are aware of EP and ensure surveillance for *C. psittaci* and institute biosecurity in cases of equine abortions and neonatal illness thereby limiting disease spread to horses and humans.

Ethical animal research: Not required: retrospective case series.

Informed consent: Not stated.

Competing interests: None declared.

Funding: none.

136 | Genetic variability of equid γ-herpesviruses detected in thoroughbred mares and their foals in Poland

K. Stasiak and J. Rola

National Veterinary Research Institute, Department of Virology, Al. Partyzantow 57, 24–100 Pulawy, Poland

Email: karol.stasiak@piwet.pulawy.pl

Background: Equid herpesvirus 2 (EHV-2) and 5 (EHV-5) are two γ-herpesviruses that are commonly detected in horses worldwide.[1] A considerable sequence variability has been demonstrated among gly-coprotein B sequences of EHV-2, and to a lesser extent for EHV-5.[2,3]

Objectives: To characterise the genomic diversity of the glycoprotein B gene of EHV-2 and EHV-5 in the nasal secretions of a cohort of Thoroughbred mares and their foals.

Study design: Cross-sectional study.

Methods: Viral DNA from selected EHV-2 and/or EHV-5 positive swabs was used as a template in conventional PCR assays targeting a 1222 bp (EHV-2) and 1339 bp (EHV-5) fragment of the gB gene. Amplified products were subjected to electrophoresis, purified and cloned into pCR™4-TOPO vectors and transformed into *Escherichia coli* competent cells. At least five clones from each successful amplifi-cation were sequenced. Phylogenetic trees were constructed using the maximum likelihood method with 1000 bootstrap replicates in

MEGA7. Sequence identity among sequences analysed in the study was calculated using the identity matrix in BioEdit software.

Results: Long PCR products were amplified from 24 EHV-2 and 29 EHV-5 PCR positive swabs tested, from which 82 and 94 clones were obtained, respectively. Sequence alignments of partial gB gene from 77 EHV-2 clones analysed showed 65.4 to 100% identity at the nucleotide level and 58.9 to 100% at the amino acid level. The genetic variability of EHV-2 sequences from mares and their foals was at the same nucleotide level (65.3%–100%). The degree of identity between sequences of 94 EHV-5 clones was higher and ranged from 89.6 to 100% and 86.7 to 100% at the nucleotide and amino acid level, respectively. Based on the phylogenetic tree, Polish EHV-2 sequences clustered within two main branches. Most of them ($n = 64$) clustered with international sequences (including Australian reference strain EHV-2.86/67) within the main branch, defined as group 1.[4] The 94 EHV-5 sequences clustered within at least three main lineages.

Main limitations: A limited number of EHV-2/EHV-5 positive swabs from the same mare-foal pairs sampled over several months.

Conclusions: EHV-2 sequences from mares and their foals showed similar, high genetic variability. Weaning does not seem to have an impact on the genetic variability of equid gammaherpesviruses among Thoroughbreds.

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Informed consent was sought from the stud manager before commencement of sampling.

Competing interests: None declared.

Funding: None.

References:

[1] Marenzoni ML, Stefanetti V, Danzetta ML, Timoney PJ. Gammaherpesvirus infections in equids: A review. Vet. Med. Auckl. 2015;6:91–101.

[2] Stasiak K, Dunowska M, Rola J. Prevalence and sequence analysis of equid herpesviruses from the respiratory tract of Polish horses. Virol. J. 2018;15:106.

[3] Stasiak K, Dunowska M, Trewick S, Rola J. Genetic variation in the glycoprotein B sequence of equid herpesvirus 5 among horses in various breeds at Polish national studs. Pathogens 2021;10:322.

[4] Sharp EL, Farrell HE, Borchers K, Holmes EC, Davis-Poynter NJ. (2007) Sequence analysis of the equid herpesvirus 2 chemokine receptor homologues E1, ORF74 and E6 demonstrates high sequence divergence between field isolates. J. Gen. Virol. 2007;88:2450–2462.

137 | Association between fungal detection in the airways and equine asthma

P. Barbazanges[1], A. Couroucé[1,2], G. Le Digarcher[1], J.M. Cardwell[3], E. Schmitt[2,4], M.-P. Toquet[2,4], L.C. Lemonnier[1] and E.A. Richard[2,4]

[1]Department of Equine Internal Medicine, Equine Clinic, ONIRIS, National College of Veterinary Medicine, Food Science en Engineering, Nantes, France; [2]Université de Caen Normandie, BIOTARGEN UR7450, F-14000 Caen, France; [3]Department of Pathobiology and Population Sciences, Royal Veterinary College, Hatfield, Hertfordshire, UK and [4]LABEO (Frank Duncombe), Caen, France

Email: pauline.barbazanges@oniris-nantes.fr

Background: Fungi are ubiquitous in horses' environment. Their contribution to the pathophysiology of severe asthma (SA) is acknowledged, while controversies remain for mild–moderate asthma (MA).

Objectives: We hypothesised that fungi were a risk factor for asthma. Our objective was to compare different combinations of analytical methods (cytology, culture) and sampling sites (tracheal wash (TW), bronchoalveolar lavage fluid (BALF)) in relation to clinical status (control, MA, SA).

Study design: Prospective cross-sectional study.

Methods: The study population included asymptomatic racing horses in the field and horses referred to the hospital for respiratory investigations. Fungi were detected by cytology and identified by mycology on TW and pooled BALF. Chi-square tests were used for prevalence comparison between groups and association with clinical investigations.

Results: A total of 155 horses (85 MA, 35 SA and 35 controls) were included in the study. The overall proportions of fungal detection in TW ranged from 45.7% to 89.4% among groups. The prevalence of fungal detection in BALF was significantly lower by cytology for SA (5.7%) than MA horses (23.6%) and significantly higher by culture for MA horses (31.8%) than controls (8.6%). Fungal detection by culture in BALF was significantly associated with high tracheal mucus score, high neutrophil proportions in BALF and diagnosis of MA.

Main limitations: Mycology was only performed in pooled BALF and environment was not sampled.

Conclusion and clinical importance: Fungi were significantly more prevalent in the airways of MA horses than SA and/or controls. Fungal detection in TW, either by cytology or culture, was uninformative in a clinical context. Fungal detection by culture (but not cytology) in BALF represents a risk factor for MA.

Ethical animal research: Approved by the regional Ethic Committee for Clinical and Epidemiological Veterinary Research (CERVO-2020-3-V).

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: IFCE: Grant numbers CS-2020-2022-028; Fonds Eperon: Grant numbers N50-2019.

138 | Whole genome sequence analysis of the 2018 Persian onager isolate suggests subspecies lineages within the *Taylorella asinigenitalis* species

S. Kozak [1], D. Merda [2], F. Duquesne [1], M.-F. Breuil [1], C. Sévin [1], I. Mawhinney [3] and S. Petry [1]

[1]ANSES, Normandy Laboratory for Animal Health, Physiopathology and Epidemiology of Equine Diseases Unit, Goustranville, France; [2]SPAAD unit, Paris Est University, Anses, Maisons-Alfort, France and [3]Animal and Plant Health Agency, Penrith, UK

Email: deborah.merda@anses.fr

Background: In 2018, a *Taylorella asinigenitalis* strain (MCE663) was isolated from a Persian onager (endangered species of the *Asinus* subgenus) kept in a UK zoo.[1] *T. asinigenitalis*, mostly found in donkeys in which pathogenicity remains to be assessed, had previously never been reported in the UK, and MCE663 showed atypical genetic distance with isolates from the https://pubmlst.org/organisms/taylorella-spp/ database.

Objectives: To better characterise the MCE663 strain using phenotypic and genome-wide approaches.

Study design: In vitro and comparative genome analysis of microorganisms.

Methods: Gram staining, catalase and oxidase activities, slide agglutination test, IFAT and PCR were performed on MCE663 and three *Taylorella* reference strains. Carbon source utilisation (Biolog GenIII MicroPlate) and antimicrobial resistances to 12 antibiotics (disk diffusion method) were investigated. Illumina whole-genome sequencing was performed to conduct phylogeny, average nucleotide identity (ANI) and synteny analysis with 43 published *Taylorella* spp. genomes.

Results: MCE663 showed all the identifying characteristics of *T. asinigenitalis* with smaller colonies and susceptibility to all tested antibiotics. While gene organisation was conserved, genome-level phylogeny showed that MCE663 shared only 96.1% ANI with the other published genomes (which together shared 98.3% ANI).

Main limitations: Study based on the analysis of a single strain.

Conclusions: According to current cut-offs consensus for species and subspecies delineation (95% and 98%, respectively), MCE663 formed a distinct phylogroup with the other published genomes supporting the insight of a sub-lineage delineation within the *T. asinigenitalis* species [2].

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Not applicable.

Competing interests: None declared.

Funding. The French Horse and Riding Institute, IFCE (http://www.ifce.fr) and the Fonds Eperon (https://www.fondseperon.com/).

References:

[1] Mawhinney I, Florea L, Torrens N, Thurston L, Poynter R. *Taylorella asinigenitalis* in an onager in a UK zoo. Vet. Rec. 2018;183:266.

[2] Kozak S, Merda D, Duquesne F, Breuil MF, Mawhinney I, Petry S. Whole genome sequence analysis of the 2018 Persian onager isolate suggests subspecies lineages within the *Taylorella asinigenitalis* species. Vet. Microbiol. 2023. 286: 109884, https://doi.org/10.1016/j.vetmic.2023.109884

139 | Development of Disabled Infectious Single Animal (DISA)-DIVA vaccines for African Horse Sickness

R.G.P. van Gennip[1], S. Joseph[2], U. Wernery[2] and P.A. van Rijn[1]

[1]*Wageningen Bioveterinary Research (WBVR), Lelystad, the Netherlands;* [2]*Central Veterinary Research Laboratory (CVRL), Dubai, United Arab Emirates*

Email: piet.vanrijn@wur.nl

Background: African Horse Sickness (AHS) is a highly severe disease of equids (WOAH-notifiable disease, category A) caused by AHS-virus (AHSV). AHSV forms a distinct virus species within the genus *Orbivirus* (family *Sedoreoviridae*) of which bluetongue virus is the prototype orbivirus. AHSV contains a segmented genome (Seg-1 to 10) and consists of nine serotypes showing limited cross-neutralisation. Marketed live-attenuated vaccines (LAVs) have multiple point mutations in several segments and are impossible to differentiate from field virus. Importantly, their safety is debatable due to residual virulence, reversion to virulence, and reassortment events leading to virulent AHSV-variants.

Objective: To produce safe, efficacious DIVA-vaccines for all nine AHSV serotypes.

Study design: Vaccine development.

Methods: Reverse genetics[1] was used to generate AHS vaccine candidates according to the principles of "Disabled Infectious Single Animal (DISA)" and "Differentiating Infected from Vaccinated (DIVA) as shown for bluetongue virus.[2]

Results: The developed DISA-DIVA vaccine platform is based on LAV for serotype 4 with a deletion of 231 nucleotides in Seg-10 encoding dispensable NS3/NS3a protein. Exchange of Seg-2 and Seg-6 encoding serotype determining outer shell proteins VP2 and VP5 resulted in DISA-DIVA vaccine candidates for all nine AHSV serotypes. AHS-vaccine candidates, shortly named DISA1 to DISA9, are genetically stable and replicate to similar virus titres as LAV-4. The accompanying DIVA PCR-test targeting Seg-10 was also developed and differentiates all field virus variants from DISA vaccines.

Conclusions: DISA-DIVA vaccine candidates for all nine AHSV serotypes are available for in vivo studies. DISA1 to DISA9 can be safely combined to achieve broad protection, since virulent variants cannot arise due to the common LAV-backbone and the significant deletion in Seg-10. Viremia in infected equids can be specifically detected during the infectious period and beyond, irrespective of the vaccination status.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Central Veterinary Research Laboratory, Dubai, UAE; Wageningen Bioveterinary Research, Lelystad, NL; EU funding SPIDVAC.

References:

[1] van de Water SGP, van Gennip RGP, Potgieter CA, Wright IM, van Rijn PA. VP2 Exchange and NS3/NS3a deletion in African Horse Sickness Virus (AHSV) in development of Disabled Infectious Single Animal Vaccine Candidates for AHSV. J Virol. 2015;89(17). 10.1128/jvi.01052-15

[2] van Rijn, P. Prospects of next-generation vaccines for bluetongue. Front. Vet. Sci. 2019:6. 10.3389/fvets.2019.00407

140 | Safety and efficacy of African horse sickness Disabled Infectious Single Animal (DISA)-DIVA vaccines in IFNAR−/− mice

S. Utrilla-Trigo[1], L. Jiménez-Cabello[1], E. Calvo-Pinilla[1], R.G.P. van Gennip[2], P.A. van Rijn[2] and J. Ortego[1]
[1]*Centro de Investigación en Sanidad Animal (CISA-INIA, CSIC), Valdeolmos, Madrid, Spain and* [2]*Wageningen Bioveterinary Research (WBVR), Lelystad, the Netherlands*
Email: piet.vanrijn@wur.nl

Background: African Horse Sickness (AHS) is a highly severe disease of equids (WOAH-notifiable disease, category A) caused by nine serotypes of AHS-virus (AHSV) showing limited cross-neutralisation. Marketed live-attenuated vaccines (LAVs) are available but their safety cannot be guaranteed. Recently, promising AHS Disabled Infectious Single Animal (DISA) DIVA vaccines for all nine serotypes, shortly named DISA1 to 9, became available for in vivo studies.

Objective: Evaluation of the safety and efficacy of DISA vaccines in the artificial IFNAR−/− mouse model.

Study design: In vivo experiments.

Methods: Groups of five mice were vaccinated once or twice at an interval of 3 weeks with a cocktail of all nine DISA vaccines (DISA1-9) containing equal amounts of each DISA vaccine. Subsequently, mice were challenged with virulent AHSV4 or virulent rAHSV5.[1,2] Mice were monitored for clinical signs, viremia and antibodies.

Results: Mice did not develop PCR-positivity nor clinical signs but seroconverted after DISA-vaccination. AHSV-challenged control groups showed clinical signs, i.e. ruffled fur and weight loss, and developed viremia. AHSV4-challenged control mice also showed ocular discharge and a strong reduction in mobility. This control group was euthanised due to ethical reasons at 7 days post challenge. DISA-vaccinated mice did not develop clinical signs and survived AHSV challenge. Further, all DISA-vaccinated groups (prime and prime-boost) showed extremely high Ct values (low PCR-positivity) after AHSV challenge but viremia was not positive. DISA-vaccinated mice developed serotype specific neutralising antibodies.

Conclusions: DISA vaccines are safe according to this artificial animal model. The DISA1-9 cocktail is protective as studied for two representative virulent AHSV serotypes. Neutralising antibody titres indicate protection for more AHSV serotypes. Now, safety and efficacy of DISA1-9 should be studied in the equine host.

Ethical animal research: Approved by the Spanish Ethical Committee.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Wageningen Bioveterinary Research, EU Funding SPIDVAC, INIA CISA CSIC.

References:

1: van Rijn PA, Maris-Veldhuis MA, Potgieter CA, van Gennip RGP. African horse sickness virus (AHSV) with a deletion of 77 amino acids in NS3/NS3a protein is not virulent and a safe promising AHS Disabled Infectious Single Animal (DISA) vaccine platform. Vaccine 2018;36(1923). 10.1016/j.vaccine.2018.03.003.

2: van Rijn PA, Maris-Veldhuis MA, Boonstra J, van Gennip RGP. Diagnostic DIVA tests accompanying the Disabled Infectious Single Animal (DISA) vaccine platform for African horse sickness. Vaccine 2018;36(3584). 10.1016/j.vaccine.2018.05.044.

141 | Toward a better characterisation of the genetic diversity of circulating equine influenza virus strains by long-read sequencing

S. Dhorne-Pollet[1], C. Normand[2,3], M. Filipe-Ferreira[1], C. Fortier[2,3], E. Hue[2,3], B. Delmas[4], N. Pollet[5], E. Barrey[1] and S. Pronost[2,3]
[1]*Université Paris-Saclay, AgroParisTech, INRAE, UMR GABI, Jouy-en-Josas, France;* [2]*LABÉO, 1 route de Rosel, Saint Contest, France;* [3]*BIOTARGEN UR7450, Université de Caen Normandie, Caen, France;* [4]*Université Paris-Saclay, INRAE, UMR VIM, Jouy-en-Josas, France and* [5]*Evolution, Génomes, Comportement & Ecologie, CNRS, IRD, Université Paris-Saclay, 91 198 Gif-sur-Yvette, France*
Email: sophie.pollet@inrae.fr

Background: Equine influenza virus (EIV) infection is one of the most important respiratory diseases in the equine industry. Knowing viral genome variants to monitor their evolution is essential to anticipate vaccine drift.

Objectives: Develop a rapid and efficient method to identify circulating strains and characterise their complete genome for enhanced epidemiological surveillance.

Study design: Whole genome sequencing.

Methods: The genetic diversity of eight EIVs from France (2009–2023) was assessed using long-read sequencing. The two vaccine strains A/equine/South-Africa/4/2003(H3N8) and A/equine/Richmond/2/2007(H3N8) were included as controls. RNA was extracted from nasal swabs collected for clinical purposes and amplified the eight viral genomic segments by RT-PCR using primers complementary to the 5′ and 3′ ends of each segment. Equimolar pools of purified PCR products were prepared and sequencing libraries made for each strain. R10.3 flow cells operated on a MinION Mk1C device (ONT) were used and processed raw sequencing data using Dorado for basecalling and demultiplexing. Reads were filtered by size and mapped to one reference EIV genome to identify variants and assemble consensus sequences.

Results: An average of 70 928 reads per segment and strain was obtained, corresponding to a mean coverage of 46×. The genetic diversity of the strains circulating in France was evaluated, with between 86 and 287 genetic variants per strain. Consensus sequences were constructed for each EIV strain's segment and confirmed the circulation of clade 1 in France. The experiments were reproduced using a smaller R10 Flongle flow cell to quickly and reliably characterise HA and NA for the circulating strains.

Main limitations: The number of viral particles can limit sensitivity, and the design of primers limits amplification efficiency.

Conclusions: Long-read sequencing technology enables rapid and efficient characterisation of the whole genetic diversity of circulating EIV

strains. This method provides crucial information for epidemiological monitoring.

Ethical animal research: Not required: excess material from clinical samples was used.

Informed consent: Horse owners were made aware clinical material might be used for research purposes.

Competing interests: None declared.

Funding: IFCE: Equ'INFLUENZA project; LABEO.

142 | Booster effect of inactivated *Parapoxvirus ovis* on EHV-1 neutralising antibodies when co-injected with equine herpesvirus 1/4 vaccine

F. Carnet[1,2], R. Paillot[3], C. Fortier[1,2,4], E. Hue[1,2,4], L. Briot[5], F. de Geoffroy[5], P.-O. Vidalain[6] and S. Pronost[1,2,4]

[1]LABÉO, 14 280 Saint-Contest, France; [2]BIOTARGEN, Normandie University, UNICAEN, 14280 Saint-Contest, France; [3]Faculty of Science & Engineering, School of Agriculture, Animal & Environmental Sciences, Anglia Ruskin University (ARU Writtle), Lordship Road, Writtle, Chelmsford CM1 3RR, UK; [4]Université de Caen Normandie, UNICAEN, IMPEDANCELL, 14000 Caen, France; [5]Institut Français du Cheval et de l'Equitation, Plateau Technique du Pin-au-Haras, 61 310 Gouffern en Auge, France; [6]CIRI Centre International de Recherche en Infectiologie, Univ. Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, 69 007 Lyon, France.

Email: stephane.pronost@laboratoire-labeo.fr

Background: Equid herpesvirus 1 (EHV-1) is regularly responsible for outbreaks in many parts of the world, with a significant impact on the equine industry. Protection against EHV-1 relies on sanitary measures including vaccination, but the efficacy and duration of protection offered by existing vaccines is often limited and vaccines do not show protection against all forms of the disease.

Objectives: The aim of this study is to improve the vaccine efficacy and the benefit of immune stimulation with inactivated *Parapoxvirus ovis* (iPPVO[1]), a substance already marketed as an immunostimulant.

Study design: Experimental challenge.

Methods: Antibody response was measured after a semi-annual boost of EHV-1/-4 vaccine with or without iPPVO added. Carbopol-adjuvanted inactivated EHV-1/-4 vaccine was administered alone ($n = 9$) or combined with iPPVO injections at D0, D2 and D4 after vaccination ($n = 10$) to adult horses as a booster vaccination (i.e., 6 months after the last immunisation). EHV-1 neutralising antibody titres were measured by classical serum neutralisation and by a real-time neutralising assay 1, 3 and 6 months after vaccination.

Results: The results showed that iPPVO was safe and the horses had higher antibody levels than the control group injected with EHV-1/-4 vaccine alone. In contrast to horses that received the vaccine alone, antibody titres increased or were at least maintained up to 3 months after immunisation in horses that received the vaccine plus iPPVO.

Main limitations: It should be noted that in this experimental protocol, iPPVO was not mixed and co-injected with the vaccine, but administered near the injection site of the vaccine.

Conclusions iPPVO could be used to improve vaccination protocols against EHV-1 in horses.

Key manufacturer: 1 Zylexis, Zoetis, USA https://www.zoetisus.com/content/_assets/docs/vmips/safety-data-sheets/zylexis.pdf

Ethical animal research: Approved by the CENOMEXA no 54 ethics committee (No APAFIS 2021081914235114_V3 (#32718)) and the Ministry of Higher Education, Research and Innovation.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324), the IFCE (Institut Français du Cheval et de l'Equitation) grant number Rech-CS-2022-2023-019-VacA-dEq_HVE. CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational program 2014–2020.

143 | Immunostimulating effect of inactivated *Parapoxvirus ovis* on the serological response to equine influenza booster vaccination

F. Carnet[1,2], R. Paillot[3], C. Fortier[1,2], E.S. Hue[1,2], L. Briot[4], F. de Geoffroy[4], P.-O. Vidalain[5] and S. Pronost[1,2]

[1]LABÉO, 14 280 Saint-Contest, France; [2]BIOTARGEN, Normandie University, UNICAEN, 14280 Saint-Contest, France; [3]Faculty of Science & Engineering, School of Agriculture, Animal & Environmental Sciences, Anglia Ruskin University (ARU Writtle), Lordship Road, Writtle, Chelmsford CM1 3RR, UK; [4]Institut Français du Cheval et de l'Equitation, Plateau Technique du Pin-au-Haras, 61 310 Gouffern en Auge, France and [5]CIRI Centre International de Recherche en Infectiologie, Univ. Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, 69 007 Lyon, France

Email: stephane.pronost@laboratoire-labeo.fr

Background: Equine influenza virus (EIV) is responsible for recurring outbreaks that are detrimental to the equine industry. Vaccination is key for prevention, but the effectiveness and duration of protection provided by existing vaccines is insufficient.

Objectives: To improve vaccine efficacy, the benefit of immune stimulation with inactivated Parapoxvirus ovis (iPPVO[1]) on the antibody response induced by a vaccine boost against EIV was evaluated.

Study design: Experimental vaccination challenge.

Methods: Twenty healthy mares, breed Selle-français, Anglo-Arabian, trotter and draught horses between 11 and 25 years of age were enrolled in the study. The horses were stratified into three different age groups (i.e., 11–14, 15–18 and 19–25 years) and then randomly allocated to a control group that received the semi-annual EI booster (SAB) vaccination or the iPPVO group that received the SAB vaccination plus iPPVO injections. Antibody levels were measured with the single radial haemolysis (SRH) assay at 1, 3 and 6 months post-vaccination.

Results: Results revealed that horses that received iPPVO had higher antibody levels than the control group injected with the EI vaccine alone. Although the vaccine used contains only a clade 1 and European lineage strain, the increase in protective antibodies was also observed against a clade 2 strain.

Main limitations: It should be noted that in this experimental protocol, iPPVO was not mixed and co-injected with the vaccine, but administered near the injection site of the vaccine.

Conclusions: Immune stimulation with iPPVO, a substance already marketed as an immunostimulant, could be used to improve vaccination protocols in horses and potentially other species.

Key manufacturer: 1 Zylexis, Zoetis, USA https://www.zoetisus.com/content/_assets/docs/vmips/safety-data-sheets/zylexis.pdf

Ethical animal research: approved by the CENOMEXA n-54 ethics committee (No APAFIS #29210–2 020 113 009 246 927 v3) and the Ministry of Higher Education, Research and Innovation.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324), the IFCE (Institut Français du Cheval et de l'Equitation) grant number Rech-CS-2022-2023-019-VacA-dEq_HVE. CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational program 2014–2020.

144 | LAMP: DNA/RNA amplification technology as a point of care tool to help diagnose pathogens causing respiratory diseases in horses

L. Valot, T. Thibault, M. Simonnet, D. Cormier, A. Lacaze, V. Luong, D. Schieb and L. Thiery
Enalees, 5 rue Henri Desbruères 91 000 Evry, France
Email: laurine.valot@enalees.com

Background: LAMP technology (Loop mediated isothermal AMPlification) is a NAAT technology (Nucleic Acid Amplification test) which can run at a constant temperature, unlike PCR which needs thermal cycles. This allows the use of this technology as a POC (point of care) test and results are obtained in only 30 min without the necessity to use a thermal cycler. This technology is useful to detect highly contagious pathogens causing diseases in animals and facilitates treatment or isolation of the animal immediately. In a pandemic, this technology can be useful to isolate infected animals quickly and limit dissemination of the pathogen.

Objectives: To develop LAMP tests for equine influenza, EHV-1, EHV-4 and *Streptococcus equi equi*.

Study design: Assay development and validation.

Methods: Specific LAMP primers for EHV-1/EHV-4/Equine Influenza/*Streptococcus equi equi* were designed[1] and LAMP reactions were performed on pathogen genomes and on nasopharyngeal swab samples submitted for clinical purposes and extracted by our own specific quick extraction. The performances of these tests were evaluated in our lab and compared to PCRs performed in external labs.

Results: LAMP tests have a limit of detection for amplification between 1 and 40 copies/reaction dependant of the test. The limit of detection of the method (quick extraction and LAMP test) is similar or better of the limit of detection of the method (extraction on column and PCR test) obtained in a conventional analysis laboratory. LAMP tests which were designed were specific (>95% specificity) and sensitive for the target (>90% sensitivity).

Main limitations: Tests were performed in different labs and not all published genomes were tested.

Conclusions: LAMP technology is really fast (<30 min) and can be used as a point of care test. LAMP tests are a new generation of DNA/RNA amplification tests which are specific, sensitive, performed at point of care of a sick animal and can give a result in 30 min. These parameters allow use of this technology to analyse potential pathogens quickly and treat or isolate the animal immediately.

Key manufacturer: 1 www.enalees.com

Ethical animal research: Not required: excess material from clinical samples was used.

Informed consent: Not stated.

Competing interests: All authors are Enalees employees.

Funding: Corporate financial support from Enalees.

145 | LAMP: DNA amplification technology as a point of care tool to help diagnosis of pathogens causing Piro-like diseases in horses

A. Zocevic, T. Thibault, L. Valot, M. Simonnet, L. Melo, D. Cormier, A. Lacaze, V. Luong, D. Schieb and L. Thiery
Enalees, 5 rue Henri Desbruères 91 000 Evry, France
Email: aleksandar.zocevic@enalees.com

Background: LAMP technology (Loop mediated isothermal AMPlification) is a NAAT technology (Nucleic Acid Amplification test) which can run at a constant temperature, unlike PCR which needs thermal cycles. This allows the use of this technology as a POC (point of care) test and results are obtained in only 30 min without the necessity to use a thermal cycler. This technology is useful to detect highly contagious pathogens causing diseases in animals and facilitates treatment or isolation of the animal immediately. In a pandemic, this technology can be useful to isolate infected animals quickly and limit dissemination of the pathogen.

Objectives: To develop LAMP tests for pathogens causing equine piroplasmosis.

Study design: Assay development and validation.

Methods: Specific LAMP primers for *Theileria equi*, *Babesia caballi*, *Anaplasma phagocytophilum*, were [designed1] and LAMP reactions were performed on the genomes of the pathogen and on blood samples extracted by our own specific quick extraction. The performances of these tests were evaluated in our lab and compared to PCRs performed in external labs.

Results: The LAMP tests were specific (>93%) and sensitive (>90%) for the targets. This technology is really fast (<40 min) and can be performed near a sick animal to help veterinarians make treatment decisions.

Main limitations: Tests were performed in different labs.

Conclusions: LAMP tests are a new generation of DNA/RNA amplification test which are specific and sensitive and can be performed as a point of care test, with results available in 40 min. These parameters allow use of this technology to analyse potential pathogens quickly and treat or isolate the animal immediately.

Key manufacturer: 1 www.enalees.com

Ethical animal research: Not required: excess material from clinical samples was used.

Informed consent: Not stated.

Competing interests: All authors are employed by Enalees.

Funding: Corporate financial support from Enalees.

146 | A new highly sensitive indirect ELISA for the detection of antibodies against African horse sickness virus

K. Klewer*, O. Mercier, R. Bonjour, A. Carpentier and P. Pourquier
Innovative Diagnostics, IDvet, 310 rue Louis Pasteur, 34 790 Grabels, France
Email: kristine.klewer@innovative-diagnostics.com

Background: African horse sickness (AHS) is an infectious disease of equids caused by the Orbivirus AHSV (family Reoviridae) and transmitted by *Culicoïdes* midges. AHS is endemic in sub-Saharan Africa, but outbreaks have occurred in many countries surrounding the Mediterranean Sea (e.g. Morocco, Spain, Portugal), in the Middle East and Asia. AHS is listed by the WOAH; guidelines for official recognition of disease-free status must be applied for international movements. Vaccination is prohibited in officially AHS-free countries.

Objectives: The ID Screen® African Horse Sickness Indirect ELISA kit[1] has been developed to detect antibodies against the VP7 protein, which is conserved among all 9 AHSV serotypes. The aim of the study was the evaluation of specificity and sensitivity of the ELISA.

Study design: Assay evaluation.

Methods: Diagnostic specificity and sensitivity were evaluated on 1015 sera from non-infected animals (France, Brazil, Argentina, Iceland) and 26 positive sera from different reference laboratories (EU/WOAH Reference Laboratory for AHS, Spain; the Pirbright Institute, UK; Friedrich-Loeffler-Institut, Germany). Serum samples from ruminants infected with Blue Tongue virus (BTV) or epizootic haemorrhagic disease virus (EHDV)were tested.

Results: The observed specificity of the novel ELISA was 100% (95% CI [99.6, 100.0], $n = 1015$) and the observed diagnostic sensitivity was 100% (95%CI [87.13, 100.0], $n = 26$). Sera tested in serial dilutions revealed an excellent analytical sensitivity. No-cross reaction was observed with BTV and EHDV positive sera. Evaluation of the novel ELISA was done by the EU/WOAH RL and it confirmed excellent performances of the new kit.

Main limitations: Small number of AHSV seropositive samples.

Conclusion: The novel ELISA is a reliable and easy-to-use test for the detection of AHSV VP7 antibodies. It shows very high specificity and an excellent diagnostic and analytical sensitivity. VP7 antibody detection is the prescribed WOAH method to prove freedom from AHS infection, estimation of prevalence and surveillance of a disease-free status.

Key manufacturer: 1 https://www.innovative-diagnostics.com/produit/id-screen-african-horse-sickness-indirect/.

Ethical animal research: Not applicable.

Informed consent: Not applicable, sera were purchased from reference laboratories.

Competing interests: None declared.

Funding: IDvet.

147 | A new indirect ELISA for the discrimination of anti-equine herpes virus-1 and -4 antibodies in horse sera

O. Mercier[1], S. Roche[1], A. Carpentier[1,*], K. Klewer[1], S. Pronost[2,3], G. Fortier[2,3] and P. Pourquier[1]
[1]*Innovative Diagnostics - IDvet, 310 rue Louis Pasteur, 34 790 Grabels, France;* [2]*LABÉO, Research Department, — 1 route de Rosel, 14 280 Saint-Contest, France and* [3]*Normandie University, UNICAEN, BIOTARGEN UR7450, 14 000 Caen, France*
Email: oceane.mercier@innovative-diagnostics.com;**alix.carpentier@innovative-diagnostics.com*.

Background: Equine herpes virus-1 and -4 (EHV-1 and EHV-4) infections are widespread in horse populations around the world. Both viruses are responsible for respiratory syndromes, but EHV-1 can cause more severe signs, neurological disorders and abortions. Performances of sport horses are affected as well as equine breeding, resulting in considerable economic loss.

Objectives: EHV-1 and EHV-4 are closely related, making serological diagnosis difficult by conventional methods. Therefore, the ID Screen® EHV-1/EHV-4 Discrimination test Indirect ELISA kit1 was developed as a bi-well ELISA based on specific recombinant proteins, in order to distinguish between EHV-1 and EHV-4 antibodies in equine sera.

Study design: Assay evaluation.

Methods: Diagnostic specificity and sensitivity were evaluated on VNT-pretested sera from France and Iceland. Seroconversion of 7 experimentally EHV-1 infected horses was monitored.[1] Horses were sampled between day 0 and 20 dpi. For EHV-4, eight horses were monitored during a natural EHV-4 epizootic (France). Sera and nasal swabs were collected regularly from the first day of clinical signs (day 0) until day 77. All sera were tested by VNT and ID Screen® ELISA.[1]

Results: Specificity was evaluated for EHV-1 at 99% (95% CI [96.6%–99.7%], $n = 208$). For EHV-4 it was difficult to find seronegative horses, but the few VNT negative sera tested, showed negative results by ELISA ($n = 5$). EHV-1-antibodies were detected between

13 and 17 dpi. EHV-4 antibodies were detected between 6 and 10 days following first clinical signs. No cross-reactivity was observed between EHV-1 and EHV-4 antibodies during seroconversion studies.

Main limitations: Low number of EHV-4 seronegative samples.

Conclusion: The new ELISA is easy-to-use with both EHV-1 and EHV-4 testing performed within the same analytical run, thanks to its biwell plate format. It presents excellent discriminatory capacity between EHV-1 and EHV-4 infected horses.

Key manufacturer: 1 https://www.innovative-diagnostics.com/produit/id-screen-ehv-1-ehv-4-discrimination-test-indirect/.

Ethical animal research: Approved by the Loire Valley Ethical Review Board (CEEA VdL, committee number 19, authorisation number APAFiS#22708).

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: OM, SR, AC, KK and PP are employees of IDvet.

Funding: IDvet.

Reference:

[1] Thieulent CJ, Sutton G, Toquet M-P, Fremaux S, Hue E, Fortier C, Pléau A, Deslis A, Abrioux S, Guitton E, Pronost S, Paillot R. Oral administration of valganciclovir reduces clinical signs, virus shedding and cell-associated viremia in ponies experimentally infected with the Equid Herpesvirus-1 C_{2254} Variant. Pathogens 2022;11(5):539. 10.3390/pathogens11050539.

148 | High performance freeze-dried triplex qPCR for diagnosis of equine herpesvirus-1 (EHV-1) and -4 (EHV-4) infections

L. Le Franc[1], M. Duchatellier[1], O. Mercier[2], A. Carpentier[2*], K. Klewer[2], S. Pronost[3,4], G. Fortier[3,4] and P. Pourquier[2]

[1]IDEQUI—3 rue Nelson Mandela, 14 280 Saint-Contest, France; [2]Innovative Diagnostics—IDvet, 310 rue Louis Pasteur, 34 790 Grabels, France; [3]LABÉO, Research Department, -1 route de Rosel, 14 280 Saint-Contest, France and [4]Normandie University, UNICAEN, BIOTARGEN UR7450, 14 000 Caen, France

Email: lorane.lefranc@innovative-diagnostics.com *alix.carpentier@innovative-diagnostics.com.

Background: EHV-1 and EHV-4 are common in horses globally, causing respiratory issues. EHV-1 can lead to severe signs, neurological disorders, and abortions. This affects sport horse performance and breeding, causing significant economic loss. Hence, a sensitive and specific diagnostic tool is crucial during outbreaks to determine which herpesvirus is involved.

Objectives: To develop and evaluate a freeze-dried triplex qPCR kit for EHV-1 and EHV-4 diagnosis, ID Gene Lyo™ Equine Herpes virus 1 and 4 Triplex,[1] containing an exogenous internal control.

Study design: Assay evaluation.

Methods: Limit of detection of the PCR (LD_{PCR}) was determined with a synthetic nucleic acid of EHV-1 and EHV-4. The Method Detection Limit (MDL) was determined by using equine whole blood, respiratory swabs and foetal organ samples spiked with EHV-1 (ATCC® VR-700™[2]) and EHV-4 (ATCC® VR-2230™[2]) strains separately. Specificity was evaluated with strains from respiratory virus or bacteria from equine and other mammals.

Results: The LD_{PCR} (95%) was established around 3.12 copies/PCR for EHV-1 and EHV-4. The MDL obtained for EHV-1 was: 291.5 copies/mL for whole blood and respiratory swab, and 2915 copies/mL for foetal organ. The MDL obtained for EHV-4 was 473 600 copies/mL for whole blood, respiratory swab, and foetal organ. The new qPCR kit has no cross-reactions with other pathogens except EHV-8, which is closely related to EHV-1.[1]

Main limitations: Not applicable.

Conclusion: The new freeze-dried triplex qPCR kit for EHV-1 and EHV-4 kit allows for rapid detection of both viruses simultaneously, with high reliability. It can be used for different forms of equine herpesvirus infections involving many different sample types thanks to the exogenous internal control included. It is freeze-dried, allowing for easy shipment at ambient temperature worldwide, reducing shipping costs and the environmental footprint.

Key manufacturers: 1 ID Gene Lyo™, https://www.innovative-diagnostics.com; 2 https://genomes.atcc.org/

Ethical animal research: Not applicable.

Competing interests: OM, AC, KK and PP are employees of IDvet.

Informed consent: Not applicable.

Funding: IDvet.

Reference:

[1] Garvey M, Suárez NM, Kerr K, Hector R, Moloney-Quinn L, Arkins S, Davison AJ, Cullinane A. Equid herpesvirus 8: Complete genome sequence and association with abortion in mares. PLoS One 2018;13(2):e0192301. 10.1371/journal.pone.0192301

149 | Retrospective study over 10 years (2010–2019) of equine infectious abortion in France

P. Moreau, M.-B. Romand, L. Baudet, S. Petry, J.-C. Valle-Casuso, C. Sévin and N. Foucher

ANSES, Normandy Laboratory for Animal Health, Physiopathology, Epidemiology of Equine Diseases Unit, Goustranville, France

Email: peggy.moreau@anses.fr

Background: Abortions still represent a significant economic loss in horse breeding. Our data suggest that in the regions and the horse populations studied, the frequency of infectious abortions is variable (18.7%–53.1%). No data have been available in France since 2011.

Objectives: To describe the main causes of equine infectious abortion recorded by Anses and the French National Surveillance Network of Equine Mortality (RESUMEQ) over a 10-year period.

Study design: Retrospective descriptive.

Methods: Records of 851 *post-mortem* cases of infectious abortion from different facilities carrying out necropsies of foetuses and recording their cases in the national database of the French surveillance network of equine mortality from 2010 to 2019 were reviewed. Infectious abortions were assigned into four main categories:

bacterial, fungal, mixed (bacterial and fungal) or viral and the aetiological pathogens were described.

Results: Nine facilities recorded an infectious aetiology for 1 to 335 cases, with a total of 474 abortions of 851 (55.4%). The main infectious causes were bacterial with 389 cases (82.1%), including a quarter with macroscopic placentitis and approximately 40 bacterial species were isolated. Viral infections were identified in 42 cases (8.9%), with EHV-1 identified in the vast majority (90%). Mixed and fungal infections were diagnosed in 13 (2.7%) and 7 cases (1.5%), respectively. No specific pathogens was identified in 23 cases (4.8%) despite the presence of macroscopic infectious lesions.

Main limitations: The data were only based on abortions that were examined at necropsy. It was not possible to compare facilities because case numbers in some facilities were low.

Conclusions: The rate of infectious abortions was similar to that of previous studies conducted in France, Italy and the USA, but higher than that of the United Kingdom and Canada. Proportions of bacterial and viral infections have also varied from one study to another.

Ethical animal research: Not required: retrospective analysis of clinical data.

Informed consent: Not stated.

Competing interests: None declared.

Funding: None.

150 | Transplacental transmission of *Theileria equi*

I. Ricci[1], A. Cersini[1], G.A. Marcario[1], R. Nardini[1], G. Manna[1], G. Brocherel[1], I. Congiu[1], S. Greco[1], C.A. Minniti[2], R. Conti[1] and M.T. Scicluna[1]

[1]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Via Appia Nuova 1411, 00178 Rome, Italy and* [2]*Carabinieri Corps General Head Quarters-Department for Health and Veterinary Organisation*

Email: teresa.scicluna@izslt.it

Background: Tick-borne piroplasmosis, caused by *Babesia caballi* and *Theileria equi* (EP) affects equids and may result in reproductive disorders [1], including abortions but may be underestimated when investigating these conditions, as they not regularly included in differential diagnoses.

Objectives: To improve knowledge on the vertical transmission of EP and subsequent cause of abortion.

Study design: Case report.

Methods: Following an abortion, the mare's blood and uterine swab, placenta, foetal brain, lung, spleen and liver, were tested using direct and indirect methods for the diagnosis of the principal viral and bacterial aetiological agents causing abortions, including EP. In particular, for EP, a commercial competitive serological ELISA[1] and a real time PCR protocol, amplifying a fragment of the 18S rRNA V4 hypervariable region gene[2] were used. EP PCR products were sequenced using Genetic Analyser Sequencing v5.4[2]. Sequencing alignment was performed using nucleotide Basic Local Alignment Search Tool (BLASTn).[2]

Results: *T. equi* was detected by PCR in the mare's blood, placenta and foetal organs examined. No significant positivity was obtained for other infectious agents investigated.

Main limitations: EP transplacental transmission is not direct evidence of the primary cause of abortion.

Conclusions: *T. equi* was detected in both the mare and foetal samples in the absence of other infectious agents, further enforcing the potential role of *T. equi* as a cause of abortion in equids.

Key manufacturers: 1. https://vmrd.com/; 2. Applied Biosystems, Foster City, https://www.thermofisher.com/uk/en/home/brands/applied-biosystems.html.

Ethical animal research: Not required: case report.

Informed consent: Not stated.

Competing interests: None declared.

Funding: None.

References:

[1] Leon A, Pillon C, Tebourski I, Bruyas JF, Lupo C. Overview of the causes of abortion in horses, their follow-up and management. Reprod Domest Anim. 2023;58 Suppl 2:93–101. 10.1111/rda.14406. Epub 2023 Jul 12. PMID: 37312640.

[2] Bartolome Del Pino LE, Meana A, Zini M, Cersini A. Evidence of transplacental transmission of equine piroplasms *Theileria equi* and *Babesia caballi* in an Italian breed mare. Folia Parasitol (Praha). 2023;70:2023.005. 10.14411/fp.2023.005. PMID: 36960775.

151 | Seroprevalence of West Nile virus in equids in eastern Spain (2021–2023)

B. Marí-Ros[1,2], I. Ayala de la Peña[3], I. Ruiz-Arrondo[4,5], T.A. Balmori[3], F.J. Vázquez-Bringas[5], J. Zalaya[5], C. De la Fe[6], S. Santibáñez[4], M.S. Simó[2], R. Domingo[2], S. Vega[7], S. Fuente[5], E. Jose-Cunilleras[1*]

[1]*Dept. Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, Bellaterra (Barcelona) 08193 Spain;* [2]*Depto. Medicina y Cirugía Animal. Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, Alfara del Patriarca (Valencia) 46 115 Spain;* [3]*Depto. Medicina y Cirugía Animal, Universidad de Murcia, Espinardo (Murcia) 30 100 Spain;* [4]*Center for Rickettsiosis and Arthropod-Borne Diseases, Hospital Universitario San Pedro-CIBIR, Logroño, Spain;* [5]*Dept. of Animal Pathology, Faculty of Veterinary Sciences, Instituto Universitario de Investigación Mixto Agroalimentario de Aragón (IA2), Universidad de Zaragoza, Zaragoza, Spain;* [6]*Depto. Sanidad Animal, Universidad de Murcia, Espinardo, Murcia 30 100, Spain and* [7]*Depto. Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos. Facultad de Veterinaria, Instituto de Ciencias Biomédicas, Universidad Cardenal Herrera-CEU, CEU Universities, Alfara del Patriarca (Valencia) 46 115 Spain*

Email: eduard.jose.cunilleras@uab.cat

Background: West Nile virus (WNV) is transmitted among wild birds by infected mosquitoes, and humans and horses are infected as dead-end hosts. WNV infections and neuroinvasive disease have been

reported in birds, humans and equids in Andalusia, Catalonia, and Extremadura; however, large regions in Spain remain unexplored.

Objectives: To estimate the seroprevalence and map out the geographic distribution of WNV in equids in unexplored regions of Spain where the presence of WNV vectors is very abundant.

Study design: Prospective observational field study stratified geographically.

Methods: Aragón, Navarra, La Rioja, C. Valenciana and Murcia were surveyed. Sample size in each region was estimated based on the horse population, expected seroprevalence of 5%–10%, precision of ±3%, and a confidence interval of 95%. Sampling locations were stratified by counties to ensure broad geographic distribution. Blood samples of equids (horses, donkeys, and mules) were obtained by jugular venipuncture. Serum samples were used for testing for the presence of WNV total antibodies by commercial blocking ELISA and any positive samples were sent to national reference laboratory for confirmation by sero-neutralisation.

Results: The proportion of horses seropositive against WNV by ELISA was: 4/46 (8.7%) in Aragón, 17/204 (8.3%) in C. Valenciana, 8/117 (6.8%) in Murcia, 3/44 (6.8%) in La Rioja and 0/36 in Navarra. A wide geographic distribution of seropositive horses was observed in these regions, even in villages at high altitude (i.e., near Jaca, Central Pyrenees, altitude 900 m).

Main limitations: Ongoing study with 20%–100% of planned sampling depending upon the region.

Conclusions: WNV circulation appears to be more geographically extensive than expected in eastern Spain. The low level of seroprevalence in horses suggests that relying on passive surveillance may be unreliable to detect WNV circulation in certain regions.

Ethical animal research: Institutional Ethics approval obtained, ref CEEAH-UAB 5409.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: Project funded by Ministry of Science and Innovation, Spain; "Proyectos de I + D + I 2020" ref. PID2020-113768RB-I00. Barbara Marí Ros was funded by "Conselleria d'Innovació, Universitats, Ciència i Societat Digital, Generalitat Valenciana", Spain; ref CIACIF/2022/259; and fellowship "AGAUR-FISDUR del Departament de Recerca i Universitats de la Generalitat de Catalunya" ref: 2023-FISDU-00048.

152 | Innovative purification method for equine infectious anaemia virus NGS analysis

G. Manna[1], G. Cardeti[1], R. Nardini[1], M. **Bazzucchi**[2], R. Conti[1], **G. Pietrella[1], P. Cavadini**[2], D. La Rocca[1], S. **Tofani[1] and M.T.** Scicluna[1]
[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) *via* Appia Nuova, 1411–00178 Roma (WOAH Reference Laboratory for Equine Infectious Anaemia) and [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" (IZSLER), *Via* Antonio Bianchi, 7/9, 25 124 Brescia, Italy
Email: teresa.scicluna@izslt.it

Background: Equine infectious anaemia (EIA) is a persistent viral infection of equids caused by EIA virus (EIAV), a lentivirus with a single-strand RNA genome of approximately 8.2 kb containing three major genes, gag, pol and env flanked by long terminal repeats (LTRs). Virus replicates in white blood cells, particularly in macrophages, and it integrates with the host genome, ensuring the persistence of the infection over time.

Objectives: Development of an innovative method of purification and concentration of EIAV for its characterisation using a Next Genome Sequencing (NGS) method to obtain the complete EIAV genome, including LTRs regions, that are usually difficult to obtain [1].

Study design: Method development.

Methods: The Wyoming EIAV reference strain of which several full genome sequences are available for subsequent verification in NGS analysis was used. The virus was grown on equine E. Derm cells. Supernatants were divided into two aliquots each of which was subjected to one of two different purification methods: method A using a tangential ultrafiltration by centrifugation; method B using a PEG precipitation, nucleases treatment followed by a chromatographic purification. NGS runs were performed on both preparations [1].

Results: Method A did not have an overall good performance, having an average depth coverage lower than 600, and not covering the LTR regions. With method B the average depth coverage was at least 9500 and covering part of the LTRs.

Main limitations: This novel method requires further testing for its standardisation on field strains.

Conclusions: This study suggests that the use of a purification and concentration method using gel columns followed by ion exchange resins significantly improves the resolution of an NGS analysis on viruses such as EIAV.

Ethical animal research: The samples were collected for the implementation of the 'National plan for the surveillance and control of infectious anaemia in equidae" (Ministerial Decree 2 February 2016).

Informed consent: Not stated.

Competing interests: None declared.

Funding: Institutional.

Reference:
[1] Colitti B, Coradduzza E, Puggioni G, Capucchio MT, Reina R, Bertolotti L, Rosati S. A new approach for Small Ruminant Lentivirus full genome characterisation revealed the circulation of divergent strains. PLoS One 2019;14(2):e0212585. doi:10.1371/journal.pone.0212585

153 | Alternative sites for chronic-persistent EHV-1

L.S. Goehring*, E. Olajide, A. Khan, R. Ruby and J. Janes
Department of Veterinary Science, Martin-Gatton College of Agriculture, Food and Environment, University of Kentucky, Lexington, USA
Email: l.goehring@uky.edu

Background: In a recent terminal 70 days post infection study, Equid alphaherpesvirus −1 glycoprotein B (EHV-1 gB) genome copies were detected in established latency sites of this virus – trigeminal ganglion (TG) and respiratory tract associated lymphoid tissues (RALT) as well as non-established latency site tissues including alimentary lymph nodes and (para)sympathetic ganglia.
Objective: To determine EHV-1 prevalence and genome frequencies in defined sympathetic ganglion structures (i.e., 'sympathetic trunk' ST) and alimentary lymph nodes (i.e. 'mesenteric lymph node' mesLn) among a random population of horses. This screening was done in addition to TG and RALT screening.
Study design: Cross-sectional with convenience sampling.
Methods: Samples were obtained from 89 horses submitted to the University of Kentucky Veterinary Diagnostic Laboratory for post mortem examination. Sections of formalin fixed, paraffin embedded ST and mesLn underwent DNA extraction followed by qPCR for EHV-1 glycoprotein B (gB) detection.
Results: STs of 15 of 82 horses (18.3%) contained EHV-1 (gB) genome copies, while only 3 of 54 mesLn (5.6%) were positive. TG was the most common location for gB copies (34%) followed by ST. RALT tissue was positive in 13% of cases.
Main limitations: Small sample size.
Conclusions: (Para)sympathetic ganglia are likely also part of chronic-persistent infection with EHV-1. This is similar to other *Alphaherpesvirinae* with viraemic spread (e.g. VZV). Consequences of EHV-1 positive ST in the pathogenesis of EHV-1 need to be determined.
Ethical animal research: Not required: post-mortem survey.
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: None declared.
Funding: Gluck Equine Research Foundation.

154 | Investigation of the EHV-1 genotype (N_{752}, D_{752} and H_{752}) in swabs collected from equids with respiratory and neurological disease and abortion from the United States (2019–2022)

N. Pusterla, S. Barnum, K. Lawton, C. Wademan, R. Corbin and E. Hodzic
Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA
Email: npusterla@ucdavis.edu

Background: Contemporary data on EHV-1 genotype (non-neuropathogenic or N_{752}, neuropathogenic or D_{752} and new variant or H_{752}) in clinically diseased equids is important in order to determine the frequency of these genotypes and their association with disease expression. While the interplay between viral, host and environmental factors remains quite elusive in the pathogenesis and disease expression of EHV-1, contemporary studies using diagnostic material are essential in order to understand which genotypes are circulating in specific horse populations.
Objectives: To evaluate the EHV-1 genotype (N_{752}, D_{752} and H_{752}) in swabs collected from horses with respiratory disease, abortion and neurological disease and submitted to a diagnostic molecular laboratory.
Study design: Retrospective study
Methods: A total of 297 EHV-1 qPCR-positive swabs collected from 2019 to 2022 from horses with respiratory disease (EHV-1), neurological disease (equine herpesvirus-1 myeloencephalopathy (EHM)) and abortion were tested for the three different EHV-1 genotypes (N_{752}, D_{752} and H_{752}) using qPCR allelic discrimination assays.
Results: All submissions originated from the United States and included 257 EHV-1 cases, 35 EHM cases and 5 cases of abortion. EHV-1 qPCR-positive cases were predominantly seen during winter and spring. N_{752} was the predominant genotypes detected in EHV-1 cases (87.5%), EHM cases (74.3%) and abortions (80%). D_{752} was detected less frequently in EHV-1 cases (9.3%) and EHM cases (25.7%), while H_{752} was only detected in EHV-1 cases (3.1%).
Main limitations: The present study material was based on data from samples submitted to only one diagnostic molecular laboratory.
Conclusions: While the N_{752} genotype has remained the predominant genotype affecting horses with respiratory disease and abortion, it has also become a leading genotype in cases of EHM, when compared to historical data. The new H_{752} genotype, first reported in the United States in 2021, has remained confined to a cluster of geographically and temporally related outbreaks and the data showed no emerging spread of H_{752} since it was first reported.
Ethical animal research: Not required: retrospective descriptive report.
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: None declared.
Funding: Center for Equine Health, School of Veterinary Medicine, University of California, Davis, CA, USA.

155 | Antimicrobial resistance in *Escherichia coli* of equine origin

J. Gibbons
Irish Equine Centre, Naas, Co. Kildare, Ireland
Email: jgibbons@irishequinecentre.ie

Background: Antimicrobial resistance, and multi-drug resistance (MDR) in particular, is recognised as a significant global threat to human and animal health.
Objectives: to determine the occurrence of resistance and MDR in *E. coli* of equine origin and to determine if the occurrence of resistance and MDR differed over time or by anatomical site of origin.

Study design: Retrospective cross-sectional study.

Methods: Antimicrobial susceptibility data for *E. coli* isolated from diagnostic samples of equine origin during the period 2019–2023 were retrieved from the Laboratory Management System. Only isolates with a Minimum Inhibitory Concentration (MIC) value as provided by the VITEK2 system (Biomerieux, France) were included. Samples were categorised by year of isolation and site of origin ('Faecal', 'Respiratory', 'Reproductive' and 'Other'). The proportion of resistant isolates by year and by anatomical site of origin were compared using the Z-test of proportions while the distribution of MIC values by year and by sample type were compared using the Kruskal-Wallis test.

Results: The greatest frequencies of resistance in *E. coli* of equine origin were observed to the early generation cephalosporins (>90%), trimethoprim-sulphamethoxazole (41.6%), ampicillin (41.1%) and tetracycline (36.1%). The overall frequency of MDR was 34.6%. No significant difference in the occurrence of resistance over time was seen for most antimicrobials. Isolates of reproductive origin were significantly ($P < 0.05$) less resistant to 10 of 12 tested antimicrobials and displayed significantly less MDR.

Main limitations: Data on the clinical presentation of cases and previous antimicrobial treatment were not available.

Conclusions: Antimicrobial resistance to many antimicrobials and MDR occurs frequently and consistently over time in *E. coli* of equine origin. The occurrence of resistance differs significantly by site of origin, with *E. coli* from the reproductive system displaying significantly less resistance and MDR than those from other body sites.

Ethical animal research: Research ethics committee oversight not required: retrospective analysis of laboratory data.

Informed consent: Not stated.

Competing interests: None declared.

Funding: None.

156 | Characterisation of an equine herpesvirus-1 strain using long fragment sequencing technology

D. Froger[1], V. Chesnais[2], A. Madeline[1], C. Schimmich[1], F. Lecouturier[1], D. Merda[2], N. Wiernasz[1] and J.-C. Valle Casuso[1]

[1]*ANSES Laboratory for Animal Health in Normandy, Goustranville, France and* [2]*ANSES Laboratory for Animal Health, Maisons Alfort, France*

Email: delphine.froger@anses.fr

Background: Since 2021, outbreaks of equine rhinopneumonitis, one of the clinical forms of equine herpesvirus type-1 and/or -4 (EHV-1/EHV-4) infection, have been rising sharply in Europe. EHV-1 and EHV-4 belong to the *herpesviridae* family, and more specifically to the *alphaherpesvirinae* subfamily. These viruses can cause numerous animal and financial losses in the equine industry. Various vaccines are authorised to prevent this disease, but recurrent epidemics show that vaccination can be ineffective sometimes. Molecular characterisation of the strains circulating each year could be a great help to establish links between outbreaks or take better decisions about the vaccination strategy.

Objective: To characterise currently circulating strains of EHV-1 in our region.

Study design: Phylogenetic description.

Methods: In 2022, two EHV1 infections were suspected based on observations from autopsies of aborted foetuses and were confirmed by real-time PCR. Virus isolation on cell culture was performed from tissue homogenates (liver, lung, spleen, kidney and allantois) as described in the chapter 3.6.9 of the WOAH terrestrial manual. Isolated and amplified EHV-1 strains were then sequenced using Nanopore long fragment sequencing technology with the advantage of being easy to use by laboratories.

Results: Obtaining and rapidly analysing the sequences of EHV-1 isolated in both outbreaks enabled us to be reactive and self-contained in the molecular characterisation and allow us to establish the phylogenetic analysis of both strains. The sequencing results showed that strains from recent outbreaks were related to other EHV-1 strains isolated in the UK in 2009 and 2010.

Conclusion: This new molecular technology will enable the production of more detailed reports for the competent authorities, who will then define the future EHV-1 surveillance and prevention plans, as well as alerting the equine industry more quickly to apply effective prevention measures.

Ethical animal research: Research ethics committee oversight not required: retrospective analysis of clinical data.

Informed consent: Consent was obtained from the owners of the horses sampled in this study.

Competing interests: None declared.

Funding: None.

157 | Characterisation of the antimicrobial effect of essential oils for equine veterinary medicine as a complementary therapy

E. Kokabi[1,2], A. Taiebi[1,2], P. Legendre[1], E. Hue[1,3,4], S. Pronost[1,3,4], V. Bouchart[1,5], I. Lussot-Kervern[6] and A. Leon-Seck[1,2]

[1]*LABÉO, Research Department, Saint-Contest, 14 000 Caen, France;* [2]*Normandie Université, UNICAEN, UNIROUEN, DYNAMICURE, INSERM, UMR 1311, 14 000 Caen, France;* [3]*Normandie Université, BIOTARGEN, Saint-Contest, 14 000 Caen, France;* [4]*Normandie Université, ImpedanCELL, Saint-Contest, 14 000 Caen, France;* [5]*Normandie Université, ANTICIPE, INSERM U1086, 14 000 Caen, France and* [6]*Veterinary phyto-aromatherapy network, RéPAAS, France*

Email: elena.kokabi@laboratoire-labeo.fr

Background: Essential Oils (EOs) are a therapeutic adjunct to the emergence of antibiotic resistance due to their reported antimicrobial properties [1,2].

Objectives: The antibacterial and antiviral effects of some EOs from three different suppliers were investigated, considering EOs' volatility and hydrophobicity.

Study design: In vitro experiments.

Methods: After the chemical composition determination of selected EOs, their antimicrobial activity was characterised on pathogenic bacteria and viruses Seven EOs were selected for their interest in

veterinary phyto-pharmacopoeia. Gas chromatography was used to identify and quantify their main compounds. Antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) broth dilution against four bacterial (Gram +ve and Gram −ve) wild-type and resistant strains. Impedance measurement on equine dermal cells was used to evaluate the antiviral activity of EOs against equine herpesviruses (EHV) -1 and -4.

Results: Our in-house analysis results match the purity certificates for each EO provided by the suppliers. EO composition was not affected by organic versus non-organic origin or by different batches of EO. One component is systematically and significantly in the majority for each EO (T-Test and Wilcoxon test, $p \leq 0.05$). Gram +ve strains were more susceptible than Gram -ve strains to EOs (total oils and active ingredients). Cinnamon EOs has the lowest MICs (from 0.01% to 0.4%) and tea tree and ravintsara EOs had the highest MICs (from 0.25% to 20%). The EOs tested showed strong cytotoxicity in our cell model even at low concentrations. At the maximum EO concentration that showed no cytotoxicity in our model, no antiviral effect was observed against EHV-1 or -4.

Main limitation: No gold standard methods are available for EOs antimicrobial characterisation.

Conclusions: Our study lays the scientific foundations for an evaluation of the in vitro effects of EOs to provide a better understanding of in vivo applications.

Ethical animal research: Not required: analysis of microorganisms.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: IFCE (Institut Français du Cheval et de l'Equitation) and Fonds Eperon.

References:

[1] Maccelli A, Vitanza L, Imbriano A, Fraschetti C, et al. *Satureja montana* L. Essential Oils: Chemical profiles/phytochemical screening, antimicrobial activity and O/W NanoEmulson Formulations. Pharmaceutics. 2019;12(1):7. DOI: 10.3390/pharmaceutics12010007

[2] Lisboa FP, Silvestre WP, Castro JO, Martins GV, Segabinazzi LGTM, Pauletti GF, Dell-Aqua JA. In vitro antimicrobial activity of selected essential oils against endometritis-causing microorganisms in mares. J Equine Vet Sci. 2022;110:103840. DOI: 10.1016/j.jevs.2021.103840

158 | Antibodies against the strangles vaccine component CCE inhibit both alpha-2-macroglobulin and albumin binding

J. Bjerketorp[1], K. Jacobsson[1], S. Frosth[1], B. Guss[1], L. Frykberg[1] and A.S. Waller[2]

[1]*Department of Animal Biosciences, Swedish University of Agricultural Sciences, Uppsala, Sweden and* [2]*Intervacc AB, Stockholm, Sweden*

Email: Joakim.Bjerketorp@slu.se

Background: Strangvac[1] is a multicomponent vaccine against strangles that contains fragments of eight different *Streptococcus equi* proteins.[1] Five of these eight fragments are contained in the fusion protein CCE. The C-terminal part of CCE contains the first 161 amino acids of EAG (ZAG[2]), which includes two Alpha-2-macroglobulin-binding domains and 2/3 of the albumin-binding domain.

Objectives: To measure the binding of anti-CCE antibodies to different domains of EAG and to determine if antibody-binding inhibits the ability of EAG to bind the protease inhibitor Alpha-2-macroglobulin (A2M) and horse albumin.

Study design: Fusion protein CCE was used to immunise a goat, the serum IgG fraction was first purified on protein A and then further affinity purified on CCE (Capra Science). These antibodies were used to investigate the binding to EAG domains.

Methods: Five subclones of EAG were purified as GST-fusions. CCE antibody binding to these GST-EAG fusions were investigated. In addition, the ability of these antibodies to interfere with the A2M and albumin binding of these GST-EAG fusions was tested.

Results: The CCE antibodies bound well to the A2M-binding domains, but not to the native albumin-binding domain. The antibodies inhibited EAG's interaction with A2M and albumin and could efficiently dissociate these ligands even after they had bound to EAG.

Main limitations: The CCE antibodies tested were derived from a goat and not from horses.

Conclusions: CCE antibodies efficiently dissociated A2M and albumin from EAG. Consequently, antibodies induced by the Strangvac vaccine have the potential to remove A2M and albumin from the bacterial cell surface.

Key manufacturer: 1 Strangvacc, Intervacc: https://intervacc.se/en/.

Ethical animal research: Capra Science provided the goat antibodies used in this study and have ethical approval for antibody production and are also certified according to ISO9001:2015 and EU ORGANICS farming. https://www.caprascience.com/.

Informed consent: Not applicable.

Competing interests: AS Waller is the Chief Scientific Officer for Intervacc.

Funding: JB, KJ, SF, BG and LF have received research grants from Intervacc AB.

References:

1. Guss B. et al. Getting to grips with strangles: an effective multicomponent recombinant vaccine for the protection of horses from Streptococcus equi infection. PLoS Pathog 2009;5(9). 10.1371/journal.ppat.1000584

2. Jonsson, H. et al. A protein G-related cell surface protein in Streptococcus zooepidemicus. Infect Immun, 1995;63(8). 10.1128/iai.63.8.2968-2975.1995

159 | Antiviral strategies in equines

C.V. Migné[1], E. Quéré[2], E. Hue[3,4], M. Begue[3], V. Chaillot[1], C. Beck[1], S. Lecollinet[1], P.-O. Vidalain[5], P. Dallemagne[6], S. Peggy[6], M. Coulpier[1], H. Munier-Lehmann[7], J.-C. Valle-Casuso[8], S. Zientara[1], G. Gonzalez[1] and S. Pronost[3,4]

[1]*Anses, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR 1161 Virologie, Laboratoire de Santé Animale, Maison-Alfort, France;* [2]*Ecole Nationale*

Vétérinaire d'Alfort, CHUV-Equidés, Maisons-Alfort, France; [3]LABÉO, 14 280 Saint Contest, France; [4]Normandie Université, UNICAEN, BIOTARGEN, 14000 Caen, France; [5]CIRI Centre International de Recherche en Infectiologie, Univ. Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, 69 007 Lyon, France; [6]CERMN, UR 4258 Université de Caen Normandie, France; [7]CECS, I-STEM, AFM, 91100, Evry, France and [8]Anses, Laboratory for Animal Health in Normandy, Physiopathology and Epidemiology of Equine Diseases Unit, 14 430 Goustranville, France; Mixed Technological Unit "Equine Health and Welfare—Organisation and Traceability of the Equine Industry" (UMT SABOT), France

Email: camille.migne@anses.fr

Background: Viral diseases have an economic and medical impact on equine health. The main strategies in their control are based on prevention through vaccination and/or supportive treatments. To date, there are very few antiviral molecules in the equine therapeutic arsenal, apart from valacyclovir for EHV-1.

Objectives: In order to repurpose approved drugs, FDA-approved therapeutic libraries were tested on equine cells. Two molecules have shown promising antiviral activity: CLABÉO and GANSES (anonymised molecules). The safety of these molecules in horses, including the tolerance, the pharmacokinetics and pharmacotoxicity, was assessed.

Study design: In vivo experiments.

Methods: The molecules were inoculated twice into horses at two different doses at 7 days apart (one tenth of the human therapeutic dose (1/10 DTh) and the human therapeutic dose (DTh)). Various parameters were recorded and evaluated, such as clinical, general condition, biochemical and biological check-ups and the molecules elimination kinetics. Eight healthy mares were included and assigned into two groups. Pharmaco-toxicity was mainly assessed using haemato-biochemical monitoring before injection, 24 h post-injection (pi) and 1 week pi. Pharmacokinetics were assessed using blood and urine samples taken at several time points.

Results: Blood tests showed the horses to be in good general condition, with the exception of neutropenia observed in horses given the DTh of GANSES. The molecules were measured in plasma and urine. GANSES was detectable up to 24 h in urine and was no longer detectable very quickly in plasma (<4 h pi). CLABÉO was detectable up to 24 h in the urine of some ponies and at 6 h in the plasma.

Conclusions: These results showed that the molecules are well tolerated by horses. Further studies are needed to test the antiviral spectrum of GANSES and CLABÉO against equine pathogens as respiratory viruses or arthropod- borne equine encephalitis viruses.

Ethical animal research: Approved by the CENOMEXA No54 ethics committee (No APAFIS #30663-202103251639113_v6) and the Ministry of Higher Education, Research and Innovation.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: The IFCE (Institut Français du Cheval et de l'Equitation) grant number Rech-CS-2020-010-SAVE_SATELLITE.

160 | Acute pulmonary haemorrhage in five adult horses: an unusual presentation of equine leptospirosis

C. Decoster[1], E. Paulussen[1], L. Lefere[1], K. Vanderperren[2], G. van Loon[1] and A. Dufourni[1]

[1]University of Ghent, Large Animal Internal Medicine, Salisburylaan 133, Merelbeke, 9820, Belgium and [2]University of Ghent, Faculty of Veterinary Medicine, Department of Medical Imaging, Salisburylaan 133, Merelbeke, 9820, Belgium

Email: celin.decoster@ugent.be

Background: Leptospirosis is a widespread zoonotic, infectious disease characterised by various clinical manifestations including abortion, stillbirth, and liver and kidney dysfunction. *Leptospira* infection associated pulmonary haemorrhage has been documented in human patients and in foals. This atypical presentation has not yet been documented in adult horses.

Objective: This report describes five adult horses which presented with acute respiratory distress and bilateral epistaxis due to pulmonary haemorrhage.

Study design: Case series.

Clinical records: Five adult horses were presented with acute respiratory distress and bilateral epistaxis between 2021 and 2024 at the Veterinary Teaching Hospital. Blood analysis revealed anaemia (PCV 18%–32%), severe azotaemia (Creatinine 241–1792 μmol/L—BUN 17.2–65.5 mmol/L), and thrombocytopenia ($n = 2$, 7–48 K/μL). Lung ultrasound revealed widespread B-lines bilaterally. Thoracic radiography showed a generalised miliary pattern consistent with pulmonary haemorrhage. On endoscopic examination, haemorrhagic fluid was present in the trachea. Urinary PCR and serological testing using microscopic agglutination confirmed *Leptospira* infection. Treatment with poly-ionic fluids, diuretics, antimicrobials (penicillin or oxytetracycline) and non-steroidal anti-inflammatory drugs resulted in clinical improvement in four out of five horses and discharge from the hospital within 2 weeks. One horse was euthanised because of financial constraints.

Main limitations: Lack of follow up.

Conclusions: Acute respiratory distress and pulmonary haemorrhage due to leptospirosis has been documented in foals but was only rarely associated with haemorrhagic fluid in the trachea. In adult horses, bilateral epistaxis as a result of severe pulmonary haemorrhage seems to be more prominent. These findings highlight the importance of including leptospirosis in the differential diagnosis for equine pulmonary haemorrhage in adult horses, especially in the presence of azotaemia.

Ethical animal research: Not required: retrospective case series.

Informed consent: Not stated.

Competing interests: None declared.
Funding: None.

161 | Chronic-persistent EHV-1 infection in horses

E. Olajide, A. Khan, J. Janes, R. Ruby and L. Goehring
Department of Veterinary Science, University of Kentucky, Lexington, USA
Email: edward.olajide@uky.edu

Background: Equid alphaherpesvirus 1 (EHV-1) is an important viral pathogen of horses worldwide. It holds significance due to clinical manifestations of respiratory disease, abortion/neonatal death and myeloencephalopathy (EHM), that emerge during or after acute infection. Chronic-persistent (CP) infection, sometimes referred to as latency is suggested to be a permanent, life-long infection. Its establishment likely starts with the first respiratory tract infection. EHV-1 is known to establish CP in trigeminal ganglia (TG) and in respiratory tract associated lymphatics (RAL).
Objective: To determine EHV-1 prevalence and genome frequencies in TG and RALs among different age groups of horses within a random population.
Study design: Cross-sectional.
Methods: Samples were obtained from 89 horses submitted to University of Kentucky Veterinary Diagnostic Laboratory for diagnostic postmortem examination. DNA was isolated from paraffin embedded fixed tissues followed by qPCR for EHV-1 glycoprotein B (gB) detection. Horses were grouped according to age: [I: ≤ 23 months ($n = 26$); II: 2–10 years ($n = 34$); III > 10 years ($n = 29$)]. All tissues were also tested for Equid alphaherpesvirus 4 (EHV-4). IBM SPSS statistical software 26.0.0, and the Chi-square test was used to investigate age related association and distribution pattern.
Results: In total, 30 TG and 12 RAL samples were positive for EHV-1 gB while 63 TG and 8 RAL samples were positive for EHV-4. There was no association between tissue sites. Group I gB prevalence was less than groups II or III; prevalence between the latter were similar.
Main limitation: Small sample size which limits independent confirmation.
Conclusions: Here, it was rare to find EHV-1 gB in group I horses, while EHV-4 was commonly detected. This corresponds with the clinical impression that EHV-4 dominates in this age group. TG appears to be the predominant tissue site. EHV-1 CP prevalence is likely lower than previously suggested yet geographical differences may exist.
Ethical animal research: Not required: post-mortem survey.
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: None declared.
Funding: The Gluck Equine Research Foundation.

162 | AmpliSeq approach using third-generation sequencing technologies for equine infectious anaemia virus characterisation

N. Wiernasz, D. Froger, F. Lecouturier and J.-C. Valle-Casuso
ANSES, Laboratory for Animal Health in Normandy, Physiopathology and Epidemiology of Equine Diseases Unit, 14 430 Goustranville, France
Email: norman.wiernasz@anses.fr

Background: Equine Infectious Anaemia (EIA) is a lifelong persistent infection affecting all equids, transmitted mainly by hematophagous insects. With no treatments available and an almost worldwide distribution, some countries have adopted systematic euthanasia of every infected equid as prophylactic measures. Today, in the context of a global market, EIA is of significant importance for the equine industry and thus is notifiable to the World Organisation for Animal Health. Its aetiological agent is a macrophages tropic lentivirus, which like its family cousins (e.g., HIV and SIV), can integrate its genome into that of its target cell. In addition, lentiviruses possess significant genetic variability, making the development of molecular biology detection methods difficult [1].
Objective: To develop molecular biology tools for EIA virus characterisation.
Study design: Assay development.
Methods: To study viral strains' genetic proximity, their DNA is usually extracted from postmortem tissues, such as the spleen or liver. It then undergoes Nested PCR amplification targeting the gag gene, followed by amplicon sequencing by the Sanger method. Although affordable for most laboratories, Sanger sequencing has the disadvantage of possessing a low sensitivity by requiring high concentration of pure and excellent quality DNA template. To overcome these limitations, we have coupled the production of gag amplicons from Nested PCR with a sequencing strategy based on Oxford Nanopore technology.
Results: We succeeded in recovering gag sequences from challenging tissue samples collected from previous outbreaks in France. This technical approach allowed us for the first time, to characterise the gag sequence from the buffy coat fraction of blood samples collected from an alive animal.
Conclusion: This may represent a new step in studying the genetic evolution of EIA virus without requiring animal euthanasia.
Ethical animal research: Not required: excess material from clinical surveillance samples were used.
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: None declared.
Funding: Chaire d'Excellence Region Normandie.
Reference:
[1] Cook RF, Leroux C, Issel CJ. Equine infectious anaemia and equine infectious anaemia virus in 2013: A review. Vet. Microbiol. 2013;167:181–204.

163 | **Implementation of a "One Health" territorial network for operational research following the emergence of the West Nile and Usutu arboviruses in New Aquitaine in 2022**

G. Gonzalez[1], C. Bigeard[1,2], C. Migné[1], N. Chevalier[4], M. Depecker[4], T. Helle[1], M. Dumarest[1], S. Martin-Latil[1], T. Touzet[2], C. Marcillaud-Pitel[5], C. Lupo[5], A. Fontaine[6], X. de Lamballerie[6], L. Pezzi[6], S. Zientara[1], A. Leblond[7], A. Duvignaud[8] and D. Malvy[8]

[1]*ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR Virologie, Laboratoire de Santé Animale, F-94700 Maisons-Alfort, France;* [2]*ENSVI-FVI VetAgro Sup, F-69280 Marcy-L'étoile, France;* [3]*Direction Départementale de la Protection des Populations de Gironde, F-33520 Bruges, France;* [4]*Clinique de Conques, Centre hospitalier Vétérinaire équin, F-33420 Saint-Aubin-de-Branne, France;* [5]*RESPE,14 280, Saint-Contest, France;* [6]*Aix Marseille Université, CNR Arbovirus, Unité des Virus Emergents, UVE, IHU Méditerranée Infection, Marseille, France;* [7]*UMR EPIA (Epidémiologie des Maladies Animales et Zoonotiques), INRAE, VetAgro Sup, Université de Lyon, 69 280 Marcy L'Etoile, France and* [8]*Département Maladies infectieuses tropicales, CHU de Bordeaux, Bordeaux, France; INSERM 1219, Université of Bordeaux, Bordeaux, France*

Email: gaelle.gonzalez@anses.fr

Background: The emergence of West Nile (WNV) and Usutu viruses (USUV) is a threat to human and veterinary health. Ideally, surveillance for these agents should enable early detection of their circulation in a given area. The integrated "One Health" approach aims to inform public decision-makers, in order to prevent or control their circulation and limit the burden. WNV and USUV are two flavivirus transmitted in a bird-mosquito-bird cycle. Present since the 1960s in the Mediterranean region, WNV was unexpectedly detected in the Nouvelle-Aquitaine region at the end of 2022, with the notification of the first equine cases in Gironde. There is evidence of USUV circulation in wild birds in the Nouvelle Aquitaine region.

Objectives: To characterise the emergence and circulation of WNV and USUV in Aquitaine using a One Health approach.

Methods: One Health operational research initiatives have been developed to characterise the emergence and circulation of these arboviruses in the Nouvelle Aquitaine ecosystem. A consortium of regional and national partners produced coordinated scientific data in the human, veterinary and entomological sectors. Horses and mosquitos trapped at stables were sampled.

Results: We have (i) detected the intense circulation of WNV and USUV in a territorial corridor to the north of the Gironde, bordering Charente Maritime and (ii) validated an innovative technology for the early detection of viruses in mosquitoes.

Main limitations: the study was limited to stables chosen because WNV positive equine cases were diagnosed in 2022.

Conclusions: These data were decisive in ensuring the safety of blood donations in Charente Maritime, where no human WNV cases had been yet detected. This demonstrates the importance of implementing integrated One Health surveillance to prevent and anticipate future outbreaks of WNV in mainland France.

Ethical animal research: Approved by the Ethics Committee for Clinical Research (ComERC) of Veterinary School of Alfort (ENVA) (Agreement number: 2023-06-23).

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: ANSES and CNR.

164 | **Development of a real-time recombinase-aided amplification method for the rapid detection of *Streptococcus equi* subsp. *equi***

H. Zu[1]*, R. Sun[1,3,*], J. Li[1], M. Wang[1], W. Guo[1,2**] and X. Wang[1,2,**]

*Haoyu Zu and Rongkuan Sun contributed equally to this work

[1]*State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150 069, China;* [2]*Institute of Western Agriculture, The Chinese Academy of Agricultural Sciences, Changji 831 100, China and* [3]*College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210 095, China*

Email: MuTouZu@outlook.com

Background: *Streptococcus equi* subspecies *equi* (*S. equi*) is the pathogen causing severe respiratory infections in horses and is an important factor in global biosecurity concerns due to its antibiotic resistance and current limitations in diagnostic technologies, highlighting the urgent need for rapid, accurate, and accessible diagnostic tools.

Objectives: This study aimed to establish the efficacy and reliability of real-time recombinase polymerase amplification (RAA) for rapidly detecting *S. equi*. and compare its performance with traditional real-time PCR methods.

Study design: Assay development.

Methods: Primers and probes were designed within the conserved region of *eqbE* gene, and these were carefully screened. Following optimisation of reaction temperatures and times, the sensitivity and specificity of the method were assessed. Ultimately, the performance of this approach was compared to real-time PCR to evaluate its detection capabilities. Specific primers and probes targeting the *eqbE* gene of *S. equi* were designed. The real-time RAA detection method was optimised for field application. Sensitivity and specificity were assessed, and its diagnostic performance was compared to traditional real-time PCR on 98 clinical nasal swab samples from horses.

Results: Analytical evaluations indicated a detection limit of 10 copies per reaction, showing exceptional analytical specificity only for *S. equi*. Clinical evaluations showed a concordance rate of 96.94% between real-time RAA and real-time PCR methods.

Main limitations: The risk of sample contamination increased due to low operational temperatures, necessitating strict procedural control.

Conclusion: Real-time RAA presents a promising tool for rapid and accurate on-site diagnosis of strangles, with significant advantages in equipment and time efficiency compared to real-time PCR. Further validation in diverse environments is recommended.

Ethical animal research: Not required: excess material from clinical samples was used.
Informed consent: Not stated.
Competing interests: None declared.
Funding: The National Key Research and Development Project of China (grants number 2020YFE0203400) and The Natural Science Foundation of Heilongjiang Province (grant number TD2022C006).

165 | Restriction of lentivirus infection by proteins encoded by the equine genome

X.-F. Wang, X. Yin, Y. Tang, Y. Lin, S. Ji, H. Ren and X. Wang*
State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences, Harbin, China
Email: wangxuefeng@caas.cn

Background: Cellular restriction factor is a general term for the class of host proteins that have the ability to restrict viral replication. Previous studies from our group have identified a number of equine host proteins, including tetherin,[1] viperin,[2] schlafen 11,[3] Mx2[4] and SAMHD1,[5] which inhibit the replication of lentiviruses such as equine infectious anaemia virus (EIAV) or human immunodeficiency virus type 1 (HIV-1) at different steps of their replication cycle.
Objective: To assess the roles of selected cellular restriction factors in EIAV replication.
Study design: Literature review.
Method: Our recent studies are summarised.[1–5]
Results: Equine tetherin localises to the cell surface and physically tethers viral particles to block the release of EIAV, HIV-1 and SIV. Restriction of EIAV by equine viperin occurs at multiple steps of viral replication, including inhibition of Gag release and inhibition of viral particle production and entry by disruption of the synthesis of Env and the receptor protein ELR1. Equine SLFN11 inhibits EIAV and HIV-1 replication by preventing viral Gag protein synthesis in a codon usage-dependent manner via its binding of transfer RNAs (tRNAs). Equine Mx2 blocks EIAV, HIV-1 and simian immunodeficiency virus (SIV) by targeting the viral capsid to inhibit the nuclear entry of viral cDNAs. Restriction of EIAV replication by equine SAMHD1 occurs through its dNTPase activity at the reverse transcription step. We also found that EIAV Env antagonises equine tetherin by specifically interacting with the protein and re-localising it from the cell surface to the intracellular compartment, and that EIAV Rev antagonises equine SAMHD1 by downregulating its expression via a lysosomal pathway.
Main limitations: in vitro results may not reflect in vivo physiological conditions.
Conclusions: This work characterises the antiviral activity of equine cellular restriction factors and demonstrates that lentiviruses employ diverse strategies to counteract innate immune restrictions. further studies are needed to determine the extent to which these host proteins inhibit lentiviral replication under physiological conditions in vivo.
Ethical animal research: Not applicable.
Informed consent: Not applicable.
Competing interests: None declared.
Funding: None.
References:
[1] Yin X, Hu Z, Gu Q, Wu X, Zheng Y-H, Wei P, Wang X. Equine tetherin blocks retrovirus release and its activity is antagonised by equine infectious anaemia virus envelope protein. J Virol. 2014;88(2):1259–1270.
[2] Tang Y, Na L, Zhu C-H, Shen N, Yang F, Fu X-Q, Wang Y-H, Fu L-H, Wang J-Y, Lin Y-Z, Wang X-F, Wang X, Zhou JH, Li C-Y. Equine viperin restricts equine infectious anaemia virus replication by inhibiting the production and/or release of viral Gag, Env, and receptor via distortion of the endoplasmic reticulum. J Virol. 2014;88(21):12296–310.
[3] Lin Y, Sun L-K, Zhu D-T, Hu Z, Wang X-F, Du C, Wang Y-H, Wang X-J, Zhou J-H. Equine schlafen 11 restricts the production of equine infectious anaemia virus via a codon usage-dependent mechanism. Virology. 2016;495:112–121.
[4] Ji S, Na L, Ren H, Wang Y, Wang X. Equine myxovirus resistance protein 2 restricts lentiviral replication by blocking nuclear uptake of capsid protein. J Virol. 2018;92(18):e00499-18.
[5] Ren H, Su C, Guo M, Wang X-F, Na L, et al. Equine lentivirus counteracts SAMHD1 restriction by Rev-mediated degradation of SAMHD1 via the BECN1-dependent lysosomal pathway. Autophagy. 2021;17(10):2800–2817.

166 | Development and validation of a sandwich ELISA for equine IL-1β

S. Ma#, X. Guo#, D. Liu, W. Guo, K. Guo, R. Sun, Y. Lin* and X. Wang*
State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences, Harbin 150 069, China
#These authors contributed equally.
Email: masaiwen777@163.com

Background: Evaluating IL-1β levels is crucial for diagnosing and treating diseases in horses and donkeys. However, the absence of precise detection methods for IL-1β remains a challenge. There is an urgent need for reliable IL-1β detection techniques to improve diagnostic accuracy and therapeutic strategies in equine healthcare.
Objective: Develop IL-1β-specific monoclonal antibodies and then established an antigen capture ELISA (acELISA) method.
Study design: Assay development.
Methods: The immunogen was meticulously designed to target the highly immunogenic conserved regions of mature IL-1β (mIL-1β). After immunising mice, monoclonal antibodies were rigorously prepared and screened. Subsequently, antibodies exhibiting high reactivity and specificity were selected for the establishment of an acELISA.
Results: The IL-1β assay exhibited a limit of detection ranging from 200 to 10 000 pg/mL, meeting clinical detection requirements. The

repeatability and reproducibility of the assay were both less than 5%, indicating an acceptable level of variation. Subsequently, 84 samples from horses and 68 samples from donkeys were collected and tested in parallel with a commercially available kit. These results showed no disparity between the in-house and a commercial ELISA kit for detecting IL-1β concentration in horse sera. Importantly, our ELISA method enabled the detection of IL-1β levels in donkeys, overcoming the limitations of commercially available assays.

Main limitations: The absence of a reference value for IL-1β in donkeys of normal health status may have a potential impact on the determination of positive samples.

Conclusion: These findings suggest that the newly developed acELISA provides a feasible and reliable analytical method for detecting IL-1β in horse and donkey samples.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences.

Informed consent: Not stated.

Competing interests: None declared.

Funding: National Key Research and Development Program of China (2023YFD1802500) and the National Natural Science Foundation of China (32 330 103 and 32 372 985).

167 | Novel serological method to diagnose equine rhinopneumonitis based on an indirect ELISA

D. Liu#, X. Zhao# and X. Wang*

State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China

Email: Liudiqiu@caas.cn

*Corresponding author: wangxiaojun@caas.cn.

Background: Equine rhinopneumonitis (ER) is a historically-derived term and caused by equine herpesvirus-1 (EHV-1) and (or) equine herpesvirus-4 (EHV-4) infection via the respiratory tract as the primary route. Diagnostic methods for ER are useful to confirm population and individual freedom from infection, contribute to eradication policies, confirm clinical cases, undertake surveillance for infectious prevalence and to detect immune status in individual animals or populations post-vaccination based on identification of the agent and detection of immune responses. Serological testing of paired sera can be utilised for diagnosis of ER in horses, displaying greater efficiency and convenience than virus isolation, PCR or histopathological examination.

Objectives: A novel iELISA was designed and developed to detect and discriminate antibodies targeting EHV-1 and EHV-4.

Study design: Assay development.

Methods: Antigenic regions unique to EHV-1 and EHV-4 were analysed, screened and selected as coating material for ELISA. These antigenic regions are absent in EHV-3, EHV-6, EHV-8 and EHV-9. Chessboard tests further optimised coating and blocking formulations, the dilution and reaction course of specimens and secondary antibodies, the time phase of colour development and stopping reaction, and cut-off value. For in vitro validation we used positive sera from clinical cases and negative sera from healthy horses sampled for a surveillance programme.

Results: The iELISA detected EHV-1 or EHV-4 positive but not negative sera and lacked cross reactions between these two viruses. No reactivity to seropositive samples of other pathogens including EIAV, EIV, EAV and EHV-8 was detected. This ELISA protocol operates at room temperature within 50 minutes, exhibiting a higher level of stability, repeatability and specificity comparable to serum virus neutralising (SVN) assays, with 100% compliance rate. The kit based iELISA can be kept at 4°C for at least 12 months.

Conclusions: the iELISA showed unique specificity for EHV-1 or EHV-4 in seropositive horses. It is simple and time-saving as it avoids the complexity of serum-virus neutralisation assays and complement fixation tests. This protocol can match SVN tests with high specificity and through-put.

Ethical animal research: Approved by the Committee on the Ethics of Animal Experiments of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences.

Informed consent: Not stated.

Competing interests: None declared.

Funding: National Key Research and Development Program of China (2023YFD1802505); National Natural Science Foundation of China (32272973).

168 | Equine hepacivirus: phylogenetic analysis of Italian horse sequences highlights a fourth subtype candidate

G. Pacchiarotti[1], R. Nardini[1], A. Cersini[1], R. Conti[1], D. La Rocca[1], G. Manna[1], G. Brocherel[1], G. Bruni[1], M. Sala[1], E. Sezzi[1], G. Terracciano[1], N. Cavaliere[2], S. Costarelli[3], C. De Martinis[4], C. Di Pancrazio[5], F. Gobbo[6], M.L. Mandola[7], G. Purpari[8], A. Ruiu[9], M. Tonni[10] and M.T. Scicluna[1]

[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri," via Appia Nuova, 1411 Rome, Italy; [2]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, via Manfredonia, n. 20, Foggia, Italy; [3]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati," Via G.Salvemini, 1, Perugia, Italy; [4]Istituto Zooprofilattico Sperimentale del Mezzogiorno Via Salute, 2 – Portici (Naples), Italy; [5]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale," Campo Boario Teramo, Italy; [6]Istituto Zooprofilattico Sperimentale delle Venezie Viale dell'Università 10, Legnaro (Padua), Italy; [7]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, Turin, Italy; [8]Istituto Zooprofilattico Sperimentale della Sicilia Via Gino Marinuzzi 3 – Palermo, Italy; [9]Istituto Zooprofilattico Sperimentale della Sardegna, Via Duca degli Abruzzi n. 8 Sassari, Italy and [10]Istituto Zooprofilattico Sperimentale

della Lombardia e dell'Emilia Romagna "Bruno Ubertini" Via Bianchi, 9, Brescia, Italy
Email: roberto.nardini@izslt.it

Background: Equine hepacivirus (EqHV) is a hepatotropic virus (genus *Hepacivirus*, family *Flaviridae*) with biomolecular prevalences worldwide, ranging from <1% to 18.2%. This virus generally causes subclinical hepatitis in horses, occasionally evolving into a chronic state. In a recent study in Italy, the national biomolecular prevalence was estimated at 4.27% (77/1801) [2].

Objectives: Assessing the phylogenetic relationships of the sequences obtained within the national horse prevalence study to the three described EqHV subtypes (1-2-3).

Study design: Molecular analysis.

Methods: The sequences for the NS3 conserved region of the viral genome obtained from the aforementioned PCR positive samples, [3] were employed in phylogenetic analysis. Sequences were obtained by performing the Sanger method and MEGA11 software (Tamura–Nei model) was used to infer the evolutionary tree with 1000 replicates to test its consistency. Several equine hepacivirus sequences were downloaded from Genbank to corroborate the discrimination of the three subtypes.

Results: The NS3 sequence (approximately 500 bp) was obtained for 35 PCR products out of the 77 positive samples. In agreement with the literature available, the majority of the sequences belonged to subtype 1 (30/35, 85.7%), followed by subtype 3 (4/35, 11.4%), and subtype 2 (1/35, 2.9%). Potential transmission clusters were observed (supported by 99% of bootstrap) in Sardinia, Italy. Of note is a group of sequences which seem to cluster in a fourth subtype which has never been reported.

Main limitations: The candidate fourth subtype must still be confirmed with full genome sequencing and analysis.

Conclusions: This study confirms the extensive circulation of the EqHV within the Italian territory. Moreover, it highlights a fourth subtype candidate, requiring further studies. EqHV remains relevant in a scenario of equine infectious diseases to be considered in clinical practice.

Ethical animal research: The samples were collected for the implementation of the 'National plan for the surveillance and control of infectious anaemia in equidae" (Ministerial Decree 2 February 2016).

Informed consent: Not stated.

Competing interests: None declared.

Funding: Italian Ministry of Health RC LT1018.

References:

[1] Burbelo PD, Dubovi EJ, Simmonds P, Medina JL, Henriquez JA, Mishra N, Wagner J, Tokarz R, Cullen JM, Iadarola MJ, Rice CM, Lipkin WI, Kapoor A. Serology-enabled discovery of genetically diverse hepaciviruses in a new host. J Virol. 2012;86(11): 6171–6178. 10.1128/JVI.00250-12.

[2] Nardini R, Pacchiarotti G, Svicher V, Salpini R, Bellocchi MC, Conti R, Sala MG, La Rocca D, Carioti L, Cersini A, Manna G, the Equine Hepatic Viruses Consortium, Scicluna MT. First national prevalence in Italian horse population and phylogenesis highlight a fourth sub-type candidate of equine hepacivirus. Viruses 2024;16(4): 616. 10.3390/v16040616.

[3] Schlottau K, Fereidouni S, Beer M, Hoffmann B. Arch Virol. 2019;164(2):391–400. doi: 10.1007/s00705-018-4077-2.

169 | Using viral metagenomics to identify viruses associated with fever in horses in Scandinavia

A.-L. Blomström[1], H. Choletti[1], S. Hanche-Olsen[2], I. Risnes Hellings[2], S. Hansen[3] and M. Riihimäki[1,4]
[1]*Department of Animal Biosciences, Swedish University of Agricultural Sciences;* [2]*Department of Companion Animal Clinical Sciences, Norwegian University of Life Sciences;* [3]*Department of Veterinary Clinical Sciences, University of Copenhagen;* [4]*Department of Clinical Sciences, Swedish University of Agricultural Sciences*
Email: anne-lie.blomstrom@slu.se

Background: Viral infections pose a threat to equine welfare and can cause great economic losses for the equine industry. Thus, knowledge about viruses circulating in the equine population with the potential to cause disease is of importance. New technologies such as viral metagenomics combining high-throughput sequencing with bioinformatic analysis allows an unbiased approach to identify all viruses in a sample simultaneously.

Objectives: To determine the complete viral composition in samples collected from horses with fever in order to improve the diagnosis of clinical cases with unknown aetiology.

Study design: Cross sectional.

Methods: Samples were collected from horses (>60) in Scandinavia for high-throughput sequencing and bioinformatic analysis. Nucleic acid enriched for viruses through filtration, rRNA depletion and nuclease treatment was extracted from the collected samples (nasal swabs and serum/plasma). High-throughput sequencing (Illumina and/or Nanopore) and bioinformatic analysis were used to identify and genetically characterise viruses.

Results: Viruses were found in all investigated sample pools. In nasal swabs, expected viruses such as different equine herpes virus types were identified. Equine herpes virus-2 was the most abundant and a near complete genome was obtained. Other viruses were also identified such as e.g. adeno-, papilloma- and picornaviruses. Interestingly, some of these identified viruses showed very low sequence similarity to previously known viruses e.g. picornavirus sequences with only around 30% protein identity. On the contrary, in sera, the main viruses identified were equine pegivirus and Torque teno equus virus 1 (TTeV1). The near complete TTeV1 genome was obtained showing high similarity to a TTeV1 strain from the USA.

Main limitations: Pooled samples rather than individual horses were examined.

Conclusions: The study provides an overview of the different viruses circulating in horses with fever in Scandinavia. It also identifies

previously uncharacterized equine viruses whose role in disease is unknown and renders further investigations.

Ethical animal research: Approved by the local Research Ethics Committee.

Informed consent: Informed consent was given by the horses' owners.

Competing interests: None declared.

Funding: The Swedish foundation for equine research (project number H-20-47-555).

Antimicrobial Stewardship Policy: The work does not investigate nor used any quinolones, extended spectrum beta-lactam antimicrobials (such as 3rd/4th generation cephalosporins) or macrolides.

170 | Explaining the role of the veterinary hospital microbiome in equine antimicrobial resistance

E. Herring[1], E. Beasley[1], N. Noyes[2], P. Morley[3] and B. Burgess[1]

[1]*University of Georgia, College of Veterinary Science, Athens, Georgia, 30 602–0002, USA*; [2]*University of Minnesota, Veterinary Population Medicine, Minneapolis, Minnesota, USA and* [3]*Colorado State University, Veterinary Clinical Sciences, Fort Collins, Colorado, 80 523, USA*

Email: brandy.burgess@uga.edu

Background: Available evidence in horses suggests that exposure to a hospital environment, even without direct exposure to antimicrobial drugs (AMD), increases the risk of acquiring AMR organisms. However, full characterisation of how bacteria and resistance determinants are shared between hospitalised patients and their surrounding environment is lacking. Understanding mechanisms which promote the development and maintenance of resistant flora in horses can allow a tailored approach to emergence prevention.

Objectives: To characterise faecal microbiome and resistome of hospitalised horses with AMD exposure, hospitalised horses without AMD exposure, and their healthy stablemates; characterise the microbiome and resistome of the hospital environment; and evaluate relationships between taxonomic and functional profiles of environmental and equine faecal metagenomes.

Study design: Matched case–control study.

Methods: Eighty horses were enrolled: hospitalised horses with AMD exposure ($n = 19$), hospitalised horses without AMD exposure ($n = 21$), and farm-matched stablemates without hospital or AMD exposure ($n = 40$). Samples were collected at regular intervals from hospitalised horses and housing area, and a single faecal sample from each stablemate. DNA was extracted and processed for 16S rRNA sequencing and target-enriched metagenomic sequencing. Data were analysed for differences in relative taxa, resistance determinants, and microbial diversity between study groups and sample types.

Results: The study population had a median age of 13.5 (range: 4–25) and included horses under the care of either surgery (75%) or medicine (25%) services. Horses were hospitalised for a median of 3 days (range: 2–30), and within the AMD group, received antimicrobials for a median of 2 days (range: 1–12).

Main limitations: Convenience sampling may reduce generalisability of results.

Conclusions: This study provides novel insights into the ecology of antimicrobial resistance in equine hospitals, paving the way for further investigations into the impact of environmental hygiene practices on the microbiome and resistome of hospitalised patients.

Ethical animal research: Approved by IACUC prior to study initiation.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: Morris Animal Foundation and Grayson-Jockey Club Research Foundation.

171 | Generation of equine infectious anaemia virus (EIAV) full length molecular clones

S.L. McGowan, F. Steinbach and B. Choudhury

Animal and Plant Health Agency, Department of Virology, Addlestone, KT15 3NB, UK

Email: sarah.mcgowan@apha.gov.uk

Background: EIAV, a persistent infectious disease of equids, is found globally and is a WOAH notifiable disease. EIAV can be subclinical and has fluctuating viremia levels thus serological diagnosis is the most reliable method of detection following seroconversion. Serology mainly targets antibodies toward EIAV p26 and it's been proposed that mutations in p26 reduce detection rates, especially for the AGIDT. The impact of such mutations on the infectivity of EIAV is unclear.

Objective: To generate full-length EIAV molecular clones with and without mutations in p26 which can be used to assess viability in vitro.

Study design: Golden gate cloning.

Method: Golden gate cloning was used to assemble four adjoining fragments covering the entire EIAV genome. The designed fragments were synthesised and pre-cloned into donor vectors, then simultaneously cleaved and assembled into high and low copy number vectors by *AarI* Type IIs restriction enzyme and T4 DNA ligase respectively. Invitrogen™ One Shot™ Stbl3™ chemically competent *E. coli*[1] were transformed with the assembled clones and colonies containing the correct insert were cultured, extracted, and confirmed by sequencing.

Results: Two full-length molecular clones, with and without mutations in p26, were successfully created.

Main limitations: The cloning of EIAV is complex due to the long terminal repeats and instability issues.

Conclusion: After multiple attempts, the limitations were overcome by using golden gate cloning and *E. coli* cells designed to reduce homologous recombination of direct repeats. It was possible to achieve stable EIAV clones in both high and low copy number vectors. The two resulting viruses will be rescued to compare their ability to grow in vitro, but it may be that a virus with significant mutations in p26 is no longer fit to replicate.

Key manufacturer: 1 Thermo Fisher Scientific, UK.
Ethical animal research: Not applicable.
Informed consent: Not applicable.
Competing interests: None declared.
Funding: Department for Environment, Food & Rural Affairs.

172 | Investigation of large volume samples for the detection of *Streptococcus equi* ssp. *equi* using a novel point-of-care PCR assay

S. Anaya[1], P. Naranatt[1], B.R. Buchannan[2], A. Catalano[3], T. Chipman[3], A. Vitomirov[1] and E. Mendonsa[1]
[1]*Fluxergy, Irvine, California, USA;* [2]*Brazos Valley Equine Hospital, Navasota, Texas, USA and* [3]*Ocean State Equine Associates, North Scituate, Rhode Island, USA*
Email: sanaya@fluxergy.com

Background: Quantitative PCR (qPCR) assays for the detection of *Streptococcus equi* ssp. *equi* (*S. equi)* in nasal swabs, and large volume samples such as nasopharyngeal washes (NPW) and guttural pouch lavages (GPL), are considered the molecular 'gold standard' for diagnosis of strangles.[1] A novel point-of-care (POC) PCR assay has recently been validated for rostral nasal secretions, however, the assay has yet to be validated using large volume samples, often used to determine shedding status post-infection.
Objectives: To evaluate a POC PCR assay for the detection of *S. equi* in large volume samples collected from horses with and without clinical signs and to compare the results against the gold standard of qPCR.
Study design: Cross-sectional.
Methods: Large volume samples were collected from 90 horses involved in various strangles' outbreaks. Large volume flushes consisted of the administration of a minimum of 50 mL of sterile saline in the nasopharynx or the guttural pouches. The samples were tested directly via a commercial *S. equi* POC PCR assay. The POC PCR assay has a limit of detection of 277 *S. equi eqbE* target genes and a testing time of 50 minutes. The samples were further processed for nucleic acid purification and qPCR testing using a previously validated assay targeting the *S. equi eqbE* gene. Nucleic acid purification and qPCR testing took approximately 3 h.
Results: Overall agreement between the *S. equi* POC PCR assay and qPCR assay was determined for 90 samples (89.9%; 80 PCR-negative and 10 PCR-positive samples). Discrepant results were documented for 9 samples (10.1%; 6 false positive and 3 false negative samples).
Main limitations: Large volume samples were centrifuged, with the pellet used for the testing of *S. equi* by qPCR.
Conclusions: The testing of large volume samples for *S. equi* yielded an acceptable agreement between the POC PCR assay and the molecular gold standard of qPCR. The *S. equi* POC PCR assay can be run stall-side, requires no processing of the collected samples and results are available within 50 minutes. The acquisition of results in real-time allows for rapid treatment (guttural pouch lavages and topical administration of antimicrobials) and reduces the number of post-diagnostic visits.
Ethical animal research: Not required: excess material from clinical samples was used.
Informed consent: Not stated.
Competing Interests: Authors Selina Anaya, Pramod Naranatt, Andrej Vitomirov, and Eric Mendonsa are all employed by Fluxergy Inc.
Funding: Fluxergy Inc.
References:
[1] Newton JR, Verheyen K, Talbot NC, et al. Control of strangles outbreaks by isolation of guttural pouch carriers identified using PCR and culture of *Streptococcus equi*. Equine Vet J 2000;32:515–526.
[2] Willis AT, Barnum S, Pusterla N. Validation of a point-of-care polymerase chain reaction assay for detection of *Streptococcus equi* subspecies *equi* in rostral nasal swabs from horses with suspected strangles. Can Vet J. 2021;62(1):51–54. PMID: 33390599; PMCID: PMC7739398.

173 | Molecular detection of infectious respiratory agents in nasal swabs of Thoroughbred racehorses in Korea

S.-K. Lee
Veterinary Center, Korea Racing Authority, Gwacheon 13 822, Republic of Korea
Email: tman1230@naver.com

Background: Infectious respiratory diseases frequently affect Thoroughbred racehorses, impacting their performance.
Objectives: To identify circulating infectious respiratory agents in Thoroughbred racehorses through PCR testing of nasal swab samples and assess their influence on race outcomes.
Study design: Cross sectional.
Methods: Nasal swab samples were collected from Thoroughbred racehorses on race days and tested for equine influenza (EI), *Streptococcus equi* subspecies *equi* (*S. equi*), equine rhinitis viruses A (ERAV) and B (ERBV), equine herpesviruses (EHV) -1 and -4 using qPCR, and EHV-2 and -5 using nested PCR. Race results were categorised into three levels based on the rank ratio among participants. These results were then analysed alongside the test findings.
Results: A total of 185 samples were collected from clinically normal racehorses, with none testing positive for EI, S. equi, ERAV, ERBV, EHV-1, or EHV-4. EHV-2 was detected in 34 horses (18.4%, 34/185), and EHV-5 was found in 62 horses (33.5%, 62/185). In comparison with race results, the detection of EHV-5 was statistically significantly associated with race performance in this study ($p = 0.04$).
Main limitations: Nasal swabs were randomly collected from race participants, and the sample size was limited. Further investigation with a larger sample size and inclusion of all race participants may be necessary.
Conclusions: This study suggests that EHV-5 may impact the performance of Thoroughbred racehorses.
Ethical animal research: Approved by the Ethical Committee of Korea Racing Authority.
Informed consent: Horse trainers gave consent for their animals' inclusion.

Competing interests: None declared.
Funding: None.

174 | Western equine encephalomyelitis in Argentina after 35 years: The importance of an early and specific diagnosis

M.S. Tordoya [1,2], F. Alamos [1,2], V. Vera [1], C. Gabaglio [1], J. Schammas [1,2], K. Trono [1,2], M. Barrandeguy [3] and M. Aldana Vissani[1,2,4]
[1]*Instituto de Virología CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), Dr. Nicolás Repetto y De Los Reseros s/n°, Hurlingham B1686LQF, Buenos Aires, Argentina;* [2]*Instituto de Virología e Innovaciones Tecnológicas (IVIT), CONICET, Buenos Aires, Argentina;* [3]*Plataforma de Salud Animal, INIA La Estanzuela, Colonia, Uruguay and* [4]*Instituto de Investigación en Veterinaria, Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador, Buenos Aires, Argentina*
Email: vissani.aldana@inta.gob.ar

Background: Alphavirus encephalomyelitis caused by Eastern, Western and Venezuelan equine encephalitis viruses (EEE, WEE and VEE), are mosquito-borne infections that cause severe neurological disease of variable lethality in horses and humans in the Americas. Thus, an on-time, reliable diagnosis is crucial for the application of preventive and control measures not only in horses but also in humans. In November 2023, an outbreak of neurological disease was registered in horses all over the country, starting in the north and reaching the southern provinces of Argentina over a 4-month period.
Objectives: To communicate the virological findings on clinical samples received at the Equine Virology Laboratory.
Study design: Descriptive study.
Methods: Thirty-four cases of horses with neurological signs and four cases of non-equine species showing compatible clinical signs, were received. Samples from equine cases [32 brains, 33 organs (other than brain) and 19 cerebrospinal fluid (CSF)] and non-equine cases (four brains) were analysed by RT-PCR for the detection of alphaviruses. A discriminative real time RT-PCR for EEE or WEE was performed on alphavirus-positive samples. On the negative samples, a differential diagnosis for equine herpesvirus-1 (EHV-1) and West Nile Virus (WNV) was carried out by PCR and RT-PCR, respectively.
Results: WEE was detected in 74% (25/34) of equine samples but only in brain and organ samples and not in CSF. All the horses in which WEE was confirmed had no history of previous vaccination. Neither EHV-1 nor WNV was detected. WEE was detected in the brain of a sheep.
Main limitations: The lack of appropriate samples (neural tissue) in some cases.
Conclusions: The outbreak of neurological disease in Argentina was due to the re-emergence of WEE infection. Accurate and on-time diagnosis was critical to alert animal and human health authorities (One Health). Phylogenetic analyses and serologic studies are in progress.
Ethical animal research: Not required: retrospective case series.
Informed consent: Not stated.
Competing interests: None declared.
Funding: INTA and INTA-HARAS Agreement.

175 | Retrospective study of equid herpesvirus-1 excretion in clinically healthy horses before international travel

M.S. Tordoya[1,2], V. Vera[1], F. Alamos[1,2], C. Gabaglio[1], M. Barrandeguy[3] and M. Aldana Vissani[1,2,4]
[1]*Instituto de Virología, CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina;* [2]*Instituto de Virología e Innovaciones Tecnológicas, UEDD, CONICET, Buenos Aires, Argentina;* [3]*Plataforma de Salud Animal, INIA La Estanzuela, Colonia, Uruguay and* [4]*Instituto de Investigación en Veterinaria, Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador, Buenos Aires, Argentina*
Email: vissani.aldana@inta.gob.ar

Background: Equid herpesvirus-1 (EHV-1) is ubiquitous in equine populations worldwide. Events of infection/reinfection or reactivation from latency lead to respiratory disease, abortion, neonatal losses and myeloencephalopathy (EHM) in horses. During the last few years, EHM outbreaks have been reported in many countries, including Argentina, causing significant economic losses. Recent reports reveal that the excretion rate of EHV-1 in clinically healthy horses can reach 27% in the context of an EHM outbreak, while it ranges from 0% to 4% when not related to an EHM outbreak.
Objectives: To determine the frequency of EHV-1 detection in clinically healthy horses not related to an EHM outbreak.
Study design: Retrospective cohort study.
Methods: Nasopharyngeal swabs (NS; $n =$: 270) from horses, obtained during the pre-export quarantine between 2016 and 2024, were analysed by an EHV-1/EHV-4 multiplex PCR.
Results: EHV-1 was detected in one of the NS analysed, representing 0.37% (1/270). Although the detection of EHV-4 was not the objective of this study, two NS tested positive for this virus.
Main limitations: The reduced number of samples analysed.
Conclusions: The detection rate of EHV-1 in healthy horses not related to an EHM outbreak was similar to the ones reported previously. Even though this study has been done retrospectively, there were no reports of clinical signs related to EHV-1 infection during the pre-quarantine stage or at the countries of destination. Regarding EHV-4, the finding of excretion of virus in adult healthy horses constitutes additional evidence of the ubiquity of this virus in the horse population worldwide but, so far, this virus seems to pose no risk for comingling horses as it does not cause abortion or neurological disease. Finally, this study provides information on the detection rate of EHV-1 in clinically healthy horses and reinforces the need to maintain good management practices and an adequate vaccination program.
Ethical animal research: Not required: excess material from samples taken for export screening was used.
Informed consent: Not stated.
Competing interests: None declared.

Funding: This work was supported by INTA and INTA-HARAS Agreement.

176 | C2254 in ORF30 and G713 in ORF11, two EHV-1 variants of interest, what happened to them?

C. Normand[1,2], H. Virevialle[3], C. Fortier[1,2], G. Sutton[1,4], F. Carnet[5], P.-H. Pitel[1], E. Hue[1,2], A. Couroucé[2,6] and S. Pronost[1,2]
[1]LABÉO, 14 280 Saint-Contest, France; [2]Normandie Université, UNICAEN, BIOTARGEN, 14280 Saint-Contest, France; [3]Cabinet Equiveto, 13 420 Genenos, France; [4]Université de Montréal, H3C 3 J7 Montreal, Quebec, Canada; [5]Anses Ploufragan 22 440 Ploufragan, France and [6]Cisco-Oniris, 44 000 Nantes, France
Email: stephane.pronost@laboratoire-labeo.fr

Background: *Varicellovirus equidalpha1* (EHV-1) is an endemic horse pathogen worldwide responsible of respiratory epidemics, abortions and myeloencephalopathy (EHM). Since 2019, EHM epizooties have been described and for some of them, the reports focus on two variants identified by a specific nucleotide mutation: C2254, because this position in ORF30 is receiving particular attention from the scientific community and G713 in ORF11 because this nucleotide was used as a tracer during and after an important EHM outbreak in Europe (Valencia, Spain).
Objectives: To identify the presence of C2254 and G713 mutations since 2019.
Study design: Laboratory surveillance and literature survey.
Methods: 98 samples testing positive for EHV-1 in France between 2019 and 2023 were typed by PCR to discriminate A/C/G nucleotides in ORF30/2254 and A/G nucleotides in ORF11/713. Sequencing was also performed to confirm the data. An analysis of literature data for 2019–2024 was carried out to identify these two "hot spots."
Results: Since the first identification of C2254 (ORF30), it has been found in only 3 nasopharyngeal swabs from another equestrian centre in France. To date, no epidemiological link has been established between the 2 centres. This mutation was reported the following year in the United States, and this is the only time we know of. Concerning G713 (ORF11), no samples with no direct link outside the period of the Valencia epidemic were detected either before or after. The mutation was reported in three studies in Europe.
Main limitations: Lack of whole genomes to compare these 2 variants.
Conclusions: To date, only two studies have reported the detection of the C2254 mutation (ORF30). This study also shows that the Valencia strain G713 (ORF11) has not been found in France since the outbreak, and this has enabled it to be identified in connection with the crisis in three other studies.
Ethical animal research: Not required: analysis of microorganisms.
Informed consent: Not stated.
Competing interests: None declared.
Funding: LABÉO.

177 | Next generation probiotics: A novel strategy for controlling *Salmonella* associated diarrhoea in horses

B. Lamichhane and Y.A. Helmy*
Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky, Lexington, Kentucky, USA
Email: bibek.lamichhane@uky.edu

*Corresponding author: yosra.helmy@uky.edu

Background: *Salmonella* is a common cause of diarrhoea in equines, infecting horses of all age groups. The infection is treated using antibiotics like fluroquinolones. Increasing antimicrobial-resistant *Salmonella* has necessitated the development of alternative therapeutics. Next-generation probiotics (NGPs) are derived from the normal microbiota and have not been used against any pathogen.
Objectives: To identify NGPs with high effectiveness against *Salmonella*.
Study design: In vitro experiments.
Methods: After screening, the effect of selected NGPs was evaluated on growth, biofilms, intracellular survival, and expression of virulence associated genes of *Salmonella* in vitro. 38 probiotics strains were screened for their effect on *Salmonella* growth using an agar well diffusion assay and NGPs with high growth inhibition selected for further development. Selected probiotics were co-cultured with *Salmonella* in liquid media. Their effects on biofilm formation and preformed biofilms and on *Salmonella* adhesion, invasion, and survival in intestinal cells were evaluated. Additionally, NGPs' influence on *Salmonella* virulence genes was examined using RT-PCR. The experiments were replicated at least twice, and results were statistically analysed using two-way ANOVA with Tukey analysis.
Results: All probiotics effectively inhibited *Salmonella* growth, and the top 7 probiotics were selected for further development. All selected candidates significantly inhibited *Salmonella* growth in liquid media and showed high inhibition of biofilm formation and preformed biofilms. They significantly ($p < 0.05$) inhibited *Salmonella* adhesion, invasion, and intracellular survival in human intestinal cells. RT-PCR analysis revealed a significant downregulation of genes associated with virulence factors, colonisation, motility, and quorum sensing of *Salmonella*.
Main limitations: The study is only focused on in vitro models, which may not fully replicate in vivo. **Conclusion:** NGPs are promising alternatives to antibiotics for combating *Salmonella* infections in horses. Future research will focus on continued in vitro evaluation and assessing the efficacy of the top two candidates in vivo in foals.
Ethical animal research: Not required: analysis of microorganisms.
Informed consent: Not applicable.
Competing interests: None declared.
Funding: Dr Yosra A Helmy's startup fund at Maxwell H. Gluck Equine Research Center, University of Kentucky.

178 | Antigenic mapping of African horse sickness virus

O.A. Elbohy[1], K. Pitchers[1], Y. Lemmer[2], M. O'Kennedy[2] and J.M. Daly[1]

[1]*One Virology, Wolfson Centre for Virus Research, Sutton Bonington Campus, University of Nottingham, UK and* [2]*Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa*

Email: svzoe@exmail.nottingham.ac.uk

Background: African horse sickness virus (AHSV) is endemic in sub-Saharan Africa, where it causes disease ranging from mild to severe and often fatal. The co-circulation of nine serotypes (ASHV-1 to AHSV-9) is a major challenge to diagnosis and control of AHSV by vaccination.

Objectives: To determine cross-reactivity of antibodies against each of the nine AHSV serotypes using antigenic cartography.

Study design: In vitro experiments.

Methods: *Nicotiana benthamiana* leaves were infiltrated separately with transformed AGL-1 containing nine AHSV VP2 DNA constructs. The transiently-expressed VP2 proteins were extracted and purified. Reference equine sera[1] against the nine AHSV serotypes were tested by indirect ELISA using the recombinant AHSV VP2 proteins as target antigens. A 3D antigenic map was generated based on the antibody titres detected by indirect ELISA using R software (Racmacs package).

Results: The antigenic distances between serotypes 1, 2 and 6 revealed a closer antigenic relationship among them. Also serotypes 5, 7 and 8 were clustered close to each other on the map, while serotypes 3, 4 and 9 were positioned at the greatest distance from each other and all other serotypes. Some of the antigenic relationships are concordant with the genetic relationships (e.g., serotypes 1 and 2 and 5 and 8 are genetically most similar and serotype 4 is genetically distinct). However, the position of serotypes 6 and 7 on the antigenic map are in contrast with their genetic relationships.

Main limitations: The results of cross-reactivity using the whole virus and recombinant VP2 proteins will likely differ, and the equine sera were raised against different strains to the VP2 proteins expressed in plants.

Conclusions: The study provides insight into cross-reactivity between the nine plant-expressed VP2 proteins that could help with design of vaccines against AHSV.

Key manufacturers: 1 Equine reference antisera were purchased from The Pirbright Institute.

Ethical animal research: Approved by the University of Nottingham School of Veterinary Medicine and Science Committee for Animal Research and Ethics (CARE)—reference number 3453 211 006.

Informed consent: Not applicable.

Competing interests: None declared.

Funding: This work was funded by the Horserace Betting Levy Board (reference vet/prj/799).

179 | RNA Viral threats: Unravelling NEV and SARS-CoV-2 dynamics for one health

A. Vieira-Pires[1], R. Barroso[1,2], A. Antunes [2,3], J. Ramalho-Santos [4,5] and I. Fidalgo-Carvalho [1,6,7]

[1]*Equigerminal, S.A., Rua Eduardo Correia, 13, 3030–507 Coimbra, Portugal;* [2]*Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, s/n, 4169–007 Porto, Portugal;* [3]*CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450–208 Porto, Portugal;* [4]*University of Coimbra, CNC-UC Center for Neuroscience and Cell Biology, CIBB, Rua Larga, 3004–504 Coimbra, Portugal;* [5]*University of Coimbra, Department of Life Sciences, Faculty of Sciences and Technology, 3000–456 Coimbra, Portugal;* [6]*University of Coimbra, Institute for Interdisciplinary Research, (III UC), PhD Programme in Experimental Biology and Biomedicine (PDBEB) CNC-UC Center for Neuroscience and Cell Biology, CIBB, Rua Larga, 3004–504 Coimbra, Portugal and* [7]*Departamento de Ciências Agrárias e Tecnologias, Escola Superior Agrária de Coimbra, 3045–601 Coimbra, Portugal*

Email: isabel@equigerminal.pt

Background: Amid rising zoonotic diseases from mutable RNA viruses, vigilant surveillance is paramount. Our pre-pandemic focus was on New Equine Virus (NEV), a novel lentivirus, investigating its potential as 'Pathogen X'. The advent of SARS-CoV-2 shifted our lens, concurrently probing both viruses, thereby deepening insights into their One Health impacts, especially in horses and pets.

Objective: To proactively monitor emerging RNA viruses, pinpoint 'Pathogen X' candidates, and devise diagnostic, antiviral, and vaccine tools, emphasising NEV and ongoing SARS-CoV-2 surveillance in horses and pets.

Study design: Assay development and cross-sectional surveillance.

Methods: NEV was isolated from EIAV-discordant horse cell lines and subsequently cultivated across a range of different cell types. ELISA kits for NEV and SARS-CoV-2 were developed for horses, dogs, and cats. We utilised qPCR, RT-qPCR, LC–MS/MS, and pursued expansive sero-epidemiological assessments.

Results: NEV displayed distinctive cytopathic effects in vitro. While NEV's genome did not align with EIAV, its proteomic profile showed similarities to HIV-1. The successful isolation of NEV led to the creation of specialised ELISA and RT-qPCR kits, revealing NEV's widespread presence in horses with varying seroprevalence rates: 51/678 in Portugal, 17/160 in Ireland, 3/40 in Brazil, 4/38 in the US, 5/22 in Germany, and 1/4 in France. In Portuguese pets, NEV antibodies appeared in 3/73 cats but in none of the 152 dogs. Post-SARS-CoV-2, our RBD-based-ELISA and RT-qPCR enabled pets' and horses' monitoring, finding 15/69 cats, 7/148 dogs seropositive, but none of the 152 horses. Intriguingly, 11/22 seropositive animals likely contracted the virus from humans, with several cats transmitting it to each other.

Main limitations: The primary limitations include the fact that NEV is a newly identified lentivirus, with an unknown but likely extended incubation period. There is also limited understanding of its transmission routes and pathogenesis, and other challenges associated with a novel virus affecting horses. Additionally, the absence of longitudinal data hampers understanding of its transmission dynamics over time.

Conclusion: NEV, a novel lentivirus akin to HIV-1 in its proteomic profile, has established its epidemiological footprint beyond Europe. Concurrent SARS-CoV-2 findings in pets highlight their zoonotic risk, stressing the essentiality of integrated human-animal health strategies and underlining public health's pivotal role in pre-empting pandemics.

Ethical animal research: Approved by the Equigerminal Institutional Review Board under the project code AWEC20201022 approved on 22 October 2020. The study complies with the EU general data protection regulation (GDPR) requirements.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: One or more of the co-authors have an approved patent related to the New Equine Virus (NEV) WO2015094001A1.

Funding: The European Regional Development Fund (ERDF) of the European Union, through Operational Program PO Centro 2020 of Portugal 2020 under the reference Centro-01–9247-FEDER-38.121 managed by the ANI- Agência Nacional de Inovação and Equigerminal, S.A. internal project with the reference EQG004-IDI-0008.

180 | Assessing benzimidazole resistance in horse strongylids by in vitro and in vivo methods

A. Königová[1], M. Babják[1], T. A Kuzmina[1,2], Y. Syrota[1,2], L. Burcáková[1,3] and M. Várady[1]

[1]Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Kosice,04001, Slovakia, [2]I. I. Schmalhausen Institute of Zoology NAS of Ukraine, B. Khmelnytsky street, 15, Kyiv, Ukraine and [3]University of Veterinary Medicine and Pharmacy in Kosice, Komenskeho 73, Kosice, 04181, Slovakia

Email: konig@saske.sk

Background: Widespread distribution of anthelmintic resistance (AR) in strongylids requires sensitive indicators for early AR detection in farms.

Objectives: To perform a survey on the occurrence of benzimidazole (BZ) AR in strongylids in Slovakia using in vitro *egg* hatch test (EHT) and larval development test (LDT), and in vivo faecal egg count reduction test (FECRT).

Study design: Longitudinal field and laboratory trial.

Methods: Of 735 horses examined in Slovakia, 77 were selected for EHT, LDT, and FECRT to detect BZ resistance in strongylids. The results were analysed as described previously[1].

Results: The EHT indicated the presence of AR with an exceeded threshold value of 0.1 µg/mL thiabendazole (TBZ) in 57.1% of samples. The random forest algorithm demonstrated that the egg survival rates at TBZ doses of 0.1, 0.3, and 0.05 µg/mL were the most important predictors for detecting BZ resistance. The FECRT revealed BZ resistance in 88.6% of the horses (FECRT<90%). The LDT succeeded only for 44 samples, in which cyathostomin infective larvae were found in TBZ concentrations ≥0.08 µg/mL in 81.8%. Comparing the results of all three tests (LHT, LDT, and FECRT) revealed no statistically significant agreement among their results (Light's Kappa test; $p = 0.884$). Regression analysis indicated that horse age and farm size factors would potentially be associated with developing AR in our study; these factors could explain 8.6% of the variation in the results.

Main limitations: Study was limited to a small number of infected horses.

Conclusions: In vitro EHT could serve as a BZ resistance detection method in case of the impossibility of performing the in vivo FECRT; LDT was unsuitable as an individual field method for AR detection in horse farms.

Ethical animal research: Not required: non-invasive.

Informed consent: Not applicable.

Competing interests: Owners gave consent for their animals' inclusion.

Funding: VEGA grant 2/0099/22 and EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia, project No. 09I03-03-V01-00015 and 09I03-03-V01-00046.

Reference:

[1] Dobson RJ, Griffiths DA, Donald AD, Waller PJ. A genetic model describing the evolution of levamisole resistance in Trichostrongylus colubriformis, a nematode parasite of sheep. IMA J Math Appl Med Biol. 1987;4(4):279–293. doi: 10.1093/imammb/4.4.279

181 | A systematic review of mitigation strategies to reduce veterinary teaching hospital zoonotic disease transmission

N.V. Jasper[1], B.A. Burgess[1], E. Feyes[2], T. Gull[3], G. Perkins[4], J. Stull[5] and J.T. McClure[5]

[1]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA, USA; [2]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA; [3]University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, Missouri, USA; [4]Department of Clinical Sciences, Cornell University College of Veterinary Medicine, Ithaca, New York, USA and [5]Department of Health Management. Atlantic Veterinary College, University of Prince Edward Island, Canada

Email: nicelle.jasper@uga.edu

Background: Preventing transmission of zoonotic diseases is an ever-present challenge in veterinary practice, and even more so in educational settings when training students and working with novice practitioners. In fact, nearly 50% of AVMA-accredited veterinary teaching hospitals (VTHs) report zoonotic infections among personnel.[1] What is more, zoonotic agents are two times more likely to be emerging pathogens[2]; something that was all too real in 1994 with the emergence of Hendra virus in Australia, which had devastating effects on both horses and veterinarians.

Objectives: To summarise the current understanding of the zoonotic prevention efforts in educational settings.
Study design: Systematic review.
Methods: The Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) was used to perform a systematic review and quality assessment of the literature. Relevant articles were identified in Agricola, CAB Abstracts/CAB Archive, Global Health, and Web of Science from 1970 to 2023. Manuscripts were managed Microsoft Excel®, Endnote®, and Covidence® software. Descriptive statistics performed.
Results: Twenty-four studies met inclusion criteria representing many study designs (cohort, cross-sectional, experimental), with a spectrum of evidence quality, and multiple practice settings (academic, private practice).
Main limitations: This review was limited to published literature in four databases; evidence provided was generally considered to be weak; and study heterogeneity precluded the ability to perform a meta-analysis.
Conclusions: Study results suggests that while veterinarians attempt to prevent zoonotic disease transmission, there is a lack of robust evidence to determine which mitigation strategies are effective. Study heterogeneity may reflect the absence of universally applied standard precautions and the need for development of evidence-based, best-practices.
Ethical animal research: Not applicable.
Informed consent: Not applicable.
Competing interests: None declared.
Funding: None.
References:
[1] Benedict, et al. Characteristics of biosecurity and infection control programs at veterinary teaching hospitals. J Am Vet Med Assoc. 2008;233:767–773.
[2] Taylor, et al. Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci, 2001;356:983–989.

182 | Selective anthelmintic treatment of horses in Sweden: Ten-year results

E. Osterman Lind, M. Holmberg and G. Grandi
Swedish Veterinary Agency (SVA), 751 89 Uppsala, Sweden
Email: eva.osterman-lind@sva.se

Background: To slow down the development of resistance to anthelmintics in equine parasites it is important to avoid unnecessary use of these drugs. Therefore, selective treatment based on faecal analyses is recommended in Sweden.
Objectives: To show how selective treatment can be used in practice to control parasites in Swedish equestrian premises.
Study design: Retrospective review of clinical data.
Methods: As part of a parasite monitoring programme, faecal samples from 935 equestrian premises were submitted to SVA in springtime between 2008 and 2017. In total, 43 330 samples were analysed for helminth eggs by quantitative or semi-quantitative flotation. Larval cultures or PCR were used to detect *Strongylus vulgaris*. Along with the results, specific advice regarding deworming and parasite control strategies were provided to premises.
Results: Between 4 and 11% of individual horses tested positive for *S. vulgaris* and the majority of these appeared to have been introduced into the herd within the previous 12 months. There were recurrent high and low strongyle egg shedders and 80% of the total output of eggs originated from 26% of the individuals. Moreover, 3%–10% of the horses shed tapeworm eggs. Based on the results of *S. vulgaris* diagnostics and strongyle egg-shedding level, 59% of the horses did not need to be dewormed.
Main limitations: The premises included were not representative of the whole country as there was an overrepresentation in central Sweden.
Conclusions: The use of antiparasitic drugs could be greatly reduced when selective treatment is practiced. However, it is important that premises establish deworming routines of newcomers to prevent the introduction of *S. vulgaris*.
Ethical animal research: Not required: clinical surveillance.
Informed consent: Equestrian premises gave their consent to use submitted material for research purposes.
Competing interests: None declared.
Funding: None.

183 | No signs of reduced anti-strongyle ivermectin efficacy in Danish horses

I.M.D. Bartholdy[1], K.S. Kristensen[2], C.E. Rendtorff[3], J. Borrye[4], M.D. Hjortflod[5], K.S.S. Meilvang[6], M.K. Nielsen[7], T.H. Pihl[8], S.M. Thamsborg[9] and K. Toft[8]
[1]Valby, Denmark; [2]Copenhagen, Denmark; [3]Store Fuglede, Denmark; [4]Copenhagen, Denmark; [5]Frederiksvaerk, Denmark; [6]Copenhagen, Denmark; [7]University of Kentucky, Veterinary Science, Gluck Equine Research Center, Lexington, Kentucky 40 546, USA; [8]University of Copenhagen, Faculty of Health and Medical Science – Large Animal Sciences, Taastrup 2630, Denmark and [9]University of Copenhagen, Department of Veterinary and Animal Sciences, Frederiksberg, Denmark
Email: thpi@sund.ku.dk

Background: Equine strongyles are ubiquitous and can cause severe health issues. Several studies world-wide have shown increasing resistance to ivermectin among strongyles in the past decade. Danish prescription-only legislation has decreased the treatment intensity.
Objective: The aims were to determine ivermectin efficacy and the strongyle egg reappearance period (ERP) in Danish horses, compare two faecal egg count techniques, and identify risk factors for faecal egg count levels.
Study design: Field efficacy study and cross-sectional survey.
Methods: Herds and horses were elected by four collaborating veterinary clinics. A total number of 299 horses from 30 herds were enrolled in the study. Faecal egg counts (FEC) were estimated using a McMaster technique as well as an automated faecal egg counting system. All horses with FEC >0 EPG were treated with ivermectin.

Ivermectin efficacy was determined with a faecal egg count reduction test (FECRT). ERP was determined by repeated faecal egg counts for a total of 8 weeks. Risk factor analysis was carried out by mixed linear regression.

Results: McMaster egg counts suggested inconclusive results from two herds and full efficacy in the remaining populations. The automated system suggested ivermectin resistance in 6 herds and inconclusive in another 8. ERP was found to be at least 8 weeks. FEC was negatively associated with increase in age (p-value <0.001), and positively associated with time since last treatment (p-value = 0.002).

Main limitations: Lack of random selection of the study population and the complicated inclusion process due to multiple study aims.

Conclusions: McMaster data indicated that ivermectin efficacy was high and ERPs exceeded 8 weeks. The egg counting techniques were in general agreement, but the automated system detected more positives at low egg count levels, which affected FECRT results. Faecal egg count levels were associated with age and time since treatment.

Ethical animal research: Approved by the University of Copenhagen, Department of Veterinary and Animal Sciences.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: The University of Copenhagen, Department of Veterinary Science and Sveastiftelsen, Denmark. Boehringer Ingelheim, Denmark, supplied ivermectin (Ivomec comp.). Parasight Systems, Lexington, Kentucky, USA, supplied an automated faecal egg count system.

184 | Exploring the molecular diversity of *Parascaris* spp. infecting horses in France

K. Bourrier[1,2], T. Guilmin[3], M. Thomas[3], G. Karadjian[3] and A. Merlin[1,2]
[1]*ANSES, Laboratory for Animal Health in Normandy, Physiopathology and Epidemiology of Equine Diseases Unit, 14 430 Goustranville, France;* [2]*Mixed Technological Unit "Equine health and welfare—Organisation and traceability of the equine industry" (UMT SABOT) and* [3]*ANSES, Laboratory for Animal Health in Maisons-Alfort, Unit in Molecular biology and immunology of parasitic diseases (BIPAR), 94 700 Maisons-Alfort, France*
Email: kenza.bourrier.ext@anses.fr

Background: Equine ascarids, *Parascaris* spp. (whitish nematodes), are prevalent parasites affecting young horses. Although, most infected equids remain asymptomatic, the larvae that migrate to the internal organs (liver, lungs) can cause coughing and hamper growth. In addition, heavy adult worm accumulation in the small intestine may lead to intestinal occlusion or rupture, resulting in fatality. These parasites have a significant impact on the health and welfare of young animals, and cause economic losses due to treatment costs. Within *Parascaris* spp., two morphologically identical but karyotypically distinguishable species exist: *Parascaris equorum* and *Parascaris univalens*. The study of karyotype is complex, requiring eggs with a single cell. In France, *Parascaris* spp. populations remain understudied.

Objective: This study aimed to describe French *Parascaris* spp. populations.

Study design: Molecular analysis.

Methods: Two exploratory methods were used: DNA amplification (targeting its1, its2, cox1, 12s, and nad5 genes) via PCR and MALDI-TOF mass spectrometry. Seventy adult *Parascaris* spp. were collected from necropsied horses or faeces of living horses (mainly in Normandy) aged <5 years from 12 farms. MALDI-TOF mass spectrometry analysed parasite head samples.

Results: While gene sequencing did not reveal clusters, protein profiles formed three distinct clusters. No effect of the farm was found, with the exception of one red cluster from a single farm. The small variations between clusters suggest that all the groups could belong to a single *Parascaris* species with potential sub-populations or different stages of development.

Main limitations: The study sample, limited to 70 *Parascaris* spp., may not reflect the full genetic diversity of the species in France.

Conclusions: In short, a fragmentary analysis did not enable us to determine the molecular diversity of the *Parascaris* species. In future, we are going to sequence the complete mitochondrial genome of the most divergent protein samples using the MinION nanopore sequencer to maximise our chances of identifying genetic differences and distinguishing species.

Ethical animal research: Not stated.

Informed consent: Not stated.

Competing interests: None declared.

Funding: French Agency for Food, Environmental and Occupational Health & Safety (Anses), The French Horse and Riding Institute and the Fonds Eperons.

185 | Assessment of the infection level of foals by *Parascaris* spp. in Normandy

K. Bourrier[1,2,3*], C. Roche[1], N. Larcher[1], G. Karadjian[3] and A. Merlin[1,2]
[1]*ANSES, Laboratory for Animal Health in Normandy, Physiopathology and Epidemiology of Equine Diseases Unit, 14 430 Goustranville, France;* [2]*Mixed Technological Unit "Equine health and welfare—Organisation and traceability of the equine industry" (UMT SABOT) and* [3]*ANSES, Laboratory for Animal Health in Maisons-Alfort, Unit in Molecular biology and immunology of parasitic diseases (BIPAR), 94 700 Maisons-Alfort, France*
Email: kenza.bourrier.ext@anses.fr

Background: *Parascaris* spp. are the most common and pathogenic parasites in foals. Unfortunately, little is known about the prevalence of *Parascaris* infection in French equids. A single study conducted in 2010 observed that 30.5% of foals aged between 3 and 9 months were infected with *Parascaris* spp.

Objective: The aim of this study was to assess the current proportion of foals infected with *Parascaris* spp. in Normandy between March and May 2024.

Study design: Surveillance study.

Methods: Foal owners were recruited through the French Horse and Riding Institute. On each farm, a maximum number of faeces samples were collected from each individual and coproscopic analyses were carried out (Mini-Flotac method). In addition, a logistic regression model was developed to assess the individual factors (age, breed, sex, and number of samples) influencing the presence of eggs in the samples.

Results: A total of 29 structures were recruited, representing 324 foals. *Parascaris* spp. eggs were identified in 18% (IC95% = [0.13; 0.25]) of the faeces of foals under 1 year old, and in 5% (IC95% = [0.03; 0.10]) of the faeces of those strictly over 1 year old. *Parascaris* spp. eggs were less frequently observed in animals over 15 months old that is consistent with the acquisition of immunity by the animals from 6 months of age. Eggs were more frequently identified in saddle horses and draught horses than in racehorses, probably due to the more frequent use of anthelmintics in the latter. The difference in the proportion of egg-positive faeces between males/females may be explained by female sex hormones, which can have an impact on parasite prolificacy.

Main limitations: the study was limited to one geographic region.

Conclusions: This study provides valuable information on the current proportion of foals infected by *Parascaris* spp. and on factors impacting the infection. It would be interesting to continue monitoring the level of infection of foals over time and to expand this study to other regions.

Ethical animal research: Not required: clinical surveillance.

Informed consent: Owners gave consent for their animals' inclusion.

Competing interests: None declared.

Funding: The French Agency for Food, Environmental and Occupational Health & Safety (Anses), the French Horse and Riding Institute and the Fonds Eperons.

186 | Air and surface samples to better assess the presence of *Rhodococcus equi* in the foal's environment

J. Manifacier-Lauks[1,2], E. Reimer[2], M.-P. Toquet[1,2], C. Vercken[3], E. Richard[1,2], A. Rincé[2]

[1]*LABÉO, 14 280 Saint Contest, France;* [2]*Normandie Université, UNICAEN BIOTARGEN, 14280 Saint Contest, France and* [3]*EQUIWAYS, 14 280 Saint Contest, France*

Email: alain.rince@unicaen.fr

Background: *Rhodococcus equi* mainly affects foals and is associated with a range of clinical presentation of which respiratory signs are the most common. Inhalation of bacteria harbouring a virulence plasmid (VapA+) is considered the main route of transmission.

Objectives: Evaluate foals' exposure to *R. equi* and *R. equi* VapA+ by looking for these bacteria in the soil, faeces, air and on the surface of mares' udders and foals' nostrils.

Study design: Samples (217) were collected between February 2023 and June 2024 from 3 stud farms in northwest France. After culturing, bacteria were detected by PCR.

Methods: Soil, faecal and surface samples were used to inoculate agar plates containing selective agents. 500 L of air were filtered on a membrane using pumps placed on mares to collect the air near their nostrils or fixed within the stables or paddocks. The membranes were then placed on agar plates. After incubation, colonies were pooled and duplex PCR targeting *choE* and *vapA* genes were performed to detect *R. equi* and *R. equi* VapA+, respectively.

Results: The bacteria were frequently detected in soil. They were also detected from the surface of udders (44.7% and 26.3% positive samples respectively for *R. equi* and *R. equi* VapA+), nostrils of foals (24.3% and 18.9%) and air collected from mares (46.2% and 28.2%) and more rarely in faeces (10.5% and 2.6%) and ambient air (3.4% and 0%). The higher proportion of positive samples for air collected from mares compared to ambient air (p-value $= 1.04 \times 10^{-4}$) demonstrates the relevance of placing this device on animals.

Main limitations: Study conducted over only 2 years and 3 studs.

Conclusions: The study highlighted that analyses of air collected from mares, the surface of their udders or foals' nostrils show good correlations and are more effective than ambient air analyses in highlighting the presence of *R. equi* and *R. equi* VapA+ in the environment of foals.

Ethical animal research: The authors attest that ethical review for this minimally invasive work is not required in their institute.

Informed consent: Authorisation from owners was obtained.

Competing interests: None declared.

Funding: Institut Français du Cheval et de l'Equitation as part of the project "Air equi."