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27th September – 1st October 2021

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Foreword and Acknowledgements

The 11th International Equine Infectious Diseases Conference (IEIDC XI) was due to be held in 2020, in the beautiful resort of Deauville, France. When planning began in 2016, who could have imagined that a coronavirus pandemic would force the International and Local Organising Committees to take the hugely disappointing decision to postpone the conference to 2021. But in the face of adversity, the equine infectious disease community has rallied round and submitted over 130 high quality abstracts so that IEIDC XI can still go ahead, albeit on-line; it will be held from 27th September to 1st October 2021. The peer-reviewed abstracts are now published in this supplement.

Since IEIDC X in Argentina five years ago and despite many countries entering stringent lockdown measures, which have also curtailed the movement of horses over the past 18 months, major outbreaks of equine infectious disease have continued unabated. These include strangles, equine herpesvirus myeloencephalopathy, influenza, contagious equine metritis, equine infectious anaemia, West Nile virus and piroplasmosis to name a few. The first outbreak of African horse sickness in horses in South East Asia was also reported in 2020, with phylogenetic typing indicating a close relationship with isolates from South Africa. Thus IEIDC XI aims to summarise these events and report the prevention and control of such diseases.

IEIDC XI is held over four days. Over the first three scientific research days, we are fortunate to have several eminent speakers, whose invited plenary presentations will address the most common equine infectious diseases, followed by live question and answer sessions. Authors of the research abstracts will also present their data as pre-recorded oral and poster formats. All will cover the major equine infectious diseases within the disciplines of bacteriology, parasitology and virology. Importantly, the conference will also host a Practitioners' Day, for which separate registration is available and this will report state-of-the-art, clinically relevant advice on the prevention, control and management of the most common equine infectious diseases. This day will include pre-recorded videos and live panel discussions followed by question and answer sessions, relating to recent outbreaks of strangles, influenza and equine herpesvirus myeloencephalopathy, as well as sessions on emerging equine infectious pathogens, anthelminthic resistance in parasites, antimicrobial stewardship and the role of the vet in international movement of horses. All of the Practitioners' Day sessions will be recorded and therefore available to registered delegates for 12 months after the conference. For details of the conference programme and registration, please see: https://eidc2021.com/ and https://www.eventbrite.com/e/11th-international-equine-infectious-diseases-conference-tickets-153703488271. The International Committee would also like to thank all sponsors for their generous support of this conference: https://eidc2021.com/current-sponsors/

The Covid-19 pandemic has changed the world to the extent that everyone is now familiar with the importance of infection control measures, quarantine and vaccination safety and efficacy in reducing clinical signs and hospitalisation. Thus this tragic event may be a timely opportunity for veterinary surgeons and scientists to re-enforce the education of horse-owners and competition organisers about prevention and control of infectious disease in their horses and other equids. The economic impact of the pandemic is also likely to have an indirect but hopefully short term, negative effect on the future funding of equine infectious disease research. As economies recover, every effort must be made to ensure continuity in equine research funding and the recruitment and nurture of the next generation of enthusiastic and talented scientists and veterinary surgeons. With this aim in mind, the equine infectious disease community will continue its efforts to improve the health and welfare of horses around the world.

For the next conference, IEIDC XII in Deauville, France (2024), the International and Local Organising Committees will pick up the reins and with the support of their colleagues, continue where planning left off – this time to organise an in-person meeting. We look forward to seeing you there!

Dr Julia Kydd
Guest Editor and Member of the IEIDC International Committee

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Bacteriology

Antimicrobial resistance/susceptibility

Oral Presentations

1 | Widespread environmental presence of multidrug-resistant *Salmonella* in an equine veterinary hospital in a horse racetrack facility

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Background: *Salmonella*, due to its nosocomial and zoonotic characteristics, is responsible for severe outbreaks in equine hospitals, with high morbidity/mortality rates, and closure of facilities. In some countries, the lack of written records and infection control programs, lead to an erroneous safety concept regarding hospital acquired infections.

Objectives: Determine and characterise the presence and diversity of *Salmonella* spp. in an equine veterinary hospital (EVH) in Chile.

Study design: Environmental and clinical screening.

Methods: Samples (545) were collected during a 1-year period from human and animal contact surfaces in an EVH located at a racetrack facility, housing an average of 1500 Thoroughbred racehorses, but providing equine health services to housed Thoroughbreds and external patients. Hospitalised patients were also sampled. Samples were submitted for *Salmonella* isolation by bacterial culture [1]. Suggestive colonies were submitted to molecular identification and classification [2,3]. Confirmed *Salmonella* isolates were assessed for antimicrobial susceptibility through disk diffusion tests [4]. Finally, *Salmonella* isolates were submitted for molecular typing by pulse field gel electrophoresis (PFGE), analysed using Bionumericsv7.5 and interpreted using Tenover's guidelines [5,6].

Results: *Salmonella* (21) was isolated from human (offices, pharmacy) and animal contact surfaces (stalls, surgery room, waterers), and from one patient. Isolates were serotyped as *S. typhimurium* (19) and *S. infantis* (3). Twenty were resistant to at least one antimicrobial class, and only two were susceptible to all antimicrobials tested. Nine multidrug-resistant (MDR) isolates of *S. typhimurium* were identified which displayed resistance to AMP-AMP-CIP-CHL-STR-GEN-SXT-TET. PFGE revealed three patterns of *Salmonella* permanently present in the environment of the hospital during our study.

Conclusions: Widespread persistent presence of MDR-*Salmonella* at an equine hospital on a racetrack facility highlights the necessity of biosecurity protocols to prevent a general dissemination of the pathogen and ensure the safety of horses and people.

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electrophoresis: Criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**, 2233–2239.

Ethical animal research: The study was approved by the local Research Ethics Committee.

Informed consent: Not stated.

Competing interests: None declared.

Sources of funding: This study was entirely supported by FONDECYT N° 11140108 and a Regular Project UNAB DI-1300-16/RG.

2 | Decreased susceptibility of human and equine *Pseudomonas aeruginosa* isolates to a major component of disinfectant

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Background: Didecyldimethylammonium chloride (DDAC) is a quaternary ammonium compound used in several disinfection products in veterinary and human hospital environments. In human medicine, strains of *Pseudomonas aeruginosa* (PSAE) show a decreased susceptibility to DDAC according to the NFEN-13727+A2 referential, by incompletely understood mechanisms. Decreased susceptibility to antibiotics and disinfectant agents is a major concern to public health, as it allows pathogens to persist in their environment. Based on a "One health" approach, our global project aims at a better understanding of the resistance mechanisms to disinfectants and antibiotics for PSAE.

Objectives: To evaluate the existence of PSAE strains with DDAC decreased susceptibility among animals in Normandy, France.

Study design: *In vitro* analysis of micro-organisms.

Methods: Between 1996 and 2020, 183 strains isolated from animal samples (including 146 equines) were provided by our reference laboratory and routine diagnostic service. Minimum inhibitory concentrations (MICs) of DDAC were determined using a broth microdilution method. Decreased susceptibility was defined as MIC≥64mg/L, corresponding to the concentration of DDAC in a disinfectant solution according to the manufacturer's instructions.

Results: During 1996–2015, all strains tested were sensitive to DDAC, whereas for 2017, 2018, 2019 and 2020 respectively 20%; 26.3%; 6.5% and 12% of strains had decreased susceptibility. Overall, 10.4% of the strains had this decreased susceptibility phenotype and 84.21% of them were equine. Equine respiratory and genital samples showed the most decrease in sensitivity, representing respectively 42.11% and 36.84% of all strains.

Main limitation: From 1996 to 2015, only 23 strains were isolated; from 2017 and 2018, only strains showing an antimicrobial resistance phenotype were selected.

Conclusion: Decreased susceptibility of PSAE to DDAC is present in the veterinary industry. Genomic analyses are underway to determine the relatedness of these strains and to understand the mechanisms involved.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: Conseil Régional de Normandie and Ministère de l'Agriculture (EcoAntibio) support this study.

Poster Presentation

3 | Antimicrobial susceptibility of pathogenic bacteria in Thoroughbred racehorses in Japan

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Background: Antimicrobial resistance (AMR) in bacteria is a global problem. In equine practice, monitoring of AMR and antimicrobial stewardship are required.

Objective: We investigated the antimicrobial susceptibility of pathogenic bacteria in Thoroughbred racehorses in Japan.

Study design: *In vitro* analysis of micro-organisms.

Methods: We examined 382 isolates including 90 of *Escherichia coli*, 58 of *Pseudomonas aeruginosa*, 96 of *Staphylococcus aureus*, and 138 of *Streptococcus equi* subsp. *zooepidemicus*. These isolates were obtained from diseased horses between 2001 and 2018. MIC was measured using the microbroth dilution method, following the Clinical Laboratory Standard Institute (CLSI) guidelines. Determination of extended spectrum ß-lactamase (ESBL)-producing *E. coli* and methicillin-resistant *S. aureus* (MRSA) were performed in accordance with the CLSI guideline.

Results: Antimicrobial resistance patterns varied in the *E. coli* isolates. No fosfomycin-resistant isolates were observed. A few *E. coli* isolates were resistant to ceftiofur, gentamicin, and enrofloxacin, and the ceftiofur-resistant isolates produced ESBL. No resistance was observed in recent *P. aeruginosa* isolates. Gentamicin resistance was observed only in the isolates obtained before 2003. Thirty-two (33%) of the 96 *S. aureus* isolates were MRSA. The number of MRSA isolates had drastically increased since 2011. No *S. aureus* isolates were resistant to trimethoprim-sulfamethoxazole combinations or vancomycin. All the *S. zooepidemicus* isolates were susceptible to ß-lactams, but one-third of the isolates were resistant to minocycline.

Main limitations: Some isolates were obtained from horses undergoing antimicrobial treatment.
Conclusion: In Japanese Thoroughbred racehorses, increased AMR is a concern for *E. coli* and *S. aureus* but not for *P. aeruginosa* or *S. zooepidemicus*. Since there are effective agents against *E. coli* and *S. aureus* with AMR, adequate selection and prudent use of these agents are important to control infectious bacterial diseases.
Ethical animal research: The study was approved by Research Planning Committee of Japan Racing Association, number 2018-3263-07.
Informed consent: Not applicable.
Competing interests: None declared.
Source of funding: Japan Racing Association.

Chlamydia psittaci

Oral Presentations

4 | *Chlamydia psittaci*: a suspected cause of abortion and fatal neonatal illness in horses within Victoria, Australia

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Background: A wide variety of pathogens may cause abortion and neonatal illness in horses. Reports of the zoonotic pathogen *Chlamydia psittaci* (*C. psittaci)* in equine abortion in one state of Australia has highlighted the importance of greater vigilance for this bacterium in aborted equine tissues, in terms of both diagnostic accuracy and zoonotic potential.
Objectives: To determine the presence of *C. psittaci* in samples submitted from cases of equine abortions over the 2018-19 foaling season in Victoria, Australia as part of a subsidised diagnostic scheme.
Study design: Descriptive cadaver study.
Methods: Quantitative PCR targeting a 460 bp region of the 16S rRNA gene of *Chlamydiaceae* and histopathological analysis of tissues where available.
Results: *Chlamydia psittaci* was detected in the lung and fetal membranes of two aborted fetuses and one weak foal from two different studs in North-East Victoria (NEVic), Australia. The abortions occurred in Friesian mares sharing a paddock. The Thoroughbred foal died soon after birth. Both studs were in a rural area with many eucalyptus trees and a variety of bird species present. The level of *C. psittaci* DNA genome copies in the lung and fetal membranes of the aborted or weak foals, in the absence of other infectious abortigenic pathogens suggest that *C. psittaci* had a causative role.
Main limitations: Although *C. psittaci* was detected using PCR techniques in a variety of tissues, poor tissue preservation prevented further confirmation using histopathology or immunohistochemistry.
Conclusions: The detection of *C. psittaci* in these cases is consistent with the recent detection of *C. psittaci* in association with equine abortion and neonatal illness in New South Wales, a state sharing Victoria's northern border. This finding highlights the importance of considering *C. psittaci* as a possible cause of equine abortion or neonatal illness and underlines the potential of zoonotic pathogens in these cases.
Ethical animal research: Research ethics committee oversight not required: retrospective analysis of clinical data.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: None.

5 | *Chlamydia psittaci*: not a pathogen to "horse around with" in Australia

S. Anstey[1]; D. Lizárraga[1,2]; S. Nyari[1]; G. Chalmers[3]; J. Carrick[4]; C. Chicken[5]; C. Jenkins[6]; N. Perkins[7]; P. Timms[1] and M. Jelocnik[1,*]
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Background: Late-term foal loss due to the avian pathogen *Chlamydia psittaci* recently emerged as a threat to the Australian Thoroughbred industry.
Objectives: To better understand the epidemiology of this pathogen, 228 pregnant mares and their newborn foals from 14 stud farms in Australia were followed.
Study design: Longitudinal.
Methods: We evaluated *C. psitta13ci* prevalence in the equine and avian reservoirs, and investigated potential risk factors, including EHV-1 co-infection and weather events. Molecular epidemiology explored the relationship between host, strain type and disease in the Australian setting. Screening for *C. psittaci* was performed using species specific qPCR assays, while strain characterisation utilised multilocus sequence typing (MLST) and genotyping (*omp*A). Risk analyses utilised multiple logistic regression modelling.
Results: Our study cohort recorded no *C. psittaci* abortion events, yet we detected *C. psittaci* in 13.2% (30/228) of healthy newborn foals. Risk factor analysis found that winter foaling had a significantly higher risk of *C. psittaci* infection than spring foaling (adjusted

Odds Ratio = 15.83; p < 0.001). There was a significant correlation between historical Chlamydial abortion cases and frost events (Spearmans' rho = 0.44; *P*=0.002). In mares, co-infection with EHV-1, being a maiden mare, absence of a prophylactic vaginal suture, interventions in the last trimester and residing on a farm with a prior history of *C. psittaci* abortion posed no higher risks to infection in the newborn foal. Molecular epidemiology of all recorded *C. psittaci* abortions (2016-2019) revealed a successful clonal and potentially tissue trophic strain (ST24).

Main limitations: This research was conducted on Thoroughbred farms, where individual variation in sampling may have occurred.

Conclusions: This research informs management strategies to prevent loss of foal life and zoonosis. It is recommended that *Chlamydia psittaci* in added to equine abortion diagnostic panels in Australia. Furthermore, revision of the terminology of Psittacosis to Chlamydiosis will be a move towards a One Health approach.

Ethical animal research: Ethics approval for this study were granted by the University of the Sunshine Coast (ANE1939/ANA19149).

Informed consent: Informed consent was obtained from all participating farms.

Competing interests: None declared.

Sources of funding: Agrifutures Thoroughbred Horses Program (project number PRJ-011402) awarded to PT, MJ, JC, CC and the Australian Research Council Discovery Early Career Research Award (DE190100238) awarded to MJ.

6 | Rapid isothermal assays for detection of equine pathogens *Chlamydia psittaci* and Equine Herpes Virus 1

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Background: *Chlamydia psittaci* has recently emerged as an equine abortigenic pathogen causing significant losses to the Australian Thoroughbred industry, while Equine herpesvirus-1 (EHV-1) is a well-recognised abortigenic agent.

Objectives: We validated *C. psittaci* and new EHV-1 Loop Mediated Isothermal Amplification (LAMP) assays and evaluated their use in a clinical setting.

Study design: Assay validation.

Methods: *C. psittaci* and EHV-1 LAMP assays were validated against diagnostic laboratory reference assays and used to test 'real-world' clinical samples. We also applied the *C. psittaci* LAMP assay for Point-of-Care (POC) testing in an equine hospital. LAMP assays were performed in a real-time fluorometer, or on a heating block using colorimetric mix for "naked eye" end-point detection. Comparison to reference assays was performed using qPCR assays.

Results: The analytical sensitivity of *C. psittaci* and EHV-1 LAMPs was determined as one and 10 genome equivalents per reaction, respectively. Compared to the reference diagnostic qPCR assays, the *C. psittaci* LAMP showed specificity of 97.5%, and 98.86% agreement, while EHV-1 LAMP showed 100% specificity, and 91.43% agreement. When testing rapidly processed clinical samples, both *C. psittaci* and EHV-1 real-time and colorimetric LAMP assays were highly congruent with each other, with Kappa values of 0.638 - 0.906. In the clinical setting, the *C. psittaci*-LAMP assays using rapidly processed swabs (with no DNA extraction) were easy to perform by technicians with no prior molecular experience, with the overall congruence between the LAMPs and the qPCR assays ranged between 90.91 - 100%.

Main limitations: The reference qPCR assays were run at two different laboratories, so additional comparison testing with a larger sample set is required.

Conclusions: This study describes reliable POC options for the detection of *C. psittaci* and EHV-1. Testing 'real-world' samples in an equine clinical setting, represents a proof-of-concept that POC isothermal diagnostics can be applied to rapid disease screening in the equine industry.

Ethical animal research: Approval for testing and use of equine mucosal swabs was granted by the University of the Sunshine Coast (ANE1719/ANE1939/ANA19149).

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: AgriFutures Australia as part of the AgriFutures Thoroughbred Horses Program (project number PRJ-011174) awarded to CJ, MJ and JC and the Australian Research Council Discovery Early Career Research Award (DE190100238) awarded to MJ.

Clostridium

Poster Presentations

7 | Chilean Patagonia: Equine grass sickness cases

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Background: Equine grass sickness (EGS) is a multi-system neuropathy affecting horses, characterised by degeneration of autonomic

neurons and stasis of the gastrointestinal tract [1]. The precise aetiology remains unknown and both historical and recent evidence supports a contributory role for *Clostridium botulinum*. In the Patagonian Andes Forest Ecoregion equines graze over prairies with abundant species of low forage value and invasive exotic plants, some with toxic components.

Objective: To monitor and characterise the histopathology of cases of grass sickness in a herd of 170 horses, donkeys and mules.

Study design: Case series.

Methods: The feeding system was direct grazing in the pasture and supplementation based on bales of grass and oats, applied directly to the pastures. The mares and their foals are managed at pasture all year round. The diagnosis of equine grass sickness was made by histopathological analysis of nerve ganglia and by compatible macroscopic findings.

Results: From May 2019 to March 2021, 17 cases of equine dysautonomia were reported, in 11 mares and 6 mules. Animals withdrew from the group; had difficulty in eating, stopped defecating and developed very poor body condition, adopting a narrow base stance. All the animals were refractory to the treatments used. In the large colon, there were hard and dry stools, detached mucous membranes and purplish congested intestinal walls. Histopathological changes in the coeliacomesenteric and stellate were neuronal degeneration, pyknosis, central chromatolysis and cytoplasmic eosinophilia.

Main limitations: No attempt to culture *C. botulinum* was made.

Conclusions: Equine grass sickness is present in the Patagonian Andes Forest ecoregion of Chile.

Reference

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Ethical animal research: Research ethics committee oversight not required by this journal: case series.

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: Universidad Santo Tomás, Servicio Agrícola y Ganadero.

8 | *Clostridioides difficile* in necropsied equidae: isolation and characterisation of the CloDifEqui collection of strains

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Background: *Clostridioides difficile* is a sporulating anaerobe and a major entero-pathogen of both humans and animals. It produces toxins and is responsible for post-antibiotic diarrhoea and colitis that are difficult to treat and can be recurrent. In animals, the importance of *C. difficile* is not well evaluated. In cases of diarrhoea, its presence is not necessarily tested for. Moreover, diagnosis of *C. difficile* infection is difficult, because toxinogenic strains can be asymptomatically carried, if they do not produce toxins. They nevertheless remain able, after spread and transmission, to cause new, and notably community-acquired, infections.

Objectives: The project aimed to i) build up and characterise a collection of *C. difficile* strains isolated from necropsied equidae, and ii) evaluate the frequency of *C. difficile* carriers in this whole equine population, together with its prevalence as a cause of infectious disease.

Study design: Post-mortem survey.

Methods: The presence of *C. difficile* was systematically monitored in equideae that had been necropsied at ANSES since 2019, whatever the cause of their death. Animal data (individual, clinical, epidemiological, cause of death) were registered and intestinal contents sampled to search for *C. difficile*. *C. difficile* strains were isolated and stored, forming the CloDifEqui collection. They were characterised by molecular genotyping and phenotyping. The intestinal contents of equideae displaying a toxinogenic strain were analysed for the presence of the toxins, in order to establish a diagnosis of infection.

Results: Twenty strains were isolated in 80 necropsies.

Conclusions: *C. difficile* is prevalent in equine intestinal contents. Further work on molecular epidemiology and to compare these findings with the animal data is needed.

Ethical animal research: The study was approved by the scientific committee of Institut Français du Cheval et de l'Equitation (IFCE).

Informed consent: Animal owners gave consent for inclusion in the study.

Competing interests: None declared.

Sources of funding: This work was funded by IFCE (Institut Français du Cheval et de l'Equitation), ANSES and INRAe.

Mycoplasma

Oral Presentations

9 | Characterisation of *Mycoplasma* spp. isolated in 2020 from respiratory tract samples of horses in France

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Background: With more than 100 species colonising animal hosts, bacteria of the genus *Mycoplasma* include important livestock pathogens. They are wall-less and considered as the smallest organisms

capable of autonomous growth *in vitro*. Because their isolation can be laborious, they often lag behind other bacteria in terms of general knowledge and diagnosis. In horses, three species, namely *Mycoplasma (M.) equirhinis*, *M. pulmonis* and *M. felis*, have been reported from respiratory tract samples, with an unknown pathogenic role but a potential implication on poor-performance [1-2]. In our laboratory, amongst equine respiratory samples submitted routinely for diagnosis, 15% per year were positive by real-time PCR (rt-PCR) for *Mycoplasma* spp. but the corresponding species have not been explored yet.

Objectives: To evaluate the prevalence of different *Mycoplasma* species in respiratory clinical samples and characterise their intra-species diversity.

Study design: Retrospective clinical study.

Methods: Respiratory samples (n = 687, 68% tracheal wash, 26% bronchoalveolar lavage and 6% others) collected in 2020 were analysed by culture, rt-PCR and identified by 16S rRNA sequencing. Isolates were subtyped when necessary.

Results: To date, 126 samples (18.3%) were positive by rtPCR and 30 strains were isolated (still ongoing). A majority of *M. equirhinis* (42/126 samples and 24/30 strains) was identified, while *M. pulmonis* and *M. felis* have been detected only sporadically so far.

Main limitations: A gold standard method for *Mycoplasma* spp. detection and identification in equine samples would be helpful. Clinical characterisation is not complete.

Conclusions: A Multi Locus Sequence Typing scheme is being developed to analyse the genetic heterogeneity of *M. equirhinis* isolates. Subtypes will be examined in the light of their clinical history gaining the first insight into the potential pathogenic role of *M. equirhinis*.

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

Informed consent: Explicit study consent was not stated but owners were aware that samples could be used for research activities.

Competing interests: None declared.

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

10 | Possible association of *Mycoplasma equirhinis* with respiratory signs in Japanese Thoroughbred racehorses

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Background: *Mycoplasma equirhinis* was isolated in culture or detected using PCR methods from tracheal wash (TW) samples from horses with respiratory signs [1]. *Mycoplasma* infections are thought to be associated with inflammatory airway diseases of horses [2]; however, their pathogenicity is still unclear. There was an outbreak of a respiratory syndrome in Thoroughbred racehorses in Japan during the autumn of 2018, and Mycoplasmas were frequently isolated in TW samples from these cases.

Objectives: To elucidate the cause of the respiratory signs seen in 2018.

Study design: Epidemiological survey.

Methods: TW samples were collected from 40 cases with respiratory signs. Bacterial species and viruses related to respiratory diseases of horses in TW were investigated by loop-mediated isothermal amplification [3] and PCR methods respectively. *Mycoplasma* species were isolated on PPLO agar from TW samples after selective growth in PPLO medium. Isolates were identified using 16S rRNA gene sequencing analysis and specific PCR for *M. equirhinis*. TW samples from sporadic cases with respiratory signs in 2019 - 2020 and from healthy horses in Japan were also examined to isolate Mycoplasmas.

Results: *Mycoplasma equirhinis* was isolated from 16 of 40 cases (40.0%), whereas other pathogenic bacteria were detected from five cases. No viruses were detected. The isolation rate of *M. equirhinis* in the outbreak cases was higher than in sporadic cases with respiratory signs (13.6%, 3 of 22), and healthy horses (13.5%, 5 of 37).

Main limitations: This study was limited to an epidemiological survey.

Conclusions: The isolation rate of *M. equirhinis* was notably high, suggesting it may be related to the observed respiratory signs.

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Ethical animal research: This study was approved by the Research Planning and Ethics Committee of the Equine Research Institute with accession number 2018-3263-07.

Informed consent: Not stated.

Competing interests: None declared.
Source of funding: Japan Racing Association.

Rhodococcus equi

Oral Presentations

11 | Biosecurity audit and tailored grassland and facility biosecurity measures to reduce the occurrence of Rhodococcal infection in breeding centres

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Background: *Rhodococcus equi* (*R.equi*) VapA+ is a facultative pathogen that could induce pneumonia in foals below 6 months of age. Rhodococcal infection may be severe and is a recognised threat to the equine breeding industry. Infected foals may require antibiotherapy for several weeks with associated issues such as side effects and/or bacterial resistance. To date, no vaccine is commercially available, and protection induced by hyperimmune plasma has limitations.
Objectives: To validate a tailored biosecurity approach to reduce disease occurrence in equine breeding centres/farms.
Study design: Proof of concept; field biosecurity audit, design/implementation of tailored biosecurity measures and longitudinal annual monitoring (up to 3 years).
Methods: 5 breeding centres (from 50 to 200 breeding mares) with a disease history ranging from no cases to almost 80% per year were selected. A biosecurity audit was conducted in each centre to determine existing measures. A tailored biosecurity programme (including grassland and facilities management, animal/human/vehicle flows etc.) was subsequently designed with owners and veterinarians to support/strengthen existing measures. Soil samples were semi-quantitatively analysed to identify areas with high concentration of *R. equi* VapA+.
Results: Bacterial analyses were successfully used to map "at risk" areas and to adapt foals' paddock allocation, animal movement flows and grassland management accordingly. The combination of measures such as regular disinfection protocols, faeces removal, annual reseeding, liming, watering and use of heavy sand, reduced disease occurrence in all previously affected centres when implemented (e.g. from 49 suspicious/confirmed cases prior to audit to 2 cases per year for 2 years). Audit and annual monitoring also increased general awareness about other equine infectious diseases.
Main limitations: The audit was standardised but implemented measures were centre-specific. Confirmation of results will require a larger number of centres and standardisation.
Conclusions: Tailored grassland and facility management could help to reduce the occurrence of Rhodococcal infection in the field.
Ethical animal research: No animal samples were taken.
Informed consent: Not applicable.
Competing interests: R. Paillot reports no competing interests. C. Vercken is providing commercial equine biosecurity services.
Source of funding: This research study was funded by the IFCE (Institut Français du Cheval et de l'Equitation), ref Project CS-2019-21, which covered all associated costs (e.g. audit, analysis, reporting etc).

12 | Changes over time in total IgG and vapA-specific IgG in mares and in their foals after *Rhodococcus equi* hyperimmune plasma administration

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Background: *Rhodococcus equi*-specific hyperimmune plasma (Re-HIP) is used prophylactically to prevent *R. equi* pneumonia. Changes over time of *R. equi*-specific antibodies in mares and foals remain poorly defined.
Objectives: 1) Evaluate the changes over time in total IgG and vapA-specific IgG after Re-HIP administration, 2) Compare foal results to those of the mare.
Study design: Pilot prospective study.
Methods: Serum was collected from mares (at foaling, 3 weeks, and 3 months after foaling) and their foals (24 h of life before Re-HIP, 48 h of life post 2 L of Re-HIP given IV, 3 weeks and 3 month of age). All Re-HIP bags were sampled. Total IgG and vapA-IgG were evaluated using ELISA. Repeated measures ANOVA (ANOVA on ranks) and t-test (Mann-Whitney test) were used for analysis.
Results: Neither IgG nor vapA-IgG change in mares over time. Foal IgG did not increase significantly after Re-HIP and decreased ($p < 0.05$) over time. Foal VapA-IgG increased after Re-HIP ($p < 0.001$) and returned to baseline values by 3 months of age. Total IgG did not differ between mares and foals before or after Re-HIP and was higher in mares by 3 weeks ($p = 0.04$) and 3 months ($p < 0.001$) after foaling. VapA-IgG was higher ($p = 0.2$) in mares before Re-HIP but was higher ($p < 0.05$) in foals at every timepoint thereafter. There was a large variation in total IgG (median 53, range 26-420 ng/mL) and vapA-IgG (median 27.4 x 10^3, range 19 to 41.5 x 10^3 EU) in HIP.
Main limitations: The number of animals included is limited. One Re-HIP product was evaluated.

Conclusion: While total foal IgG did not increase after 2 L of Re-HIP IV, vapA-IgG did. The total and vapA-IgG present in the Re-HIP was highly variable. Total and vapA-IgG does not appear to change in mares over time.

Ethical animal research: This project was approved by the University of Guelph's Institutional Animal Care Committee.

Informed consent: Managers gave consent for animals' inclusion.

Competing interests: None declared.

Sources of funding: Funding was provided by an internal grant from Washington State University and Equine Guelph, OVC.

13 | Plasma lipidome and metabolome of healthy and *Rhodococcus equi re*-infected foals over time

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Background: Foals with pulmonary ultrasonographic lesions consistent with *Rhodococcus equi* (*R. equi*) are treated with antibiotics because those at risk of developing pneumonia cannot be recognised early. Candidate biomarkers identified using an omics approach (lipidomics and metabolomics) may aid targeted treatment.

Objectives: To 1) Describe the changes that occur in foals' omics (lipidome and metabolome) due to ageing (birth to 8 weeks) and 2) Compare these results with those observed in foals after experimental infection with *R. equi*.

Study design: Pilot experiment.

Methods: Healthy new-born foals (n = 9) were infected with *R. equi* intratracheally during the first week of life. Foals were treated with antibiotics if they developed clinical pneumonia (n = 4, "clinical group") or were closely monitored if they showed no clinical signs of disease (n = 5 "subclinical group"). An unchallenged group (n = 4) was also included. All foals were free of disease (transtracheal wash fluid cytology and culture as well as thoracic ultrasonography) by 8 weeks of life. Plasma omics was determined weekly for 8 weeks using LC-MS and GC-MS.

Results: Both, ageing and experimental infection altered the foals' plasma omics as demonstrated by multivariate statistical analysis. The intensities of 31 lipids and 25 metabolites were altered by ageing and 12 lipids and 28 metabolites were changed by infection ($p < 0.05$). Furthermore, 9 lipids and 20 metabolites changed by more than 2-fold between the clinical and subclinical groups.

Main limitations: Low number of foals and experimental infection with *R equi*.

Conclusions: Ageing and *R. equi* infection induced changes in the plasma omics of foals. These results provide the background for future work in the discovery of early biomarkers of *R. equi* pneumonia. Early identification of foals at risk of developing clinical pneumonia is key to decreasing antimicrobial use and development of drug-resistant strains.

Ethical animal research: This project was approved by the University of Kentucky's Institutional Animal Care and Use Committee.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: Internal grant from Washington State University.

14 | Bacteriophages from Australian horse farms targeting *Rhodococcus equi*

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Background: *Rhodococcus equi* is a ubiquitous saprophyte with significant disease implications in foals. Emerging antimicrobial resistance and no vaccine has prompted research into novel strategies to control rhodococcosis. Viruses (bacteriophages) that kill *R. equi* have been isolated previously and could be exploited to mitigate *R. equi* on horse farms.

Objectives: To isolate and characterise bacteriophages infective for *R. equi* from NSW Thoroughbred farms and explore the genetic features related to host susceptibility to these viruses.

Study design: Observational study.

Methods: Bacteriophages were isolated and purified from farm soil and equine faeces by enrichment with *R. equi* culture, centrifugation and filtration of specimen supernatants. Host range was determined by plaque assays. Pulsed-field gel electrophoresis of bacteriophage stocks confirmed purity and approximate genome size. Viral structure was visualised through transmission electron microscopy. *R. equi* strains and bacteriophages were further characterised by whole genome sequencing (WGS) analysis, e.g. BLAST, Kraken, and multi-locus sequence typing (MLST).

Results: From 102 specimens, 21 bacteriophages were purified and 14 were characterised further. Host range differed ranging from highly specific (n=1) to broader spectrum (n=16); however, all

displayed tail structure typical of the *Siphoviridae* family. WGS of 11 bacteriophages produced genomes ranging from 46kb to 77kb. High sequence homology with previously reported *R. equi* bacteriophages was seen for five isolates, whilst others appeared to be novel. There were different degrees of susceptibility to phage infection across *R. equi* strains; however, no association with MLST type was identified.
Main limitations: Small sample size of bacteriophages and *R. equi* isolates considering the abundance and diversity of both.
Conclusions: Bacteriophages are easily sourced from Australian horse farms but vary in range of infectivity against *R. equi*. Genotyping of *R. equi* through MLST is unlikely to predict sensitivity to bacteriophages and further work is required to identify loci responsible for variation in host resistance.
Ethic animal research: Procedures were approved by the University of Sydney Animal Ethics Committee (Project Number 1319).
Informed consent: Farm managers gave informed consent.
Competing interests: None declared.
Sources of funding: AgriFutures Australia (PRJ-012079) with internal funding from the University of Sydney, including a bequest from the Murray Bain Horse Fund granted by the Sydney School of Veterinary Science.

Poster Presentation

15 | Tulathromycin and tulathromycin/rifampicyn to treat subclinical *Rhodococcus equi* pneumonia in foals

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Background: *Rhodococcus equi* (*R. equi*) pneumonia is an infectious disease of foals with a major financial impact on the horse-breeding industry. Control of *R. equi* at endemic farms relies on early detection of disease using thoracic ultrasonography plus antimicrobial therapy for all foals with pulmonary lesions. The recommended treatment is a combination of erythromycin, azithromycin, or clarithromycin, and rifampicyn, but adverse effects have been extensively reported (diarrhoea, respiratory distress and fatal hyperthermia). Tulathromycin has been thus suggested, but its effectiveness is still controversial.
Objectives: Investigate the effect of tulathromycin and tulathromycin/rifampicyn as a treatment for *R. equi* subclinical pneumonia in foals.
Study design: Retrospective clinical study.
Methods: The study included 1930 foals, from 11 foaling seasons (2009 to 2020), at a Thoroughbred farm in Buenos Aires, Argentina, where *R. equi* is endemic. Foals were screened by ultrasonography every 15 days starting at 5 weeks of age. Foals with one or more abscesses > 2 cm or many areas of consolidations were treated with tulathromycin if <3 cm, and with tulathromycin/rifampicyn if >3 cm or if lungs were further compromised.
Results: Of 1930 foals, 550 (28.5%) developed subclinical pneumonia attributed to *R. equi*; tulathromycin was used in 392 (71.3%) and the remaining 158 (28.7%) were given tulathromycin/rifampicyn. The average period of treatment with tulathromycin was 3 weeks (SD: 1), and with tulathromycin/rifampicyn 3.9 weeks (SD: 1.2) with a survival rate of 100%. The size of pulmonary abscesses reduced in all foals and none of them developed clinical pneumonia, hyperthermia or other adverse effects. No evidence of antimicrobial resistance was found.
Main limitations: The outcome of subclinical Rhodococcal pneumonia in foals without treatment was not assessed.
Conclusions: Tulathromycin, as monotherapy or combined with rifampicyn, is a reliable alternative for the treatment of foals with *R. equi* subclinical pneumonia.
Ethical animal research: Research ethics committee oversight not required: retrospective analysis of clinical data.
Informed consent: Not stated.
Competing interests: None declared.
Sources of funding: This work was supported by INTA - HARAS Agreement.

Oral Presentation

16 | The type of anticoagulant used for plasma collection significantly affects *in vitro Rhodococcus equi* assays

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Background: Anticoagulants (AC) used for plasma collection can negatively impact bacterial growth. Hyperimmune plasma (HIP) is used for prophylaxis against *R. equi* and the efficacy of *Rhodococcus equi*-specific HIP against *R. equi* is usually evaluated *in vitro*. However, the effect that the AC used may have on *R. equi* growth has not been evaluated.
Objective: To establish the effect that AC used in veterinary medicine (ACD, K_2EDTA, Li Heparin, and Na Citrate) have on *in vitro R. equi* growth.
Study design: Experimental study.

Methods: Brain Heart Infusion (BHI) containing *R. equi* was mixed with BHI-AC, horse plasma collected with different AC, or control. CFUs were counted at different timepoints. *R. equi* was opsonised with plasma using each AC and RAW264.7 macrophages were infected. Infected cells and CFU were counted at different times post-inoculation. Normality was assessed using Shapiro-Wilks test, variance using Levene's test. Data were analysed using repeated measures two-factor ANOVA, significance was set at $p<0.05$.

Results: There was no direct effect of ACD, Li Heparin or Na Citrate on *R. equi* growth. These three products significantly ($p < 0.001$) delayed growth for 12h post-opsonisation but there was no AC effect. Intracellular *R. equi* growth was significantly lower in Na Citrate ($p = 0.02$) 72 h post-infection. In contrast, direct contact with K_2EDTA completely inhibited the formation of *R. equi* colony forming units by 12 h as well as the intracellular growth at all the time points evaluated.

Main limitations: Only one strain was used for this study (*R. equi* #ATCC 103+).

Conclusions: K_2EDTA had a pronounced direct effect and resulted in intracellular growth inhibition whereas Na Citrate delayed intracellular *R. equi* growth. The use of ACD and Li Heparin appear to be more appropriate choices for the selected *in vitro* assays.

Ethical animal research: Plasma used for this study was obtained from horses from the WSU research herd.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: CVM Intramural Research Fund, WSU.

Streptococcus equi subsp. *equi*

Oral Presentations

17 | Globetrotting strangles: the unbridled national and international transmission of *Streptococcus equi* between horses

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Background: *Streptococcus equi* subspecies *equi (S.equi)* is the cause of the highly contagious equine respiratory disease 'strangles'. Approximately 10% of recovered animals can persistently carry the bacteria and transmit it to naïve animals. The global movement of horses is an ideal mechanism for widespread transmission to geographically distant locations.

Objectives: Utilise whole-genome sequence data to disentangle the transmission of *S. equi* and subsequent outbreaks of strangles.

Study design: *In vitro* analysis of micro-organisms.

Methods: Isolates (n = 670) of *S. equi* were recovered from clinical samples submitted to multiple collaborating clinics and institutions globally. Following species confirmation, isolates underwent whole-genome sequencing using Illumina technology. Sequence reads passing quality control measures were assembled and uploaded to Pathogenwatch, which assigned a phylogeny based upon sequences of core genome alleles. Population structure was inferred using the population mixture analysis in BAPS.

Results: BAPS clustered the isolates into six different clusters (BAPS 1-6) and showed dominant lineages in different geographical areas but also global transmission within the clusters. Sub-groups within the clusters highlighted multiple outbreaks at local, national and international scales and highlighted population structures and transmission dynamics within single locations. For example, four different strains collected over just seven months were identified in a single location. Sequence data also identified a statistically significant decline in BAPS-5 since 2010.

Main limitations: Pathogenwatch has shown its utility in investigating *S. equi* transmission and population structure. However, it is based upon a curated set of 1286 core genome loci. Further investigations will need to be conducted using the full spectrum of data available from whole-genome sequencing.

Conclusions: Pathogenwatch was used as a tool to rapidly identify and visualise the whole-genome sequence data of a large *S. equi* dataset. The data demonstrate widespread transmission of multiple *S. equi* lineages and provide strong evidence that asymptomatic carrier horses are perpetuating this dissemination.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Explicit owner consent was not stated but isolates were archived for further research with permission from submitting veterinarians.

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

Sources of funding: The Horse Trust, Estate of Paul Mellon Foundation, Alice Noakes Memorial Charitable Trust, Ivo Trust, Tattersalls, Elise Pilkington Charitable Trust, European Breeders Fund, Serth and Gates Charity, Margaret Giffen Charitable Trust, Payne Gallwey Charitable Trust, Stafford Trust, Marjorie Coote Animal Charity Trust, Beryl Evetts and Robert Luff Animal Welfare Trust and The Anne Duchess of Westminster's Charitable Trust. SW was funded by a grant from the Sir Peter O'Sullevan Charitable Trust. JRN was supported through a combined contribution to the AHT's Equine Infectious Disease Service from the Horserace Betting Levy Board, Racehorse Owners Association and Thoroughbred Breeders' Association. HW is funded by a grant from the Petplan Charitable Trust (S19-741-780). RC and OP were supported by the New Zealand Equine Research Foundation. SR, JP and MTGH were supported by Wellcome Trust (grant number 098051).

18 | Conservation of antigen sequences across a global population of *Streptococcus equi*

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Background: *Streptococcus equi* (*S. equi*) can be differentiated into six Bayesian-analysis-of-population-structure (BAPS) groups using core genome polymorphisms. However, conservation of *S. equi* genomes coding for antigens in the Strangvac protein-subunit vaccine has not been determined.

Objective: To define the diversity of Strangvac vaccine antigens in a diverse *S. equi* population.

Study design: *In vitro* analysis of micro-organisms.

Methods: Genomic antigen sequences of 1026 *S. equi* isolates from 19 countries between 1955 and 2019 were analysed. Predicted amino acid sequences of SEQ0256(Eq5), SEQ0402(Eq8), SEQ0721(EAG), SEQ0855(SclF), SEQ0935(CNE), SEQ0999(IdeE), SEQ1817(SclB), SEQ2101(SclC) (in Strangvac) and SeM were extracted from 1026 assembled genomes and compared.

Results: The predicted amino acid sequences of SclF, SclI and IdeE were identical across all 1026 genomes. CNE was truncated in the genomes of six (0.6%) isolates. SclC was absent from one genome and another encoded a single P^{85} to L substitution. EAG was truncated in two genomes. Eq5 was truncated in four genomes and 137 genomes encoded a single I^{201} to L substitution. Eq8 was truncated in three genomes, one genome encoded four amino acid substitutions (E^{212} to G, E^{214} to G, A^{218} to D and L^{223} to I) and 726 genomes encoded a single H^{225} to Y substitution at the final amino acid. Therefore, at least 1579 (99.9%) of 1580 amino acids in Strangvac were identical in 1009 (98%) genomes, and all genomes had identical amino acid sequences for at least six of the eight Strangvac antigens. For comparison, 86 different amino acid changes were identified within the N-terminal 107 amino acids of SeM encoded by this collection and 26 (2.5%) isolates encoded truncated forms of SeM.

Main limitations: The majority (655, 64%) of isolates in this study were recovered from horses in the UK.

Conclusions: The predicted amino acid sequences of antigens in Strangvac, but not SeM, were highly conserved across this collection of *S. equi*.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not stated.

Competing interests: J.-I. Flock and A.S. Waller are employed by Intervacc AB. B. Guss is a member of the board of Intervacc AB.

Sources of funding: The Horse Trust, Estate of Paul Mellon Foundation, Alice Noakes Memorial Charitable Trust, Ivo Trust, Tattersalls, Elise Pilkington Charitable Trust, The European Breeders Fund, Serth and Gates Charity, Margaret Giffen Charitable Trust, The Payne Gallwey Charitable Trust, Stafford Trust, Marjorie Coote Animal Charity Trust, Beryl Evetts and Robert Luff Animal Welfare Trust and Anne Duchess of Westminster's Charitable Trust. RJN was supported by the Horserace Betting Levy Board, Racehorse Owners Association and Thoroughbred Breeders' Association. HW is funded by a grant from the Petplan Charitable Trust (S19-741-780).

19 | Argentinian *Streptococcus equi* subsp. *equi* isolates clustered in the same group according to cgMLST

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Background: Strangles is a worldwide infectious disease that affects the upper respiratory tract of horses and is caused by *Streptococcus equi* subsp. *equi* (*S. equi*). Recently, a high number of *S. equi* isolates obtained from 19 countries were analysed using the Pathogenwatch core genome Multilocus Sequence Typing (cgMLST) web bioresource. All of the isolates recovered from Argentina (n=15) belonged to the same cluster and were closely related to a limited number of isolates from the United Kingdom and the United Arab Emirates.
Objective: To study genome diversity among Argentinian *S. equi* isolates and define their epidemiological relationships.
Study design: Epidemiological investigation using genomic analysis.
Methods: Genomic DNA from 44 isolates of *S. equi* were sequenced on a NextSeq 500 sequencer system (Illumina) and analysed within Pathogenwatch alongside the 15 previously described Argentinian *S. equi* genomes and the genome of strain 4047, which was included as a reference. The dendrogram was reconstructed from pairwise cgMLST scores using the APE package.
Results: Argentinian genomes were closely related and differentiated into three clusters. The *S. equi* 4047 genome was an outlier. Most of the isolates recovered from the same outbreak were closely related with some exceptions: UBA21Gd (*seM*-131) and UBA22Gd (*seM*-132) strains isolated from Daireaux in 2012 and UBA27Gd (*seM*-135) and UBA28Gd (*seM*-135) strains isolated from Trenque Lauquen in 2012. All of the isolates obtained from carriers during 2013 clustered in the same group. The strain UBA8Md that was isolated from a guttural pouch empyema belonged to a different cluster.
Main limitations: Further outbreak investigation and genome sequencing will strengthen the dataset.
Conclusions: Although *S. equi* isolates are closely related, the high resolution provided by cgMLST enabled their differentiation and the identification of genetically related strains affecting horses in Argentina.
Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on material collected previously during clinical procedures.
Informed consent: Not stated.
Competing interests: A.S. Waller is employed by Intervacc AB.
Sources of funding: PICT-2018-0242, UBACYT 20020190200042BA and UBACyT-20020170100537BA.

20 | Characterisation of *S. equi* strains by whole genome sequencing isolated from 2016 to 2019 in France

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Background: The prevalence of strangles, one of the most frequently diagnosed infectious diseases of horses worldwide, remains underestimated in France, despite causing economic losses to the equine industry. *Streptococcus equi* subsp *equi* (*S. equi*) is the causative agent and its persistence in guttural pouches of sub-clinical carriers plays an important role in the spread of the disease.
Objectives: The aim of this study was to use whole genome sequencing to understand the genetic relationships of *S. equi* isolates recovered in France from 2016 to 2019 and compare them with international data.
Study design: Longitudinal field study.
Methods: The French Network for epidemio-surveillance of equine diseases (RESPE), collected 55 isolates from 23 horses implicated in 13 different outbreaks. DNAs from 18 of these strains were extracted, purified and whole genome sequenced according to Illumina recommendations. Genomes were analysed using Pathogenwatch and visualised in Microreact.
Results: All isolates were classified as ST-179, recognised as "classic" *S. equi*. Genomes clustered into BAPS2 (Bayesian Analysis of Population Structure) but fell into two groups. Sequences in the first group, from different outbreaks (two in 2016 and one in 2017), differed from only some SNPs across the core genome and their closest relatives were previously recovered from horses in the United Arab Emirates. The second group were most closely related to other strains from France, Belgium, Spain, Sweden and the United Kingdom.
Main limitations: Not all available isolates have been sequenced to date and the history of the horses from which these isolates were derived requires further investigation.
Conclusions: Genomic analyses are undergoing to understand the differences between isolates from horses with acute strangles and sub-clinical carriers, by highlighting differences in genes involved in virulence, resistance, persistence or evasion of the immune system.
Ethical animal research: Research ethics committee oversight not required: retrospective analysis of clinical data.
Informed consent: Owners gave consent for their animals' inclusion
Competing interests: None declared.
Sources of funding: This study is supported by IFCE (Institut Français du Cheval et de l'Equitation), Fonds Eperon and Conseil Régional de Normandie.

21 | Preliminary investigation of the molecular epidemiology of *Streptococcus equi* in the United Kingdom 2016-2019

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Background: Combining epidemiological with pathogen-genomic data can help determine the most likely infectious transmission pathways within populations. For endemic diseases like strangles, caused by *Streptococcus equi*, molecular epidemiology could provide new insights into disease spread, informing development of effective preventive measures.
Objective: Collate epidemiological and pathogen-genomic data from the Surveillance of Equine Strangles (SES) Laboratory Network and characterise the genetics of *Streptococcus equi* isolates, enabling targeted epidemiological investigation.
Study design: Genomic surveillance.
Methods: *Streptococcus equi* isolates (n=356) submitted between 2016-2019 underwent whole genome sequencing (WGS). Accompanying epidemiological data, including submitting veterinary practice location, were obtained from laboratory submission forms. WGS data were assembled and then phylogenetic analysis was undertaken and epidemiological metadata added using the Centre for Genomic Pathogen Surveillance platform Pathogenwatch.
Results: Phylogenetic analysis of 356 *Streptococcus equi* isolates revealed 41 clusters of genetically identical isolates, 33 of which had isolates collected from ≥2 horses. For illustration, the largest cluster comprised 10 isolates recovered from 9 horses, submitted to two laboratories from 7 veterinary practices, based in 6 English regions during 2018. The first chronological detection of this cluster was in May 2018 from a carrier investigated after a positive iELISA serology test (Staffordshire). Isolates from this cluster were subsequently detected from single cases in September (Sussex), October (Suffolk), late-November and early-December (Essex, same practice) and mid-December (Northumberland). Identical isolates were also recovered from a horse in December (Cambridgeshire) and from the same horse and two others later that month (Staffordshire, single practice).
Main limitations: Time between sampling and WGS of isolates created a 'lag-phase' between outbreaks and genetic analysis.
Conclusions: Enhanced surveillance in collaboration with diagnostic laboratories in the UK, alongside the application of molecular epidemiology, has identified clusters of genetically identical *Streptococcus equi* isolates. Future investigations will likely enable the identification of transmission events between horses.
Ethical animal research: Approved by the Animal Health Trust's Clinical Research Ethics Committee (REF: 01-2017E) and the Royal Veterinary College's Clinical Research Ethical Review Board (URN 2020 1973-2).
Informed consent: Not stated.
Competing interests: A. Waller is employed at Intervacc.
Sources of funding: The Horse Trust and SEIB.

22 | Surveillance of equine strangles in the United Kingdom between 2015 and 2019 based on laboratory detection of *Streptococcus equi*

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Background: Strangles, a highly infectious bacterial disease caused by *Streptococcus equi*, remains a global problem in horses. Despite impacting equine populations and its ability to spread through international movement of horses, infection with *Streptococcus equi* is not recognised as a listed disease by the World Organization for Animal Health. Control of strangles is challenging due to limited surveillance, lack of awareness and/or failure to comply with biosecurity recommendations and pre-movement testing. Improved knowledge of laboratory diagnoses integrated with temporo-spatial analysis would permit the optimisation of biosecurity protocols and help decrease disease incidence.
Objective: Describe epidemiological data gathered from laboratory-confirmed diagnoses of strangles based on the detection of *Streptococcus equi* across the UK between 1 January 2015 and 31 December 2019.
Study design: Retrospective clinical series.
Methods: Seven UK laboratories reported strangles diagnoses within the study period based on identifying *Streptococcus equi* via agent detection assays from field-based practitioner-submitted samples. Associated clinical history and animal signalment were collected where provided, and descriptive analysis undertaken.
Results: Within the study period, 1617 laboratory-confirmed diagnoses from samples submitted by 315 veterinary practices were reported. Of these, 51.6% were from swab samples and 44.0% from

guttural pouch lavages. Diagnoses were primarily based on qPCR alone (59.6%), qPCR and culture (35.8%), or culture alone (4.6%). A total of 1791 clinical signs were reported among 713 diagnoses; nasal discharge (31.3%) and pyrexia (20.5%) were most frequent. Regions with the highest number of positive diagnoses were North Yorkshire (n=75, 4.6%), Staffordshire (n=71, 4.4%) and West Sussex (North-East) (n=63, 3.9%).

Main limitations: Limited and/or missing clinical history and signalment on laboratory submission forms restricts the completeness of the data.

Conclusions: This study provides insights into clinical features of and veterinary approaches to laboratory diagnosis of strangles in UK horses and details the epidemiology of strangles on a level previously unreported.

Ethical animal research: Approved by the Animal Health Trust's Clinical Research Ethics Committee (REF: 01-2017E) and the Royal Veterinary College's Clinical Research Ethical Review Board (URN 2020 1973-2).

Informed consent: Not stated.

Competing interests: A. Waller is employed at Intervacc.

Sources of funding: Surveillance of Equine Strangles is funded by the Horse Trust and SEIB.

23 | Improving the detection of horses exposed to strangles within Australia

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Background: The frequency of SeM antibody positive serum samples from Australian horses tested using the AHT Strangles dual antigen iELISA is typically lower than results reported in the UK.

Objectives: To measure and compare the levels of *se*M gene transcription in a diverse panel of *Streptococcus equi* ss *equi*.

Study design: *In vitro* analysis of micro-organisms

Methods: Twelve clinical isolates were selected to represent different clades for comparison with the Type strain Se4047 (UK, BAPS-5), Pinnacle IN vaccine variants (H+ and H-) and the SeM negative control, Se4592v. RT-qPCR was used to measure *se*M transcription compared with Se4047 and normalised to 4 x 10^5 copies of the core genes, *gyr*A, *gmk*, *pro*S.

Results: The mean copy number of *se*M in Se4047 (1.52 x 10^6, SD: 3.40 x 10^5) was significantly higher than all of the other isolates (P <0.05). BAPS1 isolate, USA09_12, genetically related to the Pinnacle IN vaccine, had the lowest levels of transcription (6.19 x 10^3 copies, SD: 3.85 x 10^3). The BAPS3 Australian isolate, Skyright (Mean: 6.71 x 10^5 copies, SD: 8.64 x 10^4), also had significantly lower levels of *se*M transcription compared to other isolates from Europe and the UAE, but similar levels to both of the Pinnacle IN vaccine variants.

Main limitations: The next step is to examine whether *se*M transcription corresponds with the levels of expression of the SeM protein.

Conclusions: Transcription of *se*M differed across the 12 diverse isolates and may explain why serum samples from Australian horses infected with *S. equi* contained lower levels of anti-SeM antibodies. The similarity observed between AusSkyright and the attenuated Pinnacle IN vaccine variants could suggest Australian isolates are less virulent than UK4047 and other isolates featured in this study.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: Payne-Gallwey Charitable Trust.

24 | *Streptococcus equi* subspecies *equi* avidity ELISA: a useful tool to detect carriers?

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Background: An indirect ELISA is often used to identify horses that have been exposed to *Streptococcus equi* (*S. equi*). The test can also be used as a screening test to detect carriers, although there are variable data regarding the sensitivity of this assay for this purpose. PCR testing of guttural pouch lavages is considered the gold standard for detecting carriers, but this requires expensive equipment, facilities and expertise and is also time consuming, costly and an invasive procedure. After a positive ELISA result, measuring the avidity of antibodies may be a good predictor for carrier status and provide an indication of the time of infection. Avidity assays are based on the fact that the first antibodies synthesised after an antigenic challenge or primary infection have a lower affinity for the antigen than those produced later on.

Objectives: To develop an avidity ELISA to distinguish between acute and chronic *S. equi* infections and between carriers and non-carriers.

Study design: Assay development.

Methods: An avidity ELISA was developed with antigen A of the enhanced iELISA using a range of Guanidine HCl concentrations. Serum panels from the Netherlands, UK and Sweden were tested and results were expressed as relative avidity index (RAI). Serum panels originated from longitudinally sampled well defined cohorts and diagnostic submissions with follow-up results regarding carrier status.

Results: A reproducible avidity ELISA was developed and standardised and ELISA positive sera showed significant variation in RAI values. However, no correlation was detected between chronicity of infection on the one hand and carrier status on the other.

Main limitations: OD values of samples from well-defined cohorts had declined, maybe due to long term storage or several cycles of freeze-thawing.

Conclusions: This avidity ELISA does not appear to have added value for the detection of *S. equi* carriers or discrimination between recent and older infections.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on archived material collected previously during clinical procedures.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: Internal funding GD Animal Health.

25 | Nasopharyngeal microbiomes in carrier donkeys shedding *Streptococcus equi* Subspecies *equi*

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Background: Several strangles outbreaks have been reported in donkey farms in China in the last few years and pose a great threat to health, production, and the welfare of donkeys. The microbiome of the nasopharynx may provide insights into the health of the upper respiratory tract. There has been no study investigating the nasopharyngeal microbiome in healthy donkeys, nor in donkeys shedding *Streptococcus equi* subsp. *equi* (*S. equi*).

Objectives: This study aimed to compare nasopharyngeal microbiomes in healthy and carrier donkeys using 16S rRNA gene sequencing.

Study design: Cross-sectional.

Methods: Nasopharyngeal samples were obtained from 16 donkeys recovered from strangles (group S) and 14 healthy donkeys with no history of strangles exposure (group H). Of those sampled, 7 donkeys were determined to be carriers with positive PCR and culture results in group S. In group H, all 14 donkeys were considered free of strangles based on the history of negative exposure, negative results of PCR and culture. Samples from these 21 donkeys were used for microbial analysis. The nasopharyngeal microbiome composition was compared between the two groups.

Results: As reported previously [1], at the phylum level, relative abundance of Proteobacteria was predominantly higher in the *S. equi* carrier donkeys than in healthy donkeys ($P < 0.01$), while Firmicutes and Actinobacteria were significantly less abundant in the *S. equi* carrier donkeys than in healthy donkeys ($P < 0.05$). At the genus level, *Nicoletella* was detected in the upper respiratory tract of donkeys for the first time and dominated in carrier donkeys where it is suspected to suppress other normal flora of URT microbiota including *Streptococcus* spp., *Staphylococcus* spp., and *Corynebacterium* spp.

Main limitations: The sample size was relatively small.

Conclusions: The nasopharyngeal microbiome in *S. equi* carrier donkeys still exhibited microbial dysbiosis, which might predispose them to other airway diseases.

Reference

[1] Zhu, Y., Chen, S., Yi, Z., Holyoak, R., Wang, T., Ding, Z. and Li, J. (2021) Nasopharyngeal Microbiomes in Donkeys Shedding Streptococcus equi Subspecies equi in Comparison to Healthy Donkeys. *Front. Vet. Sci.* 8:645627. https://doi.org/10.3389/fvets.2021.645627

Ethical animal research: All procedures involving animals were conducted in compliance and within the license (No.AW11110120 2-2-0) granted by the Animal Welfare and Ethics Committee of China Agriculture University.

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

Competing interests: T. Wang and Z. Ding were employed by the company Dong-E-E-Jiao Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Source of funding: Introduction of Talent Research Start-up Fund from China Agricultural University (grant number 31051017).

26 | Strangles screening pre- and post-import of horses into the United Arab Emirates: A review of 5604 horses imported between 2018-2019

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Background: Strangles infection either through active infection or carriers presents a significant management challenge to the international movement of horses and has welfare and economic consequences. A large shipment of horses with active strangles infection had major consequences in 2016 so new strategies were looked at to prevent the reoccurrence.

Objectives: To ascertain whether a modified testing protocol using the dual antigen iELISA to detect anti-*S. equi* antibodies provided appreciable benefits in reducing the risk of importation of a horse infected with strangles. Using a large pool of horses meeting the inclusion criteria, to compare the pre-import testing and post-import results of this group to establish the relative success or failure of this proposed protocol.

Study design: Retrospective clinical study.
Methods: A review of 5604 horses imported into the United Arab Emirates in 2018 and 2019 was undertaken. Clinical records and laboratory reports from 5604 horses which met the criteria for inclusion were analysed.
Results: Utilising this modified screening approach, the risk of importing a horse which subsequently tested positive for strangles by PCR in this timeframe was 0.07%.
Main limitations: Limiting factors include the lack of pre-import data for horses which tested positive and were not disclosed to the author. The effect of stress through international travel of these horses and the relative increase in antigen values as a result requires further analysis.
Conclusions: The dual antigen iELISA provides a robust assay to identify anti-*S. equi* antibodies serologically, but for international movement it is not always possible to temporally compare the assay in the same laboratory. Nevertheless, the modified approach detailed above provides a valuable screening tool.
Ethical animal research: Research ethics committee oversight not required by this journal: retrospective analysis of clinical data.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: None.

27 | *Streptococcus equi* subspecies *equi* seroprevalence in different subpopulations of the Dutch horse sector

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Background: Strangles, caused by *Streptococcus equi*, is one of the most frequently diagnosed infectious equine diseases worldwide. In recent years, an indirect ELISA has been developed and validated to identify horses that have been exposed to *S. equi*. This method has been applied to evaluate the prevalence of exposure to *S. equi* in several countries with seroprevalence estimates ranging between 8-42%.
Objectives: To estimate the seroprevalence of strangles in the Dutch equine population.
Study design: Retrospective surveillance.
Methods: 462 serum samples from various subpopulations (slaughterhouse sera, sera from export horses, sera collected in the context of WNV surveillance, and sera from foals and yearlings (6-18 months of age) were tested.
Results: In total, 5.6% of these sera were positive (OD ≥0.5) and another 5.6% of these sera were borderline (OD 0.3 or 0.4). The prevalence of seropositive horses differed in the various subpopulations: 10% of the slaughterhouse sera (n=100), 11% of sera from export horses (n=110), 3% of sera collected in the context of WNV surveillance (n=144), and none (0%) of the sera from foals and yearlings (n=82) tested positive.
Main limitations: Because sera were anonymised prior to testing, no individual follow-up was possible and the infection status of seropositive horses could not be determined.
Conclusions: The seroprevalence in export horses was comparable to seroprevalences reported for Sweden, the Irish Thoroughbred population, UK and Israel, but lower than seroprevalences reported for Croatia and Poland. The seroprevalence in slaughter horses (tail of the market) was comparable to seroprevalences reported for working horses in Lesotho and Ethiopia, but much lower than for unregulated horses in Ireland. However, the 3% seroprevalence for smaller premises in the Netherlands (livery yards and riding schools with <20 horses) was significantly lower than expected and the seroprevalence in young horses was 0%.
Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on archived material collected previously during clinical procedures, at abattoirs and for surveillance programmes.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: Internal funding GD Animal Health.

28 | Functional activities of antibody responses following vaccination of ponies with a multicomponent subunit vaccine against strangles

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Background: *Streptococcus equi* employs a range of virulence mechanisms to attach to equine tissue and subvert the host's immune response.
Objectives: To determine if blood sera from ponies vaccinated with Strangvac inhibited the ability of *S. equi* to bind collagen and degrade immunoglobulin.
Study design: *In vitro* experiments.
Methods: Serum samples were collected from ponies vaccinated during safety and efficacy studies. *S. equi* was treated with 4-fold dilutions of sera and added to collagen-coated micro-titre plates for 2 hours at 37°C. Unbound bacteria were removed by washing and remaining bacteria were stained with crystal violet. Cleavage of immunoglobulin by recombinant IdeE was measured by Western blot following incubation for 16 hours at 37°C with 5-fold serial dilutions of sera.

Results: Sera from ponies significantly inhibited the binding of *S. equi* to collagen after the third dose of Strangvac was given at 3 (P=0.02) or 12 (P<0.0001) months after second vaccination. However, sera taken 12 months post-second vaccination, but before third vaccination did not inhibit *S. equi* binding to collagen. Similarly, sera taken from ponies after vaccination with a third dose of Strangvac significantly inhibited the ability of IdeE to cleave immunoglobulin regardless of whether the third dose was administered at 3 (P=0.005), 6 (P=0.03) or 12 months (P=0.03) after second vaccination. IdeE activity was also significantly impaired by sera taken from ponies immediately prior to third vaccination at 3 (P=0.007) and 6 (P=0.05), but not 12 months (P=0.1) after second vaccination.

Main limitations: Low numbers of ponies (4 per group) received third vaccination at 6 months and 12 months post-second vaccination in this experiment.

Conclusions: Strangvac induced a serum antibody response that neutralised two key virulence functions of *S. equi*, persisted for up to 6 months post-second vaccination and was restored after a third vaccination up to 12 months after the previous dose.

Ethical animal research: Ponies were vaccinated and blood serum samples were collected under the auspices of a Home Office Project License and following ethical review and approval by the Animal Health Trust's Animal Welfare and Ethical Review Body (RPP 01_08).

Informed consent: Not applicable.

Competing interests: J.-I. Flock and A.S. Waller are employed by Intervacc AB. B. Guss is a member of the board of Intervacc AB.

Sources of funding: Intervacc AB.

Streptococcus equi subsp. *zooepidemicus*

Oral Presentations

29 | Endometritis in donkeys associated with *Streptococcus equi* subspecies *zooepidemicus* infection

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Background: Endometritis has recently become one of the most important infectious diseases in donkey breeding farms where biosecurity precautions are inadequate. However, so far, no research has been conducted to identify the causative agents.

Objectives: To identify the causative agents of endometritis in donkeys in intensive breeding farms.

Study design: Cross-sectional.

Methods: One breeding farm was selected as an endometritis research model for the isolation and identification of causative agents and epidemiological investigation. PCR-based techniques were used to detect *Taylorella spp* and *S. zooepidemicus*. Bacterial isolation was performed to identify potential causative agents by matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS). The sensitivity of bacteria to antibiotics was conducted by the disc diffusion method and used to guide treatment.

Results: Based on clinical observations, 17 of 84 (20.2%) jennies exhibited milky and purulent vaginal discharge after repeated breeding. The remaining 67 jennies and unbred female donkeys (n=29) did not show any clinical signs of oestrous behaviour/endometritis. PCR results showed all 17 affected donkey mares and 31 of 67 (46.3%) donkeys without clinical signs were positive for *S. zooepidemicus* infection. Based on the PCR diagnosis results, the infection rate was 57.1% in donkey mares at this farm and the presence of *S. zooepidemicus* was significantly associated with disease (P<0.0001, two-tailed Fisher's exact test). None of 29 female donkeys that had not been used for breeding tested positive for *S. zooepidemicus* and none of the donkeys tested positive for *Taylorella spp*. Uterine lavage with penicillin and streptomycin proved to be an effective treatment.

Main limitations: This study was conducted on only one farm.

Conclusions: Endometritis associated with *S. zooepidemicus* infection in donkeys led to a greater understanding of the causative agent and the epidemiological characteristics of endometritis in intensive donkey breeding farms.

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

Informed consent: Not stated.

Competing interests: None declared.

Sources of funding: Weifang Science and Technology Development Program (2020GX039), the Open Project of Shandong Collaborative Innovation Center for Donkey Industry Technology (3193308), Natural Science Foundation of Shandong Province (ZR2020MC182), Shandong Provincial Youth Innovation and Technology Support Program (2019KJF018), Shandong Provincial Modern Agricultural Industry Technology System (SDAIT-27) and National Natural Science Foundation of China (31902265).

30 | Molecular characterisation of *Streptococcus equi* subsp *zooepidemicus* isolated from endometritis in horses from Buenos Aires, Argentina

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Background: *Streptococcus equi* subsp *zooepidemicus* (*Sez*) is an opportunistic pathogen. It is part of the microbiota of the lower genital tract in horses and is the most isolated pathogen causing endometritis in mares. It has a high genetic diversity and it may be isolated in numerous pathologies and in different animal species, even in humans. Multilocus sequence typing (MLST) is frequently used to characterise bacterial isolates of this microorganism.
Objectives: To genetically characterise the strains of *Sez* circulating in Buenos Aires province, Argentina.
Study design: Genetic analysis of micro-organisms.
Methods: Thirty isolates obtained from the uterus of mares with reproductive problems between 2005 and 2017 were studied. The MLST analysis and the sequencing of the obtained DNA were carried out. Alternative patterns of descent were identified using the eBURST algorithm – goeBURST [1].
Results: Fourteen isolates had a sequence type (ST) previously described in the database which had been isolated from various anatomical locations in equines, humans, bovines and canines. Only six isolates shared three different STs. Sixteen isolates had novel alleles, and of these, only two ST were identical. When the possible phylogenetic relationships were analysed, some similarity was observed between the alleles in twelve isolates, and only four possible clonal complexes of between two and three ST each were observed.
Main limitations: The small number of strains.
Conclusions: The strains of *Sez* isolated from the uterus of mares from Buenos Aires province, were not closely related and these isolates were also found in other animal species and in other locations. The high genetic diversity of *Sez* observed, could be related to the wide distribution of this microorganism in horses and in other animal species.
Reference:
[1] Francisco, A., Bugalho, M., Ramirez, M. *et al.* (2009) Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach. *BMC Bioinformatics* **10**, 152.
Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on material collected previously during clinical procedures.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: UBACYT 20020190200389BA.

Poster Presentations

31 | Identification of *Streptococcus zooepidemicus* strains from an outbreak of respiratory disease in Welsh mountain ponies

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Background: *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) is often recovered from horses with respiratory disease, but standard strain typing methods are insufficient to resolve the identity of pathogenic strains.
Objectives: To identify *S. zooepidemicus* strains from cases of respiratory disease that share identity/near identity across the ~1.8Mb core genome.
Study design: Cross sectional.
Methods: Cases were 6-8 month-old Welsh mountain ponies with signs of respiratory disease, including coughing, nasal discharge and pyrexia. Nasopharyngeal swabs from cases were tested for equine influenza virus (EIV), equine herpesvirus-1 and -4 (EHV-1/EHV-4), and *S. zooepidemicus* by qPCR. For 31 isolates of *S. zooepidemicus* recovered, multi-locus sequence types and variation across the core genome were determined in Pathogenwatch (https://pathogen.watch/).
Results: None of the nasopharyngeal swabs tested positive for EIV or EHV-1, one (5%) tested positive for EHV-4, and 18 (90%) tested positive for *S. zooepidemicus*. The 31 sequenced isolates comprised nine different multi-locus sequence types (STs): ST43 (n=2), ST49 (n=2), ST103 (n=6), ST106 (n=5), ST118 (n=6), ST150 (n=1) ST300 (n=3), ST366 (n=1) and ST418 (n=5). The mean core genome variation of differing STs was 29,666 nucleotides; approximately 2% of the core genome. However, the core genomes within ST43, ST106, ST118 and ST418 were identical and those of ST103 differed by a maximum of 1 nucleotide; which together comprised 77% of sequenced isolates.
Main limitations: No challenge of naïve ponies with potential disease-causing *S. zooepidemicus* strains, extensive viral serology or screening of healthy ponies was conducted.
Conclusions: Comparison of the core genome sequences of these isolates provided evidence that five strains of *S. zooepidemicus* (ST43, ST103, ST106, ST118 and ST418) were actively circulating in ponies. The much higher prevalence of *S. zooepidemicus* compared to common viral pathogens, suggests this pathogen may have the potential to cause the observed respiratory signs.

Ethical animal research: Approved by the Animal Health Trust's Animal Welfare and Ethical Review Body (RPP 01_08).
Informed consent: Not applicable.
Competing interests: J.-I. Flock and A.S. Waller are employed by Intervacc AB. B. Guss is a member of the board of Intervacc AB.
Source of funding: Intervacc AB.

32 | Antimicrobial sensitivity of *Streptococcus equi* subsp *zooepidemicus* isolates from Buenos Aires, Argentina

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Background: Endometritis causes many economic losses and is the most common cause of infertility in mares. The most frequently isolated pathogen is generally *Streptococcus equi* subsp *zooepidemicus (Sez)*. Treatment includes β-lactams as first line antibiotics. Recently, the resistance of *Sez* to different antibiotics has increased around the world. In Argentina, to date, there is no epidemiological surveillance information of antibiotic resistance in this pathogen.
Objectives: To determine the susceptibility of isolates of *Sez* to several antimicrobial agents.
Study design: *In vitro* analysis of micro-organisms.
Methods: Antibiotic susceptibility tests were performed on *Sez* isolates that were sent to two laboratories between 2005-2017. Thirty isolates obtained from the uteri of mares with reproductive problems were studied. The minimum inhibitory concentration (MIC) was determined according to the recommendations of CLSI [1] for *Streptococcus* spp. β-haemolyticus. MIC was performed by the agar dilution method for PEN, ceftazidime (CAZ), erythromycin (ERY), clindamycin (DA), tetracycline (TET) and enrofloxacin (ENR) and for trimethoprim-sulfamethoxazole (TMS) by the broth dilution method.
Results: All isolates were susceptible to TMS, PEN and ERY. Resistance rates were: 40% to CAZ and DA, 3% to TET and 100% to ENR.
Main limitations: The *Sez* isolates were recovered from the northwest of Buenos Aires Province.
Conclusions: These results provide evidence that ongoing epidemiological surveillance of *Sez* isolates and their microbial susceptibility is necessary to avoid the selection of resistant strains.
Reference
[1] Clinical and Laboratory Standards Institute (2018) CLSI VET08. 4°ed

33 | Effect of different carbohydrate sources in the production of biofilm of *Streptococus equi* subsp. *zooepidemicus*

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Background: The zoonotic bacterium *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*) is considered part of the normal microbiota of nasopharyngeal and vaginal mucus of healthy horses, and it may cause upper respiratory tract, opportunistic infections and endometritis. The ability of *S. zooepidemicus* to produce biofilm has been previously reported. Biofilm may be considered as a virulence factor that allows bacteria to persist in different niches, especially in the equine endometrium. Some *S. zooepidemicus* strains use different sugar pathways which may confer a nutritional advantage over other commensal microorganisms. Also, sugar metabolism diversity might improve the biofilm production.
Objective: To study the effect of sucrose, mannose and galactose in the biofilm production of *S. zooepidemicus*.
Study design: *In vitro* analysis of micro-organisms.
Methods: Biofilm production was evaluated by a colorimetric method. Isolates were grown in Tryptone Soya Broth with the addition of galactose, sucrose and mannose on 96 well microplates at 37°C for 24 h, 48 h and 72 h in microaerophilic conditions. Biofilm production was measured after violet crystal staining and alcohol elution at λ=570 nm (DINEX MRX Revelation).
Results: Differences in the *S. zooepidemicus* biofilm formation were observed at different times with the addition of the carbohydrates studied, especially for galactose and mannose.
Main limitations: Only *S. zooepidemicus* biofilm production *in vitro* was performed in this study.
Conclusions: These results are important to expand our knowledge of the nutritional environment for *S. zooepidemicus* biofilm production. The role of biofilm and sugar utilisation, in the future, may be used for development of new treatments.
Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on material collected previously during clinical procedures.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: UBACYT 20020190200389BA.

34 | Genomic analysis of a *Streptococcus dysgalactiae* subsp. *equisimilis* strain associated with mastitis in an 8-day-old Arabian filly

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Background: *Streptococcus dysgalactiae* subsp. *equisimilis* (SDE) is a beta-haemolytic *Streptococcus*, which is frequently reported in human infections. SDE is part of the microbiota of skin and mucous membranes in horses and causes opportunistic infections. Although mastitis in an equine is uncommon, when it occurs, it is often associated with beta-haemolytic *Streptococci* spp. Mastitis in fillies was previously described in two clinical cases, one of them caused by the *Streptococcus dysgalactiae* group and the other by a beta-haemolytic *Streptococcus*.

Objective: To study the genome of a SDE strain isolated from a filly suffering mastitis.

Study design: *In vitro* analysis of micro-organisms.

Methods: An 8-day-old Arabian filly presented with clinical mastitis during January 2020 in Buenos Aires, Argentina. Purulent discharge was cultured on a blood agar plate, identified by API 20 Strep (bioMerieux) and sequenced on a NextSeq 500 sequencer system (Illumina). DNA sequence reads were assembled using SKESA and a pipeline script in Ridom SeqSphere+ (minimum of 30-fold coverage across the genome). The genome was annotated using RAST and analysed using ARTEMIS and Galaxy.

Results: The CRESAL27508 strain was identified as SDE. The MLST alleles (*gki* 23, *gtr* 26, *murI* 20, *mutS* 23, *recP* 38, *xpt* 42, *atoB* 24) represented a novel sequence type (ST) closely related to ST-231. Antibiotic resistance genes were found, which encoded the multidrug resistance protein ErmB, the multidrug resistance efflux pump PmrA and the efflux transporter BmrC. Loci encoding phosphoenolpyruvate-protein phosphotransferase systems (PTS) were found, related to hyaluronate-oligosaccharide, cellobiose, fructose and ascorbate utilisation.

Main limitations: No experimental challenge of horses was performed to induce mastitis and confirm causality.

Conclusions: CRESAL27508 is proposed to be an example of the horse-specific *S. dysgalactiae* genomovar. Of note, the closely related ST-231 was isolated from the uterus of a mare in Italy.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on material collected previously during clinical procedures.

Informed consent: Not stated.

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

Sources of funding: PICT-2018-0242, UBACYT 20020190200042BA and UBACyT-20020170100537BA.

Taylorella equigenitalis

Oral Presentations

35 | Sequential incidence of bacterial venereal pathogens following topical antimicrobial treatment for *Taylorella equigenitalis*

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Background: *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are reportedly venereally transmitted pathogens and their risk-association following topical antimicrobial treatment of the external genitalia of stallions is undefined.

Objectives: To monitor the sequential incidence of *P. aeruginosa* and *K. pneumoniae* in stallions subsequent to topical antimicrobial treatment of their external genitalia for *T. equigenitalis*.

Study design: Consecutive controlled case series.

Methods: Swabs for bacterial culture were obtained from genital predilection sites of all resident stallions (n=32) at the South African Lipizzaner Centre, including *T. equigenitalis*-positive and subsequently treated (n=23) and *T. equigenitalis*-negative and untreated (n=9), respectively. Sampling on five occasions over a 12-month period followed topical treatment with either 0.2% nitrofurazone- (n=13) or 1% silver sulphadiazine-containing emollients (n=10). Range to elimination of *T. equigenitalis* was 9-65 days without a difference between the two agents. There were nine untreated stallions and one was bred by natural mating during the observation period.

Results: One-month post-treatment, 10/14 treated stallions tested positive for either *P. aeruginosa* (n=6) or *K. pneumoniae* (n=4), with one positive for both. Eight were negative by the second month, nine by four months and one remained positive for *K. pneumoniae* at 12 months. Untreated stallions showed one positive (*K. pneumoniae*) at one-month and thereafter none were positive until 12-months, with four *K. pneumoniae*-positive.

Main limitations: No stallions were swabbed for bacterial culture prior to the *T. equigenitalis* outbreak.

Conclusions: There was a transient and similar association of treatment with isolating *P. aeruginosa* and *K. pneumoniae*, arguably challenging the duration of persistence of the carrier state in stallions. Interestingly, although untreated stallions were all initially negative for both bacteria, 44% subsequently swabbed positive for *K. pneumoniae* after 12 months, and only one was bred during that time. Although a small sample size, these findings support further

investigation of the risks of topical treatment and persistence of carrier status for bacterial venereal pathogens in stallions.

Ethical animal research: Approved by the National Department of Agriculture, Forestry and Fisheries, Republic of South Africa.

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

Competing interests: None declared.

Sources of funding: Funding for this study was provided by Equine Research Centre, University of Pretoria, South Africa and the Department of Agriculture, Forestry and Fisheries, Republic of South Africa.

36 | Comparison of molecular testing methods for detecting *Taylorella equigenitalis*

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Background: *Taylorella equigenitalis* is a causative bacterium of contagious equine metritis (CEM), which usually results in temporary infertility in mares. A rapid and reliable diagnostic method is needed to prevent the spread of CEM.

Objective: To compare seven molecular testing methods (one quantitative PCR [qPCR], four conventional PCR [cPCR], one semi-nested PCR, and one LAMP) to detect *T. equigenitalis* for detection limit and positive number of samples from experimentally infected cases.

Study design: Experimental assay comparison.

Methods: The detection limits of the seven molecular assays were evaluated using five *T. equigenitalis* strains. Assays were performed three times with triplicate samples of each strain that had been serially diluted 10-fold. The detection limits of each assay, in which 95% of the diluted samples were positive, were calculated using the Reed and Muench method. To obtain clinical samples, two Thoroughbred horses were experimentally infected with *T. equigenitalis* and a total of 42 genital samples (e.g. clitoral sinus and fossa) were collected at intervals for two weeks. After extracting genomic DNA, each type of molecular assay was conducted.

Results: Of the seven assays, qPCR showed the lowest 95% detection limit (0.77 fg/reaction), followed by LAMP (0.89 fg/reaction) and semi-nested PCR (6.42 fg/reaction), while those of some of the cPCRs were > 100 fg/reaction. In clinical samples, qPCR and semi-nested PCR showed the highest positive numbers (33 out of the 42 samples), but some of the cPCRs detected only two or seven positive results.

Main limitation: No naturally infected samples were used.

Conclusion: The detection limits and positive numbers of infected samples were shown to vary greatly according to the molecular method employed. Our results indicate that the use of sensitive molecular assays such as qPCR and semi-nested PCR is important for the efficient detection of *T. equigenitalis* in clinical samples.

Ethical animal research: This study was approved by the Animal care committee of the Equine Research Institute with accession number 16-9.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: Japan Racing Association.

Poster Presentations

37 | Overview of spatio-temporal distribution inferred by multilocus sequence typing of *Taylorella equigenitalis* isolated worldwide from 1977 to 2018 in equidae

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Background: Contagious equine metritis (CEM), a highly contagious bacterial venereal infection of equids, caused by *Taylorella equigenitalis*, is of worldwide concern and occurrence must be notified to the World Organisation for Animal Health (OIE).

Objectives: To assess the distribution of MLST genotypes from a large population of *T. equigenitalis* strains mainly originating from several European countries.

Study design: *In vitro* analysis of micro-organisms.

Methods: Comparison of 367 *T. equigenitalis* strains using MLST according to the geographical origin, isolation year and equine breed.

Results: The strains were divided into 49 sequence types (STs) grouped into three major and three minor clonal complexes (CCs), and 11 singletons. Overall, the genetic heterogeneity was low (0.13 STs/strain) despite the wide diversity of geographical origins (n=16), isolation years (1977-2018) and equine breeds (n=18). Current major STs and CCs already existed before 1998. Previous data associated the major CC1 with the first CEM outbreaks in 1977-1978 in the UK, Australia and the USA, and revealed its circulation in France. Our

study confirms its circulation in France from 1992 to 2018 and its distribution in Spain and Germany. In addition to CC1, relationships between non-European and European countries were observed only through ST4, ST17 and ST30. Within Europe, several STs emerged with cross-border circulation, in particular ST16 and ST46 from the major complexes CC2 and CC8.

Main limitations: A retrospective analysis of a higher number of strains isolated outside Europe would allow an exhaustive picture of the original CEM situation.

Conclusions: This study provides new insights into the molecular epidemiology of *T. equigenitalis* in Europe and constitutes a baseline for monitoring the spread of CEM outbreaks [1].

Reference

[1] Duquesne F. *et al*. (2020) Overview of spatio-temporal distribution inferred by multi-locus sequence typing of *Taylorella equigenitalis* isolated worldwide from 1977 to 2018 in equidae. *Vet. Microbiol.* Mar. **242**, 108597.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: ANSES and the European Commission through DG SANTE funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness.

38 | MALDI-TOF MS for the differentiation of *Taylorella equigenitalis* and *Taylorella asinigenitalis*

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Background: Contagious equine metritis (CEM) is an equine venereal disease caused by *Taylorella equigenitalis*, a slow-growing capnophilic Gram-negative coccobacillus. CEM is a notifiable disease to the World Organisation for Animal Health (OIE). Diagnosis is based on the isolation and identification of *T. equigenitalis* by conventional bacteriology [1]. Misidentification with *Taylorella asinigenitalis* is observed because phenotypic tests discriminate insufficiently.

Objectives: Matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) is becoming an indispensable tool in clinical microbiology; its performance is evaluated for the CEM diagnosis.

Study design: *In vitro* analysis of micro-organisms.

Methods: Eighty-five *T. equigenitalis* and 28 *T. asinigenitalis* strains were selected on their MLST genotype. MALDI-TOF analyses were performed from three colonies isolated per strain on chocolate agar after 48 and 72 h of incubation at 37±2°C and 7% CO_2 in air. Three sample preparation procedures were tested: *(i)* direct colony spotting, *(ii)* addition of formic acid on direct colony spotting, and *(iii)* total protein extraction spotting. Sixteen in-house *Taylorella* reference spectra were generated to expand the commercial Bruker database.

Results: MALDI-TOF is a reliable tool for the species-level identification and differentiation of *T. equigenitalis* and *T. asinigenitalis*. Direct spotting of 48-h colonies was the most efficient protocol and the easiest to implement in a clinical setting [2].

Main limitations: Commercial databases must be expanded with *T. asinigenitalis* reference spectra to achieve the expected *T. asinigenitalis* identification.

Conclusions: MALDI-TOF is a useful and rapid diagnostic tool to differentiate CEM from suspicious colonies isolated according to OIE methods [1].

References

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Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: ANSES and the European Commission through DG SANTE funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness.

General

Diagnosis and reporting of equine infectious disease

Oral Presentations

39 | Review of equine diagnostic tests recommended in the World Organisation for Animal Health (OIE) Terrestrial Manual

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Background: In order to provide a standardised approach to the diagnosis of diseases and to facilitate health certification for trade,

the OIE Terrestrial Manual standards include internationally agreed laboratory diagnostic techniques.

Objective: To review the type of tests recommended in the equine disease-specific chapters of the Manual for each of the six most common purposes for diagnostic techniques.

Study design: Literature appraisal.

Methods: A database was compiled of the test methods and their purpose; the (1) population freedom from infection, (2) certification of freedom from infection for trade/movement, (3) eradication of disease, (4) confirmation of a diagnosis of suspect or clinical cases, (5) estimation of prevalence (surveillance) and (6) determination of immune status post-vaccination. Only tests that scored +++ "recommended" or ++ "suitable" by the OIE equine disease experts were included.

Results: Preliminary analysis of recommended tests in the OIE Manual clearly demonstrated the increasing replacement of agent detection by traditional methods such as bacterial culture/virus isolation (30%) and antigen detection (11%) in agent detection across the five relevant purposes for equidae, with PCR (54%). Similarly, the majority of recommended tests in the OIE Manual for detection of the immune response are ELISA tests (38%) which are frequently commercially available. However, some classical techniques for example virus neutralisation (15%) and the complement fixation test (13%) continue to be well represented due, for example, to their sensitivity or proven track record. In contrast, novel technologies such as microarrays and isothermal amplification techniques are slow to gain acceptance.

Main limitations: It is unclear if the majority of the tests recommended are validated to OIE standard.

Conclusions: To assist those who consult the Manual, consideration could be given to the inclusion in each chapter of a section devoted to test application, including the use of combinations of tests particularly for trade/movement.

Ethical animal research: Research ethics committee oversight not required by this journal: Data available in the public domain.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: Funding was provided by the Department of Agriculture, Food and the Marine, Ireland.

Acknowledgements The authors would like to thank Evelyn Ryan MSc. for her assistance with the compilation of tables.

40 | Development and delivery of an interactive international equine infectious disease collating and reporting platform

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Background: With the intention of keeping stakeholders informed and facilitating timely disease control and prevention, the International Collating Centre (ICC), based in the UK, has been collating and sharing reports on equine disease outbreaks since 1987.

Objectives: To develop an online interactive disease collating and reporting platform, with near real-time updating, national and regional mapping and period-specified searching and summarising capabilities.

Study design: Database and associated interactive reporting platform development.

Methods: A cloud-based PostgresSQL database was developed with a PostGIS module to facilitate both descriptive and spatial data entry and analysis. Web-based dashboards were developed in R using the Shiny package. Information from diagnostic laboratory confirmed disease incidents were obtained from a range of existing international and country-specific equine disease reporting systems and ICC-registered country contacts and laboratories.

Results: The online web interface http://www.jdata.co.za/iccviewer, populated with all ICC reports issued since 1 January 2019, was launched in August 2019. The website provides an interactive archive able to generate aggregated reports in multiple formats; as tables of total reports of each disease from each country or as interactive maps showing either country- or region-level distributions. Specific outbreak listings can also be derived from searches with key epidemiological information available in each report. Furthermore, since its launch, individual ICC disease reports have been issued in near real-time, once a day, by email to its subscriber list, with embedded links to specific disease incident reports in individual countries.

Main limitations: Equine infectious disease information is only available for inclusion in ICC reports for a limited number of countries, is provided voluntarily and laboratory confirmations are not subject to consistent quality control protocols.

Conclusions: The ICC website now provides a comprehensive resource of collated equine infectious disease diagnoses internationally since 1 January 2019 and is being maintained in near real-time.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: International Thoroughbred Breeders' Federation members' contributions support the costs of the International Collating Centre.

In vitro models

Oral Presentations

41 | New dimensions of equine intestinal organoids to model host-microbe interactions *in vitro*

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Background: The recent development of stem-cell derived intestinal organoids has emerged as a new powerful tool to study host-microbe interactions *in vitro*. While organoid cultures have become standardised in human and mouse, there is a limited description of equine intestinal organoids. To date, there is no paper describing procedures for two-dimensional equine organoid culture, which is necessary to study luminal host-microbe interactions.
Objectives: To develop methods for culture of equine 3D intestinal organoids and organoid-derived 2D monolayers, that are responsive to microbial stimuli.
Study design: *In vitro* experiments.
Methods: Procedures for crypt isolation from equine intestine and their differentiation into organoids and organoid-derived 2D monolayers were developed. The responsiveness of the two types of cell cultures was assessed after exposure to microbial mimics. Equine organoids were generated from crypts cultured in a 3D supporting medium containing intestinal niche growth factors. The organoids were characterised using morphology and gene expression of cell-type specific markers. To generate 2D monolayers, the organoids were dissociated and cultured in flat-bottomed plates or transwell inserts. The 2D monolayers were exposed to TLR agonists and analysed for their expression of cytokine genes.
Results: Transcriptional analysis of the organoids confirmed the presence of enterocytes, stem-, paneth-, proliferative-, enteroendocrine-, goblet- and tuft cells. The transfer to 2D monolayers slightly modified the relative expression levels of the cell type markers, indicating a decrease of goblet- and paneth cells in the monolayers. Exposure to Pam3CSK4, Poly I:C and LPS induced the pro-inflammatory cytokines TNF-α and IL-8, while FliC only induced TNF-α. In addition, Poly I:C up-regulated TGF-β and IL-33.
Main limitations: Access to reagents for phenotypic characterisation of equine stem cells.
Conclusions: The equine organoid-derived 2D monolayers show both genetic and functional similarities with the equine intestine making it an interesting and reproducible *in vitro* model to delineate host-microbe interactions.
Ethical animal research: Approved by the regional committee for animal experimentation in Uppsala, Sweden (ID: 5.8.18-15533/2018).
Informed consent: Not applicable.
Competing interests: None declared.
Sources of funding: Swedish-Norwegian Foundation for Equine Research (H-16-47-193), the platform SLU Future animals, nature and health at the Swedish University of Agricultural Sciences (SLU ua 2019.4.2 – 3814) and Formas (2019-00809).

42 | Establishment of a three-dimensional primary cell culture model of equine papillomavirus type 2 infection

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Background: There is strong evidence that equine papillomavirus type 2 (EcPV2) infection is causally associated with genital squamous cell carcinoma (SCC) and precancerous lesions in equids. The early viral genes E6 and E7 are assumed to drive transformation of infected keratinocytes, but the mechanisms underlying neoplastic progression remain to be elucidated. Thus far, no equine cell culture model allowing in-depth study of EcPV2-induced carcinogenesis exists.
Objective: The aim of this study was the establishment and evaluation of a three-dimensional (3D) primary cell culture model of genital EcPV2 infection.
Study design: *In vitro* experiments.
Methods: 3D raft cultures were generated from keratinocytes of equine penile perilesional skin, plaques and SCCs of three EcPV2-infected equine patients. Raft cultures were compared to corresponding histologic tissue sections. Histology, PCR, *in situ* hybridisation and immunohistochemistry were employed to comparatively assess rafts vs. corresponding tissue sections with regard to morphology, presence of EcPV2 DNA, presence and location of E6/E7 transcripts, and immunohistochemical expression of pan-cytokeratin, vimentin, p53, Ki67, and MCM7.
Results: Raft cultures from perilesional skin harboured only a few EcPV2-positive (EcPV2+) cells and accurately reflected the differentiation process of normal skin. Rafts from EcPV2+ penile plaques were structurally organised but showed early hyperplasia. Rafts from EcPV2+ SCCs exhibited pronounced hyperplasia and marked dysplasia. All raft cultures expressed the epithelial marker pan-cytokeratin, while the mesenchymal marker vimentin was only rarely present in SCC rafts. Levels of EcPV2 oncogene transcription and the expression of the tumour and proliferation

markers p53, Ki67 and MCM7 positively correlated with neoplastic progression.

Main limitations: The raft cultures were established from a small number of horses.

Conclusion: Established 3D raft cultures authentically reflect major features of corresponding *ex vivo* material, thus constituting a valuable new research model for the study of EcPV2 infection and viral-induced neoplasia in the horse.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on archived material collected previously during clinical procedures.

Informed consent: Owners gave informed consent for use of samples for research.

Competing interests: None declared.

Source of funding: The study was funded by Vetsuisse Faculty University of Zurich's competitive Joint Appointments.

Other pathogens

Oral Presentations

43 | Seroprevalence of equine piroplasmosis, equine infectious anaemia and equine influenza in zebras and rhinoceros from Kenya and Tanzania

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Background: Limited information exists regarding prevalence of major infectious diseases (OIE equine listed) in members of the *Perissodactlya* order, like wild equids (*Equus grevyi)* and rhinoceros (black -*Diceros bicornis* and white - *Ceratotherium simum*).

Objectives: To perform a serological survey in Kenya and Tanzania national parks and national reserves on zebras and rhinoceros and investigate the seroprevalence of Equine Infectious Anaemia Virus (EIAV) combined with a survey on equine piroplasmosis (both *Theileria equi* and *Babesia caballi*) and equine influenza (EI) and compare two EIAV ELISAs.

Study design: Retrospective serosurvey.

Methods: Sera (fresh or banked), from 284 animals were tested (135 females, 149 males including 69 rhinoceros, 215 zebras) from 8 locations in Kenya and 3 locations in Tanzania using commercially available ELISA kits. Two kits from different manufacturers were employed for EIAV testing.

Results: Rhinoceros showed no serological evidence of previous exposure to any of the tested diseases. All zebra samples were negative for evidence of exposure to EIAV. Piroplasmosis was found in all regions ranging from 3.9% for *B. caballi* to 94.2% for *T. equi*. EI seroprevalence in zebras was 9.3% (N=20), all from Serengeti, Tanzania.

Main limitations: Limited sample size and lack of animal history.

Conclusions: EIAV might not be present or has a very low prevalence in wild African *Perissodactlya* from Kenya and Tanzania. The seroprevalence of equine piroplasmosis in our study is in line with previous reports in the area, with reactions against the antigens used probably representing exposure to closely related organisms. Almost 10% of the tested Grevy's zebra had previous exposure to Type A influenza viruses, suggesting they could represent a potential reservoir for this virus.

Ethical animal research: Samples were provided by Tanzania Wildlife Research Institute, with approval from Wildlife Research Ethics Committee of TAWIRI and by Kenya Wildlife Service

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: RUSVM Research Center "One Health Center for Zoonoses and Tropical Veterinary Medicine" - Grant number: 770510-65119.

44 | Topography of the respiratory, oral, and guttural pouch bacterial and fungal microbiotas in horses

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Background: Respiratory disease has a large economic impact on the equine industry. It has been reported that the equine lower respiratory tract microbiota is different in states of health and disease; however, the bacterial and fungal composition of the healthy equine respiratory tract has not been studied in detail.

Objective: To characterise the bacterial and fungal microbiotas present along the upper and lower equine respiratory tract.

Study design: Prospective, controlled, cohort study.

Methods: Eleven upper and lower respiratory tract anatomical locations were sampled in 11 healthy Argentinian Thoroughbred horses from the same herd using a combination of swabs, protected specimen brushes, and saline washes. DNA was extracted from each sample and negative control, and the 16S rRNA gene (V4) and ITS2 region were sequenced. Community composition, alpha-diversity, and beta-diversity were compared among sampling locations.

Results: Fungal species richness and diversity was highest in the nostrils. There was more spatial heterogeneity in bacterial composition than fungal communities. The pharyngeal and arytenoid microbiotas were most similar to the distal tracheal bacterial and

fungal microbiota in healthy horses and therefore may serve as the primary source of bacteria and fungi to the lower respiratory tract.

Main limitations: A limitation of 16S sequencing is the ability to accurately identify the microbes constituting the microbiota to a clinically relevant taxonomic level. Future studies might consider using whole genome shotgun sequencing for enhanced detection of bacterial species, increased detection of diversity, and improved accuracy of species detection.

Conclusions: The pharynx is an important location that should be targeted when doing equine respiratory microbiota research.

Ethical animal research: The University of Calgary Veterinary Animal Care Committee approved the study (#AC17-0036).

Informed consent: Explicit written owner informed consent was obtained from the horses' agent.

Competing interests: None declared.

Source of funding: Calgary Chair in Equine Sports Medicine.

45 | Characterisation of equine rotavirus G-genotypes from diarrhoeic foals in Argentina

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Background: Equine rotavirus A (ERVA) is the major cause of diarrhoea in foals under four months old. Based on the VP7 and VP4 outer capsid proteins (both of which contain the viral neutralisation determinants), ERVA strains are classified into G- and P-genotypes respectively, G3P[12] and G14P[12] being the most predominant worldwide. While G3P[12] ERVA was the predominant genotype in Argentina since 1992, the incidence of G14P[12] ERVA has steadily increased following its first detection in 2000, particularly during 2006 and 2007. From 2008-2014, both genotypes have been circulating in our territory with a cyclical pattern.

Objectives: To characterise the G-type of ERVA strains circulating in Argentina between 2016-2020.

Study design: Analytical time series study.

Methods: A total of 179 faecal samples corresponding to 42 foal diarrhoea outbreaks were analysed and ERVA-positive samples were characterised by a G3/G14 multiplex genotyping qPCR.

Results: Equine RVA was detected in 23% (41/179) of the samples and in 33% (14/42) of the reported outbreaks of diarrhoea. Overall, 76% (31/41) of ERVA strains were classified as G14 while 24% (10/41) were classified as G3 genotype. Based on annual frequencies, a higher incidence (>50% of ERVA strains) of G14 was noted during 2016-2019, with a predominance of G3 strains in 2020 (80% of ERVA strains).

Main limitations: Small number of samples analysed.

Conclusions: The detection rate of ERVA presented during this 4-year study period is similar to previous reports. The higher but cyclic incidence of G14 ERVA strains could be due to vaccine-associated immune pressure, as current commercial vaccines widely used during late gestation only include a G3 ERVA strain.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not stated.

Competing interests: None declared.

Sources of funding: This work was supported by INTA and INTA -HARAS Agreement.

46 | Characteristics of cases of nocardioform/mucoid placentitis in Central Kentucky from the 2019-2020 Foaling Season

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Background: Nocardioform/mucoid placentitis is a unique type of placentitis with significant variation in disease incidence between years. The 2019-2020 foaling season in Central Kentucky documented a total of 338 cases of mucoid/nocardioform placentitis.

Objectives: Present data from cases of mucoid placentitis from the 2019-2020 foal crop from central Kentucky.

Study design: Retrospective clinical study.

Methods: Cases with a pathologic diagnosis of mucoid placentitis were evaluated for fetal and placental weight, umbilical cord length, sex, breed, aerobic culture and PCR results and site and size of abnormal areas of the chorioallantois.

Results: 130 fetuses had lesions compatible with a mucoid placentitis. Average fetal weight was 28.4 kg. Average placental weight was 6.6 kg. Aerobic placental culture grew a gram-positive branching bacillus in 84 cases. Thirty-eight cases were PCR positive for *Amycolatopsis sp*., 67 positive for *Crossiella equi*, 9 positive for both. The majority of cases (78.4%) occurred in January, February and March. Average lesion size was 52.4cm^2. 208 placenta-only submissions were received. The average weight was 7.1kg. 49 were PCR positive for *Crossiella equi*, 89 for *Amycolatopsis sp*. and 6 positive for both. Aerobic culture grew a gram-positive branching bacillus in 119 cases. 69% of cases occurred in February and March. Average lesion

size was 29.8 cm^2. 31 foals had neonatal complications or small size reported. Thirty-six foals were reported to be 'normal or healthy'. 10 cases of premature placental separation and 3 of dystocia were recorded.

Main limitations: Sample set and size was determined by submissions from owners and veterinarians to the UKVDL. Not all data was available for each case.

Conclusions: Nocardioform/mucoid continues to be a significant cause of foal mortality and morbidity. Findings in the 2019-2020 foal season provide additional data and highlight differences between cases of fetal loss and fetal survival.

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

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: None.

Poster Presentations

47 | Seroprevalence of equine infectious anaemia virus (EIAV), equine arteritis virus (EAV) and West Nile virus (WNV) in donkeys in eastern Algeria in 2015

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Background: Viral diseases are responsible for important economic losses within the equine population. Worldwide, the viruses most commonly described as pathogenic in donkeys are equine infectious anaemia virus (EIAV), equine arteritis virus (EAV), and West Nile virus (WNV). In eastern Algeria, no data on EAV, EIAV and WNV circulation is available in donkeys.

Objectives: The aim of this study was to investigate, for the first time in eastern Algeria (Batna province), the prevalence rate of EAV, EIAV and WNV antibodies in a large number of donkeys.

Study design: Cross-sectional study.

Methods: 201 sera were collected from donkeys aged between 4 months and 30 years in 2015. Serological analysis of the sera for the presence of EAV, was performed using the virus neutralisation test, as described by the World Organisation for Animal Health. The presence of antibodies against EIAV and WNV was tested by commercially available ELISAs.

Results: The prevalence of EAV antibodies in this Algerian donkey population was 18.4% (37/201). Among the 201 samples screened for EAV, 124 (61.7%) were negative and 40 (19.9%) were cytotoxic for RK-13 cells even after different treatments were applied to reduce sera cytotoxicity. Antibodies against WNV were found in 4 samples (1.99%). EIAV antibodies were not detected in any samples.

Main limitations: Cytotoxicity can be mistaken for viral cytopathic effect and this has led to increasing difficulties in test interpretation of EAV serology.

Conclusions: This study is the first to describe the circulation of EAV in the Algerian donkey population. Further studies are necessary to isolate and obtain molecular characterisation of EAV and WNV from donkeys in Algeria.

Ethical animal research: The authors confirm that ethical review is not required from this work in their region.

Informed consent: Animal owners gave consent for sample collection.

Competing interests: None declared.

Source of funding: None.

48 | High sero-prevalance of equine trypanosomosis, equine infectious anemia and equine piroplasmosis in a herd of semi-wild horses from North Argentina

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Background: Northern Argentina hosts equine populations living in preserved natural areas and extensive breeding conditions. Horses can be in contact with wildlife considered to be a potential reservoir of horse pathogens and/or potential disease vectors.

Objectives: To assess the exposure of horses from a herd in northern Argentina to different vector-borne pathogens [1].

Study design: Retrospective serological survey.

Methods: Sera were collected from 20 horses on a farm in Chaco province. Their general health was recorded and the serological tests performed according to the OIE Terrestrial Manual.

Results: Most of these horses were in good health but a few showed fever, neurological signs or emaciation. Potential vectors (ticks, horse flies, *Culicidae* and vampire bats) were present. This serological survey revealed that 100% (20/20) horses were positive for equine infectious anaemia (EIA), 100% (18/18) for West Nile fever (WNF), 53% (10/19) for surra and 45% (9/20) for equine piroplasmosis (*Babesia equi*). Four horses were seropositive for all four infections. Tested horses were seronegative for equine viral arteritis

(EVA), Eastern equine encephalomyelitis (EEE), Venezuelan equine encephalitis (VEE), Western equine encephalomyelitis (WEE) and glanders.

Main limitation: This survey was conducted on a small number of animals from a single farm.

Conclusions: This study illustrates the need for effective application of surveillance programs and control measures for equine diseases in northern Argentina and constitutes, to our knowledge, the first report of horses simultaneously seropositive for EIA, WNF, surra and equine piroplasmosis.

Reference

[1] Hébert, L., Polledo, G., Lecouturier, F., Giorgi, M., Beck, C., Lowenski, S., Laroucau, K., Büscher, P., Hans, A. and Becù, T. (2021) Serological evidence of equine infectious anaemia, West Nile fever, surra and equine piroplasmosis in a herd of horses in northern Argentina. *Veterinary Parasitology: Regional Studies and Reports* (In press) https://doi.org/https://doi.org/10.1016/j.vprsr.2021.100566.

Ethical animal research: Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on samples collected for a preventative health care programme.

Informed consent: The animals' owner gave consent for their inclusion.

Competing interests: None declared.

Sources of funding: This work was supported by ANSES and the European Commission through DG SANTE funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness.

49 | What have we learned from 7 years of equine rhinitis B virus qPCR testing in nasal secretions from horses with respiratory signs?

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Background: Equine rhinitis B virus (ERBV) has been given little attention by practitioners compared to other respiratory viruses, mainly because of the lack of diagnostic modalities and association with clinical disease.

Objectives: The objective of the study was to determine the frequency of detection of ERBV in nasal secretions from 6,568 horses with acute onset of respiratory signs.

Study design: Retrospective survey.

Methods: ERBV-positive qPCR results from nasal secretions submitted to a molecular diagnostic laboratory from 2013 to 2019 were reviewed. To determine the rate of ERBV infection in healthy horses, nasal secretions from 356 equids collected from 2012 to 2018 for clinical purposes were included in the study.

Results: A total of 333 ERBV qPCR-positive samples (5.1%) were detected with increasing yearly frequency since the introduction of the assay in 2013. In comparison, only three of 356 (0.8%) healthy horses tested qPCR-positive for ERBV. Median age for ERBV qPCR-positive horses was 3 years of age, and fever, coughing and nasal discharge were the most common signs reported. Further, co-infections with other respiratory pathogens were reported in 73 (21.9%) of ERBV qPCR-positive samples.

Main limitations: Study limitations related to the lack of information pertaining to various prevalence factors evaluated in this study. Another study limitation was the lack of true controls originating from the same location and same time as the index cases.

Conclusions: ERBV is a commonly detected respiratory virus from nasal secretions of young horses presenting with fever, nasal discharge and coughing.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on archived material collected previously during clinical procedures.

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: Equine Infectious Disease Research Laboratory.

50 | Pathological findings following experimental challenge with *Burkholderia pseudomallei* (equine melioidosis)

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Background: Melioidosis and Glanders caused by *Burkholderia pseudomallei* and *Burkholderia mallei* respectively are very similar equine diseases and therefore the OIE present them in one chapter of its Terrestrial Manual in 2018. However, differences exist between both diseases. *B. pseudomallei* has an affinity to moist and wet environments and urine should be collected when dealing with melioidosis.

Objective: To characterise the clinical signs, pathology and serological responses associated with experimental infection of horses with *B pseudomallei* (melioidosis) and to compare with those reported for glanders (*Burkholderia mallei).*

Study design: *In vivo* experiment.

Methods: Horses were infected with *B. pseudomallei* and swabs taken from urinary bladder, lung, nasal septum and all other organs. Serum samples were also collected to perform Complement fixation tests.

Results: None of the infected horses displayed lesions in the nasal septum nor conchae and therefore nasal discharge is rarely observed. If nasal discharge is observed, it originates from severe lesions of the lung and not from the head. Typical pyogranulomas were, however detected in the lungs and other organs indistinguishable from reported glanders pathology. Serological diagnosis of melioidosis was unreliable.

Main limitations: Data was compared with reported *B. mallei* pathology.
Conclusions: Clinical and pathological differences do exist between glanders and melioidosis. However, glanders and melioidosis cannot be differentiated based on current diagnostic tests for glanders laid down in the OIE manual.
Ethical animal research: An ethical committee comprising of veterinarians from the CVRL and the UAE Ministry of Environment and Climate Change (MOCCAE) approved the study.
Informed consent: Not applicable.
Competing interests: None declared.
Source of funding: The horses involved in this study were donated by HH Sh Mohammed Bin Rashid Al Maktoum, Vice President and Prime Minister of the UAE and Ruler of Dubai, under whose patronage CVRL Dubai is functioning.

Parasitology

Cyathostomins

Oral Presentations

51 | Prevalence of anthelmintic resistant cyathostomins in Prince Edward Island, Canada

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Background: Cyathostomins are the most common parasites of adult horses worldwide. Due to widespread anthelminthic overuse, cyathostomins have developed varying degrees of resistance to benzimidazoles, pyrimidines, and macrocyclic lactones. There have been no equine anthelmintic resistance (AR) surveys for cyathostomins in Atlantic Canada.
Objectives: Investigate the prevalence of strongyle egg shedding, cyathostomin AR and egg reappearance periods (ERP) for pyrantel pamoate and ivermectin in Prince Edward Island (PEI), Canada.
Study design: Observational cohort study.
Methods: To identify treatment cohorts, preliminary faecal egg counts (FEC) were performed on 270 horses (14 farms). Horses with ≥ 200 eggs per gram (EPG) were enrolled for treatment (n=101) and faecal culture and larva identification. Median (range) horses/farm was 7.5 (4-11) and 6.0 (4-8) for pyrantel pamoate and ivermectin treatment groups, respectively. Horses were treated with 6.6 mg/kg of pyrantel pamoate orally (n = 101). Faecal egg counts were conducted every two weeks for 8 weeks post treatment. Once FECs were ≥ 200 EPG, horses received 0.2 mg/kg of ivermectin orally (n = 80), and FEC were performed every 2- 3 weeks for 7 weeks. Faecal egg count reduction tests (FECRT) and ERP evaluated treatment efficacy.
Results: Pyrantel pamoate AR was detected on 5/14 farms and no ivermectin AR was detected. ERP occurred at 4-6 weeks and 7-9 weeks for pyrantel pamoate and ivermectin, respectively. Faecal culture detected large strongyles on 5/14 farms, which accounted for 0.3% of strongyle type eggs cultured. The prevalence of *Strongylus vulgaris* among individual horses in this study was 2.8% and was detected on 2/14 farms.
Main limitations: Small sample size and potential for selection bias.
Conclusions: These findings allow us to educate owners and veterinarians on appropriate anthelmintic protocols in PEI and can be used as a baseline for continued monitoring of ERP and AR in this region.
Ethical animal research: Ethical approval was granted by the University of Prince Edward Island, Animal Care Committee (ACC).
Informed consent: Owners gave informed consent for their animals' inclusion.
Competing interests: None declared.
Source of funding: Sir James Dunn Animal Welfare Centre, Atlantic Veterinary College, University of Prince Edward Island, Canada.
Acknowledgements: The authors wish to thank Dr Gary Conboy for his morphological expertise during larvae identification. In addition, we would like to thank Dr Carly Lilley and Cynthia Mitchell for their technical support during sample collection and analysis.

52 | Comparing ITS-2 and COI as DNA barcode markers for the most common cyathostomin species based on comparative phylogenetic analysis

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Background: Worldwide, cyathostomins are ubiquitous in the large intestine of horses and other equids. The high prevalence, potentially fatal infections and widespread anthelmintic resistance supports the

view that cyathostomins are currently the most important parasites in equines. The current morphological-taxonomic classification includes 50 recognised species. It has been shown that this classification may diverge from molecular phylogenetic systematics.

Objectives: The establishment of a genomic reference database is the basis for reliable and practical species' identification and is indispensable for further research projects.

Study design: Descriptive parasite genomics.

Methods: Nuclear ribosomal DNA second internal transcribed spacer (ITS-2) and partial mitochondrial cytochrome C oxidase subunit I (COI) sequences were generated for 20 cyathostomin species. Maximum likelihood trees were calculated for COI, ITS-2 and the concatenated data. Specimens from different host species and geographical regions were obtained.

Results: As previously described, cryptic species for *Cylicostephanus (Cys.) minutus* and *Cys. calicatus* were found. Despite the assignment to different genera, a close relationship between *Coronocyclus (Cor.) coronatus* and *Cys. calicatus* was ascertained. A well-defined monophyletic group is recognisable for the species of the genus *Cyathostomum*. *Cylicocyclus (Cyc.) nassatus* and *Cor. labiatus* form two different haplotype groups for nuclear and mitochondrial sequences. However, a cryptic species complex was excluded due to the free mixing of the nuclear and mitochondrial haplotype groups. Within species comparatively homogeneous sequences were determined for specimens *of Cys. longibursatus*, *Cys. goldi*, *Cylicodontophorus (Cyd.) bicoronatus*, *Cyathostomum (Cya.). catinatum* and *Cyc. ashworthi*, while sequences of *Cyc. insigne*, *Cyc. leptostomus*, *Cyathostomum (Cya.) pateratum* specimens were assigned to more than one distinct cluster. No host-specific or geographical differences were found in the investigated specimens.

Conclusions: Molecular analysis invalidates the taxonomic positioning for some genera which is based on their morphological characters. The complete molecular taxonomic assignment of cyathostomins can only be deciphered with further molecular data.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures or previous research approved by the regional administration (LaGeSo Berlin, A 0237/14).

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project Number 251133687/GRK 2046; Deutscher Akademischer Austauschdienst (DAAD, German Academic Exchange Service)—funding program 57210259, and Freie Universität Berlin TAK received funding from Research Stays for University Academics and Scientists, 2016. ML received funding for her traineeship at the Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Germany by the Erasmus + program (Contract Number 035448 86/SMT/2017) of the European Union.

53 | Complete anthelmintic failure in treating cyathostomins

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Background: Despite widespread anthelmintic resistance in equine cyathostomins, macrocyclic lactones have generally maintained efficacy despite heavy usage. However, in 2020, we reported clear resistance to this class in a group of yearlings imported from Ireland to the US.

Objectives: The objective was to study anthelmintic efficacy in the same equine operation and collect efficacy data on all anthelmintic classes.

Study design: Retrospective clinical report.

Methods: Data were collected as part of routine parasite monitoring on a Thoroughbred operation in Central Kentucky, USA. For the 2021 season, the farm imported 50 yearlings from Ireland, while another 45 were home bred. Parasite faecal egg counts were determined on the day of anthelmintic treatment and again 14 days post treatment. Anthelmintics used were ivermectin (200 μg/kg), oxibendazole (10 mg/kg) and pyrantel pamoate (13.2 mg/kg). Egg counts were determined with the Ovassay technique and fecal egg count reductions were calculated using a hierarchical Bayesian analysis.

Results: In February 2021, ivermectin was administered to all yearlings. The efficacy among the US bred was 100% (CI: 99.0-100), but only 55.9% (40.8-67.9) among the imported yearlings. A follow-up ivermectin treatment among 13 yearlings returned a 31.8% reduction (15.1-54.4). In addition, oxibendazole administration in five yearlings reduced egg counts by 82.5% (43.3-99.9), while strongyle egg counts increased in all seven yearlings treated with pyrantel pamoate.

Main limitations: This study examined one equine operation and data may not represent the resistance situation in other operations and locations.

Conclusions: The data confirms recently reported findings of macrocyclic lactone resistance in cyathostomin isolates imported from Ireland. Furthermore, the data indicate that resistance is established to the two other anthelmintic classes as well. As no new anthelmintic classes are expected for equine usage in the foreseeable future, this is very concerning.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed samples collected during clinical procedures.

Informed consent: Horse owners' gave consent for their animals' inclusion.

Competing interests: None declared.

Source of funding: None.

Poster Presentation

54 | Control of cyathostomin infections in horses: is the 200 eggs per gram threshold really relevant?

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Background: Cyathostomins are considered as grazing horses' most prevalent and pathogenic parasites. To limit their impact on equine health and avoid the emergence of anthelmintic resistance, individual faecal egg counts (FEC) are commonly used to select the high egg shedders for anthelmintic treatment, with the usual threshold of 200 eggs per gram (epg). However, the link between egg excretion and (i) medical consequences of infection and (ii) exposure to infective larvae remains unclear.
Objectives: To optimise the use of FEC for selective treatment, the relationships between FECs and (i) general health indicators and (ii) pasture infectivity level were assessed.
Study design: Observational field study.
Methods: FEC was monthly measured in 3 groups of adult horses (n=68) between March (turn-out) and October (housing) 2020 and compared with pastures' infectivity level (number of L3 per kg dry herbage-DH), diarrhoea score, body condition score (BCS) and weight.
Results: Before turn-out, no infective larvae were counted on pastures but a peak of FEC was observed in the 3 groups (48-67% of animals per group ≥200 epg), likely related to the emergence of L4 from the intestinal mucosa. During the grazing season, peaks of pasture infectivity levels were observed in August in group 1 (63,347 L3/Kg.DH) and in October in groups 2 and 3 (78,876 and 16,263 L3/kg.DH, respectively). During these infectivity peaks, few animals showed a FEC ≥200 epg, but those peaks were preceded by excretion peaks 2 months before (39-65% of animals per group ≥200 epg). No correlation was found between FEC and diarrhoea score, BCS or weight.
Conclusions: The results suggest that the peak of Cyathostomin L3 excretion could be a good predictor of the increase of infectivity of pastures, but the individual threshold of 200 epg appears to lack sensitivity and specificity to detect horses suffering the most from infection.
Ethical animal research: The study was approved by the Ethics Committee of Normandy (France), DGRI agreement APAFIS#2020041615267486.
Informed consent: Not stated.
Competing interests: None declared.
Sources of funding: The Parasit'SimEq project is funded by the French horse and riding institute (IFCE), the "Fonds Eperon" and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES).

Leptospirosis

Oral Presentations

55 | Leptospirosis on a horse farm in central Italy

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Background: Although Leptospira infection is not considered the main cause of equine abortion, serological surveys indicate horses as maintenance host for *Leptospira interrogans serovar Bratislava* and potential accidental hosts for other *serovars* [1]. Most infections are asymptomatic, but it is possible to observe clinical signs including stillbirth and abortion especially in the last term of pregnancy [1].
Objectives: to describe serological and biomolecular findings relating to leptospirosis on a horse farm. Horses were living on pasture, in the province of Viterbo, Italy and the investigations were conducted after sporadic abortions had occurred during autumn 2019.
Study design: Outbreak report.
Methods: Serological investigations for leptospirosis were carried out by the microscopic agglutination test (MAT) [2] on all horses on the farm.
Results: Of the 37 horses tested, 12 were *Leptospira interrogans* positive and of these, 7 had high agglutinating titres predominantly for Pomona and Bratislava serovars. *Leptospira* spp. was detected by real time PCR in fetal organs [3].
Main limitations: MAT has two important limitations, first is that for a diagnosis of certainty, it is necessary to evaluate a four-fold antibody titre increase, between acute serum and convalescent serum which is not always possible; second is that a diagnosis can be missed in case the serovar causing the infection is not among the panel used for the serodiagnosis.
Conclusions: Diagnosing leptospirosis as a cause of abortion or stillbirth, although sporadic, is essential; the farm may still be at a risk of infection, in the short and long term, particularly if the appropriate measures of therapy and prophylaxis are not undertaken.
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Ethical animal research: Research ethics committee oversight not required by this journal: retrospective analysis of clinical data.

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: Not applicable.

56 | Acute renal failure after pathogenic leptospira infection in a mare in southern Chile

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Background: A 2-year-old Holsteiner-Thoroughbred cross mare was referred to the Equine Hospital for assessment of worsening neurological signs. Up until 2 weeks before presentation, the mare was healthy, when moved to a new farm. After arrival she was treated for a mild corneal injury for 10 days with systemic phenylbutazone and topical eye drops. Treatment was discontinued 4 days before presentation.

Objective: To determine the cause of neurological signs.

Study design: Case report.

Methods: Clinical findings, clinical biochemistry and haematology, and MAT analysis for titers to *Leptospira interrogans were extracted from medical records*.

Results: At admission, the mare showed profuse sweating, discomfort, altered consciousness, head pressing and incoordination. Signs of dehydration (6-7%) and encephalopathy were evident. Severe leukocytosis with neutrophilia, and hyperfibrinogenemia and hypoalbuminemia, increased serum creatinine (293 umol/L) and BUN (32 mmol/L) were present. Urine specific gravity was 1.010. Our working diagnosis was acute renal failure with secondary uremic encephalopathy. Administration of nephrotoxic drugs and sporadic pathogenic-leptospira-abortions in the region the mare was moved to, were considered as possible triggering causes. Titers (MAT analysis) for *Leptospira interrogans* serovar Autumnalis were 1:200 and 1:3200 for day 1 and 14, respectively. Urine was PCR positive to pathogenic-leptospiras on presentation, and negative 14 days later, confirming the diagnosis of acute renal failure after pathogenic-leptospira infection. The mare was stabilised and treated aggressively. Over the next days, neurological signs resolved, the kidneys slowly recovered function and the mare recovered completely 10 days after admission. The farm to which the mare was moved was visited. All horses (15) were healthy, most had low titers against pathogenic-leptospiras (12/15), while 2 had high titers (1:3200).

Main limitations: Single case report, urine analysis from in-contact horses with high titers was not performed.

Conclusions: This is the first report of acute renal failure after pathogenic-leptospira infection in horses in southern Chile. Previous administration of NSAIDs possibly played a role in severity of disease.

Ethical animal research and informed consent: Research ethics committee oversight not required by this journal: retrospective analysis of clinical data.

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: Partially funded by Proyectos de Instalación VIDCA 2020 nr. INS-INV-2020-25, Universidad Austral de Chile.

Parascaris

Oral Presentations

57 | Characterising the *Parascaris* spp. microbiome

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Background: The microbiome is an important component of health in many organisms. Parasitic nematodes have distinct microbiomes from their host and also within different parasite organs that contribute to overall fitness and can be exploited for parasite control. Only few parasitic nematodes have had their microbiomes characterised, and none of these infect equines.

Objectives: The objective of this research was to characterise the microbiome of the gonad and intestine of *Parascaris* spp. adult male and female specimens and compare it to the microbiome of their environment, the foal jejunum. Deciphering the unique parasite microbiome will provide the first step in developing novel parasite control strategies.

Study design: Cross sectional.

Methods: *Parascaris* spp. were collected from foals at necropsy and a 16S metagenomic analysis was completed to identify different bacteria taxa present in the specimens. Adult parasites (46 total; 24 male, 22 female) were collected and dissected to remove gonads and intestines. Next generation metagenomic sequencing was completed and data analysed using a bioinformatics pipeline to determine relative abundance, alpha diversity, and beta diversity.

Results: *Lactobacillus* was the most common genus, found in 83% of samples, and two genera, *Reyranella* and *Aminobacter*, were found in parasite samples but not horses. There were significant differences in alpha diversity between male and female worms overall ($p = 0.0005$), as well as between male and female gonads ($p < 0.001$).

Beta diversity indicated distinct communities for intestinal and gonad samples, particularly the male gonad.

Main limitations: This study examined one population of *Parascaris* spp., and local environment may impact microbiomes for different parasite populations. Future research comparing various populations is therefore necessary.

Conclusions: *Parascaris* spp. gonads have a microbiome distinct from their equine host and harbour unique microbiota not found in horses that may provide an opportunity for future control options.

Ethical animal research: All foals were humanely euthanised as part of a regular research program under the University of Kentucky IACUC protocol 2012-1046.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: National Center for Veterinary Parasitology Research Grant (Oklahoma, USA).

58 | *Parascaris univalens* – first case of fenbendazole treatment failure in Sweden, with enhanced efficacy of a combination treatment with ivermectin

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Background: Resistance to anthelmintic drugs is an increasing problem in the equine roundworm *Parascaris univalens*. Combination treatment with anthelmintic drugs from different chemical groups has been proposed as an alternative control strategy where failure of individual drugs is documented.

Objectives: To investigate the efficacy of fenbendazole on foals naturally infected with *P. univalens*. In addition, the efficacy of a combination treatment of fenbendazole and ivermectin was studied in foals with co-infection of *P. univalens* and cyathostomins.

Study design: Field trial.

Methods: The clinical efficacy of the treatments was examined by Faecal Egg Count Reduction Test (FECRT). Foals positive for *P. univalens* only were treated with fenbendazole oral paste (Axilur® Intervet) and foals with a co-infection of *P. univalens* and cyathostomins were treated with fenbendazole and ivermectin (Ivomec® Boehringer Ingelheim) oral pastes.

Methods: Foals between three and six months old located on two separate farms, A (43 foals) and B (26 foals) on a breeding stud in central Sweden were included. 46 foals received fenbendazole treatment, 35 foals on farm A and 11 foals on farm B. 23 foals were treated with fenbendazole and ivermectin, eight foals on farm A and 11 foals on farm B.

Results: FECRT of fenbendazole treatment showed a 73% reduction of *P. univalens* eggs on farm A and 88% on farm B. The combination treatment of fenbendazole and ivermectin increased the reduction of *P. univalens* eggs to 92% on farm A and 99% on farm B.

Main limitations: Small study population. The FECRT method shows high variability.

Conclusion: First case of fenbendazole treatment failure in Sweden, indicating the presence of triple anthelmintic resistant *P. univalens* populations. A combination treatment of fenbendazole and ivermectin improved the reduction of egg counts of *P. univalens*. The combination of fenbendazole and ivermectin in resistant *P. univalens* populations is promising but needs further validation.

Ethical animal research: Research ethics committee oversight was not required for this research according to the Swedish National Board of Agriculture (SJVFS 2019:9, L159).

Informed consent: Written owner informed consent was obtained for all included animals.

Competing interests: None declared.

Sources of funding: Swedish research council FORMAS (grant number 942-2015-508) and by the Swedish-Norwegian Foundation for Equine Research (grant number H-20-47-556).

Protozoans

Oral Presentations

59 | Epidemiological situation of equine piroplasmosis in Spain: where is the risk?

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Background: *Theileria equi* (*T. equi*) and *Babesia caballi* (*B. caballi*) cause equine piroplasmosis (EP), one of the most important tick-borne diseases of horses worldwide, due to its negative impact to the equine industry [1]. Although infections with these protozoa have been reported for years in Spain, epidemiological studies have only been carried out in certain regions [2].

Objectives: To determine the prevalence of these parasites in asymptomatic horses in Spain and to identify potential risk factors associated with seropositivity to EP.

Study design: Cross sectional.

Methods: Horses registered in Spain in 2013 were used to calculate the sample size; a random stratified sampling was carried out by autonomous community. A questionnaire was used to collect data on factors associated with EP seropositivity. A total of 740 horses were tested by serological (cELISA and complement fixation test) and molecular methods. Risk factors were identified computing two independent logistic regression models with the collated data.

Results: Antibodies against EP were detected in 42.9% of horses, whereas 30.3% were EP positive by PCR. *T. equi* was significantly more prevalent than *B. caballi* and the highest (sero)prevalence was detected in the north of Spain. Risk factors related to EP were horse age, ticks and contact with cows, whereas tetanus vaccination and fairs' attendance were associated with lower risk.

Main limitations: A possible source of bias in the selection of horses in each premises by the participating vets should be taken into account.

Conclusions: Horses in Spain have a significant risk of infection with EP, varying between areas and depending on management factors. Appropriate prevention measures should be considered to reduce and control the infection.

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Ethical animal research: Approved by the Ethical Committee of the Universidad Complutense de Madrid.

Informed consent: Horse owners gave consent for their animals' inclusion.

Competing interests: None declared.

Source of funding: EC was supported by the Grants for Predoctoral Researchers of the Complutense University of Madrid (CT27/16).

60 | Evaluation of a rapid diagnostic method for equine piroplasmosis using a flow cytometry-based method

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Background: Equine piroplasmosis (EP) is a protozoan disease of equids, caused by infection with *Theileria equi* (*T. equi*) and *Babesia caballi* (*B. caballi*). Infected horses present various signs, including anaemia, because the protozoa parasitise red blood cells (RBCs). The diagnosis of EP is currently performed using microscopic, molecular, and serological methods. Each of these techniques has its advantages and disadvantages, around specificity, sensitivity, simplicity, and cost.

Objectives: We evaluated the utility of the Prototype XN-30 haematology analyser, which utilised flow cytometry (FCM), for the rapid diagnosis of EP.

Study design: Development of a diagnostic method.

Methods: *T. equi* and *B. caballi* were cultured *in vitro*. The numbers of infected RBCs (iRBC#) and ratio of infected RBCs (iRBC%) were counted by using a prototype XN-30 (Sysmex, Kobe, Japan) and by microscopic examination. The prototype XN-30 was assessed for limit of blank (LoB), limits of detection (LoD), quantitation (LoQ), linearity, carryover and precision.

Results: The prototype XN-30 was able to detect infected RBCs (iRBCs) in approximately 1 minute. To investigate the reliability of the prototype XN-30, iRBC% determined by the prototype XN-30 analysis and microscopy examination were compared. The correlation of iRBC% was high ($R^2 > 0.9$). LoB was 5 cells/µl. The LoDs and LoQs were 12 cells/µl and 21 cells/µl for *T. equi* and 27 cells/µl and 53 cells/µl for *B caballi* respectively. Linearity was good ($R^2 > 0.9$). Carryover never exceeded 0.05%. To assess the precision of the prototype XN-30, the coefficient of variation (CV) was calculated: it proved <5%.

Main limitations: *In vitro* evaluation.

Conclusions: FCM was able to recognise and count iRBCs, reporting the infection ratio in approximately 1 minute. These findings indicate that FCM may be useful for EP diagnosis, especially for the monitoring of infected horses and for screening tests.

Ethical animal research: This study was approved by the Animal Care Committee of the Equine Research Institute with accession number 20-17.

Informed consent: Not applicable.

Competing interests: YT, MS and ST are employees of Sysmex.

Source of funding: Japan Racing Association and Sysmex.

61 | Molecular detection of 7SL-derived small RNA is a promising alternative for trypanosomosis diagnosis

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Background: Equine trypanosomosis is a complex of infectious diseases caused by parasites of the subgenus *Trypanozoon*: *Trypanosoma equiperdum* (causative agent of dourine), *Trypanosoma brucei* (nagana) and *Trypanosoma evansi* (surra). Due to the absence of a vaccine and the lack of efficacy of the few available chemotherapies, these diseases represent a major health and economic problem for international equine trade. To control that threat, development of innovative and sensitive diagnostic techniques remains crucial. In this context, it has been recently shown that a small RNA derived from the 7SL gene (7SL-sRNA) is produced in high concentrations in sera of cattle infected with several *Trypanosoma* subspecies.

Objectives: Determine whether 7SL-sRNA could serve as a marker of *Trypanosoma* infection in equids.

Study design: Diagnostic test development using archived sera.

Methods: Using negative sera from field and positive sera from mares experimentally infected with *T. equiperdum*, we analysed the sensitivity and the specificity of the 7SL-sRNA detection and the stability of the 7SL-sRNA.

Methods: 7SL-sRNA were amplified with a *Trypanozoon*-specific double-step RT-qPCR.

Results: The 7SL-sRNA signal was detected prior to the seroconversion of the experimentally infected horses. There was a rapid loss of 7SL-sRNA one day post-trypanocide treatment of infected animals. The 7SL-sRNA RT-qPCR allowed early detection of treatment failure, revealed by glucocorticoid-induced immunosuppression. The specificity of this assay was confirmed on 63 sera from horses seronegative for dourine. The 7SL-sRNA signal remained stable in sera during at least 7 days of storage (at 4°C, room temperature or 30°C).

Main limitations: Tested sera derived from experimental infections, so it will be crucial to further evaluate the sensitivity of this method with positive sera from acute and chronic natural infections.

Conclusions: The detection of a strong and consistent 7SL-sRNA signal even during sub-patent parasitaemia, highlight the promising nature of this new diagnostic method for equine trypanosomoses.

Ethical animal research: All experimental procedures were ethically approved by the Loire Valley ethical review board (DGRI agreement APAFIS#2015010908456425).

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: LH, CL, TG, AH and MV were supported by ANSES, the European Commission through DG SANTE funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness, the Regional Council of Normandy and the GIS Centaure Recherche Equine. FG and LM were supported by a core grant to the Roslin Institute by the UK Biotechnology and Biological Sciences Research Council (grant number BBS/E/D/20002173).

62 | Diagnosis of equine trypanosomosis (dourine, nagana and surra): towards reconciling phylogeny and pathologies

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Background: Equine trypanosomosis is a complex of infectious diseases called dourine, nagana and surra, which are caused by closely related species of *Trypanosoma* (*Trypanosoma equiperdum*, *Trypanosoma brucei* and *Trypanosoma evansi*, respectively). The classification of these parasites as independent species or subspecies has been long debated and current taxonomy does not acknowledge the complex evolutionary relationships between them [1]. As a consequence, the diagnosis of equine trypanosomosis remains a challenge.

Objectives: Identify the phylogenetic relationships between the different causative agents of equine trypanosomosis.

Study design: Phylogenetic analysis of existing database sequences.

Methods: Publicly available genome data of different lineages of trypanosomosis infectious agents were analysed, to study their evolutionary relationships.

Results: Whole genome comparisons confirm that *T. equiperdum* and *T. evansi* strains are each separated in two distinct lineages which arose independently on at least four separate occasions from a diverse *T. brucei* population. This observation highlights that *T. evansi* and *T. equiperdum* species are polyphyletic and can be assigned in at least four independent lineages, such that their species' names do not describe the evolutionary relationships between these strains. One isolate, typed as *T. equiperdum*, was found to be a closer relative to *T. evansi*, highlighting the risk of using pathognomonic descriptors for species' assignment.

Main limitation: Precise information on transmission modes and host spectrum of these parasites would help to complete these data.

Conclusions: Using phylogenetic analysis, we suggest the current taxonomy requires modification, reconciling phylogeny and pathology in equine trypanosomosis agents. Taking these considerations into account is an essential element for the control and the development of diagnostic tools for equine trypanosomosis.

Reference

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Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: LH and MV were supported by ANSES, the European Commission through DG SANTE funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness, the Regional Council of Normandy and the GIS Centaure Recherche Equine. KM held a Wellcome Trust Investigator award grant (103740/Z/14/Z) and GO held a Wellcome Trust PhD studentship to G. Oldrieve (108905/B/15/Z).

63 | Investigation of the bi-weekly administration of diclazuril on the antibody kinetics to *Sarcocystis neurona* in healthy adult horses

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Background: The bi-weekly administration of diclazuril (Protazil®) at half the current label dose has been shown to produce steady-state plasma drug concentrations known to inhibit *Sarcocystis neurona in vitro*; this pathogen is associated with equine protozoal myeloencephalitis (EPM).
Objectives: To determine if bi-weekly administration of diclazuril at half the label dose would reduce seroprevalence and magnitude of titers to *S. neurona* in horses naturally exposed to *S. neurona*.
Study design: Randomised prospective study.
Methods: Twenty adult horses were moved from a low-risk exposure environment to a farm with a high exposure rate to *S. neurona* in their horse population. The horses were randomly assigned to either a treatment or a control group. Treatment consisted of the administration of 0.5 mg/kg BWT of diclazuril pelleted top dress every 3-4 days for 12 months. Prior to initiation of treatment and monthly thereafter, blood was collected for the detection of antibodies to *S. neurona* using a quantitative immunoassay. Further, trough plasma diclazuril levels were determined every 60 days.
Results: All 20 horses remained healthy during the entire study period and *S. neurona* seroprevalence was similar between the two groups. Treated horses displayed significantly lower titers throughout the treatment period ($P < 0.05$). All treated study horses had detectable plasma trough diclazuril levels throughout the study and the levels were above the concentration known to inhibit *S. neurona in vitro* (1.0 ng/mL).
Main limitations: Investigating antibody titers to *S. neurona* is a proxy for infection. Further, no data are yet available to determine the preventive effect of bi-weekly diclazuril administration on the development of EPM.
Conclusions: The administration of diclazuril pelleted top dress at half the label dose twice weekly was able to maintain low antibody titers to *S. neurona* in healthy adult horses naturally exposed to *S. neurona*. Further, trough diclazuril levels were in excess of the minimal concentration known to inhibit *S. neurona*.
Ethical animal research: IACUC protocol was approved for the study.
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: FB, CB, DC, EG, BC, CS and WV are employees of Merck Animal Health.
Source of funding: Merck Animal Health.

Strongylus vulgaris

Oral Presentations

64 | Rejuvenating tradition: Evaluation of the larval culture method for diagnosis of *Strongylus vulgaris*

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Background: *Strongylus vulgaris (S. vulgaris)* is a strongyle type parasite infecting horses, and capable of causing severe pathogenicity and even death. Currently, larval culture and identification is the only diagnostic method commercially available in all countries. This historical technique requires 10-14 days of culturing.
Objective: To evaluate the traditional culture length and identify if a shorter culture period would be suitable for diagnosing *S. vulgaris* infection.
Study design: Assay modification.
Methods: Fresh faecal samples were collected from a horse previously identified to be infected with a patent *S. vulgaris* infection, and a faecal egg count between 500-1000 eggs per gram faeces. All copro-cultures were prepared immediately following sample collection. Cultures were maintained at room temperature and moistened as necessary. Ten samples were examined every day for 15 days, including the day of collection (no culture). All larvae present were identified and enumerated.
Results: No larva were present on the day of collection. After 5 days of culture, third stage larvae (L3) were present, most of which were identified as *Triodontophorus* spp. The first positive *S. vulgaris* larvae presented after 6 days of incubation, with an average positivity rate of 2.4%. After 7 days, the average positivity rate was 34.3%, which was significantly higher than day 6 ($p<0.0001$). There was no significant difference in the proportion of *S. vulgaris* larvae beyond 7 days of culture.
Main limitations: Only a horse of moderate egg shedding level was examined, results may differ for low or high egg shedders, or those with lower proportions of *S. vulgaris*.
Conclusions: One week of culturing is sufficient for identification of *S. vulgaris* larvae, which is shorter than the traditionally described method.
Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples.
Informed consent: Not applicable.
Competing interests: None declared.
Source of funding: Eastern KY University: University Funded Scholarship Grant Competition

65 | The application of Calibrated Automated Thrombogram and plasma Thromboelastography in horses with migrating *Strongylus vulgaris* larvae

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Background: Migration of *Strongylus vulgaris* causes haemostatic imbalances. Global haemostatic tests might be a useful diagnostic aid in evaluation of horses with gastrointestinal (GI) disease in areas with a high prevalence of *S. vulgaris*.
Objective: To assess if plasma-Thromboelastography (pTEG) and Calibrated Automated Thrombogram (CAT) assay can distinguish horses with migrating *S. vulgaris* without clinical disease from horses with GI disease caused by migrating *S. vulgaris* and horses with GI disease of other causes.
Study design: Cohort-study.
Methods: Citrated platelet poor plasma from 5 groups of horses were analysed with the two assays using tissue factor as the activator; clinically healthy horses (n=10), horses with mild (n=9) and severe GI-disease (n=15), *S. vulgaris* associated GI-disease (Sv-GI) (n=6) and *S. vulgaris* infected horses without GI-disease (Sv) (n=10). Groups were compared with ANOVA or Kruskal-Wallis test.
Results: pTEG identified significantly increased coagulability when examining α, K, maximum amplitude and G in the Sv-GI and Sv groups compared to healthy and mild GI-disease groups, but no significant difference between the severe GI-disease, Sv-GI and Sv groups. The CAT assay identified a potential decrease in coagulability by a significantly decreased lag time and time to peak in the mild and severe GI-disease groups compared to the Sv group, but no significant difference between the remaining groups.
Main limitations: Small number of horses.
Conclusion: The two global haemostatic tests demonstrated moderate contradictory results in the horses with *S. vulgaris*. These findings call for further investigations of pTEG and CAT in horses with migrating *S. vulgaris*.
Ethical animal research: Approved by the ethical board of the Department of Veterinary Clinical Sciences at the University of Copenhagen and the University of Kentucky Animal Care and Use Committee.
Informed consent: Owners gave consent for their horses' inclusion.
Competing interests: None declared.
Source of funding: Danish Horse Levy Foundation.

66 | Idiopathic peritonitis versus non-strangulating intestinal infarction associated with *Strongylus vulgaris* infection

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Background: Since the implementation of selective anthelmintic treatment in 2007 in Sweden, there has been an increase in *Strongylus vulgaris* prevalence. This could result in a subsequent increase in non-strangulating intestinal infarction (NSII) peritonitis due to thrombus formation secondary to *S. vulgaris* larval migration. Differentiating cases with NSII peritonitis and idiopathic peritonitis is important for optimal treatment and accurate prognosis but difficult, as they present with similar signs of colic and systemic inflammation.
Objectives: To compare the outcome and identify early indicators that can differentiate cases with NSII and idiopathic peritonitis.
Study design: Retrospective clinical study.
Methods: Patient records from three equine referral hospitals in Sweden during 2017-2020 were reviewed. All NSII cases were confirmed by autopsy or pathological assessment of resected intestinal tissue. Cluster analysis was used to explore associations between demographic parameters, clinical parameters (heart rate, respiratory rate, mucous membranes, colic signs, rectal temperature, rectal palpation), laboratory parameters (peripheral blood and abdominal fluid parameters) and outcome. Univariate analysis (chi^2 test) was used to assess significance of observed associations.
Results: Horses with idiopathic peritonitis had a 98% survival rate with medical treatment while 80% of the NSII cases were euthanased. Horses with NSII presented more often during the winter with more severe signs of colic ($p<0.001$) and were less responsive to medical treatment within 48 hours. Abnormal rectal findings and increased peritoneal protein concentrations were strongly associated with NSII ($p<0.001$ respectively).
Main limitations: The retrospective design resulted in missing data and owner reluctance to consent to surgery in NSII cases due to the poor prognosis may have influenced survival rates in the NSII group.
Conclusions: Horses presenting with septic peritonitis during the winter months, which are unresponsive to medical treatment within the first 48 hours and with a palpable mass in the area of the pelvic flexure, may be cases of NSII.

Ethical animal research: Research ethics committee oversight not required: retrospective analysis of clinical data.
Informed consent: Explicit owner informed consent for inclusion of animals in this study was not stated.
Competing interests: None declared.
Source of funding: The Swedish-Norwegian Foundation for Equine Research.

67 | Differentiation of *Strongylus vulgaris*-associated non-strangulating intestinal infarctions from idiopathic peritonitis and acute colitis

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Background: Horses with non-strangulating intestinal infarction (NSII) are often misdiagnosed as idiopathic peritonitis or acute colitis. Early diagnosis of NSII is essential to ensure immediate surgical intervention and secure survival.
Objective: To compare admission data of horses with NSII to horses with idiopathic peritonitis and acute colitis in order to aid clinicians in their diagnostic workup of horses with NSII.
Study design: Retrospective cohort study.
Methods: Comparison of clinical and laboratory admission data and *Strongylus vulgaris* antibodies in horses admitted with NSII, idiopathic peritonitis and acute colitis at University of Copenhagen during 2009-2018.
Results: 231 horses were included with the three diagnoses (NSII, n=48; idiopathic peritonitis, n=29; acute colitis, n=154). Compared to the two other groups, the NSII group more often had an increased volume of gastric reflux, a mass related to the intestines identified at rectal palpation, and increased *S. vulgaris* antibodies. *S. vulgaris* antibody concentrations >25% (percent of a positive control) had a likelihood ratio of 5.3 and 67% sensitivity and 88% specificity for diagnosis of NSII. Horses with NSII were typically less systemically affected on the clinical and paraclinical presentation and had more peritoneal reaction (higher peritoneal fluid protein, white blood cells and peritoneal fluid lactate/blood lactate ratio) than the acute colitis group but were indistinguishable from the idiopathic peritonitis group.
Main limitations: Due to its retrospective nature, the study was flawed by inconsistent sample collection resulting in missing values.
Conclusions: *S. vulgaris* antibodies could be an aid in diagnosing NSII, but its use is hampered by the high false- negative rate and the lack of a stall-site test system. Based on these results and our previously demonstrated high mortality in NSII which is managed conservatively, exploratory laparotomy is recommended in horses with peritonitis of unknown aetiology in areas were *S. vulgaris* is prevalent.

Ethical animal research: Ethical approval obtained from the ethical board of the University of Copenhagen Large Animal Teaching Hospital.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: The Danish Horse Levy Foundation (Hesteafgiftsfonden) has covered expenses for *S. vulgaris* antibody analyses.

Diagnosis and Control

Oral Presentations

68 | Automated parasite faecal egg counts: Limiting the room for error

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Background: Faecal egg counts (FECs) are the keystone of parasite control programs, but precision, sensitivity, and specificity differ between various manual counting methods and their modifications. Automated FEC systems aim to improve these measures' diagnostic success, in addition to decreasing training requirements and variability introduced by different analysts.
Objectives: To 1) compare precision between McMaster (MM), Wisconsin (MW), and two automated systems using particle shape analysis (PSA) or machine learning (ML) algorithms; 2) estimate sensitivity and specificity using a Bayesian analysis; 3) determine how training effects analysts' ability to perform FECs.
Study design: Assay comparison.
Methods: Equine faecal samples were screened in triplicate using Mini-FLOTAC and placed into negative, 1-200, 201-500, 501-1000, and ≥1001 eggs per gram of faeces (EPG) categories. Precision analysis was completed by performing ten counts per horse per method and a coefficient of variation (CV) was calculated for each sample. CVs for each method were compared using ANOVA. Sensitivity and specificity were estimated using a Bayesian analysis. The effect of training on analyst variability was completed by recruiting three untrained analysts and having each of them perform ten repeated counts at each FEC level for each method. They were then formally trained, and the protocol repeated to calculate analyst variability and variance due to analyst.
Results: CV for samples >200 EPG was significantly lower for MM than MW (p=0.001); MW than PSA (p=<0.001); and ML than MW (p=<0.001). Sensitivity was >98% for all methods, and specificity was highest for CC/PSA. Training minimally effected PSA and

ML, but significantly reduced variability between analysts for MM (p=0.01). Most variance was due to subsample rather than analyst.

Main limitations: Negative samples may have been false negatives due to the screening method.

Conclusions: Automated FEC methods are comparable to manual methods and reduce the need for training.

Ethical animal research: Samples were collected as part of a regular research program under the University of Kentucky IACUC protocol 2012-1046.

Informed consent: Not applicable.

Competing interests: MN and PS both hold stock in MEP Equine Solutions, a company that is manufacturing an automated parasite egg counting technique. PS is also an employee.

Source of funding: None.

References

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Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not stated.

Competing interests: None declared.

Sources of funding: University of Naples Federico II.

69 | Comparison of three methods for the diagnosis of *Oxyuris equi* infection in horses

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Background: Horses are parasitised by several intestinal helminths such as strongyles, ascarids, tapeworms and pinworms. *Oxyuris equi*, the horse pinworm, causes perineal irritation (tail rubbing, broken hairs and excoriated skin) [1]. Faecal Egg Count is the routine method for the diagnosis of intestinal parasite in equids [2]; however, pinworm eggs are rarely observed in faecal samples because *O. equi* females deposit eggs in the perianal region [1].

Objectives: The aim of this study was to compare three diagnostic techniques namely Mini-FLOTAC, Proudman test and Scotch test for detecting *O. equi* eggs.

Study design: Assay comparison.

Methods: Scotch test, Mini-Flotac technique and Proudman test were performed on 2,857 horses to determine technique diagnostic accuracy. Two Scotch tests were performed for each horse and faeces were collected directly from the rectum. The faecal samples were analysed in duplicate using both Mini-FLOTAC and Proudman test with a Sheather's saturated sugar solution. Considering all three methods used, a total of 17,142 assays was performed.

Results: Overall, *O. equi* eggs were found in 125 (4.4%) horses. Of these 91 (72.8%) were positive only by Scotch test; 19 (15.2%) by Scotch test and Mini-Flotac; 5 (4.0%) by Scotch test and Proudman test; 7 (5.6%) only by Mini-Flotac and 3 (2.4%) only by Proudman test.

Main limitations: Underestimation of *O. equi* prevalence due to the egg laying pattern.

Conclusions: The number of horses positive for *O. equi* eggs was higher using the Scotch test, thus showing that it is a more sensitive method for diagnosis of pinworms than FEC and Proudman test.

70 | Molecular detection of *Strongyloides westeri* in Australian Thoroughbred foals

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Background: Horses can be infected with a variety of gastrointestinal nematodes (GINs) at various stages of their life. *Strongyloides westeri* (*S. westeri*) is one of the GINs that can infect foals of up to 16 weeks of age. The trans-mammary transmission is one of the main routes of infection for foals and the adult parasite resides in the proximal part of the small intestine, leading to variable pathogenicity. The epidemiology of *S. westeri* in Australia is largely unknown. Further, molecular techniques have never been employed for detection of *S. westeri* in horses.

Objectives: This study was conducted as a part of the national project on GINs of Australian Thoroughbred horses and aimed to assess the utility of a molecular-phylogenetic method for the detection of *S. westeri* in the faeces of foals.

Study design: A pilot study on molecular based detection of *S. westeri*.

Methods: Faecal samples were collected from a foal of less than two months of age and eggs of *Strongyloides* sp. were detected using the modified McMaster technique. DNA was extracted from purified eggs and a partial fragment of the small subunit of the nuclear ribosomal DNA (18S) was characterised using polymerase chain reaction, DNA sequencing and phylogenetic methods.

Results: Microscopic examination of faeces revealed small ellipsoidal eggs typical of *Strongyloides* sp. The 18S sequence generated by PCR in this study revealed 98.4% identity with that of a reference sequence of *S. westeri* available from GenBank. Phylogenetic analyses revealed a polyphyletic clustering of *S. westeri* sequences.

Main limitations: Larval culture was not performed from collected samples and availability of only one partial 18S sequence of *S. westeri* available in Genbank were the main limitations.

Conclusion: These results show the occurrence of *S. westeri* in Australian Thoroughbred horses and warrants future large-scale studies to assess the prevalence of the parasite in Australia.

Ethical animal research: The project was approved by Office for Research Ethics and Integrity Animal Ethics committee (Ethics ID No: 1914965.1).

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

Competing interests: None declared.

Source of funding: AgriFutures Australia (PRJ-011191) and Thoroughbred Breeders Australia.

Poster Presentation

71 | A survey of helminth control practices on large horse farms in Argentina

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Background: Since nematodes have developed anthelmintic resistance (AR) in Argentina, it is essential to look for sustainable control strategies. However, information on deworming practices is very limited.

Objectives: To describe parasite control practices and identify prevalence of factors known to influence AR.

Study design: Questionnaire survey.

Methods: Eighty veterinarians from 85 stud farms were asked to complete a questionnaire on parasite control practices covering Thoroughbred, polo, criollo and mixed breed horses.

Results: Parasite control was studied in 17,363 horses, with median by farm = 204.2 (48-1860). In all farms, grazing conditions were present and co-grazing with cattle occurred in 73%. Just two facilities (2%) removed faeces from the pastures (frequency = once a week). Horses on most premises were drenched with anthelmintics at a set interval (more than 65% = three to four times a year) and none carried out selective treatments. Ivermectin was the most common anthelmintic (96% of the farms) followed by benzimidazoles (50%), pyrantel (9%) and combinations of nematodicides (moxidectin/oxfendazole; 7%). Extra-label use of ivermectin was common, with 82% of the farms using it at least in some horse categories. Approximately 20% of the farms carried out faecal egg counts (FEC) but just two of them used it as a method to decide the need for anthelmintic treatment and only one to evaluate drug efficacy in a faecal egg count reduction test.

Main limitations: Convenience sampling of respondents may not be representative of the anthelminthic practices used by the wider population of equine stud farm veterinarians.

Conclusions: In Argentina, current parasite control is based almost exclusively on massive administration of macrocyclic lactones with high off-licence use of ivermectin. Adoption of FECs to decide and determine the effectiveness of treatments is poor. Treatment protocols need updating and evidence-based guidelines for reducing further development of AR should be developed.

Ethical animal research: Research ethics committee oversight not required: survey of veterinarians.

Informed consent: Participation of responders to the survey was taken as consent.

Competing interests: None declared.

Source of funding: Universidad Católica de Córdoba, Argentina.

Virology

African Horse Sickness Virus

Oral Presentations

72 | All nine African horse sickness virus serotypes isolated from horse fatalities in Kenya over the last twenty years (2000-2020)

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Background: Knowledge of the current serotypes of African horse sickness in circulation in Kenya is important to inform future vaccination strategies.

Objectives: To re-evaluate the AHS situation in Kenya and determine if all serotypes can still be found and thus inform future vaccination strategies.

Study design: Samples from 102 horses and one donkey were analysed over a span of 20 years.

Methods: The AHS viruses were isolated from unclotted whole blood, from lung lymph nodes as well as from lung and spleen from dead necropsied horses originating from different areas of Kenya. EDTA blood was withdrawn from the jugular vein in the early stages of the disease and necropsy performed in some cases. Identification

of the virus was by virus isolation from the original sample and PCR on archived culture supernatant.

Results: Over a period of 20 years, all 9 AHS serotypes were isolated. Serotypes were isolated in tissue culture and the recovery of the virus sometimes needed 7 sub cultures on BHK cells until a proper cytopathic effect had developed.

Main limitations: With our investigations, we anticipated finding clusters of AHS, which would have enabled us to use single serotype vaccines for the control of an outbreak, but unfortunately this was not achieved.

Conclusions: All 9 AHSV serotypes still occur in any zone of Kenya, so there is a need to develop an inactivated AHS vaccine which contains all 9 serotypes.

Ethical animal research: Research activities at CVRL Dubai are monitored by an Ethics Committee consisting of veterinarians from the CVRL and the UAE Ministry of Environment and Climate Change (MOCCAE).

Informed consent: Owners gave consent for animals' inclusion.

Competing interests: None declared.

Source of funding: Kisima Farm, Kenya which belongs to one of the co-authors (SS) and CVRL.

73 | Feeding patterns of *Culicoides*

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Background: *Culicoides* Latreille (Diptera: Ceratopogonoidae) biting midges are of veterinary and economic importance worldwide. Numerous studies have been done throughout the world to clarify vector-host relationships by studying *Culicoides* host preferences via blood meal analysis. Studies have concentrated on *Culicoides* as a vector for livestock associated diseases. Since 1904, AHSV has been seen in our canine population and until recently it was believed that dogs only become infected by ingesting infected meat and therefore, little emphasis has been placed on determining whether *Culicoides* will feed on dogs.

Objectives: The objectives of this study were to ascertain the species-specific feeding behaviour of *Culicoides*.

Study design: Field survey.

Methods: Onderstepoort light traps and carbon dioxide baited traps were set weekly near different species of animals over the period of a year. The collected *Culicoides* were sorted, and the blood meals of blood engorged females were analysed. The *Culicoides* were identified to species level and by parity using light microscopy. Blood meal analysis of 310 blood engorged females was achieved by amplifying a species-specific fragment of the mitochondrial cytochrome *b* gene. This method allowed the accurate detection of multiple species from a single blood meal.

Results: *Culicoides* show a preference for feeding on horses at the Faculty of Veterinary Science at Onderstepoort. It was also shown that they will feed on dogs although to a much lesser extent than livestock species. The majority of blood meals analysed were from *C. imicola* but interestingly, all three *C. leucostictus* which were analysed had fed on dogs.

Main limitations: Light traps are only effective at night and carbon dioxide traps were not effective in collecting *C. imicola*.

Conclusions: *Culicoides* collected at Onderstepoort feed on multiple domestic species including dogs. *C. leucostictus*, previously described as being ornithophilic, feed on dogs and this warrants further investigation.

Ethical animal research: Research ethics permission obtained from the University of Pretoria.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: Equine Research Centre, Faculty of Veterinary Science, University of Pretoria.

Poster Presentation

74 | Inactivated African horse sickness vaccines

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Background: African horse sickness (AHS) is a viral disease of equids caused by a member of the genus *Orbivirus*, family *Reoviridae*. Prevention and control of the disease are based on live, attenuated vaccines and midge control. Studies show that the only commercially available live attenuated AHS vaccine is not safe. There is a need to develop a safe AHS vaccine to protect horses from the disease in case of an outbreak in the UAE.

Objectives: To establish an inactivated AHS vaccine containing all nine serotypes in the Central Veterinary Research Laboratory (CVRL), UAE and undertake vaccine safety and immunogenicity experiments in horses.

Study design: A total of 39 horses kept in a desert isolation area in Dubai and 77 horses kept in the Laikipia area of Kenya were subcutaneously or intramuscularly vaccinated with the CVRL AHS inactivated vaccine. Primary vaccination was followed by the administration of a booster and annual doses.

Methods: Blood samples were regularly withdrawn to detect antibody as measured by ELISA and virus neutralisation testing. Additionally, EDTA blood was tested every second day for 14 days post each vaccination for the presence of AHS virus or its RNA.

Results: A high titre humoral immune response was detected in horses which were simultaneously vaccinated with all nine serotypes. Antibodies remained detectable for one year. Horses

previously vaccinated with the Onderstepoort live attenuated vaccine responded rapidly and strongly to the production of protective antibodies. No replicating AHSV or viral RNA was detected after vaccination.

Main limitations: No AHSV challenge infection was carried out.

Conclusions: Inactivated AHSV vaccines containing all nine serotypes are safe, with no risk of reversion to virulence and are immunogenic. Further investigation is therefore warranted.

Ethical animal research: Research activities at CVRL Dubai are monitored by an Ethics Committee consisting of veterinarians from the CVRL as well as from the UAE Ministry of Environment and Climate Change (MOCCAE).

Informed consent: Owners gave consent for animals' inclusion.

Competing interests: None declared.

Source of funding: Kisima Farm, Kenya, which belongs to one of the co-authors (SS) and CVRL.

Equine Herpesvirus-1 and -4

Oral Presentations

75 | EHV-1 neurological outbreak during a show-jumping competition: a clinical and epidemiological study

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Background: A total of 753 horses were involved in the CES Valencia (Spain) Spring Tour 2021. Due to an EHV-1 outbreak, the competition was cancelled and the site was locked down with 157 horses from 15 different nations staying on site.

Objectives: Describe epidemiological, clinical, diagnostic, treatment and outcome data on a population of horses staying in Valencia.

Study design: Retrospective clinical study.

Methods: From the 157 horses on site, 60 horses were followed (32 mares, 24 geldings and 4 stallions) and 67 were sampled with nasopharyngeal swabs sent to Labeo.

Results: From the 60 horses, 10 showed no signs (no fever, no neurological signs). A total of 50 horses showed fever between 38.6 and 41.2°C which lasted for 4.0±2.1 days. Of these, 60% showed no further signs and 40% showed neurological signs with 8 horses hospitalised, of which 2 died. Neurological signs included either ataxia, urinary problems with bladder atony and lack of tail tone. For the mares, 75% showed fever and of these 50% showed neurological signs. For the geldings, 91.7% showed fever and of these 31.8% showed neurological signs. For the 4 stallions, all showed fever and one showed neurological signs. The mean duration between the last day of fever and the beginning of neurological signs was 1.05±1.32 days. There were 33 vaccinated horses (31 had a booster less than 6 months prior to the show): 96.9% showed fever of which 45.4% showed neurological signs. Among the 27 non-vaccinated horses, 66.7% showed fever of which 27.8% showed neurological signs. EHV-1 was detected by qPCR, genotyped as A2254 (ORF30) and isolated on cell culture.

Main limitations: Not all horses on site were included leading to potential selection bias.

Conclusions: These data were collected in a real outbreak situation and give interesting information about clinical findings in relation with epidemiological data such as sex or vaccination status for example.

Ethical animal research: This study was performed during an EHV-1 outbreak in a showjumping competition. The data obtained from the riders and the samples taken from the horses were done as normal clinical conditions.

Informed consent: Yes.

Competing interests: None declared.

Source of funding: French Equestrian Federation, Labeo Frank Duncome, ONIRIS.

76 | Major Equine Herpesvirus - 1 epizootic in Europe: Identification of a marker for epidemiological surveillance

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Background: Equine herpesvirus type-1 (EHV-1) is an important threat to the equine industry, as illustrated by the ongoing outbreak of neurological disease that was initially reported during a large equestrian event in Valencia, Spain in 2021. Horses returning from this event to their training yards have contributed to the spread of the virus to nine other European countries and to Qatar.

Objectives: To design a "tracking" marker in order to specifically follow the dissemination of the Valencia strain, in EHV-1 infected horses with no known epidemiological link with the Valencia outbreak.

Study design: Strain isolation and genome sequences' comparison.
Methods: 67 nasopharyngeal swab samples from horses stationed in Valencia were analysed by EHV-1 qPCR and a rapid A/G/C_{2254} (ORF30) typing test. Positive samples were used for strain isolation *in vitro*, and Multilocus Sequence Typing (MLST).
Results: 19/67 (28%) samples were EHV-1 positive (all 19 typed as A_{2254}). Two strains (FR/Valencia1/2021 and FR/Valencia2/2021) were successfully isolated and characterised by MLST as belonging to clade 10. Analysis of the MLST ORF sequences revealed a mutation at position 713 of the ORF11 (A713G) in FR/Valencia1/2021 and FR/Valencia2/2021, when compared with reference strains Ab4 and V592. This A713G mutation was not present in 104 ORF11 sequences obtained from Genbank (strains from the UK, USA, China, Australia, Belgium, New-Zealand, Japan or India), or 131 and 14 ORF11 sequences from strains isolated in Ireland or France, respectively. This marker allowed subsequent confirmation of suspicious epidemiological links in EHV-1 cases.
Main limitation: The limited number of ORF11 sequences available in some countries.
Conclusions: Although the existence of this mutation in other field strains cannot be excluded, its absence in the 249 ORF11 sequences analysed, suggests that this SNP constitutes an interesting epidemiological marker to identify horses infected with the EHV-1 strain which was associated with the outbreak in Valencia.
Ethical animal research: Research ethics committee oversight not required by this journal: retrospective analysis of clinical data.
Informed consent: Explicit study consent was not stated but owners were aware that samples could be used for research activities.
Competing interests: None declared.
Sources of funding: Fonds Eperon (N39-2019) and by GIS CENTAURE.

77 | Decreased virus-neutralising antibodies against equine herpesvirus type 1 in nasal secretions of horses after 12-hour transportation

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Background: Reactivation of latent equine herpesvirus type 1 (EHV-1) and type 4 (EHV-4) and subsequent disease outbreaks have been associated with long distance transportation.
Objectives: To assess the effects of 12-hour transportation on immune responses to EHV-1 and EHV-4 in horses.
Study design: *In vivo* experiments.
Methods: Six healthy Thoroughbreds with transport experience were transported in commercial trucks, repeating the same 3-hour route four times. Possible replication of EHV-1 and EHV-4 was monitored by real-time PCR of nasal swabs and peripheral blood mononuclear cells (PBMCs), and changes in systemic and mucosal antibodies were investigated. Blood samples for cortisol measurement were taken before departure and every three hours. Nasal swabs, PBMCs, nasal wash and serum samples were collected before departure, at unloading, 2 and 6 days after arrival.
Results: Cortisol concentration increased significantly after 3 and 6 hours of transport ($P < 0.05$). No evidence of viral replication was observed, and serum virus neutralisation (VN) titers for EHV-1 and EHV-4 were unchanged, except for one horse that showed a 4-fold decrease in titer against EHV-1 after transportation. Urea and total IgA concentration in nasal washes increased significantly after transportation ($P < 0.05$), while total IgA/protein ratio was unchanged. A transient, ≥4-fold decrease in VN titers for EHV-1 in nasal wash concentrates was observed in 4 out of 6 horses after transportation (geometric mean titer declined from 202 to 57, $P < 0.05$). VN antibodies against EHV-4 in nasal secretions were not detected at any timepoint.
Main limitations: Cell-mediated immunity was not investigated.
Conclusions: Twelve hours transportation caused acute stress in horses, although viral replication was not observed. VN antibody titers against EHV-1 in nasal secretions decreased temporarily after transportation, suggesting suppression of VN capacity in the nasal mucosa may contribute to susceptibility to EHV-1 after transportation.
Ethical animal research: Approved by the animal care and ethics committees of the Equine Research Institute of the Japan Racing Association with accession number 19-28 and Charles Sturt University with project number A19264.
Informed consent: Not applicable.
Competing interests: None declared.
Source of funding: Japan Racing Association.

78 | Protective immunity against equine herpesvirus type 1 is associated with antibody responses to the vaccine candidate Ab4ΔORF2

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Background: Equine herpesvirus type 1 (EHV-1) induces respiratory infection, abortion, and neurologic disease with significant impact.
Objectives: Deletion of the virulence factor-encoding open reading frame 2 from the neuropathogenic EHV-1 strain Ab4 yielded a new vaccine candidate, Ab4ΔORF2, which was analysed for safety and efficacy *in vivo*.

Study design: Randomised double-blinded vaccination/challenge study.

Methods: Clinical and immunological outcomes of primary experimental infections of horses with Ab4, or Ab4ΔORF2 compared to non-infected controls were assessed (n=8/group). All horses were challenged with EHV-1 Ab4 intranasally nine-months later. Clinical outcomes and correlates of protection were analysed.

Results: Primary infection with the vaccine candidate Ab4ΔORF2 reduced fever and minimised nasal virus shedding after infection compared to Ab4, while Ab4ΔORF2 established cell-associated viraemia similar to Ab4. The horses' immune responses were similar after infection with Ab4 or Ab4ΔORF2 and were dominated by EHV-1-specific antibodies in nasal secretions and serum. After the challenge infection, previously uninfected horses (Control/Ab4) displayed fever and clinical disease, shed high amounts of virus, and developed viraemia. In contrast, 5/8 (Ab4ΔORF2/Ab4) and 3/8 (Ab4/Ab4) horses previously infected with Ab4ΔORF2 or Ab4, respectively, were fully protected from Ab4 challenge infection as indicated by the absence of fever, clinical disease, nasal virus shedding, and viraemia. All of these outcomes were significantly reduced in the remaining, partially protected 3/8 (Ab4ΔORF2/Ab4) and 5/8 (Ab4/Ab4) horses. Protection was linked to pre-existing and rapidly boosted nasal EHV-1-specific antibody responses, that likely neutralised EHV-1 Ab4 at the respiratory entry site. Protection was also associated with EHV-1-specific serum antibodies pre-challenge.

Main limitations: A limited number of horses and no cell mediated immunity were studied. However, the protective nature of pre-challenge intranasal antibodies agreed with a previous vaccination/challenge study [1].

Conclusions: Robust protection from challenge infection emphasises Ab4ΔORF2 as a vaccine candidate. EHV-1-specific antibodies are strong correlates of protection against EHV-1 infection.

Reference

[1] Perkins, G., Babasyan, S., Stout, A.E., Freer, H., Rollins, A., Wimer, C.L. and Wagner, B. (2019) Intranasal IgG4/7 antibody responses protect horses against equid herpesvirus-1 (EHV-1) infection including nasal virus shedding and cell associated viremia. *J. Virol.* **531**, 219–232. https://doi:https://doi.org/10.1016/j.virol.2019.03.014

Ethical animal research: The animal protocol was approved by the Institutional Animal Care and Use Committee at Cornell University (protocol #2011-0011).

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: Agriculture and Food Research Initiative Competitive Grant no. 2015-67015-23091, US Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA), Harry M. Zweig Memorial Fund for Equine Research at Cornell University, USDA/NIFA grants #2005-01812 and #2015-67015-23072.

79 | Development of an assay using real time cell analysis for the measurement of equid herpesvirus 1 specific neutralizing antibody in horses after experimental infection or field vaccination

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Background: Equid herpesvirus-1 (EHV-1) is one of the most important horse pathogens, which induces respiratory disease, myeloencephalopathy, abortion or still-birth. The measurement of EHV-1 neutralising (EHV-1-VN) antibodies using a neutralisation assay (NA) gives an indication of the immune response of horses in the field.

Objectives: To develop a real time neutralisation assay (RTNA) using the xCELLigence® technology for the measurement of EHV-1-VN antibodies in serums from EHV-1 experimentally infected or vaccinated horses.

Study design: Assay development and field trial.

Methods: The RTNA based on E. Derm cells was developed using serums (n=48) taken daily (from Days 0 to 18 post-infection) from ponies experimentally infected with a field EHV-1 strain. This method was further evaluated using field serums (n=63) taken during an EHV-1/-4 vaccination campaign. RTNA results were compared to those obtained with the conventional EHV-1 NA on RK13 cells. Two-fold serial dilutions of serums were incubated with a defined concentration of the KyD EHV-1 strain before infection of E. Derm cells. The normalised cell index was monitored pre- and post-infection using the xCELLigence® technology. Conventional NA was performed using the same experimental methodology on RK13 cells and cytopathic effects were measured 72 hours post-infection.

Results: Serum titres which induced 50% of EHV-1 neutralisation (NT_{50}) were determined using a dose-response approach. The increase of the EHV-1 NT_{50} was similar to the increase in titre measured with the conventional NA (R^2=0.79). Seroconversion was detected between Days 0 and 18 post-infection for all infected ponies with both methods. The NT_{50} values measured for EHV-1/-4 vaccinated horse serums were also positively correlated with conventional NA titres (R^2=0.83).

Main limitations: EHV-1/-4 cross-reactivity will require further investigation. Each assay used different cells.

Conclusion: While the conventional NA is time consuming, requires subjective measurements and provides semi-quantitative discrete

ranked titres (e.g. 4, 8 etc.), the EHV-1-VN RTNA is an automated, sensitive and objective measurement that reports continuous titres.
Ethical animal research: All experimental procedures were approved by the Loire Valley ethical review board (CEEA VdL, committee number 19).
Informed consent: Informed consent was given by the horses' owners.
Competing interests: None declared.
Sources of funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324), the IFCE (Institut Français du Cheval et de l'Equitation) grant number 2017-008, CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

80 | Detection of *Equid herpesvirus-1* in serum samples collected from infected horses

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Background: Abortion and myeloencephalopathy are caused by *Equid herpesvirus-1* (EHV-1) infection as a consequence of its transmission to susceptible organs by viraemia. Since EHV-1 circulates in the bloodstream in a cell-associated manner, serum samples collected from infected or febrile horses are rarely used for virus detection.
Objectives: To determine the usefulness of horse serum samples for the detection of EHV-1.
Study design: Assay assessment.
Methods: Archived sera and peripheral blood mononuclear cells (PBMCs) collected daily from three horses that had been experimentally inoculated with EHV-1 during the subsequent two-week observation period [1] were investigated. Acute-phase serum samples collected from 40 febrile (≥ 38.5°C) horses, including 11 serologically confirmed field EHV-1 cases were also examined. Real-time PCR was used to detect EHV-1 in the samples.
Results: EHV-1 was detected in experimental PBMC and serum samples for 6 to 7 days (from post-infection day [PID] 5 or 6 to 11 and sporadically in one sample at PID 1) and 5 to 7 days (from PID 5 or 7 to 11), respectively. Six of 11 acute-phase serum samples collected from field EHV-1 cases were positive for the virus, whereas the rest of the field sera were negative.
Main limitation: The presence of cell-free intact particles of EHV-1 in horse sera was unclear.
Conclusions: EHV-1 was detected almost simultaneously in PBMC and serum samples collected from experimentally infected horses. Additionally, more than half of the field acute-phase sera collected from EHV-1 infected horses tested positive for the virus, which suggests that the pyrexia observed in these horses was caused by viraemia. These results show that serum samples collected from EHV-1 infected or febrile horses can be used to detect the virus if PBMC samples are not available.

Reference

[1] Bannai, H., Nemoto, M., Tsujimura, K., Yamanaka, T., Kokado, H., Kondo, T. and Matsumura, T. (2018) Comparison of protective efficacies between intranasal and intramuscular vaccination of horses with a modified live equine herpesvirus type-1 vaccine. *Vet. Microbiol.* **222**, 18–24.

Ethical animal research: This study was approved by the Research Planning and Ethics Committee of the Equine Research Institute with accession number 2018-3263-07.
Informed consent: Owner consent was obtained for all field samples.
Competing interests: None declared.
Source of funding: Japan Racing Association.

81 | Generation of EHV-1 pseudotype virus for cell tropism studies and virus-neutralising assays

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Background: Equid herpesvirus-1 (EHV-1) can cause respiratory disease, abortion, neonatal death and myeloencephalopathy. Thus, EHV-1 represents a threat to the equine industry. EHV-1 exhibits 12 glycoproteins on its surface envelope, but those important for cell entry/host immune responses remains partially unclear. To investigate the contribution of these glycoproteins, pseudotype viruses (PVs) may provide a useful study tool.
Objectives: Generate high titre EHV-1 PV particles for cell tropism studies and develop tests for virus-neutralising (VN) antibody detection in naturally/experimentally infected horses.
Study design: Assay development.
Methods: 5 EHV-1 glycoprotein gene sequences were obtained from an aborted fetus strain isolated during a large EHV-1 outbreak in France in 2010. Sequences were synthesised and subcloned into expression vectors and employed in lentivirus PV generation. PVs were utilised in a Pseudotype Virus Neutralisation Test (PVNT), a sensitive technique to measure levels of specific VN antibodies. Serum samples (n=48) tested were taken longitudinally (Days 0 to 18 pi) from ponies experimentally infected with EHV-1, compared with uninfected controls (n=4). Plasmids expressing PV components' genes were co-transfected into HEK293T/17 using polyethylenimine (PEI). PV production and quantification were assessed by fluorescence and luminescence, respectively. For PVNT, two-fold serial dilution of equine sera were incubated with PV and target cells. As for

traditional VN tests, the antibody titre was expressed as the highest serum dilution causing 50% inhibition (IC50).

Results: Titres of EHV-1 PV were optimised and PVNT successfully performed and compared with a conventional EHV-1 VN assay (r=0.82).

Main limitations: Cross-reactivity studies with other EHVs need further investigation.

Conclusions: Functional EHV-1 PVs can be generated using a minimum of four glycoproteins gB, gD, gH and gL. The addition of gC neither enhances PV production nor is essential for cell entry. EHV-1 neutralising antibodies can be quantified in experimentally infected horse sera.

Ethical animal research: The use of sera was authorised by the Loire Valley ethical review board (CEEA VdL, committee number 19).

Informed consent: Not stated.

Competing interests: None declared.

Source(s) of funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324) and the University of Kent.

82 | Oral administration of valganciclovir reduces clinical signs, virus shedding and cell-associated viraemia in ponies experimentally infected with equid herpesvirus-1

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Background: Equid alphaherpesvirus-1 (EHV-1) is a frequent respiratory pathogen of the horse, causing mild disease and occasionally myeloencephalopathy (EHM) or abortion. Current vaccines reduce the nasopharyngeal excretion and dissemination of the virus and therefore the extent of an epizooty, but their efficacy against secondary forms of diseases (abortion and EHM) is either limited or remains untested respectively. Several antiviral compounds are active against EHV-1 *in vitro* but no pharmaceuticals are licenced for *in vivo* treatment to date.

Objectives: To measure the *in vivo* efficacy of antiviral compounds, starting on the day of experimental infection of the target species with EHV-1 (C2254), as assessed by any reduction of clinical signs, virus shedding and viraemia.

Study design: Randomised semi-blinded experiment.

Methods: Four ponies were treated with valganciclovir (VGCV, the oral prodrug of ganciclovir [GCV]) at 6.5 mg/kg bodyweight, three times on day 1 and twice daily until day 14 inclusive. Four other ponies received a placebo. All ponies were experimentally infected with a field EHV-1 strain (5e07 $TCID_{50}$/pony). Clinical signs of disease, virus shedding and blood/cell associated viraemia were recorded and measured for 3 weeks.

Results: Serum GCV concentration was maintained above the EC_{50} (0.153 μg/mL) for at least 15 days. The overall cumulative clinical score was significantly reduced in VGCV treated ponies when compared with controls ($p<0.009$; pyrexia duration, nasal discharge and coughing). Infectious EHV-1 shedding measured on RK13 cells was significantly reduced in the VGCV treated group when compared with the control group between D+1 and D+12 ($p=0.006$). Blood and cell-associated viraemia were also both significantly reduced in the VGCV treated group ($p=0.02$ and 0.03, respectively). All ponies seroconverted after infection.

Main limitations: Due to animal management procedures, blinding was not possible for clinical evaluation.

Conclusions: Oral administration of valganciclovir for 14 days from the first day of experimental EHV-1 infection induced no noticeable side effects but significantly reduced clinical signs, virus shedding and cell-associated viremia.

Ethical animal research: All experimental procedures were approved by the Loire Valley ethical review board (CEEA VdL, committee number 19, authorisation number APAFiS#22708).

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324), the IFCE (Institut Français du Cheval et de l'Equitation) grant number 2017-008, CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

83 | Identification of antiviral compounds against equid herpesvirus-1 using Real-Time Cell Analysis: screening of 2,891 molecules

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Background: EHV-1 infections cause respiratory, neurological and reproductive diseases. Despite preventive measures such as vaccination, the frequent occurrence of EHV-1 infection remains a major threat to the horse industry. Furthermore, there is no approved antiviral therapy to treat infected horses.
Objectives: Identification of new antiviral compounds effective against EHV-1 by repositioning drug molecules, followed by the evaluation of any synergistic effects between the most potent candidates.
Study design: *In vitro* analysis of micro-organisms.
Methods: Using an impedance assay based on the Real-Time Cell Analysis (RTCA) system, 2,891 compounds were screened on E. Derm cells infected with EHV-1. Antiviral potency was evaluated on three different cell lines and against three EHV-1 strains (ORF30 2254 A/G/C). Combination analysis was performed with RTCA technology using MacSynergy II methods and confirmed using isobologramm and Chou-Talalay methods.
Results: 22 of 2,891 compounds were found to reduce EHV-1 replication and cytopathic effects *in vitro*. Decitabine (EC_{50} = 0.5 μM), valganciclovir (EC_{50} = 1.7 μM), ganciclovir (EC_{50} = 2.7 μM), aphidicolin (EC_{50} = 3.4 μM), idoxuridine (EC_{50} = 4.9 μM) and pritelivir (EC_{50} = 12.6 μM) were the most potent compounds. These six compounds were active regardless of the cell line or the strain used. With a synergy volume of 63.24 μM^2%, the decitabine/valganciclovir combination was the only one exhibiting synergistic effect. Deoxycytidine reverts the antiviral effect of decitabine, thus suggesting some competition at the level of nucleoside phosphorylation by deoxycytidine kinase and/or DNA synthesis.
Main limitations: Further investigation is required to validate *ex vivo* and *in vivo* the antiviral efficacy of these compounds.
Conclusions: Deoxycitidine analogues, like decitabine, were demonstrated for the first time to be potent molecules inhibiting the replication of EHV-1. Thus, they represent a new promising chemical series against EHV-1 for future *in vivo* studies.
Ethical animal research: Not applicable.
Informed consent: Not applicable.
Competing interests: None declared.
Sources of funding: LABÉO, IFCE (Institut Français du Cheval et de l'Equitation, project AMIE), Fonds Eperon (project N87-2014, N07-2015, N07-2016, N13-2017 and N62-2017), Région Normandie (CPER R25 P3) and CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

84 | Characterisation of neuropathogenic and non-neuropathogenic variants of Equid alphaherpesvirus-1 in Argentina

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Background: Equid alphaherpesvirus-1 (EHV-1) is a ubiquitous pathogen of horses, which may induce mild respiratory disease, abortion, neonatal death and myeloencephalopathy. A single nucleotide polymorphism in the EHV-1 DNA polymerase (ORF30 A2254G, N752D) has been widely associated, although not exclusively, with neuropathogenicity. Recently, a new genotype has been described (ORF30 C2254, H752). Information regarding strains circulating in Argentina between 1996 and 2008 showed that 93% of the EHV-1 strains isolated from aborted fetuses were A2254 genotype and the remaining 7%, G2254.
Objectives: To analyse the ORF30 genomic region of EHV-1 isolated from abortion samples in order to characterise the strains circulating in Argentina between 2008 and 2020.
Study design: Descriptive study.
Methods: A total of 971 samples from aborted fetuses submitted to the laboratory for virological diagnosis were analysed by PCR. EHV-1 positives samples were analysed by qPCR assay for allelic discrimination of EHV-1 strains with A2254 and G2254 polymorphisms.
Results: EHV-1 was detected in 4.73% (46/971) of the samples, with 97.8% (45/46) and 2.2% (1/46) characterised as "non-neuropathogenic" (A2254) and "neuropathogenic" (G2254) genotype, respectively. The sole G2254 strain detected, corresponded to a sporadic case of abortion not associated to neurological disease. One case with EHV-1 characterised as A2254, was part of an abortion storm with a previous (1 month before) case of neurological disease in a two-year-old horse. However, central nervous system tissue from this horse was negative for EHV-1. One EHV-1 positive sample could not be genotyped as G2254 or A2254.
Main limitations: The current q-PCR has been not designed to detect the new C2254 genotype.
Conclusions: Results showed that both EHV-1 genotypes A2254 and G2254 still circulate in Argentina, in the same proportions as those described previously. These results could contribute to a better understanding of the relationship between the presence, or absence of a point mutation in the ORF 30 of EHV-1.
Ethical animal research: Research ethics committee oversight not required by this journal: retrospective analysis of clinical data.

Owner informed consent: Explicit study consent was not stated but owners were aware that samples could be used for research activities.

Competing interests: No competing interests have been declared.

Source of funding: INTA and INTA-Haras agreement.

85 | Molecular investigation of the prevalence of Equine alphaherpesvirus-1 infection in healthy postpartum mares

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Background: Equine alphaherpesvirus-1 (EHV-1) is considered the most important equine viral cause of abortions. The detection of EHV-1 in mares with virus-negative EHV-1 aborted fetuses presents a diagnostic challenge for the clinical confirmation of EHV-1-associated abortions. Although virus-negative EHV-1 aborted fetuses have been found in naturally-occurring and experimentally-induced EHV-1 infections [1,2], it has been questioned whether healthy mares may shed the virus due to stress associated with foaling.

Objectives: Investigate 1) whether clinically healthy broodmares shed EHV-1 or develop EHV-1 viraemia during parturition and 2) whether EHV-1 can be detected in placentas of clinically healthy foals.

Study design: Pilot prospective study.

Methods: Nasal and vaginal swabs, EDTA blood, and placental tissue from 50 broodmares collected within 24 hours after foaling were tested for the presence of EHV-1 strains using a RT-PCR assay [3]. Mares' age, breed, parity, history of abortions or medical issues during pregnancy, and vaccination history for EHV-1 were recorded.

Results: A total of 36 Standardbred, 12 Thoroughbred and 1 Quarter horse and 1 Warmblood mare with a median age was 8 years (range: 4 to 21) were monitored. The median parity was 2 (range: 1 to 13 events). Twenty mares were vaccinated against EHV-1 during pregnancy, while 30 mares were unvaccinated. Three mares were reported to have placentitis during pregnancy. None of the samples (n = 400) tested positive for EHV-1 strains.

Main limitations: The animals included in the study originated from only a few farms in southern Ontario.

Conclusion: EHV-1 was not detected in placental tissues, blood and nasal or vaginal secretions by molecular testing in postpartum healthy mares. Therefore, detection of EHV-1 in similar samples from aborted mares is probably clinically significant.

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Ethical animal research: This project was approved by the University of Guelph's Institutional Animal Care Committee.

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

Competing interests: None declared.

Source of funding: Ontario Animal Health Network.

Poster Presentations

86 | Identification and *in vitro* and *in vivo* characterization of a new equid herpesvirus-1 DNA polymerase (ORF30) genotype associated with a C2254 / H752 strain in French horses

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Background: Equid herpesvirus-1 is one of the most common viral pathogens affecting horses and is associated with respiratory disease, abortion, neonatal and neurological disease. A single point mutation in the DNA polymerase gene (ORF30: A2254G, N752D) has been associated with neuropathogenicity although this is not a consistent finding in clinical outbreaks. An EHV-1 strain carrying a new genotype (C_{2254}/H_{752}) was isolated from an outbreak in France in 2018, involving 82 frequently vaccinated horses, two of which showed neurological signs of disease.

Objectives: To characterise the new ORF30 C_{2254} EHV-1 strain.

Study design: Retrospective screening of field samples with *in vitro* and *in vivo* characterisation.

Methods: The C_{2254} EHV-1 strain was isolated *in vitro* on RK13 cells and characterised by Multi Locus Sequence Typing (MLST). A probe was designed for specific C_{2254} genotype detection by qPCR. An *in vitro* antiviral assay (ganciclovir, aciclovir and aphidicolin) was carried out on EEK cells using Real-time Cell Analysis and viral load

quantification. Four 10-month-old Welsh mountain ponies were experimentally infected with the C_{2254} EHV-1 strain (5e07 $TCID_{50}$/pony).
Results: The C_{2254} EHV-1 strain displayed typical EHV-1 features *in vitro* and was classified as clade 10 by MLST. Retrospective screening of 204 EHV-1 positive samples collected since 2016 did not reveal the presence of the C_{2254} mutation outside the outbreak of origin (i.e. 2018). No difference in sensitivity to ganciclovir and aphidicolin between the A_{2254}, G_{2254} and C_{2254} strains was detected *in vitro*. Both C_{2254} and A_{2254} strains were more sensitive to aciclovir than the G_{2254} strain, based on viral load quantification (p-value <0.05). A rapid onset of marked respiratory disease, with persistent virus shedding and cell-associated viraemia, were measured after experimental infection *in vivo*.
Main limitations: No available field/experimental information about the abortigenic potential.
Conclusion: The C_{2254} EHV-1 strain showed similar characteristics to other EHV-1 strains.
Ethical animal research: All experimental procedures were approved by the Loire Valley ethical review board (CEEA VdL, committee number 19, authorisation number APAFiS#22708).
Informed consent: Not stated.
Competing interests: None declared.
Sources of funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324), the IFCE (Institut Français du Cheval et de l'Equitation) grant number 2017-008, CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

87 | Temporal variability between detection of fever and positive PCR from nasal and blood samples in a 2016 outbreak of equine herpesvirus myeloencephalopathy in California

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Background: Outbreaks of equine herpesvirus myeloencephalopathy (EHM) are difficult to mitigate. Biosecurity strategies rely on clinical monitoring of at-risk horses, through twice daily temperature recording and PCR testing of whole blood and nasal swabs at the time of initial fever or neurologic disease detection. To release horses from quarantine as soon as possible, horses are often sampled at the first sign of a fever very early in the course of disease. Health officials involved in mitigating EHM outbreaks have indicated they observed initial tests to be negative and have thus required follow-up samples be tested.
Objectives: To describe a 2016 outbreak of EHM in LA County, California, in order to determine the number of EHV-1 infected horses which were initially negative on PCR of nasal swab and/or whole blood testing samples, but which subsequently tested positive.
Study design: Retrospective outbreak investigation.
Methods: Records obtained by the California Department of Food and Agriculture were reviewed to obtain information regarding the number of horses on the premises, individual exposure, quarantine duration, timing of initial fever, sample collection, and diagnostic test results.
Results: Of the 725 horses housed on the affected premises, 330 were considered exposed and subsequently quarantined. Fifteen horses were confirmed positive for EHV-1 on PCR of blood and/or nasal swab on at least one timepoint. Of the 15 positive horses, 8 had signs consistent with EHM. Six horses were negative on PCR of nasal swab and/or whole blood samples at the time of initial fever detection, but positive on retest 1-4 days later.
Main limitations: Retrospective nature of the study of one outbreak incident. Our goal is to collect similar data from multiple outbreaks to determine if this observation is repeatable.
Conclusions: This EHM outbreak illustrates the limitations of single-timepoint PCR testing of horses undergoing intensive temperature monitoring during an outbreak.
Ethical animal research: Research ethics committee oversight not required by this journal: retrospective analysis of clinical data.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: None.

88 | Seroprevalence of equine herpesvirus-1 (EHV-1) and herpesvirus-4 (EHV-4) in Moroccan horse populations

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Background: Equine herpesvirus-1 (EHV-1) and -4 (EHV-4) are common equine pathogens, causing significant economic losses and a negative impact on equine welfare. In Morocco, the equine industry is important for the country's socio-economic development (0.61% of the country's Gross Domestic Product, more than 30,000 people employed). The Moroccan horse population is estimated at 110,000

horses with around 4,000 births every year. Epidemiological information about EHV-1/-4 circulation in Morocco is limited. The last investigation dates from 1996 and reported an EHV-1/EHV-4 seroprevalence of 35%.

Objectives: To determine the seroprevalence of EHV-1 and EHV-4 in horse populations of Morocco and to measure the antibody titres in vaccinated horses, under field conditions, with a monovalent EHV-1 vaccine.

Study design: Field serological study.

Methods: Blood samples were collected from 405 horses including 163 unvaccinated and 242 vaccinated animals and were tested using a commercial type-specific enzyme-linked immunosorbent assay (ELISA) and a virus neutralisation (VN) test.

Results: Overall 17% of horses tested positive for EHV-1, 12.8% in the unvaccinated and 21.8% in the vaccinated groups, respectively. All samples were positive for EHV-4 when tested with the type-specific ELISA. The EHV-1 VN test showed that 86.4% of samples were positive with an average antibody titer of 1:24 in the unvaccinated group. In the vaccinated group, the VN test revealed a mean antibody titer of 1:50 for EHV-1 and 1:47 for EHV-4.

Main limitations: Samples may not represent the entire population and cross-reactivity of the assays.

Conclusions: EHV-1 and EHV-4 are endemic in the horse populations of Morocco, with differences between regions. Furthermore, horses vaccinated with a monovalent EHV-1 vaccine had low antibodies titers. This study highlights the necessity to establish control strategies based on management of horses and re-evaluation of the vaccine and the vaccination protocol.

Ethical animal research: The study protocol was approved by the Royal Equestrian Society (Société Royale d'Encouragement du Cheval, SOREC)

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

Competing interests: None declared.

Source of funding: SOREC and Agronomy and Veterinary Institute Hassan II.

89 | *In vitro* evaluation of nine antiviral compounds for their potential effect against equid herpesvirus-4

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Background: Equid herpesvirus-4 (EHV-4) is a frequent respiratory pathogen of the horse. Occasional strains of EHV-4 sporadically induce abortion or neonatal death and although not clearly demonstrated, its involvement in neurological forms has been suggested. Despite prophylactic approaches using vaccines, resurgence of EHV-4 infection still constitutes an important threat to the horse industry. Yet very few studies have been conducted on the search for antiviral molecules against EHV-4 *in vitro*.

Objectives: To assess the effectiveness of 9 antiviral compounds against EHV-4.

Study design: *In vitro* experiment.

Methods: Using Real-Time Cell Analysis (RTCA) of CCL26 cell line (African green monkey kidney) infected with EHV-4, 9 compounds with antiviral properties against EHV-1 were screened. CCL26 cells were infected with EHV-4 405/76 reference strain (VR2230) at a MOI 0.24 in the presence of 8 concentrations (50 to 0.39 µM) of each selected compound: aciclovir, ganciclovir, valganciclovir, decitabine, idoxuridine, pritelivir, cidofovir, aphidicolin and vidarabine. Formation of cytopathic effects was monitored by RTCA (xCELLigence and Incucyte®) and the viral load was quantified by qPCR. EC_{50} values for both xCELLigence and qPCR methods were determined.

Results: EC_{50} values showed that seven molecules have an antiviral potency to prevent infection of CCL26 with EHV-4 *in vitro*. Aphidicolin was the most potent compound with an EC_{50} value of 1.63±0.76 µM measured by xCELLigence and 0.25±0.12 µM when measured by qPCR. Nucleoside analogs aciclovir and vidarabine were not efficient in preventing infection of CCL26 with EHV-4.

Main limitation: Results need to be confirmed on different equine cell lines and with different EHV-4 strains isolated from the field.

Conclusions: Seven antiviral compounds (ganciclovir, valganciclovir, decitabine, idoxuridine, pritelivir, cidofovir and aphidicolin) prevent EHV-4 cytopathic effect in CCL26 cells *in vitro*. Aciclovir, the most widely used antiviral *in vivo* against alpha-herpesviruses, does not appear to be effective against EHV-4 *in vitro*.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: LABÉO, IFCE (Institut Français du Cheval et de l'Equitation), Fonds Eperon (SAVE, HVE4 IRCP), Région Normandie (CPER R25 P3) and CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

Equine Herpesvirus-3

Oral Presentations

90 | Diagnosis of equid alphaherpesvirus-3 before mating: its contribution to outbreak control

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Background: Equine coital exanthema (ECE), caused by equid alphaherpesvirus-3 (EHV-3), is a venereal, highly contagious disease, characterised by the formation of papules, vesicles, pustules and ulcers on the external genitalia of mares and stallions.
Objectives: To describe an outbreak of ECE which occurred in a Thoroughbred farm, during the 2020 breeding season.
Study design: Case report.
Methods: On 18 August, a breeding farm in Buenos Aires, Argentina, with four stallions (one shuttle) and 400 booked mares (100 from the farm; 300 incoming mares for breeding), reported that between 80-90% of their own mares, most of them previously sutured *post partum*, presented with lesions typical of ECE: most were mild, like small "cracks" in the rectal sphincter. EHV-3 was confirmed by qPCR, thus strict hygienic and biosecurity measures were promptly established. Also, an EHV-3 negative qPCR on perineal-genital swabs (PGS) was required for all mares with a minimal suspicious lesion prior to mating.
Results: From booked mares, ECE clinical signs were observed in 47%, 12%, 6% and 4% during August, September, October and November, respectively. In all, 76 samples were taken, and 28 mares (37%) were EHV-3 positive, of which 16 (57%) had high viral load. The percentages of EHV-3 positive/booked mares each month was 17%, 1%, 4%, 3%, respectively. None of the stallions showed clinical evidence of infection, none were positive for EHV-3 in the PGS performed.
Main limitations: Outcome of the outbreak without intervention could not be evaluated.
Conclusions: Procedures performed on mares during the postpartum management (rectal palpation and suturing) and during ultrasonography and rectal palpation before mating, could have favoured the introduction, spread or reactivation of EHV-3 from latency. Rectifying management measures and the timely implementation of EHV-3 diagnostic screening before mating were critical to minimise any expansion of the outbreak and avoid stallions' infection.
Ethical animal research: Research ethics committee oversight not required: case report.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: INTA and INTA-HARAS Agreement.

91 | Topical ganciclovir treatment for mares naturally infected with equid alphaherpesvirus-3: case report

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Background: Equid alphaherpesvirus-3 (EHV-3) is the aetiological agent of equine coital exanthema (ECE). Therapeutic treatment of affected mares with 0.01% topical ganciclovir under experimental conditions significantly reduces duration of virus shedding.
Objectives: To evaluate the effectiveness of topical ganciclovir (1%) in mares naturally infected with EHV-3.
Study design: Case report.
Methods: Six mares with ECE lesions and high EHV-3 shedding loads (as determined by qPCR) during an ECE outbreak, were segregated. Three mares were treated by daily application of 10 gr ganciclovir (1%) administered topically on perineal and external genital areas, over 10 consecutive days or until 3 days of EHV-3 negative results while the other three mares were untreated. Perineal-genital swabs (PGS) were obtained before the application of the cream and analysed by EHV-3 qPCR for detection and quantification of viral loads.
Results: Typical lesions of ECE were observed in all six mares, being mild in two of them, like small "cracks" in the rectal sphincter. Two of three treated mares, with initial viral loads of 1.33 and 4.98 Log_{10}[TCID50%/ml], cleared viral excretion at 24 h, while the third mare, with 2.54 Log_{10}[TCID50%/ml], cleared it after two applications of topical ganciclovir (48 h). No adverse effects were observed in treated mares. In the untreated mares, one mare cleared viral excretion at day seven, with an initial load of 1.33 Log_{10}[TCID50%/ml], and the remaining two continued viral excretion until day 19 (initial PGS of 1.33 and 4.98 Log_{10}[TCID50%/ml]).
Main limitations: The small number of cases prevents statistical analysis.
Conclusions: Treatment with 1% topical ganciclovir, once a day, contributes to the clearance of EHV-3 shedding and may reduce the

time mares and stallions are segregated from reproduction in the face of an ECE outbreak.

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

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: INTA and INTA-HARAS Agreement.

92 | Circulation and characterisation of Equid herpesvirus-3 strains in France between 2010 and 2021

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Background: Equine coital exanthema (ECE) is an acute viral infection of horses caused by equid herpesvirus-3 (EHV-3). The presence of the virus and its economic impact is well described in different countries such as Argentina or Japan. However, even though it is included in the HBLB code of practice (UK) and in the list of diseases monitored by the RESPE (FR), only a few studies have been performed in Europe.

Objectives: To determine the circulation of EHV-3 in France and measure the efficacy of new antiviral compounds against EHV-3.

Study design: Retrospective field study and *in vitro* experiments.

Methods: 71 field samples from suspected ECE cases, and collected between 2010 and 2021, were analysed by qPCR (DNA polymerase gene) and phylogenetic analysis (gG gene) was performed on 10 positive samples. Antiviral compounds (aciclovir, ganciclovir, valganciclovir, decitabine, idoxuridine, pritelivir, cidofovir, aphidicolin, vidarabine) previously described with antiviral properties against EHV-1, were evaluated using Real-Time Cell Analysis (RTCA) on equine dermal cells with the reference strain EHV-3 VR3522, cytopathic effect monitoring (microscopy) and viral load quantification (qPCR).

Results: 16/71 (22%) suspected ECE cases were confirmed positive by PCR with 0 to 3 cases per year. French strains were clustered into two of the three observed phylogenic groups, but there was no correlation between the phylogeny and the time of collection or location. Aphidicolin was the most effective compound with an EC_{50} value of 2.43±0.02 µM (RTCA) and 1.63±0.48 µM (qPCR). Pritelivir and cidofovir were less potent molecules against EHV-3 (EC_{50} >50 µM).

Main limitation: Antiviral compounds were tested against the reference strain only.

Conclusions: This study demonstrates for the first time the presence of EHV-3 over a twelve-year period in France. *In vitro* studies show the antiviral efficacy of aphidicolin.

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

Informed consent: Explicit study consent was not stated but owners were aware that samples could be used for research activities.

Competing interests: None declared.

Sources of funding: LABÉO, IFCE (Institut Français du Cheval et de l'Equitation), Fonds Eperon (SAVE, OVERLORD N12-2017), Normandy County Council (17E01598/17EP04324), Région Normandie (CPER R25 P3) and CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

Other Equine Herpesviruses

Oral Presentations

93 | Equid alphaherpesviruses 8 and 9: strain isolation, ORF30 sequencing and antiviral assay

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Background: While rare, Equid alphaherpesvirus (EHV) infections could be devastating for exotic endangered species which are part of conservation programmes. When such an infection occurs in an animal park setting, antiviral treatments may be available as a therapeutic option. Such an approach was recently attempted in a French zoo to save two Grevy's zebras infected with EHV-9 and displaying severe respiratory and neurological disease. Valaciclovir was administered, with mixed results obtained. Several studies have determined *in vitro* antivirals' efficacy against EHV-1 in recent years. While EHV-9 but also EHV-8 show strong genetic homology with EHV-1, these EHVs remain largely uncharacterised.

Objectives: To highlight the genetic proximity of field EHV-1, EHV-8 and EHV-9 strains and compare their *in vitro* sensitivity to antiviral compounds previously described as efficacious against EHV-1.

Study design: *in vitro* analysis of micro-organisms.

Methods: Field EHV-8 and EHV-9 strains were isolated (from donkey and Grevy's zebra, respectively) and cultured *in vitro*. ORF30 sequences and antiviral sensitivity were compared with field and reference EHV-1 strains. Complete ORF30 genes were sequenced and compared with 33 EHV sequences referenced in GenBank (18

EHV-1, 6 EHV-9, 7 EHV-8, 1 EHV-3 and 1 EHV-4). Seven antiviral molecules (ganciclovir, valganciclovir, decitabine, idoxuridine, pritelivir, aciclovir and aphidicolin) were tested using Real-time Cell Analysis (xCELLigence®).

Results: ORF30 phylogenetic analysis confirmed the genetic proximity of EHV-1, -8 and -9. While aphidicolin, ganciclovir and valganciclovir showed clear *in vitro* antiviral activity against the EHV-1 and EHV-8, aciclovir was found to be the least effective compound tested.

Main limitations: Due to poor reproducibility, the EHV-9 antiviral assay will require some adjustments (cell lines).

Conclusions: This is the first report of *in vitro* tests of an antiviral compound panel against EHV-8/-9. While field use of valaciclovir is anecdotally reported against EHV-1 and other EHVs, other compounds may provide greater effectiveness.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: Fonds Eperon OVERLORD N12-2017, Normandy County Council (17E01598/17EP04324), the IFCE (Institut Français du Cheval et de l'Equitation) grant number 2017-008, CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational program 2014-2020.

94 | Equine multinodular pulmonary fibrosis in a Thoroughbred with pituitary pars intermedia dysfunction

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Background: Despite a strong association between equine multinodular pulmonary fibrosis (EMPF) and equine herpesvirus-5 (EHV-5), the vast majority of horses infected with this gammaherpesvirus remain free of clinical disease. Pituitary pars intermedia dysfunction (PPID) is well described in older horses resulting in greater susceptibility to infectious agents. Both disease processes are considered the result of acquired immune dysfunction.

Objectives: To investigate a cause of pulmonary disease in a 21-year-old Thoroughbred stallion with clinical signs and laboratory diagnosis consistent with PPID.

Study design: Case report.

Methods: Details of clinical investigation, clinical pathology (serum ACTH determination), gross post-mortem and histopathological examination were reviewed.

Results: Pathological findings in the lung included a severe pneumonia that was interstitial, nodular and fibrosing, multifocally extensive to coalescing with type II pneumocyte hyperplasia and intra-histiocytic intranuclear viral inclusion bodies; consistent with equine multinodular pulmonary fibrosis (EMPF). Enlargement of the pituitary gland was confirmed histologically as an adenoma of the *pars intermedia*. Cross section of the hooves revealed downward rotation of the third pedal bone more pronounced in the forelimbs, consistent with chronic laminitis.

Main limitations: No cytokine analysis to demonstrate putative Th2 pro-fibrotic immune response.

Conclusions: This is the first report of a horse suffering from EMPF with confirmed PPID. The association of EHV-5 infection with the profibrotic condition EMPF raises questions regarding co-factors that may produce this condition in the vast minority of infected horses. Given the immune suppression associated with PPID, scrutiny of host factors leading to immune dysregulation may be worth further investigation. Further understanding of the complex aetiology of EMPF is warranted given the debilitating, often fatal, fibrosis associated with EHV-5 infection.

Ethical animal research: Research ethics committee oversight not required: case report.

Informed consent: The case was donated by the owner for post mortem investigation for diagnostic and teaching purposes.

Competing interests: None declared.

Source of funding: No external funding.

Equine Arteritis Virus

Oral Presentations

95 | Development of a high-throughput screening assay to identify equine virus arteritis replication inhibitors

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Background: During the last 60 years, the equine arteritis virus (EAV) has induced respiratory and reproductive problems in equids

after infection. EAV is a significant problem for horse breeders and the equine industry. Despite two vaccines being available in the market, the current vaccination coverage remains insufficient to prevent global outbreaks. Recently our laboratory identified the first commercial antiviral molecule that impairs EAV replication. Unfortunately, it is only approved for use in humans and small animals. This proof of concept suggests that other antiviral molecules should exist and may be approved for use in large animals.

Objective: This project aims to screen thousands of antiviral molecules to find those that impair EAV replication.

Study design: *In vitro* study.

Methods: To test thousands of molecules, a high-throughput screening (HTS) model based on ATP quantification was developed, which allows evaluation of the cytotoxicity and cellular protection of molecules. In addition, the EAV replication was measured by analysing the culture supernatants by RT-qPCR.

Results: The HTS system allowed screening of 1250 molecules from 2 chemical libraries. The results showed that 75/1250 molecules induced cellular protection after 72 hpi. Regarding the EAV replication, 64 molecules impaired EAV replication efficiently and 3/64 molecules showed an antiviral capacity for the first time.

Main limitations: *In vitro* models may not mimic the *in vivo* effects.

Conclusions: The promising molecules will require *in vivo* testing in horses and, if effective, have the potential to thus complement EVA vaccines to better control EVA outbreaks and limit viral dissemination.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of Funding: Anses, IFCE (Institut Français du Cheval et de l'Equitation), Fonds Eperon, GIS CENTAURE and Région Normandie.

96 | Outbreak of equine viral arteritis in the UK 2019

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Background: While Equine Viral Arteritis is notifiable to the OIE and in the UK, it is not an exotic disease and thus outbreaks occur sporadically across Europe, including in the UK.

Objective: To investigate an outbreak in 2019 including characterization of the causative Equine Arteritis virus (EAV) strain.

Study design: Clinical description.

Methods: Serological screening is widely used to monitor stallions prior to breeding activities and led to the initial suspicion in these cases. Further analysis of semen samples from the sero-positive stallions was carried out using PCR, sequencing and virus isolation.

Results: In the first case, three stallions (KF-G, KF-P, KF-L) from the same herd were confirmed as carriers of EAV. All three stallions were Andalusian horses that had a history of attending shows together. Contact tracing led to the identification of a fourth stallion (FR-I) in a second premise that also tested positive for EAV antibodies and subsequently for EAV in semen. Further epidemiological investigations revealed that the infections must have occurred between April 2018 and April 2019, and that while two stallions (KF-L and KF-P) were imported from Spain in December 2018, it can be excluded that either of these carried the virus to the UK [1]. Virological investigation demonstrated the close relationship between all EAV strains (genotype D), making it likely that one of the stallions (KF-P) had become infected first and spread the virus among his contacts.

Main limitations: Only a limited number of samples could be obtained, prohibiting a temporal analysis of sequence variation over time.

Conclusions: The outbreak of EAV in the UK in 2019 was caused by one initial infection of one EAV strain from genotype D [2], making it likely that the onward transmission occurred via acute respiratory shedding.

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Ethical animal research: Research ethics committee oversight not currently required by this journal: procedures were performed as part of clinical investigations.

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: DEFRA project SV3300.

Poster Presentation

97 | Purine and pyrimidine biosynthesis inhibitors efficiently suppress equine arteritis virus replication

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Background: RNA viruses are responsible for a large variety of animal infections. Equine Arteritis Virus (EAV) is a positive single-stranded

RNA virus member of the family Equartiviridae from the order Nidovirales like the Coronaviridae family. EAV causes respiratory and reproductive diseases in equids. Although two vaccines are available, the vaccination coverage of the equine population is insufficient to prevent new EAV outbreaks around the world.

Objectives: To identify therapeutic agents which might be useful in outbreak control and limit the viral dissemination.

Study design: *In vitro* model.

Methods: An *in vitro* model of EAV infection assay based in the quantification of ATP by luminometry was established. This system allows evaluation of the cytotoxicity and cellular protection of molecules and collect supernatant to measure viral replication levels of EAV by RT-qPCR.

Results: Using this assay, three molecules that impaired EAV infection in equine cells were identified: the broad-spectrum antiviral and nucleoside analog ribavirin, and two compounds previously described as inhibitors of dihydroorotate dehydrogenase (DHODH), the fourth enzyme of the pyrimidine biosynthesis pathway. These molecules effectively suppressed cytopathic effects associated with EAV infection, and strongly inhibited viral replication and production of infectious particles.

Main limitations: *In vitro* effects may not be present *in vivo*.

Conclusions: Since ribavirin is already approved in humans and small animals, and several DHODH inhibitors are in advanced clinical trials, these results may lead to the identification of molecules that impair EVA viral replication for clinical use.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: Anses, IFCE (Institut Français du Cheval et de l'Equitation), Fonds Eperon and Région Normanide.

Equine Coronavirus

Oral Presentations

98 | The Icelandic epidemic of infectious pyrexia in horses 1998: a cold case resolved?

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Background: An epidemic of infectious pyrexia of unknown aetiology swept through the isolated, native Icelandic horse population in 1998. Despite extensive restrictions, the disease spread from the primary foci all over the country during February – October 1998. Most horses were mildly affected with slightly increased body temperature and reduced appetite. However, some horses had temperatures up to 42°C and showed anorexia for some days. Approximately 0.02% of the population of 80,000 horses died due to complications like severe colic and eclampsia in mares close to parturition/lactation. Clinical signs and negative bacterial isolation, together with oro-faecal transmission strongly pointed toward a viral infection. Despite extensive investigations, no viruses known at that time to infect horses could be connected to the disease.

Objectives: To determine if this epidemic of infectious pyrexia in horses was caused by equine coronavirus (ECoV).

Study design: Retrospective, immuno-epidemiological study.

Methods: Employing a recently developed ELISA for detection of antibodies to equine ECoV together with an ECoV virus neutralisation test (VNT), for testing of serum samples in the Icelandic Horse Biobank, collected in the period 1990-2020.

Results: ECoV antibody in sample sets retrieved before, during and after the epidemic show a strong increase in seroprevalence in 1998. Antibody responses against ECoV in paired serum samples collected from 18 affected horses during the epidemic revealed both ELISA and VNT seroconversions or highly significant increases in antibody levels. The most recent samples provide evidence for ECoV still circulating in the population.

Main limitations: Lack of direct virus detection.

Conclusions: The study strongly indicated that the epidemic of infectious pyrexia in native Icelandic horses in 1998 was caused by introduction of ECoV into the immunologically naïve population. The current ECoV seroprevalence between 40 and 60% seems to prevent serious outbreaks.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on archived material collected previously.

Informed consent: Not stated.

Competing interests: None declared.

Sources of funding: Keldur and Royal GD.

99 | Evaluation of the potential risk to horse racing in Japan of equine coronavirus infection

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Background: Equine coronavirus (ECoV) causes fever, anorexia, lethargy and gastrointestinal signs in horses. In 2020, an outbreak of ECoV infection occurred in 41 horses including Thoroughbreds in Japan.

Objectives: To estimate the potential risk of ECoV infection in Japanese Thoroughbred racehorses.

Study design: Cross sectional.

Methods: Yearlings at a rearing farm and racehorses at two training centres were investigated from 2017 to 2020. In each year, sera

were collected in August, December and the following April from yearlings (n = 50 to 56/year) and March–August, November and the following May from racehorses (n = 156 to 216/year). They were subjected to virus neutralisation test for ECoV. A ≥ 4-fold increase in titres between paired sera was regarded as indicative of infection.
Results: In the yearlings, the proportion of infected horses from August to December and from December to the following April was 60.9% and 5.6%, respectively. In the racehorses, 78.4% had titres of ≥ 1:8 when they first entered the training centres. The infection rate in the cool season (November to May, 11.5%) was higher than that in the warm season (March - August to November, 3.1%) (Chi-square test, $p < 0.001$). The proportion of ECoV-infected horses with fever or gastrointestinal signs was 15.0% in yearlings and 6.2% in racehorses.
Main limitations: Clinical records of racehorses did not cover the period during their absence from the training centres.
Conclusions: ECoV circulated in both racehorses and yearlings in Japan, but the periods in which a higher infection rate was observed in these two populations differed. Although ECoV infection may be partially responsible for fever and gastrointestinal signs in Thoroughbreds, the majority of infected horses were asymptomatic; therefore, the potential risk of ECoV infection to horse racing in Japan is considered to be relatively low.
Ethical animal research: This study was approved by the Research Planning and Ethics Committee of the Equine Research Institute with accession number 2018-3263-07.
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: None declared.
Source of funding: Japan Racing Association.

100 | Distribution of equine coronavirus RNA in the intestinal tracts of experimentally infected horses

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Background: Equine coronavirus (ECoV) causes fever, anorexia, lethargy and diarrhoea. Most infected horses show mild clinical signs and recover within several days. Infected horses shed a large amount of ECoV into their faeces. The main transmission route is faecal-oral, with ECoV infecting the intestinal tract.
Objectives: To elucidate the infection sites of ECoV in intestinal tracts.
Study design: Experimental challenge.
Methods: ECoV-positive faecal samples were inoculated into three horses. The horses were euthanised at 3 (Horse 1), 5 (Horse 2) and 7 (Horse 3) days post-inoculation. Tissues collected from the intestinal tracts were analysed using real-time reverse transcription-polymerase chain reaction (RT-PCR) and *in situ* hybridisation.
Results: Horses 1 and 2 showed temperatures above 38.5°C, whereas Horse 3 did not become febrile during the observation period. No severe cases were observed. All horses shed ECoV RNA from three days post-inoculation to the end of the experiment. Gross pathology revealed none of the horses to have any obvious abnormalities in their intestinal tracts. Real-time RT-PCR and *in situ* hybridisation showed that in Horse 1, ECoV RNA was detected from the jejunum to the colon and was seen in the greatest quantities in the ileum. In Horses 2 and 3, ECoV RNA was broadly detected from the duodenum to the rectum and was commonest in the caecum. In all three horses, *in situ* hybridisation showed ECoV RNA to be located on the luminal surface but not in the intestinal crypts.
Main limitations: The study did not reproduce or analyse any severe cases.
Conclusions: The results show that ECoV broadly infects the intestinal tract, suggesting that infected horses shed a large amount of virus into the faeces. ECoV was localised on luminal surfaces that showed no obvious tissue damage, which may explain why the infected horses in this study showed only mild clinical signs.
Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute with accession number 20-28.
Informed consent: Not applicable.
Competing interests: None declared.
Source of funding: Japan Racing Association.

Equine Hepacivirus and Parvovirus

Poster Presentation

101 | Intra-host analysis of hepaciviral glycoprotein evolution reveals signatures associated with persistence and clearance

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Background: Even 30 years after the discovery of hepatitis C virus (HCV), there is still no available vaccine. Reasons include the high mutation rate of HCV which allows the virus to escape immune recognition and the absence of an immunocompetent animal model for vaccine development. Phylogenetically distinct hepaciviruses have

been isolated from diverse species, each with a narrow host range: the equine hepacivirus (EqHV) is the closest known relative of HCV.
Objectives: To compare patterns of intra-host hepaciviral evolution in different species and potentially identify mutational signatures associated with transmission, immune evasion, resolution of infection, progression to chronicity and pathogenesis.
Study design: Cohort.
Methods: The viral intra-host population composition of the surface glycoproteins E1 and E2 in longitudinally sampled sera via amplicon-based deep-sequencing in a cohort of naturally as well as experimentally EqHV-infected horses, and compared them to HCV-infected patients.
Results: Differences in frequency and number of sites exhibiting intra-host variation were detected between chronic and acute EqHV infection in horses. Experimentally infected horses developed self-limiting acute EqHV infections with no pronounced transmission bottleneck or selective sweeps. The overall glycoprotein variability was higher in HCV patients compared to EqHV-infected horses. Additionally, selection pressure in HCV patients was higher, especially within the hypervariable region 1 (HVR1), while only one horse showed elevated selection pressure in the N-terminal region of E2. Furthermore, alignment of glycoprotein sequences from diverse hepaciviruses identified HVR1 as unique characteristic of the HCV E2 N-terminus: hepaciviruses from non-human species lacked this region.
Conclusion: The *in vivo* evolution of hepaciviral glycoproteins showed distinct mutational patterns in acutely and chronically infected horses, as well as differences between HCV and EqHV. These data indicate EqHV infection of horses could represent a powerful animal model for HCV vaccine design by providing insights into HCV's HVR1-mediated immune evasion strategy.
Ethical animal research: Approved by Lower Saxony's official authorities (LAVES 13/1262).
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: VIPER TiHo.

Oral Presentations

102 | Preliminary results of biomolecular investigation of equine hepacivirus in Italian horses

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Background: Equine hepacivirus A (EqHV) is a RNA virus (genus *Hepacivirus*, family *Flaviridae*), that can cause sub-clinical hepatitis in horses, occasionally evolving into a chronic disease. EqHV was reported worldwide, while data for Italy are limited [1]. Assessing its prevalence in the Italian horse population is relevant especially because of the wide use of blood products and transfusions, with potential transmission of the virus.
Objectives: Assessing the bio-molecular EqHV prevalence within different Italian horse categories.
Study design: Cross sectional.
Methods: The sampling was designed to detect, at national level, an estimated prevalence of 50% with 95% confidence level and 5% standard error; and stratified for four categories: equestrian, competition, work/meat and reproduction. Serum samples were collected by the animal health laboratory network from surveillance activities and analysed by a real-time PCR [2]. Differences between categories were evaluated by Fisher Exact Test.
Results: Up to March 2021, 1010 of 2000 samples were analysed, with a total EqHV prevalence of 4.36% (3.10-5.61), while prevalence within categories were equestrian 2.70% (1.19-4.21), competition 6.84% (3.25-10.43), work/meat 3.81% (1.22-6.40) and reproduction 6.59% (2.82-10.35). A statistical difference was detected between equestrian and both competition (p=0.02) and reproduction (p=0.03).
Main limitations: The analysis has not yet been completed and the sampled population may not reflect the target population.
Conclusions: The preliminary EqHV prevalence value estimated is similar to that previously reported in Italy [1]. Differences among production categories have not yet been fully explored.
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Ethical animal research: Research ethics committee oversight not currently required by this journal: procedures were performed as part of surveillance activities.
Informed consent: Not stated.
Competing interests: None declared.
Source of funding: Italian Ministry of Health [IZS LT 10/18].

103 | Hepatitis viruses: prevalence of equine parvovirus-hepatitis virus and equine hepacivirus in France and Australia

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Background: Recent identification of viruses considered responsible for Theiler's disease (acute hepatitis) in horses include equine parvovirus-hepatitis (EqPV-H) and the most likely of several flaviviruses, equine hepacivirus (EqHV). Both of these viruses have now been detected in North America, Asia, and Europe.

Objectives: To determine the prevalence of EqPV-H and EqHV in French and Australian horses and to analyse their respective phylogenetic relationships.

Study design: Cross-sectional.

Methods: Clinically documented sera from 188 Australian horses and 259 French horses collected between 2016-2019 were analysed. qPCRs validated according to the NF47-600 standard were used to detect EqPV-H and EqHV in sera. Sequencing for phylogenetic analysis was performed on two NS1 fragments (516 nt and 587 nt) for EqPV-H and one NS5B fragment (308 nt) for EqHV. A phylogenic network was built using a Median Joining Network algorithm.

Results: 1) EqPV-H: 12/259 (4.6%) and 6/188 (3.2%) samples from French and Australian horses were positive, respectively. Viral loads ranged from 2.3e05 to 6.5e05 copies genome/ml. 2) EqHV: 5/259 (1.9%) and 21/188 (11.2%) samples from French and Australian horses were positive, respectively. Viral loads ranged from 6.1e04 to 1.4e08 copies genome/ml. Both EqPV-H (587 nt fragment) and EqHV phylogenetic analyses showed that Australian strains were clustered, while overall distribution was more heterogenous in French horses.

Main limitations: The study population is built by accessing laboratory submissions and potentially biased. The 516 nt NS1 fragment could not be sequenced.

Conclusions: This is the first report of EqPV-H detection in both Australian and French horses. The EqPV-H prevalence was similar between the two countries (p-value=0.6) although phylogenetic differences were observed between the two populations. This is also the first reported detection of EqHV in Australian horses, with a higher prevalence when compared with French horses.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on archived material collected previously during clinical procedures.

Informed consent: Explicit study consent was not stated but owners were aware that samples could be used for research activities.

Competing interests: None declared.

Source of funding: RESPE programme 2018_pegiRESPE.

104 | Equine parvovirus-hepatitis screening in equines with histopathological liver abnormalities

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Background: There is strong evidence that equine parvovirus-hepatitis (EqPV-H) is associated with the onset of Theiler's disease, a potentially life-threatening fulminant hepatic necrosis. The virus has been detected in serum and liver tissues of affected horses. Recently, hepatotropism and persistence of viral nucleic acids in different tissues were reported. The relationship of EqPV-H to other hepatopathies beside Theiler's disease is still unknown.

Objective: The aim of this study was to evaluate the prevalence and to quantify the viral load of EqPV-H in equine livers with histopathologic abnormalities.

Study design: Retrospective case series.

Methods: Equine livers were retrospectively selected from archived, formalin-fixed, paraffin-embedded tissue samples, assigned to different groups according to their histopathologic abnormalities and screened for the presence of EqPV-H. EqPV-H was monitored by quantitative PCR. Positive samples were subjected to viral load determination by digital PCR.

Results: In total, 92 livers were included in the study and assigned to the following groups: hepatitis/cholangitis (n = 24), cirrhosis (n = 5), primary or secondary neoplasia (n = 20), metabolic/toxic disease (n = 14), congestion (n = 4), mixed abnormalities (n = 17) and normal livers (n = 8). Two out of 92 livers tested positive for EqPV-H nucleic acid and contained 3,000 and 7,000 genome equivalents per million cells according to digital PCR. Both samples originated from horses diagnosed with abdominal neoplasia and liver metastasis.

Main limitations: Due to the retrospective design of the study, numbers of included livers were only moderate and sizes of the histopathologic groups not identical.

Conclusion: EqPV-H could be detected in two livers originating from horses with abdominal neoplasia. The amount of viral nucleic acids counted would indicate rather a chronic infection or persistence of EqPV-H. In summary, this study did not provide evidence for EqPV-H being involved in other hepatopathies than Theiler's disease.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on archived material collected previously during clinical procedures.
Informed consent: Explicit study consent was not stated but owners were aware that samples could be used for research activities.
Competing interests: None declared.
Source of funding: Foundation PRO Pferd, Switzerland.

Equine Infectious Anaemia Virus

Oral Presentations

105 | Understanding the impact of genetic variation of equine infectious anaemia virus (EIAV) on serological detection

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Background: Equine infectious anaemia virus (EIAV) is the causative agent of equine infectious anaemia (EIA), a notifiable disease of equids. Diagnosis plays a crucial role in the worldwide control. Data presented at the last EIDC and subsequently published [1,2] demonstrate a broad heterogeneity of EIAV and indicated that some variants of EIAV may escape serological detection.
Objectives: The majority of EIAV serological diagnostic tests target the capsid, thus the effect of capsid mutations on the diagnosis of EIAV antibodies was investigated.
Study design: *In silica*, *in vitro* and *in vivo* experiments.
Methods: Capsid protein sequences available on GenBank were compared and individual amino acid mutations that differed from the consensus were identified. From these data, a mutated capsid incorporating a combined selection of the mutations was designed. 3D modelling showed that the final constructs' protein structure still resembled a reference EIAV capsid (strain Wyoming). Subsequently, gene synthesis followed by protein expression was used to generate a recombinant mutated capsid protein (rmp26) that was used to generate an equine antiserum. The rmp26 antisera was then tested on a variety of EIAV capsid directed serology tests.
Results: A positive EIAV result was obtained in two out of three ELISAs and by an in-house Western blot (WB), however it was found negative by the AGIDT.
Main limitations: While the design of the mutated p26 is based on an informed selection, it is unclear whether such a protein would be functional. Further, only a limited number of antisera have been tested.
Conclusions: Using the proposed state-of-the-art three-tiered testing system (ELISA, AGID, WB) for EIAV infections, it seems unlikely that mutations can escape detection in validated assays.

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Ethical animal research: All work was approved by the APHA Animal Welfare and Ethical Review under Project Licence number PP1962684.
Informed consent: Not applicable.
Competing interests: None declared.
Source of funding: Department of Environment Food and Rural Affairs (DEFRA), Grant/Award Number: SV3300.

106 | Identification and genetic characterisation of equine infectious anaemia virus in western Balkans

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Background: Equine infectious anaemia (EIA) is a viral disease, caused by the equine infectious anaemia virus (EIAV) belonging to the Retroviridae family, genus Lentivirus. EIAV infected horses are lifelong carriers and remain contagious for other horses, even in the absence of clinical signs. So far, EIAV infection has been reported among horses in many parts of the world, but there is no publication regarding the presence of EIAV in horses in Serbia.
Objective: To report, for the first time, the seroprevalence of EIAV in Serbian horses and its molecular characterisation using next generation sequencing of EIAV of horses from Vojvodina region.
Study design: Serological analysis, viral genome sequencing and phylogenetic analysis.
Methods: 316 serum underwent serological testing for EIA using agar gel immunodiffusion (AGID) tests and enzyme linked

immunosorbent assay (ELISA). Then, identification and full genome sequencing using next generation sequencing was performed from one EIA positive horse.

Results: According to the results obtained with 3 different AGID kits, 311 sera were negative and five positive for EIA. Some discrepancies were seen for the two different ELISA kits used since one exhibited the same results as AGID tests and the second gave 295 sera with negative results, 5 with a positive result and 16 with doubtful outcome. According to the phylogenetic analysis, EIAV characterised from a horse in Serbia was different from those identified so far around the world and formed a distinct and separate group.

Main limitations: Sample size is small.

Conclusions: This study demonstrates for the first time that EIAV is circulating at a low level in the horse population from the Northern part of Serbia. Interestingly, phylogenetic data indicates that a single isolated and characterised EIAV strain belongs to a new cluster.

Ethical animal research: Blood and organ collection from EIA positive horses was performed following Serbian authority regulations regarding equine infectious anaemia.

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

Competing interests: None declared.

Sources of funding: Hubert Curien Partnerships (PHC-Pavle Savic), the European Reference Laboratory for Equine Diseases other than African Horse Sickness, ANSES's own institutional resources and the Ministry of Science and Technological Development of the Republic of Serbia (Grant no. TR31071). AD and "CENTAURE project" was supported by a grant awarded by the Regional Council of Normandy and the French Ministry of Higher Education, within the framework of CPER 2015-2020 and FEDER/FSE 2014-2020.

107 | Validation of the diagnostic pathway for equine infectious anaemia

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Background: According to European Law [1], equine infectious anaemia virus (EIAV) requires measures to prevent its spreading by animal movement between Member States, and surveillance within the Union. A risk-based surveillance plan has been ongoing in Italy since 2006, based on serological monitoring of the equine population, using an ELISA as screening test and agar gel immunodiffusion test (AGID) and Immunoblot (IB) as confirmatory tests.

Objectives: Describe the validation and performance monitoring of the diagnostic pathway.

Study design: Diagnostic pathway and assay validation.

Methods: To assess the validity of the diagnostic pathway, validation of two ELISAs and the IB, comparison of six ELISAs available in Italy and repeated proficiency tests within the animal health laboratory network were employed. Pre-release approval of batches of commercial and in-house ELISAs was also performed. Two ELISAs, and the IB were validated according to OIE criteria [2]. The comparison was conducted evaluating concordance, specificity, sensitivity and coefficient of variation (CV) for repeatability and reproducibility. The last three parameters were also used for ELISA kit batch release. Proficiency tests, for ELISA and AGID, evaluated concordance of the network.

Results: From the validation results, the sensitivity and specificity values were respectively 100% and 80.8% for the competitive ELISA, 100% and 99.3% for the indirect ELISA and both 100% for IB. Comparison demonstrated all ELISAs as suitable for screening purposes, with sensitivity ranging from 98.2% to 100%, specificity constantly 100%, concordance from 0.95 to 1, CV values were below 30%. From 2016 to 2021, 47 ELISA batches met quality requirements. Proficiency testing resulted in acceptable concordance (> 0.9) within the network.

Conclusions: The described EIAV diagnostic pathway, is efficient and could be adopted by other the European member states.

References

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Ethical animal research: Research ethics committee oversight was not in place. Sera were obtained from archives held by research and diagnostic laboratories and included USDA reference sera.

Informed consent: Not stated.

Competing interests: None declared.

Source of funding: Not applicable.

Poster Presentations

108 | Isolation and identification of equine infectious anaemia virus from monocyte-derived macrophages of naturally infected horses

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Background: Equine infectious anaemia virus (EIAV) replicates in few cell cultures without producing a cytopathic effect. The equine

monocyte-derived macrophages (eMDM) allow replication suitable for isolation and phylogenetic investigations [1].

Objectives: To isolate EIAV from eMDM of naturally infected horses for phylogenetic study.

Study design: Cross sectional.

Methods: EDTA blood samples were collected from 20 serologically positive subjects. Leukocytes were separated by Histopaque® and resuspended in RPMI 1640 with antibiotics. After 18 h incubation at 37°C at 5% CO_2, medium containing 10% equine serum was added and replaced weekly for four weeks. Sixty supernatants were analysed using a nested-PCR, both from complementary-DNA and DNA [2] and two supernatants were subjected to NGS. Sequences obtained were aligned with those in GenBank.

Results: eMDM were isolated from the peripheral blood mononuclear cells of 13/20 samples. Monocytes evolved from small and rounded to cells either with pseudopodia or with irregular margins and protoplasmic extensions, before dying, usually at 3/4 weeks. EIAV was detected by nested-PCR in nine subjects and the four sequences identified belonged to EIAV-27309L/2013 (two) from Austria and to EIAV-F3/2016 from Ireland (two). NGS highlighted a correlation with French strains characterised in 2009.

Conclusions: EDTA blood represents an excellent biological matrix for EIAV isolation from eMDM of PBMCs. Sequencing detected strains from other European countries and NGS indicated that the Italian strains were similar to the French one, suggesting a wide viral circulation.

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Ethical animal research: Samples were taken as part of an epidemiological investigation, relating to the National plan for surveillance and control of infectious anaemia of equidae (Ministry of Health, Decreto 02 febbraio 2016)

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: This study was conducted within the IZSLT 09/17, research project funded by the Italian Ministry of Health.

109 | Equine infectious anaemia (EIA): Single case in Chile 2019

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Background: The clinical signs of EIA (Equine Infectious Anaemia) vary greatly and, in many cases, EIAV-seropositive horses do not show any clinical signs. Chile was considered free of EIA and the last case was observed in native animals in 1988.

Objectives: To describe the incursion of EIA into Chile.

Study design: Case report and in-contact surveillance.

Methods: Routine serology for EIA by Agar Gel Immunodiffusion (AGID) test is performed in horses during pre-export quarantine, and the AGID test was subsequently performed in 6,553 in-contact horses.

Results: In April 2019, one EIA AGID positive horse was detected; this result was confirmed by the OIE Reference Laboratory (Ames/Iowa). The horse was an 8-year-old castrated male, imported in October 2018 when it was EIAV seronegative. When sampled prior to export, the animal had no clinical signs compatible with the disease (asymptomatic) and had previously been undertaking normal equestrian activity. Direct in-contacts equines (93) all had negative EIAV results after 3 serial samplings. The EIA positive horse was euthanised on 18 May 2019. As the horse had participated in several equestrian competitions around the country, all potential in-contacts (6,553 horses) were tested but were negative.

Main limitations: The source of EIA infection was not established.

Conclusions: Despite the introduction of one EIA infected animal into Chile in October 2018, and its coexistence during 8 months with local horses, no dissemination of the infection occurred. This could be due to factors including low viraemic load of the infected horse, low density of haematophagus insects or the use of appropriate disposable equipment (hypodermic needles). To date, this imported horse has been the only case of EIA in Chile. The permanent serum surveillance of EIA and the requirement for freedom of infection for imported horses will help to maintain the EIA-free health status in Chile.

Ethical animal research: Research ethics committee oversight not currently required by this journal: procedures were performed as part of clinical investigations and surveillance protocols.

Informed consent: Not applicable.

Competing interests: None stated.

Source of funding: Universidad Santo Tomas, Servicio Agrícola y Ganadero.

Equine Influenza

Oral Presentations

110 | Descriptive epidemiology of the 2019 equine influenza epidemic in Great Britain

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Background: During 2019, an epidemic of equine influenza (EI) occurred in Great Britain (GB) and Europe.
Objectives: To describe the epidemiology of the 2019 EI epidemic within the GB equine population.
Study design: Retrospective descriptive study.
Methods: Through a well-established surveillance network, epidemiological data on laboratory confirmed EI cases were obtained from referring veterinary surgeons submitting samples to diagnostic laboratories for EI virus testing. Data on the signalment, clinical signs and vaccination status of confirmed EI cases and on the wider resident population on EI-infected premises (IPs) were collated and described.
Results: During 2019, there were 412 laboratory-confirmed cases of EI in GB, located on 234 IPs. These premises were located in 65 of 100 GB counties, with the first of two outbreak phases occurring between January and early-April followed by a second phase between late-April and August. The median age of confirmed cases (CCs) was 5 years (range 0-26 years) and sports horses made up the highest proportion of CCs (24.0%). Among CCs, 72.3% were unvaccinated and 18.0% were vaccinated. New arrivals within two weeks preceding clinical signs being noted in a CC were reported by 41.9% of IPs.
Main limitations: It is unlikely all IPs with EI were confirmed in GB in 2019. Not all infected horses in outbreaks were investigated once IPs were confirmed with EI. As data were generally collected at a single time point with limited follow up investigations performed, some data are missing.
Conclusions: During 2019, IPs had low levels of population vaccine coverage and implemented limited preventive biosecurity measures, particularly linked to horse movements. Without substantial improvements in equine biosecurity and infectious disease prevention and control, the GB equine population remains at risk of future EI epidemics.
Ethical animal research: Research ethics committee oversight not required by this journal: retrospective study of clinical and laboratory records.
Informed consent: Explicit owner consent was not stated but where exclusion from use of samples and data for anonymous surveillance was indicated on laboratory submission forms, this was honoured.
Competing interests: None declared.

Source of funding: Horseracing Betting Levy Board.

111 | Investigation of spatial and temporal aspects of the 2019 equine influenza epidemic in Great Britain

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Background: During 2019, an equine influenza (EI) epidemic occurred in Great Britain (GB).
Objectives: To describe the spatio-temporal features of the 2019 GB EI epidemic, with statistical evaluation of associated factors.
Study design: Retrospective spatio-temporal study.
Methods: Spatial and temporal evaluations of laboratory confirmed EI cases in GB in 2019 identified two temporally distinct phases of the EI epidemic (P1 and P2). The limited numbers of cases occurring in April 2019 between P1 and P2 and after September 2019 were excluded from multivariable ordinary logistic regression analyses assessing associations of different premises-level factors with P1 relative to P2.
Results: Two temporally distinct, geographically expanding phases of the 2019 GB EI epidemic were confirmed. Areas affected in P1 (1 January-30 March) occurred mainly in southern and central GB counties. Areas which had not previously had cases confirmed, such as Wales and north-eastern GB, subsequently became affected in P2 (1 May-31 August). Comparison of premises-level data between the P1 and P2 epidemic phases demonstrated that infected premises were more likely to be confirmed in P1 than P2 if they were classified as a) vaccinated (EI vaccine used routinely) *or* professional (competition, pre-training/training, racing, sales preparation) relative to being classified as non-vaccinated and non-professional (OR=2.0, OR 95%CI=0.84-4.92, P=0.116); b) vaccinated *and* professional relative to being classified as non-vaccinated and non-professional (OR=4.7, OR 95%CI=1.46-15.0, P=0.009) and c) having EI confirmed in a newly acquired animal relative to EI not attributed to a new acquisition (OR=3.8. OR 95%CI=1.89-7.53, P<0.001).
Main limitations: Not all premises infected with EI were confirmed in GB in 2019, thereby limiting available data.
Conclusions: The 2019 GB EI epidemic was biphasic, with each phase associated with different premises' population features; professional and/or vaccinated premises with recent new acquisitions in P1 compared to non-professional and/or non-vaccinated populations, with fewer new acquisitions, in P2.
Ethical animal research: Research ethics committee oversight not required by this journal: retrospective study of clinical and laboratory records.
Informed consent: Explicit owner consent was not stated but where exclusion from use of samples and data for anonymous surveillance was indicated on laboratory submission forms, this was honoured.

Competing interests: None declared.
Source of funding: Horseracing Betting Levy Board.

112 | Molecular characterisation of viruses responsible for outbreaks of equine influenza (2019-2020)

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Background: An extensive outbreak of equine influenza (EI) occurred in Ireland during 2019 which coincided with outbreaks across Europe. Sporadic outbreaks followed in January 2020.
Objective: Genetic analysis of EI viruses detected.
Study design: *In vitro* analysis of micro-organisms.
Methods: A pan-reactive influenza type A real-time RT-PCR validated to OIE standard was used for confirmatory diagnosis and sequencing of the haemagglutinin (HA) and neuraminidase (NA) genes performed when Ct<30. Genetic characterisation of selected isolates including a virus submitted from the Netherlands, was achieved by whole genome sequencing. Phylogenetic analyses were inferred using maximum likelihood. Antigenic characterisation by haemagglutination inhibition was performed using EI-specific ferret anti-sera.
Results: In 2019, 82 EI affected premises were confirmed in Ireland. Index cases had recently travelled from continental Europe for competition or breeding purposes. Sporadic outbreaks occurred on five premises in January 2020. Sequencing of HA and NA genes of viruses (2019: 47 Irish and 13 Dutch; 2020: 3 Irish) from separate premises identified the causal viruses as belonging to clade 1 of the H3N8 Florida sub-lineage. Full genome sequencing confirmed that the Irish and Dutch viruses were virtually identical and that unlike the previous Clade 1 outbreak in Ireland in 2009/10, there was no evidence of recombination. The viruses were all closely related to FC1 viruses recently circulating in North America and, to a slightly lesser extent to viruses in South America and Africa. Antigenic characterisation indicated a close relatedness to the OIE recommended FC1 vaccine strains.
Main limitations: Although some of the affected horses were vaccinated, their vaccination history was not thoroughly examined. Antigenic characterisation of virus isolates was not performed in 2020.
Conclusions: Equine influenza viruses examined were closely related to FC1 viruses circulating in mainland Europe in 2019. Antigenic characterisation did not indicate that modification to the current OIE recommendations for EI vaccine composition was justified.
Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.
Informed consent: Not applicable.

Competing interests: None declared.
Source of funding: Department of Agriculture, Food and the Marine.
Acknowledgements: The staff in the Virology Unit at the Irish Equine Centre for diagnostic testing of samples during the outbreaks.
Data accessibility statement: The whole genome sequence of A/equine/Tipperary/1/2019 was deposited in GISAID (Global Initiative on Sharing All Influenza Data, www.gisaid.org) under accession numbers EPI1398816- EPI1398823.

113 | Development and delivery of an interactive international reporting platform for equine influenza

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Background: Highly contagious equine influenza (EI) virus continues to be identified as a cause of respiratory disease outbreaks and is endemic in many countries. Successful control and prevention of EI relies largely on vaccination, with associated surveillance to provide heightened awareness of outbreak epidemiology and virology.
Objectives: To develop an online interactive information platform to describe international equine influenza outbreaks and inform stakeholders.
Study design: Database and associated interactive reporting platform development.
Methods: An online dashboard was developed in R using the Shiny web application to integrate global EI information extracted from the International Collating Centre (ICC) reporting platform along with data on UK EI outbreaks available directly from a long-established industry-supported EI surveillance programme.
Results: The online web interface Equiflunet Viewer (http://www.jdata.co.za/equiflunetviewer), populated with international EI reports since 1 January 2019, was launched in August 2019. The website provides a resource to inform stakeholders in near real-time about EI outbreaks through hosting an interactive archive able to generate aggregated period-specified EI reports for both country-level and higher resolution UK county-level laboratory confirmed EI events. Period-specified searches generate maps and tabular summaries of spatial distributions and epidemic curves of EI outbreaks. Recent notable outbreaks reported through the website include the European EI epidemic affecting Europe and the UK in 2019, which collated 374 EI reports.
Main limitations: Information on EI outbreaks is only available for inclusion in Equiflunet reports for a limited number of countries, is provided voluntarily and laboratory confirmations are not subject to consistent quality control protocols and so reported EI outbreak numbers may not reflect true EI occurrence internationally.
Conclusions: As a complimentary resource to the ICC website, the EI specific reporting platform provides a targeted insight into EI

reports internationally, which assist ongoing control and prevention of this highly infectious equine disease.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: No competing interests.

Source of funding: International Thoroughbred Breeders' Federation members' contributions support the costs of International Collating Centre.

114 | Equine influenza outbreaks in Kazakhstan in 2020

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Background: Equine influenza (EI) is a common acute respiratory infection of horses caused by the influenza A virus. The viruses currently circulating in horses worldwide belong to the H3N8 subtype. EI has occurred in Central Asian countries, including Kazakhstan, western Mongolia, India and western China, between 2007 and 2012.

Objectives: Comparative genetic characterisation of influenza A virus isolates responsible for an EI outbreak among Kazakhstan horses in 2020 and epizootic strains of 2012.

Study design: Ongoing virological surveillance of respiratory infections among equids and genetic characterization of equine influenza virus (EIV) isolates.

Methods: Nasopharyngeal swab samples were screened in reverse transcription PCR (RT-PCR), and subsequently passaged on embryonated chicken eggs for virus isolation. Determination of influenza A virus' antigenic formula was carried out using BLAST analysis of nucleotide sequences of HA and NA genes of isolates in GenBank.

Results: In 2020, a mass outbreak of respiratory disease was reported in equids on breeding farms in south and south-eastern Kazakhstan. Clinical signs included severe coughing, and the most severe cases occurred in donkeys (some fatal cases). Influenza A/H3N8 virus was isolated from affected horses and donkeys. The HA gene of EIV isolates had 98.09% similarities with a former epizootic strain A/Equine/South Kazakhstan/236/2012 and 98.25% identity with EIV strains circulating in 2015 in neighbouring countries and was related to phylogenetic sub-lineage Florida 2.

Main limitations: No systematic virological surveillance has been carried out between EI outbreaks which is necessary to analyse epizootic strain evolution.

Conclusions: EI outbreaks among horses in Kazakhstan in 2020 were caused by EIV similar to the strains responsible for the epizootics in Kazakhstan and bordering Central Asian countries in 2012-2015. Interestingly that EI outbreak among horses in Kazakhstan occurred during strict anti-COVID-19 control measures and the collapse of international travel.

Ethical animal research: Approved by the Institute of Microbiology and Virology Local Ethics Committee (Approval number: 02-12-35).

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

Competing interests: None declared.

Source of funding: Ministry of Education and Science of the Republic of Kazakhstan, project AP09258993.

115 | Validation of equine influenza real time RT-PCR test to OIE standard

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Background: In 2013, the OIE engaged in a public-private partnership with FEI and the International Federation of Horseracing Authorities (IFHA) to establish new standards to facilitate the temporary importation of horses for competition and racing purposes, with a minimum risk of infectious disease transmission.

Objective: To validate a real time RT-PCR assay for equine influenza viral RNA detection in horses, for the intended purpose of certifying freedom from infection in individual animals for trade or movement.

Study design: Assay validation.

Methods: A well-established pan-reactive influenza type A real time RT-PCR assay targeting the matrix gene was selected and a database of 1,248 samples was compiled to assess the diagnostic performance of the assay. Samples collected during outbreaks of equine influenza in Ireland (898), United Kingdom (69), Italy (2) and Turkey (3) and from populations of horses considered free of influenza, in Uruguay (185) and Singapore (91) were tested by PCR and by a nucleoprotein ELISA. Samples from subclinical and vaccinated horses were included in the positive reference population. The diagnostic sensitivity (DSe) and diagnostic specificity (DSp) of both the ELISA and RT-PCR for equine influenza virus were estimated using a 2 test-in-2 population Bayesian latent class model which assumed conditional independence in the DSe and DSp of both tests.

Reproducibility was determined in four OIE reference laboratories using the identical assay.

Results: The median diagnostic specificity of the RT-PCR and the ELISA was 99.8% and 98.7% respectively. The median diagnostic sensitivity of the RT-PCR was 99.3% in contrast to 32.2%. for the ELISA. There was 100% reproducibility of the assay by the OIE reference laboratories for clinical samples.

Main limitations: Many laboratories internationally were unable to contribute samples.

Conclusions: This validated real time RT-PCR diagnostic assay was approved by the OIE Biological Standard Commission for inclusion in the Terrestrial Manual as suitable for the purpose of certifying horses for trade or movement.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding World Organisation for Animal Health (Office International des Epizooties, or OIE), the Federation Equestre Internationale (FEI) and the International Federation for Horseracing Authorities (IFHA) (Grant number AD/SR/2015/1885).

116 | Development of real-time cell analysis methods applied to equine influenza virus: proof of concept

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Background: Equine influenza virus (EIV) is a respiratory pathogen that causes important economic losses to the equine industry. As a result, equine influenza has benefited from several technological advances in the fields of vaccine development, diagnosis and epidemiological surveillance. New Real-Time Cell Analysis (RTCA) methods (e.g. impedancemetry) allows sensitive measurement of EIV infection, tropisms and replication *in vitro*. This technological approach has now been applied to several equine viruses (e.g. equine herpesvirus; West Nile Virus) but has not been adapted to EIV yet.

Objectives: To develop a RTCA model for EIV.

Study design: *In vitro* experiments, proof of concept.

Methods: 1) Real-time EIV SeroNeutralisation assay (RSNA) was applied to equine serums (n = 5) with different Single Radial Haemolysis (SRH) antibody titres and 2) antiviral compounds activity against EIV were screened. Both assays used MDCK cells. The normalised Cell Index (CIn) was calculated after 30 minutes pre-incubation of EIV A/equine/Jouars/4/2006 (H3N8, Florida Clade 2 sub-lineage) strain with different equine serums and subsequent cell infection. SRH titres ranged from 0mm² to 252mm². Antiviral compounds Zanamivir and Memantine were used at concentrations ranging from 50 to 1.56 µg/mL. Experimental CIn were compared with control conditions (i.e. cell-culture with/without EIV).

Results: The CIn decrease induced by EIV infection was significantly reduced ($p < 0.05$) after pre-incubation with the reference EDQM serum (200mm²) and the high SRH titre serums (222 to 253 mm²). Serums with an intermediate or negative SRH titre (123 and 0 mm², respectively) did not prevent the CIn decrease induced by EIV. Zanamivir was significantly active against EIV when used at 12.5 µg/ml ($p < 0.05$). Memantine was not active against EIV at the concentrations used.

Main limitations: Limited number of serums and EIV strains tested.

Conclusions: RTCA could be used to develop new EIV neutralisation assays and to facilitate the screening of new antiviral molecules.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: LABÉO, IFCE (Institut Français du Cheval et de l'Equitation), Fonds Eperon (OVERLORD N12-2017), Normandy County Council (17E01598/17EP04324), Région Normandie (CPER R25 P3) and CENTAURE European project co-funded by Normandy County Council, European Union in the framework of the ERDF-ESF operational programme 2014-2020.

117 | CRISPR-Cas12a for detection of H3N8 equine influenza virus: preliminary results

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Background: CRISPR-Cas12a, widely used for genome editing, are RNA guided enzymes that bind and cut double-stranded DNA (dsDNA). They possess a collateral cleavage activity on single-stranded DNA (ssDNA), when it is in a context of a ternary complex formed by the Cas12a, a target specific single guided RNA (sgRNA) and the target DNA. This collateral activity can be evidenced by adding a quenched fluorescent ssDNA reporter, allowing development of a sensitive, specific and rapid method for detection of nucleic acids of different pathogens.

Objective: To evaluate CRISPR-Cas12a technology to detect equine influenza virus H3N8 (EIV) in field samples.

Study design: Assay optimisation.

Methods: Four sgRNA between 20 and 24 nucleotides in length complementary to different regions of the segment 7 (M gene) of H3N8 EIV, were designed using the CRISPRscan software, based in CAS12a predicted guides. Complete M gene of EIV H3N8 Florida clade 1 (FC1) and Florida clade 2 (FC2) representative strains were amplified by one step RT-PCR. The CRISPR-Cas12 detection step was carried out by incubating the PCR products with the CRISPR complex at 37°C, and fluorescence was measured for up to 60 minutes every 5 minutes (ssDNA FQ substrate λex = 485 nm; λem = 535 nm).

Results: All the designed sgRNA were able to detect the EIV H3N8 of FC1 while 2 of them detected FC2 representative strains, between 5 and 20 minutes.

Main limitations: Limit of detection was not established and clinical samples were not assayed.

Conclusions: CRISPR-Cas12a technology seems to be a promising system for detection of equine influenza virus, offering a convenient tool for low-resource laboratories.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: INTA and INTA-Haras agreement.

118 | Evaluation of a pseudotype virus neutralisation test for measurement of equine influenza virus (EIV) antibody responses induced by vaccination and experimental infection

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Background: Equine influenza virus (EIV) causes significant respiratory disease in horses and although primarily controlled by vaccination, major outbreaks still occur. Although neutralising antibodies (NAbs) are the main protective response, they are difficult to quantify due to poor replication of many EIV strains in cell culture. Surrogate antibody measurements are conducted via haemagglutination inhibition (HI) and single radial haemolysis (SRH) assays. EIV pseudotyped viruses (PVs) may provide a suitable alternative for virus neutralisation assays.

Objectives: To compare antibody (NAb) titres using a PV neutralisation test (PVNT) with HI titres and SRH values using a panel of equine sera.

Study design: Assay development.

Methods: EIV PV was generated via plasmid co-transfection of producer cells and supernatant harvested. Archived serum samples from a longitudinal vaccination study (n=108) and a vaccination and challenge study (n=26) were assessed by the PV neutralisation test (PVNT), HI and SRH. Full length HA gene (A/equine.Richmond/1/2007; accession #FJ195395) fragment was synthesised, cloned into a eukaryotic expression plasmid and co-transfected with plasmids for other PV components (i.e. lentivirus gag-pol and luciferase reporter) into HEK293T/17 cells using polyethylenimine (PEI). After harvest, PV production and neutralisation quantification (IC50) was assessed via target cell luminescence, following serial dilution. HI and SRH were conducted as per OIE Terrestrial Manual.

Results: PVNT results were in good agreement with SRH (100% sensitivity, 68.5% specificity) and HI (99.2% sensitivity, 49.0% sensitivity), with correlation coefficients (r) of 0.84 and 0.77 respectively. PVNT notably exhibited more sensitivity at low antibody levels [1].

Main limitations: Inter-laboratory reproducibility needs evaluating and a protective PVNT antibody level defined.

Conclusions: EIV pseudotyped lentivirus particles can be used to sensitively measure specific antibody responses in sera of vaccinated and EIV-challenged horses and could provide an effective alternative to HI and SRH tests to quantify biological neutralisation.

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Ethical animal research: Serum samples were obtained from previous studies approved by the Animal Health Trust (AHT) Ethics Review Committees.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: RK and SDD are funded by University of Kent and NT by Innovate UK (105078) and B&MG (G101404).

119 | Serological responses of equine influenza virus seronegative mature horses to large versus small combination equine influenza virus vaccines

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Background: Equine influenza virus (EIV) vaccines may include large or small combinations of other antigens. We hypothesise that antigen interference may occur with large combination vaccines.

Objective: To determine if the serological response to EIV vaccination was negatively impacted when incorporated with other vaccine antigens.
Study design: Field trials.
Methods: Three EIV vaccines were either administered as a combination with equine herpesvirus 1/4 vaccines or incorporated with their counterpart Eastern/Western equine encephalitis virus, West Nile virus, and *Clostridium tetani* vaccine. In Study 1, 90 mature EIV sero-negative horses were randomly assigned to one of four vaccination groups (n=20), and 10 horses were unvaccinated controls. Sera were collected on the day of initial vaccination (day 0) and on days 7, 21 (revaccination day), 28 and 42. In Study 2, archived sera were tested from a previous study in which EIV sero-negative horses were given two different vaccines (40 horses per vaccine group), with 20 horses as unvaccinated controls. Vaccines were administered and sera collected as in Study 1. Commercial EIV vaccines from 3 manufacturers were used and vaccines were administered by intramuscular injection. Testing for EIV-specific serum antibody titres was done by using the haemagglutination-inhibition (HI) test. Results were analysed by a linear mixed model approach for repeated measures.
Results: All vaccines showed a trend that large combination (EIV-EHV-EEE-WEE-WNV-Tet) vaccinated horses had lower HI titers against EIV when compared to the same EIV vaccines given in combination with EHV alone. This trend reached statistical significance with one of the 3 vaccines.
Main limitations: Absence of statistical significance with 2 vaccines may be due to reduced statistical power relating to group sizes or may be characteristic of the particular vaccine products.
Conclusions: Antigen interference affecting large-combination EIV vaccines may occur but the magnitude of the effect is small.
Ethical animal research: The authors have confirmed that research ethics committee oversight is not required by their institute for this study protocol.
Informed consent: Owners gave consent for their animals' inclusion.
Competing interests: VD and DA are employees of Zoetis Animal Health Inc. USA.
Source of funding: Zoetis Animal Health Inc. USA.

120 | Variation in equine vaccination practices in the United Kingdom

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Background: Vaccination is an integral to preventative equine healthcare, but there is little evidence of the current practices in the UK.
Objectives: To describe current vaccination practices and protocols advised by equine vets in the UK and compare the practices compared to manufacturers' guidelines.
Study design: Online cross-sectional survey.
Methods: An online survey was distributed through the RCVS 'Find a Vet' website and social media, targeting UK equine veterinary surgeons. The survey collected data entailing; demographics, vaccine choice, vaccination policy, adverse reactions, vaccine hesitancy and case-based scenarios. Descriptive statistical analysis was performed.
Results: Three-hundred and four questionnaires were completed; 74% respondents worked only with horses. The respondents' workload consisted of: leisure (97.4%), competition (86.2%), stud (47.7%) and racing (40.5%). The study demonstrated variation in vaccine protocols advised for use in competition and non-competing horses: 57% of respondents showed variation in advised 'booster' frequency, most commonly advising a 6-monthly vaccination in competition and annual vaccination in others. Twenty-five different sources of equine vaccination guidelines were reported. Vaccination schedules commonly did not comply with the datasheet guidance, only 7.7% of respondents complied with the advised timeframe between second and third vaccination. Adverse reactions following vaccination were encountered by 66% respondents in the last 12 months, with 2760 adverse events, though only 526 (9.1%) cases were officially reported. Common adverse events encountered were transient and included muscle stiffness (928), swelling at site of injection (837), lethargy (559) and pyrexia (355). Vaccine hesitancy was encountered by 86.4%, most commonly due to perception of unnecessary vaccination by clients, cost and concern regarding previous or anticipated adverse reactions.
Main limitations: Potential respondent bias, and potential effect of recent EI/EHV outbreaks in the UK.
Conclusions: This study of current equine vaccination practices in the UK revealed vaccination protocols advised were variable and lacked compliance with manufacturers' guidelines.
Ethical animal research: Ethics approval was sought through the University of Liverpool Veterinary Ethics Committee (VREC838).
Informed consent: Completion of the survey was taken as consent.
Competing interests: AW is supported by MSD Animal Health.
Source of funding: AW received a veterinary research bursary from MSD Animal Health.

121 | Role of the IL-33 cytokine in lung repair post equine influenza virus infection

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Background: Equine influenza virus (EIV) causes a severe respiratory disease in horses, with extensive necrosis of airway epithelium [1], regeneration of which is critical for re-establishment of lung function [2]. Interleukin-33 (IL-33), an alarmin cytokine signalling through the IL-33 receptor (IL-33R) expressed on immune cells, has been shown to promote lung repair by inducing amphiregulin (AREG) in other species [3]. To date, the role of equine IL-33 in lung repair following EIV infection is unknown.
Objectives: Evaluate the role of equine IL-33 in lung repair following EIV infection.
Study design: *In vitro* experiments.
Methods: Equine cells were infected *in vitro* with EIV for 24h and their transcriptome analysed. An *in vivo* mouse model of influenza was used to analyse IL-33, AREG and viral NP gene expression by qPCR, measure airway epithelial progenitor proliferation by flow cytometry and monitor weight loss for disease severity.
Results: In EIV-infected equine cells the IL-33R gene was significantly upregulated compared to control and correlated with disease severity (18.39-fold versus 5.20-fold increase for severe versus mild EIV disease). Furthermore, in mice, clinical resolution of EIV is apparent at 21 days post infection and correlates with viral load drop, IL-33 and AREG gene induction, and proliferation of lung epithelial progenitors.
Conclusions: The data indicate that in a horse cell line and mice, the IL-33-AREG axis is involved in lung repair post EIV infection. Next, the molecular mechanisms by which equine IL-33 drives lung tissue regeneration following EIV infection will be investigated using a combination of classical immunology, advanced cell culture technologies and next generation sequencing. This project has the potential to lead to the development of novel therapeutics to promote healthy lung repair. Developing such novel knowledge will have a significant impact on the welfare of EIV infected horses.

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Ethical animal research: All *in vivo* procedures in mice were performed under UK Home Office licenses.
Informed consent: Not applicable.
Competing interests: None declared.
Source of funding: CC is supported by a Horserace Betting Levy Board Veterinary Postdoctoral Research Fellowship (VET/2020 -1 EPDF 7).

122 | Equine influenza virus surveillance in the United Kingdom from 2019 to 2021

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Background: Equine influenza virus (EIV) is a major cause of respiratory disease in horses worldwide. Between January and September 2019, a major epidemic of EIV occurred in the UK, affecting both vaccinated and unvaccinated animals. It resulted in a 6-day cancellation of racing in February 2019 and significant economic loss to the equine industry.
Objectives: The EIV surveillance programme monitors the genetic and antigenic changes occurring in EIV circulating in the UK to identify any evidence of vaccine breakdown in the field.
Study design: Strain isolation and genome sequences' comparison.
Methods: Surveillance of EIV in the UK horse population is achieved using a sentinel practice scheme of equine practitioners who submit samples for laboratory testing from horses with suspected EIV. Nasopharyngeal swab samples submitted to the programme were tested for EIV using qRT-PCR. Positive samples were sequenced, phylogenetic analyses were completed for the haemagglutinin and neuraminidase genes, and amino acid sequences compared against current World Organisation for Animal Health (OIE)-recommended vaccine strains. Substitutions between new isolates and the corresponding vaccine strain were mapped onto the three-dimensional protein structures.
Results: From 2019 to 2021, >12,000 nasopharyngeal swabs were tested for EIV, of which >500 were positive. During the first wave of the 2019 epidemic, EIV was detected in 228 separate locations and 80 viruses were isolated in eggs from 133 swabs. Worcestershire/05469/2019 (Florida clade 1) was representative of the majority of viruses from the 2019 UK epidemic.

Main limitations: EIV surveillance is predominantly passive, relying on owners to report clinical signs and get their horses tested, meaning the actual number of outbreaks was probably higher than the number detected.

Conclusion: The results demonstrate the ability of the surveillance programme to monitor EI outbreaks. Genetic differences were observed in outbreak strains over time, although the viruses remained antigenically similar, thereby supporting the continued application of current vaccine strain recommendations.

Ethical animal research: Research ethics committee oversight not required by this journal: retrospective analysis of laboratory data.

Informed consent: Not stated

Competing interests: None declared.

Sources of funding: Horserace Betting Levy Board (HBLB), Animal Health Trust and British Horseracing Authority.

Poster Presentations

123 | Outbreaks of Equine Influenza in Italy during 2019

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Background: The current predominantly circulating Equine Influenza virus (EIV) strain is Clade 1 of the H3N8 Florida. Although in Italy there is no active surveillance, clinical reports indicate that EIV infection is present.

Objectives: Detection and characterisation of EIV strains circulating during 2019 in Italy.

Study design: Strain isolation and genome sequences' comparison.

Methods: Nasal swabs and blood were collected from horses with clinical signs consistent with EIV infection in outbreaks in Molise and Lombardia. EIV antibodies were determined by haemagglutination inhibition assay and single radial haemolysis [1]. PCR [2] positive nasal swabs were inoculated into 10-day embryonated chicken eggs and allantoic fluid was examined by the assay [1] before virus characterisation by sequencing of the haemagglutinin gene [3].

Results: All nasal swabs of 20 subjects analysed were EIV positive by RRT-PCR, with isolation and characterisation of the virus from Lombardia. Florida lineage H3N8 Clade 1 was detected, confirming data available on circulating strains but with some mutations with respect to vaccine strains.

Main limitations: EIV strains found in Molise were not characterised probably due to the small amount of virus recovered. From the serological positivity pattern, strain characterisation was not possible due to the absence of prevalent serotypes, probably related to the vaccination status.

Conclusions: Monitoring of circulating strains is necessary to verify the appropriateness of the vaccine virus composition, allowing continuous updating of the strains in circulation.

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Ethical animal research: Research ethics committee oversight not required by this journal: retrospective analysis of laboratory data.

Informed consent: Not stated

Competing interests: None declared.

Source of funding: None.

124 | Molecular characterisation of Argentinian equine influenza virus genome segments 2 and 3

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Background: Equine influenza virus (EIV) genome segments 2 (PB1) and 3 (PA) encode for two subunits of the trimeric RNA polymerase. The PB1-F2, encoded by an alternative open reading frame (ORF), contains 90 and 81 amino acids (aa) for H3N8 EIV identified before and after 1997, respectively. The PA-X is a fusion protein whose C-terminal of 61 aa is derived from a second ORF, although in some Influenza A virus a premature codon yields a truncated PA-X. Both proteins could contribute to the pathogenicity of the virus.

Objective: To analyse the molecular characteristics of genome segments 2 and 3 of EIV.

Study design: Retrospective genetic analysis study.

Methods: The PA and PB1 genes' nucleotide and aa sequences of 16 EIVs detected in Argentina were analysed, and phylogenetic trees inferred.

Results: For PB1 and PA genes, Argentinian EIV groups into three monophyletic clades, South American (SA) clade 1, SA clade 2 and Florida clade 1 (FC1), consisting of strains detected between 1993-1996, 1997-2006, and in 2012, respectively. Deduced aa sequences of the PB1-F2 of strains belonging to SA clade 2, showed a polymorphism due to a stop codon at position 238, yielding a polypeptide of

79 aa. These strains also possess a polymorphism in PA-X, characterised by a premature stop codon at position +30 resulting in a polypeptide of 29 aa. The remaining Argentinian strains had complete PB1-F2 and PA-X.

Main limitations: The relationship between the observed molecular characteristics and the pathobiology of virus strains was not assessed.

Conclusions: The described polymorphisms have been observed only in SA clade 2 Argentinian influenza strains, suggesting a local and independent evolution of PB1 and PA genes. These highlight the need for additional studies to understand the implications of such polymorphisms on viral pathogenesis and host cell responses, and also their relationship with viral fitness and perpetuation.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: The investigations and laboratory work were supported by INTA and INTA-Haras agreement.

125 | Antibody responses to a reverse genetics-derived bivalent inactivated equine influenza vaccine in Thoroughbred horses

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Background: Updating vaccine strains is important to control equine influenza. Multivalent equine influenza vaccines are available throughout the world. Previously, an inactivated monovalent vaccine derived from a virus generated by reverse genetics (RG) elicited immunogenicity in horses [1].

Objectives: To evaluate antibody responses to a bivalent vaccine derived from RG viruses in Thoroughbred horses.

Study design: *In vivo* experiments.

Methods: We generated two RG viruses possessing the haemagglutinin and neuraminidase genes from A/equine/Ibaraki/1/2007 (Florida sub-lineage clade 1) or A/equine/Yokohama/aq13/2010 (Florida sub-lineage clade 2), which have been used as vaccine strains since 2016 in Japan, based on OIE recommendations. The RG viruses were inactivated by formalin, and the haemagglutinin titre of the RG vaccine was adjusted to be the same as that of a bivalent commercial (CO) vaccine in Japan that is derived from wild-type strains. Sixteen unvaccinated yearlings (7 for the RG vaccine group and 9 for the CO vaccine group) received two doses of a primary vaccination course four weeks apart. Thirty-two vaccinated adult horses (18 in the RG-vaccinated group and 14 in the CO vaccine group) received a single dose of a booster vaccination.

Results: The patterns of haemagglutination inhibition antibody response to the primary and booster vaccinations were similar for the RG and CO groups in unvaccinated yearlings and vaccinated adult horses. The results suggest that a bivalent vaccine derived from RG viruses offers the same level of immunogenicity as a commercial vaccine derived from wild-type viruses.

Main limitations: No virus challenge study was performed.

Conclusions: RG viruses can be used in a multivalent vaccine as well as a monovalent vaccine in horses. The RG technique will contribute to the timely update of equine influenza vaccine strains.

Reference

[1] Ohta, M., Bannai, H., Kambayashi, Y., Tamura, N., Tsujimura, K., Yamayoshi, S., Kawaoka, Y. and Nemoto, M. (2021) Growth properties and immunogenicity of a virus generated by reverse genetics for an inactivated equine influenza vaccine. *Equine Vet. J.* https://doi.org/10.1111/evj.13431.

Ethical animal research: Approved by the Animal Care Committee of the Equine Research Institute with accession number 20-1.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: Japan Racing Association.

Influenza D

Oral Presentation

126 | Type-D influenza virus from bovines is not pathogenic in horses

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Background: Antibodies to Type-D influenza virus have been detected in horses, but no evidence of disease in the field has been reported.

Objective: To determine whether a Type-D influenza virus is infectious, immunogenic, and pathogenic in horses exposed by experimental challenge.

Study design: Four 2-year-old horses, seronegative for both influenza-D virus and also conventional Type A (H3N8) equine influenza, were experimentally exposed to influenza D/bovine/California/0363/2019 (D/CA/19) virus, using methods that regularly produce infection when using conventional equine influenza A virus. Clinical, virological, and serological parameters of infection were assessed following challenge.

Study design: *In vivo* experiments.

Methods: Horses were challenged by the aerosol-inhalation route with 6.25 x 10^7 TCID50/animal of influenza D/CA/19 virus that had

been propagated in MDCK cells. Horses were observed daily for clinical signs including rectal temperature, nasal discharge, coughing, lung sounds, tachycardia, and tachypnea.

Nasopharyngeal swabs were collected daily for 8 days following challenge for assessment of virus shedding by RT-PCR and by virus culture in MDCK cells. Sera were collected and tested by haemagglutination-inhibition (HI) assay.

Results: No horses exhibited clinical signs of disease. Also, all nasopharyngeal swabs were negative for virus detection by both RT-PCR and virus culture. However, all 4 horses showed evidence of an HI antibody response.

Main limitations: The failure to detect any virus shedding suggests that no viral adaptation facilitating replication in horses had occurred; but this remains a theoretical possibility.

Conclusions: A bovine-derived isolate of influenza-D virus was not pathogenic or replication-competent in horses following experimental exposure, suggesting that interspecies transmission into equids is not an important feature of the ecology of influenza-D viruses in nature.

Ethical animal research: University of Kentucky IACUC #2007-0153 for Chambers, last approved 14/05/2020.

Informed consent: Not applicable.

Competing interests: None declared.

Source of funding: University of Kentucky institutional internal accounts.

Poster Presentation

127 | Limited evidence for exposure of UK horses to influenza D virus

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Background: Influenza D virus (IDV) was first isolated from pigs but also causes respiratory disease in cattle, which are thought to be the reservoir host. Antibodies to IDV have been detected in horses, but no association with respiratory disease has been demonstrated and attachment of virus to equine respiratory tissue is limited to submucosal glands.

Objectives: Screen equine respiratory samples and serum samples for IDV RNA and antibodies, respectively.

Study design: Cross-sectional study.

Methods: Equine respiratory samples from equids with suspected infectious upper respiratory disease for which no cause has been identified were obtained from the Animal Health Trust's diagnostic service. Serum samples from Thoroughbred horses-in-training and breeding stock that would have otherwise been discarded after routine health screen laboratory testing were obtained from Newmarket Equine Hospital. A published RT-qPCR method to detect IDV RNA [1] was applied to equine respiratory samples. Several serological tests were applied to serum samples: haemagglutination inhibition (HI) and pseudotype virus neutralisation test (PVNT).

Results: No IDV RNA was detected in any of the 232 respiratory samples screened by RT-qPCR. In initial screening of 330 serum samples by HI, 1 (0.3%) was positive. Of a further 430 serum samples initially screened by PVNT, 6 (1.4%) were deemed positive.

Main limitations: Convenience samples were used for viral RNA and antibody screening. Interpretation of the HI test is subjective and non-specific inhibition can give false positive results. The PVNT described is not validated.

Conclusions: A small number of equine serum samples tested positive in IDV serological tests indicating exposure to IDV or a closely-related virus. However, there was no indication that IDV is associated with respiratory disease in equids in the UK.

Reference

[1] Faccini, S., De Mattia, A., Chiapponi, C., Barbieri, I., Boniotti, M.B., Rosignoli, C., Franzini, G., Moreno, A., Foni, E. and Nigrelli, A.D. (2017) Development and evaluation of a new Real-Time RT-PCR assay for detection of proposed influenza D virus. *J. Virol. Meth*. **243**, 31-34.

Ethical animal research: The study was conducted with approval of the University of Nottingham School of Veterinary Medicine and Science Ethics Committee (Ref. 2633 181109).

Informed consent: Explicit study consent was not stated but submitting veterinary surgeons were aware that samples could be used for research activities.

Competing interests: None declared.

Source of funding: Horserace Betting Levy Board short project grant (SPrj036).

West Nile Virus

Oral Presentations

128 | Spatial distribution and infection of West Nile virus lineages 1 and 2 in France from 2015 to 2020

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Background: Most European West Nile virus (WNV) outbreaks before 2010 were caused by WNV lineage 1 strains. The increase in WNV outbreaks in Europe since 2010, has been associated with the introduction and spread of WNV lineage 2 strains.

Objectives: We report recent French WNV outbreaks (2015-2020) in equids and the wild avifauna in the Mediterranean area and describe the emergence of WNV lineage 2 in France in 2018.

Study design: Retrospective case series.

Methods: Between 2015 to 2020, diagnostic specimens from horse and avian suspect cases were collected [1]. Suspect horse sera were tested for anti-WNV-IgM antibodies. Brains of horses and birds, suspected to be infected with WNV were subjected to WNV real time RT-PCR, virus isolation and genome sequencing.

Results: Recent intensification of enzootic WNV circulation was observed in the South of France, with most horse cases detected in 2015 (n=49), 2018 (n=13) and 2019 (n=13). A breaking point in French WNV epidemiology was reached in 2018 and coincided with the isolation of WNV lineage 2 on a moribund diurnal raptor located in a south-eastern area. This virus most probably spread from Northern Italy and caused West Nile neuro-invasive disease (WNND) in humans and the death of diurnal raptors. WNV lineage 2 emergence in France was associated with the most important human WNV epidemics identified so far (n=26, including 7 WNND cases and 2 infections in blood and organ donors). Two other major findings were the detection of WNV in areas with no or a limited history of WNV circulation (Alpes-Maritimes in 2018, Corsica in 2018-2020, Var in 2019-2020) and the distinct spatial distribution of human and horse WNV cases.

Conclusions: These new data reinforce the necessity to strengthen French WNV surveillance to better anticipate future WNV epidemics and epizootics and to improve the safety of blood and organ donations.

Reference

[1] Beck, C., Goffart, I.L., Franke, F., Gonzalez, G., Dumarest, M., Lowenski, S., Blanchard, Y., Lucas, P., de Lamballerie, X., Grard, G., Durand, G.A., Zientara, S., Tapprest, J., L'Ambert, G., Durand, B., Desvaux, S. and Lecollinet, S. (2020) Contrasted Epidemiological Patterns of West Nile Virus Lineages 1 and 2 Infections in France from 2015 to 2019. *Pathogens* **9**(11), 908.

Ethical animal research: Research ethics committee oversight not currently required by this journal: procedures were performed as part of clinical investigations

Informed consent: Not stated.

Competing interests: None declared.

Sources of funding: ANSES and the European Commission through DG SANTE funding for the Reference Laboratory for Equine Diseases other than African Horse Sickness.

129 | The sensitivity of horse surveillance in detecting West Nile and Usutu viruses in central Italy

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Background: West Nile virus (WNV) and Usutu virus (USUV) are *flaviviruses* circulating among wild birds and mosquitoes. WNV's frequent spill over to equines and humans causes from inapparent infections to serious illness. WNV and USUV can co-circulate in an overlapping geographic range.

Objectives: To describe the WNV/USUV co-circulation in central Italy and the sensitivity of the detection of the two viruses in different surveillance components.

Study design: Descriptive study.

Methods: In Italy, as in most European countries, the surveillance plan is based on active and passive surveillance in humans, birds, horses, and vectors. After a first WNV outbreak in October 2018, a reinforced surveillance was performed in 4 municipalities (512 km^2) in the Lazio region, through clinical and serological surveillance in horses and virological surveillance in horses and *Culicidae* catches.

Results: During 2018, in Lazio and Toscana, about 1300 horses, 450 mosquitoes catches and 391 birds were tested for WNV/USUV. WNV was found in 8 horses on 8 holdings of 193 equids tested in the restricted area in Lazio. The cumulative incidence was 8/18 (44%). WNV was not found in vectors or in birds. USUV was found in 17

pools of *Culex pipiens* of 56 pools from 23 mosquito catches and was not found in horses or in birds.

Main limitations: The lack of active surveillance in wild birds.

Conclusions: In the same period and geographic area, different surveillance components detected WNV only in horses and USUV only in mosquitoes. While in northern Italy, the entomological surveillance is used successfully as an early WNV warning, in central Italy, its sensitivity seemed too low to be useful in a Public Health approach. Besides, serological surveillance in sentinel horses acted efficiently as an early warning system. Further research is required to justify and adapt the surveillance activities in different epidemiological contexts.

Ethical animal research: Serum samples in horses were collected by Local Health Units official veterinarians in accordance with the European Legislation on Animal Welfare.

Informed consent: Not stated

Competing interests: None declared.

Source of funding: Not applicable.

130 | Identification of antiviral molecules against West-Nile virus by an approach combining cell imaging and equine neural cells derived from induced-pluripotent stem cells

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Background: West-Nile virus (WNV) is a member of the *Flaviviridae* family which is transmitted through an enzootic birds-mosquitoes cycle and which accidentally infects horses and humans causing neuro-invasive diseases. Vaccines are available for horses but there are no antiviral therapeutics. WNV has a worldwide distribution and is currently expanding in Europe. To overcome the lack of therapy, it is essential to identify antiviral molecules active in the brain.

Objectives: 1) develop a new *in vitro* model of equine brain cells derived from induced pluripotent stem cells (Eq-iPSCs), 2) test the sensitivity of brain cells for WNV and 3) use this biologically relevant model of WNV infection to screen a small bank of 37 molecules for their antiviral capacity.

Study design: *In vitro* experiments.

Methods: Eq-iPSCs were first induced in the neural pathway and the resulting neural progenitor cells (Eq-NPCs), cells that are capable of differentiating into neurons, were infected with WNV.

Results: Eq-NPCs were permissive to WNV in a dose-dependent manner. Optimum conditions of infection were defined and Eq-NPCs were then used to screen, by automated cell imaging, molecules selected for their antiviral capacity on various other viruses. 2'-C-Methylcytidine (2'-CMC) exhibited antiviral activity against WNV in equine brain cells. Dose effects were performed to determine its inhibitory concentration 50 (IC50 =11µM), cytotoxicity concentration (CC50 = 58µM) and its selectivity index (SI=5). The decrease in the amount of the viral genome, as shown by RT-qPCR in the supernatants of cultures exposed to 2'-CMC confirmed its inhibitory effect on WNV replication in brain cells.

Conclusions: A new *in vitro* model based on iPSCs technology and its use in phenotypic screen for the identification of antiviral molecules against WNV has been developed. *In vitro*, 2'-CMC is an antiviral molecule active against WNV in equine brain cells.

Ethical animal research: Not applicable.

Informed consent: Not applicable.

Competing interests: None declared.

Sources of funding: ENVA, IFCE.