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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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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.

References

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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 (C₂₂₅₄/H₇₅₂) 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 C₂₂₅₄ EHV-1 strain.

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

Methods: The C₂₂₅₄ EHV-1 strain was isolated *in vitro* on RK13 cells and characterised by Multi Locus Sequence Typing (MLST). A probe was designed for specific C₂₂₅₄ 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 C₂₂₅₄ EHV-1 strain (5e07 TCID₅₀/pony).

Results: The C₂₂₅₄ 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 C₂₂₅₄ mutation outside the outbreak of origin (i.e. 2018). No difference in sensitivity to ganciclovir and aphidicolin between the A₂₂₅₄, G₂₂₅₄ and C₂₂₅₄ strains was detected *in vitro*. Both C₂₂₅₄ and A₂₂₅₄ strains were more sensitive to aciclovir than the G₂₂₅₄ 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 C₂₂₅₄ 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.

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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