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

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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 (MOCCA).

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 Duncombe, 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/C₂₂₅₄ (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 A₂₂₅₄). 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 GISCENTAURE.

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*.