Trouble with Gamma-Herpesviruses

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Nasal swabs/conjunctival swabs PCR-positive for equid herpesvirus (EHV) -2 or -5 are poor indicators of disease. Testing for EHV-2/-5 in multiplex PCR panel assays is worth rethinking. PCR EHV-5 detection on peripheral blood and/or bronchoalveolar lavage fluid (BALF) from horses that presented for a respiratory disease work-up should prompt chest radiographs, especially when BAL cytology does not support the level of clinical disease/exercise intolerance. Conditions or diseases associated with EHV-5 are unlikely to benefit from (systemic or local) valacyclovir (or similar) therapy. Author’s address: Wright Markey Chair and Professor of Equine Infectious Diseases MH Gluck Equine Research Center, University of Kentucky, 1400 Nicholasville Road, Lexington, KY 40546-0099; e-mail: l.goehring@uky.edu. © 2021 AAEP.

1. Introduction

Herpesviruses are all enveloped (double-strain) DNA viruses and are divided in 3 sub-classes: alpha, beta, and gamma. Currently, there are no known beta-herpesviruses known in equids; however, several alpha-herpesviruses are known to cause disease in horses (EHV-1, -3, -4). Equid herpesvirus-2 (EHV-2) and -5 (EHV-5) are two of five currently known gamma-herpesviruses of equids. EHV-5 and EHV-2 are frequently detected in combination and seem to be more strongly associated with horses, while 3 gamma-herpesviruses, (asinine; AHV)-2, AHV-4 and -5 are more commonly found in donkeys. However, cross-over and co-existence in horses or donkeys have been observed. For decades, both gamma-herpesviruses played an unclear role in mild to, on occasions, moderate disease in horses of the respiratory tract or were associated with keratoconjunctival disease. In 2007, a novel and seriously debilitating disease was described in five horses that seemed strongly associated with EHV-5. Due to location and macroscopic appearance, the disease was named equine multi-nodular pulmonary fibrosis (EMPF). Since then, EMPF has been diagnosed as an incidental but severely debilitating disease on all continents. EHV-5 was first described in horses in Australia. Both EHV-2 and -5, when in their latent stage of chronic-persistent infection, have been found in peripheral blood mononuclear cells (PBMC), mainly in T- and B-lymphocytes. These viruses differ in their ease of recovery using viral culture techniques. While both require co-culture techniques to be isolated from PBMC, it has been easy to recover EHV-2 and notoriously difficult to recover EHV-5. With the introduction of (quantitative) PCR, the extensive prevalence of EHV-5 (and EHV-2) was discovered. Both viruses have a world-wide distribution and can even be found in isolated or remote horse (or donkey) populations. As a likely result of a parliament decision from the 1880s to
not allow the further import of mammals onto the island, the Icelandic horse population is, for example, negative for EHV-1, Strep equi spp. equi, and equine influenza; yet both gamma-herpesviruses EHV-2 and -5 (and alpha EHV-4) have been detected in Icelandic horses. This shows the longstanding pathogen and host interaction for centuries probably, which requires significant adaptation and only mild pathology during replicative phases of the pathogen, if at all.

2. Clinical Presentation

Since their detection, EHV-2 and -5 have been described as fairly benign causes of respiratory tract disease and possibly keratoconjunctivitis (KCC) in foals and young horses upon first-time exposure to these pathogens. Upon infection, there can be a fever, serous to mucoid nasal discharge, conjunctivitis, and lymphadenopathy of the mandibular/retropharyngeal lymph nodes.2 There are few single case reports published on EHV-2 and EHV-5 associated with dermatitis, lymphoma, or esophageal disease.3 In a larger number of studies, KCC has been debated as a result of EHV-2 and/or -5 infection. One large study in approx. 250 (single breed) horses, examined on four occasions at 6-month intervals, did not show an association with ophthalmic disease and equine gammaherpesviruses. Forty-five to 60% of horses with KCC were at least once positive for either EHV-2 and/or -5 on a conjunctival swab, while 13 (30%) of clinically normal horses were also positive.4 EHV-5 has been demonstrated in endometrial lavages in about 15% of a total of 60 mares with reproductive disorders,5 while less than 3% or 2%, respectively, of abortion/stillbirth/neonatal death/placentitis cases were PCR positive with EHV-2 or -5.5 EHV-5 and EHV-2 have also been identified in gastric mucosa by novel in situ hybridization technique and PCR; however, EHV-5 was not associated with ulcerative lesions.7 Several research groups have focused on the question whether EHV-2 or -5 infections are involved in inflammatory airway disease (IAD) in (young) performance horses. Houtsma and collaborators8 looked at two groups, an IAD group vs. a healthy control group at a single time point. Results suggested a positive relationship between EHV-2 presence in nasal swab and IAD. Interestingly, EHV-5 was only detected in (few) BAL samples, however, not in nasal swabs of IAD horses. The group concluded an association of gamma-herpesviruses presence and IAD in their study. Dubli-Bounoua and collaborators9 conducted their study on 50+ Standardbreds, which were followed for over two years with once-a-month collection of a nasal swab and tracheal aspirate (TA) collected via endoscopic examination. EHV-2 and EHV-5 were the most frequently identified respiratory tract viruses, often in combination, in this study. These authors found a correlation between EHV-2, coughing, and tracheal mucus volume, while EHV-5 positive samples seemed evenly distributed between affected and unaffected horses. Similar conclusions came from Sweden, where a study focused on EHV-5 presence in the respiratory tract of about 60 elite Standardbred horses and their respective performance levels.10 Horses were followed for one year with monthly sampling (15 sampling episodes), where TA and nasal swabs were collected each time. Performance status at time of sampling was determined using a standardized exercise test. At least one sample (NS or TA) per sampled horse was positive once for EHV-5 using qPCR during the observational period. Several horses had multiple samples positive for EHV-5 with highly variable quantities of viral copy numbers. However, regardless of the viral quantity, there was no association between EHV-5 presence and change in ability to perform. Viral strains of the study (and two archived strains collected from EMPF horses) were sequenced for viral gB, which allowed allocation of the Swedish strains into one of four genotypes (I – IV). Most strains were either in group I or III. As these horses were sampled multiple times at monthly intervals, it is worth mentioning that genotypes differed between monthly samplings, and the two strains from archived EMPF samples were assigned to EHV-5 genotype III.11 In 2007, EMPF was described as a novel disease of the lower respiratory tract of horses. Furthermore, EMPF appears to be the most severe clinical presentation to date consistently associated with EHV-5. Horses at a more advanced stage of disease present with tachypnea, exercise intolerance, and an undulating fever. Cytology of tracheal aspirate or BALF can be surprisingly modest regarding its cellularity. There are typically nodular opacities visible on chest radiographs, while peripheral areas of lung consolidation can be also visualized by ultrasound imaging. Lung biopsies show interstitial fibrosis with marked influx of inflammatory cells, mainly lymphocytes. In a first case series publication describing a little more than 20 cases, necropsy results revealed distinct nodular fibrosis demarcated from more normal (aerated) lung tissue, with a predominantly lymphocytic interstitial cell infiltrate.12 Remaining but deformed alveolar spaces were filled with a moderate exudate of mixed neutrophilic granulocytes and macrophages, while the remaining alveolar-like architecture is lined with cuboidal epithelial cells. An increased number of myofibroblasts has been noticed in the affected areas. Occasionally, macrophages contained viral inclusion bodies (IB), which, on electron microscopy (EM) investigation, showed viral particles. An EHV-5 specific in situ hybridization was positive for the IB and for sections of (remaining) alveolar spaces for macrophages there. PCR analysis specific for consensus herpesvirus and for equid herpesviruses -1, -2, -4, -5 was positive for EHV-5 in all 20+ cases and for EHV-2 in about one third of cases. More so, clinical disease (and macro/microscopic disease) has been successfully evoked via instillation of cultured EHV-5 that
Discussion of EMPF (Equine Multinodular Pulmonary Fibrosis)


**References**

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**Declaration of Ethics**

The Author has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

**Conflict of Interest**

The Author has no conflicts of interest.

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**References**


