Prevalence of EHV-1 in adult horses transported over long distances

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INFECTION with equine herpesvirus type 1 (EHV-1) is widespread in horse populations throughout the world; it produces well-documented syndromes and outbreaks of respiratory disease, abortion, neonatal losses and myeloencephalopathy (Van Maanen 2002). It is believed that EHV-1 circulates in a given horse population via silent or clinical infections or reactivation of latent infections. The biological source of the virus originates from a primary lytic infection or the shedding of virus following reactivation of latent virus. Latent EHV-1 has been detected in both lymphoid and neural tissues; the preferred cell in which latency is maintained is the CD5/CD8+ T lymphocyte (Slater 2007). The prevalence of latent EHV-1 infection in horses has been determined for thoroughbred broodmares in central Kentucky, USA, by Allen and others (2008). This information is important for assessment of the risk of recrudescence in a specific population of horses. Recrudescence of latent virus, with subsequent shedding of virus in nasal secretions, is a potential source of infection for naive animals, and has been implicated in outbreaks in closed populations of horses. The risk factors for recrudescence and shedding are poorly documented, but all types of stressors; for example, weaning, castration, long-distance transport, strenuous exercise or underlying disease have been suggested. Recrudescence has also been produced experimentally by the administration of immunosuppressive drugs (Edington and others 1985). Recrudescence following stress remains poorly understood, as does the role of subclinically infected shedders of the virus. This short communication describes a study to determine the prevalence of EHV-1 nasal shedding and viraemia in a population of adult horses that had undergone long-distance transport.

Three hundred and two healthy adult horses with a history of recent transportation and a mandatory quarantine period at the contagious equine metritis quarantine facility at the Center for Equine Health, University of California, Davis, USA, were enrolled in the study. Most of the horses had recently been imported from Europe. These horses had undergone air transportation to Los Angeles, a three-day quarantine period in Los Angeles, followed by a 400-mile journey in a trailer to the Center for Equine Health (estimated total travel time eight to 10 days for each horse). Horses were enrolled in the study between January 2007 and July 2008. The horses belonged to several breeds, including warmblood (186), Friesian (52), Spanish (29), pony (12), Gypsy cob (11), thoroughbred (5), Arabian (3), Missouri foxtrotter (1), draft horse (1), quarter horse (1) and Appaloosa (1). There were 250 mares, 51 stallions and one gelding. The age of the horses ranged from one to 22 years, with a median of six years (mean [sd] 6·7 [3·2] years).

Samples of whole blood and serum, and nasopharyngeal swabs collected with rayon-tipped swabs (40 cm swab; Fox Converting Swabs) were taken from the study horses when they entered the University quarantine facility. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California, and the owners' consent was requested before sample collection. Additional samples were collected from horses that showed fever, nasal discharge, coughing or acute-onset neurological disease during the quarantine period. An additional serum sample was collected from each horse at the end of the quarantine period (14 days for mares and 28 days for stallions).

Nucleic acid extraction from whole blood and nasopharyngeal secretions was performed on the day of collection using an automated nucleic acid extraction system (CAS-1820 X-tractor Gene; Corbett Life Science) according to the manufacturer's recommendations. All of the samples were assayed for the presence of the equine glyceraldehyde-3-phosphate dehydrogenase (eGAPDH) gene, the glycoprotein B (gB) gene and the polymerase (ORF 30) gene of EHV-1 using previously reported real-time TaqMan PCR assays (Pusterla and others 2009). Absolute quantitation of EHV-1 target molecules was performed using standard curves for EHV-1 and eGAPDH and expressed as EHV-1 gB gene copies per million cells.

A commercially available type-specific ELISA (Svanovir; Svanova Biotech) was used to determine the levels of antibody to EHV-1 in serum from the study horses collected when they entered and left quarantine. The ELISA can differentiate EHV-1 and EHV-4 antibodies on the basis of structural differences in the glycoprotein G (gG) of the two herpesviruses, and has been validated in field studies (Crabb and others 1995, Drummer and others 1995, Gilkerson and others 1999). Based on the manufacturers' recommendations, antibody levels were considered to be negative if the optical density of the test well was less than 0·1, doubtful if it was 0·1 to 0·2 and positive if it exceeded 0·2. Seroconversion in the horses was defined as an increase in optical density of more than 0·129 above the previous absorbance reading, as previously described for this assay by Gilkerson and others (1999).

Overall, 1 per cent of the horses arriving at the quarantine facility following transportation were PCR-positive for EHV-1 in blood or nasopharyngeal secretions. Two horses (horses 1 and 2) tested PCR-positive for the gB gene of EHV-1 in nasal secretions, and one horse (horse 3) tested PCR-positive for the gB gene of EHV-1 in whole blood (Table 1). The viral load was low and ranged from 2×10^2 to 6×10^3 EHV-1 gB genes/million cells for these three horses. The biovar of the detected virus was non-neurotropic (ORF 30 N_{752}) for all PCR-positive horses. All three horses were retested three days after the first sampling; at the second sampling, blood and nasopharyngeal secretions tested PCR-negative for the EHV-1 gB and ORF 30 genes. The three horses remained free of abnormal clinical signs during the quarantine period. One additional horse (horse 4) developed fever (40·3°C), bilateral seromucoid nasal discharge, generalised stiffness and mild distal limb oedema on day 8 of the quarantine period. No samples collected at entry to quarantine were available for this horse because its owner had not consented to the voluntary surveillance programme; however, samples were taken on the first day of illness (day 8 of quarantine) and the last day of hospitalisation. Horse 4 tested PCR-positive with EHV-1 viral loads of 1×10^2 gB genes/million cells in blood and 2×10^7 gB genes/million cells in nasopharyngeal secretions. In both samples, a non-neurotropic EHV-1 strain (ORF 30 N_{752}) was detected. This horse was normal on haematological and

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TABLE 1: Details of four horses testing positive for equine herpesvirus type 1 (EHV-1) by PCR in blood and nasopharyngeal secretions following long-distance transportation

Horse	Signalment	Date	Blood Viral load (gB gene copies/million cells)	Blood ORF 30	Nasopharyngeal secretions Viral load (gB gene copies/million cells	Nasopharyngeal secretions ORF 30
1	6-year-old warmblood mare	April 2007	Negative	Negative	200	N_{752}
2	4-year-old warmblood mare	June 2007	Negative	Negative	3000	N_{752}
3	7-year-old warmblood mare	February 2008	6000	N_{752}	Negative	Negative
4	6-year-old warmblood mare	March 2008	100	N_{752}	20,000,000	N_{752}

N_{752} Non-neurotropic biovar of EHV-1

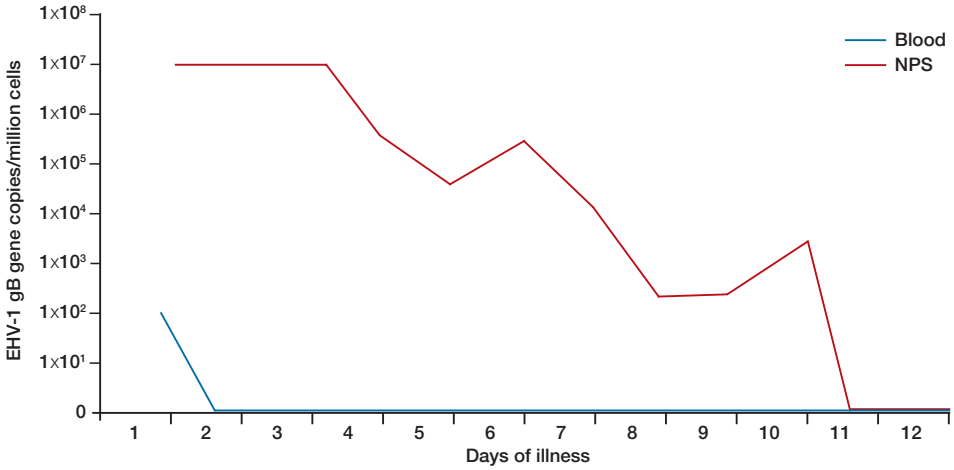

FIG 1: Equine herpesvirus type 1 (EHV-1) glycoprotein B (gB) gene loads, determined by real-time PCR, in blood and nasopharyngeal secretions (NPS) from horse 4. Day 1 represents the eighth day of quarantine

physical examination at entry to quarantine, but showed mild anaemia (haematocrit 24·7 per cent, reference range 30 to 46 per cent), lymphopenia (1·452 x 10^9 cells/l, reference range 1·6 x 10^9 to 5·8 x 10^9 cells/l) and hyperfibrinogenaemia (6 g/l, reference range 1 to 4 g/l) on the day it became acutely febrile. The horse remained PCR-positive for EHV-1 in nasopharyngeal secretions for 10 days and in blood for 24 hours (Fig 1). It was treated symptomatically and made an uneventful recovery.

A total of 46 horses (15·2 per cent) tested seropositive by EHV-1 ELISA (optical density >0·2) on admission to quarantine and remained seropositive throughout the quarantine period. Seroconversion was detected in nine horses (3·0 per cent), with an increase in optical density of more than 0·129 between the samples taken at entry to and exit from quarantine. Four of these horses were vaccinated against EHV-1/-4 at the request of the owner shortly after admission to the quarantine facility; the other five horses did not receive any EHV-1/-4 vaccine. None of the three subclinically infected horses (horses 1, 2 and 3) received rhinopneumonitis vaccine, and all remained seronegative throughout the quarantine period. However, the clinically infected horse (horse 4) did seroconvert after showing signs of disease.

These data, collected from horses undergoing long-distance transportation, showed a low level of EHV-1 infection. When the results of the PCR and ELISA tests were combined, eight of the 302 horses (2·6 per cent) had molecular or serological evidence of EHV-1 infection. The surprisingly low rate of detection of EHV-1 may have been associated with the age of the horses, the temporal association with transportation (that is, the onset and duration of viral detection following transportation-induced reactivation is unknown), previous use of vaccination, a low prevalence of latent EHV-1 or the detection limits of the molecular assays. However, the overall detection rate in the horses in the present study was similar to the detection rate recently reported from horses at show events and sales by Yactor and others (2006).

The detection of EHV-1 in three subclinically infected horses was likely to be a consequence of reactivation of latent virus or silent exposure either via direct contact with another horse or via contact with fomites. Latency and periodical reactivation of EHV-1 are key features in the epidemiology of the virus, and are responsible for its spread in horse populations (Slater 2007). The triggers for and frequency of viral reactivation are poorly understood; however, several stress factors have been associated with the reactivation of latent EHV-1 (Edington and others 1985, Slater and others 1994). Following reactivation, the virus is delivered to the respiratory epithelium, where virions are either neutralised by local mucosal immunity or establish an infection characterised by virus shedding in respiratory tract secretions and eventual development of viraemia (Slater 2007). However, even if an infection is established, it generally remains asymptomatic and results in silent shedding of the virus. The lack of clinical signs seen in horses 1, 2 and 3 in this study, the low number of viral copies determined in their blood or nasopharyngeal secretions and the lack of measurable immunological response following the detection of EHV-1 are characteristic features of reactivation of latent virus. Although temporal viral kinetics (daily viral loads determined by quantitative PCR) were not available for the three horses, the viral load measured on their admission to quarantine was too low to represent a considerable risk to other horses (Pusterla and others 2008). Viral reactivation is thought to have occurred in these horses as a consequence of suppression of their immune response secondary to stress. Epidemiologically, the mare that developed clinical signs (horse 4) is of great interest. The authors can only speculate on the source of the infection, as imported horses have ample opportunity to be exposed to infectious pathogens. In the field, incubation times of up to 10 days have been observed and are dependent on the viral strain, infective dose and host immunity (Slater 2007). The severity of respiratory signs developed by this horse was likely to indicate a primary infection; previously infected horses often display very mild clinical signs for only a short period (Kydd and others 1994, 1996). The increase in serum antibodies and the level and duration of viral shedding observed in horse 4 were consistent with a lytic infection (Slater 2007, Pusterla and others 2008, 2009). The high viral load detected in nasopharyngeal secretions from this horse highlights its potential for transmitting infection to naive herdmates. A non-neurotropic EHV-1 biovar was detected in the PCR-positive horses. This is in agreement with recent studies that showed that non-neurotropic EHV-1 strains are most commonly detected from anatomical sites of latency (Allen 2006, Allen and others 2008, Pusterla and Mapes 2008).

Positive antibody titres to EHV-1 are routinely detected after vaccination with monovalent or bivalent herpesvirus vaccines, as well as following natural exposure. Unfortunately, the serological assays that are available are unable to differentiate between vaccine-induced and infection-induced immune responses. Because the vaccine history was not known for all the horses in this study, the authors are unable to draw any conclusions as to their initial immune status against EHV-1. However, nine of the horses seroconverted during the quarantine period; seroconversion was attributed to vaccination against EHV-1/-4 in four horses, silent infection in four horses and clinical infection in one horse. The serological data complement the PCR results and highlight the pattern of silent infection commonly observed for EHV-1.

Although it is not known how often reactivation of latent EHV-1 and subsequent shedding of virus occurs in different horse populations, it appears that this occurrence is relatively rare in adult horses undergoing long-distance transportation. Even if reactivation does occur, the viral load in nasopharyngeal secretions from these horses is low and rarely represents a source of infection for susceptible horses. Awareness of infectious disease among horse owners and workers has dramatically improved following recent outbreaks that occurred at several equine venues throughout Europe and North America. It should be borne in mind that

reactivation of a latent viral infection during stressful events cannot be prevented. The random testing of horses for subclinical infection should be avoided, since practising veterinarians and regulatory officials who have positive test results may be unaware of the complexities involved in test interpretation, leading to inappropriate decision making in relation to the quarantine of equine facilities or the cancellation of competitions. However, the prevention of disease spread in high-risk populations, such as the horses in this study, can be improved by daily clinical assessment of the horses, early testing of horses showing clinical signs consistent with EHV-1 infection, and the implementation of adequate biosecurity measures around suspected or confirmed cases.

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