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Equine Herpesvirus-1 Consensus Statement

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Equine herpesvirus-1 is a highly prevalent and frequently pathogenic infection of equids. The most serious clinical consequences of infection are abortion and equine herpesvirus myeloencephalopathy (EHM). In recent years, there has been an apparent increase in the incidence of EHM in North America, with serious consequences for horses and the horse industry. This consensus statement draws together current knowledge in the areas of pathogenesis, strain variation, epidemiology, diagnostic testing, vaccination, outbreak prevention and control, and treatment.

Key words: Abortion; Horse; Immunology; Infectious diseases; Myeloencephalopathy; Respiratory tract; Viral.

Equine herpesvirus-1 (EHV-1) infection is ubiquitous in most horse populations throughout the world, and causes disease in horses and extensive economic losses through frequent outbreaks of respiratory disease, abortion, neonatal foal death, and myeloencephalopathy.^{1–4} Infections caused by EHV-1 are particularly common in young performance horses, and typically result in establishment of latent infection within the 1st weeks or months of life⁵ with subsequent viral reactivation causing clinical disease and viral shedding during

Abbreviations:

CTL cytotoxic T-lymphocyte

EHM equine herpesvirus myeloencephalopathy

EHV-1 equine herpesvirus-1

periods of stress. The relevant effects of this virus on the equine population are 3-fold. Firstly, sporadic occurrence of mild respiratory disease associated with pyrexia, principally affecting horses under 2 years of age, can lead to interruptions in athletic training programs; this is economically the least important manifestation of EHV-1 disease. Secondly, abortion occurring during the 3rd trimester of pregnancy, results in important economic losses. Thirdly, outbreaks of neurological disease (equine herpes myeloencephalopathy or EHM) cause suffering and loss of life and also lead to extensive movement restrictions, disrupting breeding or training schedules and causing management difficulties at training centers, race-tracks, and horse events. A perceived increase in the incidence of EHM outbreaks in North America in recent years has led to the proposal that it could represent an emerging disease threat.⁶

The recent increased impact of EHM in North America provided the impetus for this consensus statement. The renewed focus on EHV-1 infection and its control, new developments in our understanding of this virus and its behavior in horses, and the development of new viral detection technologies have resulted in renewed challenges for clinicians in responding to the threat of EHV-1 and to outbreaks. In an attempt to address this challenge, this statement is structured as a series of critical questions, which we believe capture the key challenges for equine clinicians and scientists. The responses seek to distill current evidence-based knowledge

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on each topic, and to identify critical areas requiring further investigation and research.

The Questions

Pathogenesis. How and why does EHV-1 infection target the pregnant uterus and CNS? Why do some horses but not others develop neurological disease?

Primary EHV-1 infection occurs at the respiratory epithelium, resulting in erosion of the upper respiratory mucosal surface and viral shedding for 10–14 days after infection, or even longer in EHM affected horses. Cell-to-cell spread results in the presence of virus in respiratory tract lymph nodes within 24–48 hours after infection.⁷ A leukocyte-associated viremia is then established, which is directly responsible for the delivery of EHV-1 to other tissues; the specific leukocyte subset(s) harboring EHV-1 remain poorly defined. The viremia can persist for at least 14 days, and is a prerequisite for EHM and abortion as it allows for transport of the virus to the vasculature of the pregnant uterus or the CNS where infection of endothelial cells occurs. This infection results in damage to the microvasculature of the CNS due to initiation of an inflammatory cascade, vasculitis, microthrombosis, and extravasation of mononuclear cells resulting in perivascular cuffing and local hemorrhage.³ The spinal cord gray and white matter are most commonly affected, with the brainstem being infrequently affected. While viremia is a common sequel to EHV-1 infection, transfer of virus to the CNS endothelium and development of EHM is not; typically some 10% of infected horses develop neurological signs during EHM outbreaks.⁸ The typical result is disseminated ischemic necrosis of the spinal cord. In contrast, abortion outbreaks can have attack rates in excess of 50%, but the underlying pathogenesis is otherwise similar to that of EHM.⁴ Viremia precipitates infection of endothelial cells in the small arterioles in the glandular layer of the endometrium at the base of microcotyledons, leading to vasculitis, microcotyledonary infarction, perivascular cuffing, and transplacental spread of virus at the sites of vascular lesions and abortion.⁹ Most commonly the fetus is virus positive; however, in some instances the virus can be restricted to the placenta.¹⁰ Uterine endothelial cells have an increased susceptibility to infection in late pregnancy¹¹ consistent with the occurrence of abortion principally in the last trimester.

The mechanism underlying CNS endothelial infection is unknown, as are the risk factors that determine its occurrence. While viral factors are certain to be important, such as the DNA_{pol} SNP described below, host and environmental factors also have a critical role.^{8,12} More is known about the pathogenesis of abortion than about EHM. Endothelial and leukocyte cell surface adhesion molecules play an important role in the infection of vascular endothelium,¹³ and their expression in the uterus appears to be associated with pregnancy.¹⁴ The regulation of the expression of adhesion molecules could be dependent on the hormonal milieu of late pregnancy. The high attack rate of abortion in the last trimester could be consistent with common features of this physi-

ological state, such as differential expression of endothelial cell surface molecules. Of the viral factors determining the occurrence of abortion, strain variation, and specifically the occurrence of the DNA_{pol} SNP seems to be of importance. In naturally occurring abortions, the association of abortion with the N₇₅₂ strain variant is very strong; far stronger in fact that the association of EHM with the D₇₅₂ strain.^{a,15} However, the difficulties associated with creating an experimental model of EHV-1 abortion in horses could only be overcome when the Ab4 virus, a D₇₅₂ strain, was used.¹⁶ Currently, there is no explanation for these different features of DNA_{pol} SNP strain variants in natural and experimental abortion.

The factors determining whether horses develop EHM after EHV-1 infection are poorly understood. It has been proposed that the magnitude of cell-associated viremia is an important factor for the development of EHM because infection with the DNA_{pol} D₇₅₂ strain leads to a higher magnitude and duration of viremia.^{12,17,18} The hypothesis that the duration or magnitude of viremia directly determines the occurrence of EHM is appealing, and there is new evidence to support it.¹² It is unlikely to be a simple relationship, as considerable differences are observed in levels of viremia produced by different DNA_{pol} D₇₅₂ strains, such as Ohio '03 and Ab4.^{18,19} It is also noteworthy that in experiments in which signs of neurological disease were described in control horses, but not in vaccinates, the level of viremia was the same in both groups.¹⁹ If the D₇₅₂ strain has an increased ability to cause EHM, the mechanisms could extend beyond simply inducing high levels of viremia.

Recently the cellular receptor of EHV-1, nectin-3, was identified and it was shown that EHV-1 can enter cells both by fusion at normal pH at the plasma membrane but also via the endosomal route.²⁰ It is possible that differential utilization of receptors and efficiency of virus entry or attachment of virus-infected lymphocytes to endothelial cells in response to host factors could be a determining factor for the occurrence of EHM.

The host and environmental factors that determine the occurrence of EHM are similarly ill defined. Older horses are generally more susceptible to EHM,^{8,12} implying a possible role for immunological status in the pathogenesis of EHM, as older horses generally demonstrate a greater interferon-gamma (IFN- γ) based cellular response to EHV-1.²¹ Nevertheless, at this time our understanding of how and why EHV-1 infection leads to EHM in some horses remains rudimentary.

Neuropathogenic strains. What are the clinical implications of the DNA_{pol} SNP (D₇₅₂ versus N₇₅₂)?

The EHV-1 genome was first reported in 1992²² and since then understanding of the 150 kb EHV-1 genome, and its 76 open-reading frames (ORFs) has considerably increased.⁴ The most important single discovery could be the association of a single nucleotide polymorphism (SNP) in the DNA polymerase (DNA_{pol}) gene and the occurrence of EHM.¹⁵ Analysis of a panel of isolates from over 100 EHV-1 outbreaks (either involving EHM or with no reported neurological signs) collected from

Table 1. Key question about D/N₇₅₂ strains

Is there a particular EHV-1 strain that causes neurological disease?	No DNA _{pol} (ORF30) variants carrying the D ₇₅₂ marker are associated with most neurological disease outbreaks
Are all outbreaks of neurological disease caused by D ₇₅₂ viruses?	No D ₇₅₂ viruses are more commonly isolated from horses that suffered from neurological disease than N ₇₅₂
Are N ₇₅₂ viruses nonpathogenic?	No N ₇₅₂ viruses are isolated from the majority of abortion outbreaks and a minority of neurological disease outbreaks worldwide
What proportion of horses carry D ₇₅₂ viruses?	Not known Available data suggest that 5–20% of EHV-1 viruses have the D ₇₅₂ genotype, but this data has been generated from a small number of studies
Is D/N ₇₅₂ testing useful?	Debatable Knowing the D/N ₇₅₂ genotype of an EHV-1 isolate is not relevant to the prevention and control of EHV-1 abortion outbreaks If an EHV-1 outbreak is associated with neurological signs, strict disease control measures should be imposed regardless of D/N ₇₅₂ genotype If an active EHV-1 infection, as evidenced by viremia and/or shedding, is diagnosed as D ₇₅₂ positive, even in the absence of neurological signs, it is possible that there could be an increased risk of a neurological disease outbreak. However, no study to date has properly tested this relationship The most important reason to perform this testing is to increase our knowledge about EHV-1 epidemiology

EHV-1, equine herpesvirus-1.

several countries demonstrated that variability of a single amino acid residue of the DNA polymerase (DNA_{pol}) was found to be strongly associated with the occurrence of EHM, with the majority of EHM outbreaks involving a strain with D₇₅₂, whereas most nonneurological outbreaks involved a N₇₅₂ strain. Importantly, this association was consistent over several decades, suggesting that DNA_{pol} variation has had a major association with EHV-1 pathogenicity for many years. These data have led to the proposal that EHV-1 viruses carrying the D₇₅₂ variant of the DNA polymerase (ORF30) have a higher risk of causing neurological disease than those with the N₇₅₂ marker.

Direct evidence supporting the hypothesis that the D₇₅₂ variant has increased neuropathogenic potential has recently been obtained.¹⁸ Targeted mutation of the D₇₅₂ to the N₇₅₂ genotype in a neurovirulent isolate resulted in attenuation of virulence, specifically reduced levels of viremia, reduced capacity to cause neurological disease and reduced severity of other clinical signs. However, both viruses were very similar to each other with regard to peak titers of virus shedding, suggesting that the 2 viruses have a similar capacity to spread in a population.¹⁸ Old horses (>20 years of age) have increased susceptibility to EHM, and in a study using an old horse model, the D₇₅₂ strain had an increased capacity to cause EHM compared with the N₇₅₂ strain.¹² What has not been analyzed so far is the efficiency and frequency of reactivation of N₇₅₂ versus D₇₅₂ genotypes.

Epidemiological data describing the prevalence of the N₇₅₂ and D₇₅₂ strains remains limited, and is presented in full in the epidemiology section below. In brief, these data suggest that the majority of EHV-1 viruses circulat-

ing in the field are of the N₇₅₂ genotype. These N₇₅₂ variants are not apathogenic and have been responsible for approximately 95–98% of abortion outbreaks in the US, UK and other countries, and between 15 and 25% of neurological outbreaks.¹⁵

Based on the above findings, we believe that the interpretation of diagnostic tests, which distinguish between DNA_{pol} genotypes, must be treated cautiously. It would be incorrect to interpret the finding of N₇₅₂ as indicative of infection with a “benign” form of EHV-1 not requiring disease control measures. It can be argued that the DNA polymerase genotype is not relevant to the management and prevention of EHV-1 abortion outbreaks. It is also clear that N₇₅₂ isolates can cause neurological disease, so any horse with neurological signs, which is suspected to be due to EHV-1 infection, should be handled with rigorous disease control measures, regardless of the D/N₇₅₂ genotype. The data are clear, however, that D₇₅₂ strains are the most common isolate from horses suffering from EHM, and in 1 study they had an increased capacity to cause EHM when administered to old horses.¹² Consequently, there may be justification for a different risk assessment in the event of detecting active D₇₅₂ infection even in the absence of neurological signs.

Epidemiology. What does the most current data tell us about EHV-1 epidemiology, and the prevalence of strain variants?

Latency and reactivation are critical features of the epidemiology of EHV-1 infection. In large equine breeding operations EHV-1 infection occurs in the 1st weeks or months of life,^{23,24} and current vaccines

and management practices cannot prevent this.^{5,25} It is thought that viral reactivation in latently infected mares leads to foal infection in this circumstance.²⁴ When horses are first infected, latency is established in both the lymphoreticular system and in the trigeminal ganglion.⁴

Estimates of the prevalence of EHV-1 infection based on viral detection technologies vary. In an European abattoir study EHV-1 was directly isolated from 24/40 (60%) horses when bronchial lymph nodes were examined, and conventional polymerase chain reaction (PCR) detected EHV-1 in 35/40 (88%) of this population.²⁶ A study of the retropharyngeal lymph nodes of aged horses in Kentucky with a magnetic bead, sequence-capture, nested PCR technique detected latent infection in 8/12 (66%) horses with an unknown EHV-1 history, and 18/24 (75%) horses, which had been deliberately infected as weanlings, 4–5 years previously.²⁷ Clearly, the prevalence of latent EHV-1 infection can be influenced by the geographic region, management practices, and other factors. Similarly, the testing technology and, importantly, the tissue sampled will have major effects on the sensitivity of testing. Nevertheless, current estimates of the prevalence of latent EHV-1 infection suggest a rate in excess of 60%, a rate that could be limited more by the detection technology than by the actual infection rate. For practical purposes, clinicians should presume that the majority of horses are latently infected with EHV-1.

Outbreaks of clinical EHV-1 disease are infrequent, and outbreaks of EHM are relatively rare.⁸ When clusters of such outbreaks arise with apparent increased frequency,⁶ this is a major cause for concern, and raises the question of what specific factors could lead to an increased incidence of EHM? The identification of a genetic marker associated with cases of EHM (DNA_{pol} D₇₅₂) has led to further speculation that EHV-1 could be becoming increasing virulent. Multiple risk factors unrelated to EHV-1 genotype influence the size and clinical presentation of EHV-1 disease outbreaks, including host and environmental factors, and in a study of unvaccinated horses in the Netherlands it was shown that breed, age, and sex influenced the risk of EHM.⁸

Conventionally, it is thought that subclinical EHV-1 infections are common in horses, resulting in frequent spread and a high risk of exposure particularly in open horse populations subject to stress and introduction of new animals.³ Recent reports of USA surveillance studies in horses exposed to the stress of transport and mixing²⁸ or by the stress of acute severe disease^b demonstrated very limited evidence of subclinical EHV-1 infection. Studies in healthy horse populations resident on farms in both Australia²⁹ and the USA³⁰ similarly demonstrated that subclinical shedding of EHV-1 was infrequent, and when it does occur it is at a very low level that might not pose a contagious disease threat to other horses.³¹ These observations question the paradigm of common subclinical transmission of EHV-1, at least in the absence of neonatal and juvenile horse populations, and suggest that spread of EHV-1 among adult horses is typically accompanied by clinical disease, either abortion or EHM.

Early descriptions of the epidemiology of the D₇₅₂ and N₇₅₂ strains are now available. In a study of submandibular lymph nodes with a sensitive magnetic bead, sequence-capture, nested PCR technique of 24 horses infected 4–5 years earlier with either D₇₅₂ and N₇₅₂ strains, when latent infection was detected it was with the same strain as was originally used to infect these horses as weanlings.²⁷ In a larger study of 132 mares examined at necropsy in central Kentucky by the same detection methodology,³² latent infection was detected in 71 (54%) mares, and of the latently infected mares 13 (18%) harbored the D₇₅₂ strain. Of these 13 mares, a total of 11 were co-infected with the N₇₅₂ strain. A recent research abstract report of a postmortem study conducted in California, examining submandibular lymph nodes and trigeminal ganglia detected latent infection in 23/153 (15%) of horses, and more commonly in the trigeminal ganglia.^c The N₇₅₂ strain was as common as the D₇₅₂ strain, and co-infection with both strains was a common observation. Taken together these observations indicate that either or both the geographical area and the detection methodology influence detection rates, that the N₇₅₂ strain may be more common, and that co-infection with both the D₇₅₂ and N₇₅₂ strains is a common event. Clearly there is ample justification for additional epidemiological studies of these strain variants.

The likely evolutionary origins of EHV-1 strains, and in particular the DNA_{pol} D/N₇₅₂ sequence variants, are a subject worthy of discussion in this context. Overall, EHV-1 has a relatively low degree of sequence variability: comparison between 2 different strains of EHV-1 demonstrated that only approximately half of the genes had amino acid coding changes, which in most cases involved only 1 or 2 residues.¹⁵ Preliminary analysis of variable genes for a selection of isolates suggested that generally sets of variable markers tended to occur together (cosegregate), consistent with a progressive accumulation of sequence changes over time leading to the divergence of several distinct EHV-1 strain groups.¹⁵ The initial study,¹⁵ focusing on neuropathogenic outbreaks, did not identify an association with a particular strain of EHV-1, but rather with variation at the single DNA_{pol} residue D/N₇₅₂. However, the ability to genetically type EHV-1 should provide a valuable tool to investigate other potential associations between strain group and disease severity or vaccine efficacy.

There are 2 notable features of the D/N₇₅₂ sequence variation. First, in contrast to the general rule of marker cosegregation, the distribution of the D or N₇₅₂ marker is not linked to any other marker tested.¹⁵ This suggests that mutations at this position from N to D (or vice versa) have occurred as independent events in multiple strain groups. It can be further postulated that the position is relatively “unstable”, such that changes from N to D (or vice versa) have occurred regularly during the evolution of EHV-1, but tend not to be stably inherited over time. Second, the position corresponding to EHV-1 DNA_{pol} 752 is highly conserved in other herpesviruses as an acidic residue (usually D).¹⁵ This suggests that the ancient, progenitor EHV-1 would have encoded D₇₅₂ and raises the hypothesis that the novel N₇₅₂ genotype has arisen, apparently uniquely in

EHV-1, due to a selective advantage. The observation that N₇₅₂ is the most common variant lends some weight to this hypothesis. The D₇₅₂ genotype is not a recent entity—it is likely to have been present at the origin of EHV-1 and is the genotype of one of the earliest isolates of EHV-1, Army 183 (isolated in 1941). Data are lacking to determine whether the prevalence of the D₇₅₂ genotype in the general horse population has been changing in recent years, or whether the prevalence varies between different breeds or geographical locations.

Finally, what is our current understanding of the origin of neuropathogenic outbreaks, specifically the role of latently infected “carrier” horses? Do horses that have been exposed to EHV-1 during a neurologic outbreak constitute a higher risk of being the source of future neurologic outbreaks? It is not currently possible to provide an answer, but the following points should be considered. Reactivation from latency, with shedding and transmission to susceptible hosts, is a defining feature of herpesviruses and is very likely to play an important role in the etiology of EHV-1 disease outbreaks. There are 2 alternative scenarios for the origin of “high risk neuropathogenic” (D₇₅₂ genotype) EHV-1 variants; either reactivation from a horse latently infected with a D₇₅₂ variant or spontaneous mutation from a “low risk variant” (N₇₅₂) to the high-risk genotype. It is possible that both events occur and given the rarity of neurologic disease outbreaks, it is very difficult to determine the relative contribution of one or the other event to such outbreaks. It seems likely that horses that are exposed to D₇₅₂ variants during a neurologic disease outbreak will become latently infected carriers of that strain,²⁷ even if they were already latently infected with an N₇₅₂ variant.³² Genetic fingerprinting of EHV-1 isolates to trace links between outbreaks and long term follow-up of horses that have been typed for latent EHV-1 carriage or with known exposure to neurologic outbreaks can help to further elucidate these issues in the future.

Risk factors for disease. What are the risk factors for horses for respiratory, abortigenic, or neurologic disease caused by EHV-1?

A number of clinical reports, outbreak investigations, and a few detailed epidemiological studies contribute to our current understanding of risk factors for EHV-1 disease.^{8,33–37} It is certain that risk is multifactorial and involves viral, host, and environmental factors. Known (1–10) and suspected (11–13) risk factors include:

1. The presence of both EHV-1 and susceptible horses in the herd.
2. The presence of an infected, shedding horse in the herd.
3. Season: the majority of EHM outbreaks occur in late autumn, winter, and spring.⁸
4. Age is a factor in the development of clinical manifestations of EHM. EHM can occur in horses of all ages,³⁴ and has been reported in weanlings, both naturally occurring³³ and experimentally induced.¹⁶ However, in a large epidemiological study conducted in the Netherlands, EHM was largely

restricted to horses over 3 years of age,⁸ and age > 5 years was similarly associated with an increased risk of EHM in a recent large North American outbreak.³⁵ In experimental infections, 1 report has described inducing EHM in 8/12 (66%) of horses over 20 years of age.¹² Respiratory disease caused by EHV-1 is infrequently observed in horses over 2 years of age.³

5. Past exposure produces a limited period of protection from re-infection, of as little as 3–6 months.¹ There are no reports of horses repeatedly affected by EHM, although the rarity of the disease may be a contributing factor. It is rare for mares to suffer from EHV-1-mediated abortion in consecutive pregnancies.³
6. Pregnancy: Abortion can occur in mares of any age, although it is largely restricted to the last trimester of pregnancy.
7. Rectal temperature: Horses with high fever (temperatures > 103.5°F), and high fevers occurring several days after the initial onset of fever, are more likely to develop EHM.³⁵
8. Introduction of horses to a herd is commonly reported before the development of EHV-1 outbreaks, and specifically before EHM outbreaks.^{8,35–37}
9. Clinical infection with the D₇₅₂ EHV-1 biovar is more commonly detected in horses suffering from EHM than infection with the N₇₅₂ biovar.¹⁵ However, in both of these studies it was clear that in a substantial proportion of EHM cases (up to 25%) the isolates subsequently made were of the N₇₅₂ biovar.
10. Breed and sex were identified as risk factors for EHM in 1 epidemiological survey, with ponies and smaller breeds less commonly affected, and females more commonly affected.⁸
11. Geographical region appears to be associated with the development of EHM. For example, the authors found only 1 published report of this condition in Australia or New Zealand,³⁸ and anecdotally there have been only a very small number of cases recorded in those countries (J. Gilkerson, University of Melbourne, personal communication, 2008).
12. Stressors: Outbreaks of EHV-1 disease are anecdotally associated with stressors including weaning, commingling, transportation, and concurrent infections.
13. Immunological status has been proposed to have an association with the development of EHM, specifically as a result of vaccination.³⁵ However, the association between increased use of vaccines and development of EHM described in that study was completely confounded by the fact that vaccination frequency was greatest in older horses, and EHM was strongly associated with greater age. It is interesting to note that the association of increased age with EHM risk is reported in populations of horses in which vaccination is not practiced.⁸ Similarly, IFN-γ immune responses to EHV-1 are increased in older horses.²¹ In experimental infections of horses > 20 years of age, the increased incidence of EHM was related to the diminished frequency of cytotoxic T lymphocyte (CTL) precursors.¹² Whether immunity

and EHM risk are causally related cannot be defined at this time, but the likely role of the immune system in the development of EHM pathogenesis strongly recommends further study of this possibility.

Diagnostic testing. What kinds of viral detection tests should I select for diagnosis, prognosis, and screening of horses for EHV-1 and its strains?

Virus culture and isolation is considered the gold standard test for making a laboratory diagnosis of EHV-1 and should be attempted especially during epidemics of EHM, concurrently with rapid diagnostic testing (PCR), in order to retrospectively be able to biologically and molecularly characterize the virus isolate.⁴ Virus culture, isolation, and identification of EHV-1 from nasal or nasopharyngeal swabs or buffy coat samples is strongly supportive of a diagnosis of EHM in a horse with compatible clinical signs. The likelihood of detecting EHV-1 during outbreaks of disease can be increased by testing in-contact horses, especially during episodes of fever. While viral culture and positive identification can be accomplished in as little as 2–3 days in a laboratory when the sample contains a high viral load, the time required to run these tests can limit their clinical utility for outbreak management.

PCR has become the diagnostic test of choice because of its high analytical sensitivity and specificity. Positive PCR results can be obtained when virus isolation is negative because of low viral load. PCR detection of EHV-1 can be routinely performed in respiratory secretions from a nasal or nasopharyngeal swab and in uncoagulated blood. In the index case, both nasal secretions and uncoagulated blood should be analyzed simultaneously, because the interpretation of the results from respiratory secretions and blood can help in assessing disease stage. For nasal samples, a recent study has shown that nasal swabs are more sensitive than nasopharyngeal swabs for EHV-1 detection.³⁹ Details of suitable sources of sampling materials are provided at the AAEP's website on infectious disease outbreak management.⁴⁰

Many conventional PCR protocols (single or nested PCR) targeting specific genes of EHV-1 have been published in recent years for the molecular detection of EHV-1.⁴ Although considerable progress has been made in developing PCR assays, the lack of protocol standardization between laboratories and the lack of standardized use of quality assurance controls remain an ongoing challenge. In general, conventional nonquantitative PCR results can be interpreted as follows:

- (a) A positive EHV-1 test result on a blood sample indicates viremia most probably resulting from an active infection. It is unlikely that latent viral infection alone will give a positive result in this test.
- (b) A negative EHV-1 test result on a blood sample indicates the absence of detectable EHV-1 viremia.
- (c) A positive EHV-1 test result on a nasal swab sample should be interpreted as indicative of the shedding of infectious virus. Quantitative PCR (ie, real-time PCR) could provide more information about the likely level of risk this shedding poses.

- (d) A negative EHV-1 test result on a nasal swab indicates the absence of detectable virus shedding.

Conventional (nonquantitative) PCR is limited in its sensitivity, and we rely on this limitation to distinguish between the presence of infectious virus (high and detectable viral presence) and latent infection (low and typically non-detectable virus). The sensitivity and specificity of conventional PCR is typically ill defined, so the possibility of an erroneous result or interpretation is typically present. The random testing or screening of healthy horses for EHV-1 by conventional PCR should therefore be avoided. Testing should instead be reserved for cases where there are clinical grounds to suspect EHV-1 infection.

Advances in technology and the use of novel EHV-1 PCR platforms, such as real-time PCR, allow for more sensitive detection, greater specificity, and calculation of viral loads.^{17,31,41,42} Determination of viral load³¹ can offer important advantages as it can allow for better characterization of disease stage, assessment of risk of exposure to other horses and monitoring of response to treatment. However, this test is not routinely offered by veterinary diagnostic laboratories. In general, quantitative (real time) PCR results should be interpreted with consultation from the testing laboratory. Some laboratories only report positive/negative test results, and their interpretation is generally the same as for conventional PCR testing as described above, with the caveats that these tests are generally much more sensitive, faster, and typically more specific. When viral loads,³¹ or interpretive results of the test are offered, it is possible to distinguish between horses that are shedding high or low amounts of virus in nasal secretions, and to estimate the risk they pose to other horses. Similarly, the magnitude of the viremia can be determined, and inference can be made about the severity of the infection and the risk of progression.

Consequent to the identification of the DNA_{pol} SNP (D₇₅₂/N₇₅₂),¹⁵ real-time PCR tests have been developed that can distinguish these 2 biovars,⁴³ and testing is commercially available. However, the interpretation of genotyping of field isolates needs care, as 15–24% of EHV-1 isolates from horses with confirmed EHM do not have the D₇₅₂ marker.¹⁵ The detection of the D₇₅₂ marker is most commonly made in horses suffering from EHM. Whether detection of the D₇₅₂ marker in an EHV-1-infected horse when no cases of EHM have yet occurred leads to a prediction of an increased risk of developing EHM has yet to be determined, although the perception that such a finding increases risk does influence treatment decisions for some clinicians. Whatever the risk status is, it is important to remember that the absence of the D₇₅₂ marker does not preclude the development of EHM. The detection of latent EHV-1 infection is likely of no clinical diagnostic significance in the great majority of instances, independent of the biovar identified.

Serologic testing which demonstrates a 4-fold or greater increase in serum antibody titer, by serum-neutralizing (SN) or complement-fixation (CF) tests, on acute and convalescent samples collected 7–21 days apart provides presumptive evidence of EHV-1 infection.¹ Practically, in

the midst of an outbreak, detection of rising virus-neutralizing antibodies in paired serum samples can be used to screen for horses that were exposed to the virus. Although serologic testing has limitations in confirming a diagnosis of EHV-1 infection in an individual horse, testing of paired serum samples from in-contact horses is recommended because a proportion of both affected and unaffected in-contact horses seroconvert, providing indirect evidence that EHV-1 is the etiologic agent. Interpretation of the results of serologic tests is complicated by the fact that the SN, CF, and ELISA tests in use at most diagnostic laboratories do not distinguish between antibodies to EHV-1 and EHV-4. A specific ELISA test based on the C-terminal portion of glycoprotein G of both viruses has been developed and is valuable in the investigation and management of disease outbreaks in the future.⁴⁴ This assay is not commercially available in North America.

Cerebro-spinal fluid analysis is often supportive of an EHM diagnosis and can be of value while waiting for the PCR test results. Xanthochromia is typical in horses with EHM. In addition, increased protein concentrations with or without a monocytic pleocytosis are additional typical findings. EHV-1 antibody titer in CSF is not diagnostic during the acute phase as it reflects leakage of blood into the CSF as a result of EHM lesions. Because of the low numbers of virus particles in CSF, it is usually unrewarding to perform PCR tests on this sample. Histopathology on brain and spinal cord is an essential method for confirming EHV-1 infection in a horse with suspected EHM. Vasculitis and thrombosis of small blood vessels in the spinal cord or brain are consistent histopathological changes associated with EHM. Virus antigen detection in the CNS is routinely performed via immunohistochemistry, in situ hybridization and PCR testing.

In conclusion, molecular assays have supplanted the more cumbersome and time-consuming diagnostic modalities for the routine clinical diagnosis of EHV-1. One of the main drawbacks of PCR testing has been the lack of standardized protocols between laboratories and the need for a consensus on the interpretation of the results of these molecular diagnostic techniques. In the future, diagnostic laboratories should consider reporting quantitative information regarding EHV-1 viral loads in blood and nasal/nasopharyngeal secretions because this information can facilitate management of EHV-1 outbreaks. Although molecular technology has become more complex in its interpretation, the information gained from such assays will help prevent disease spread and maximize treatment options in affected animals.

In summary, the general recommendations to document active EHV-1 infection include:

- Uncoagulated blood and nasal swab for PCR analysis (preferentially quantitative real-time PCR assays should be used). Some laboratories prefer EDTA as an anticoagulant, as heparin may interfere with PCR reactions.
- Uncoagulated blood and nasal swab for virus isolation of EHV-1 when clinical signs and PCR results are suggestive of infection.

- Paired-serum samples collected 15–21 days apart for serology—VN assay and ELISA for specific virus antigen when available.
- In the absence of clinical signs consistent EHV-1 infection, use of current diagnostic methods, including real-time PCR, as a screening test is not recommended.

Vaccination. How, and when should I use current commercially available vaccines to control EHV-1 infection and disease?

Vaccination remains the optimal means to prevent infectious diseases in many circumstances; however, there is no evidence that current vaccines can prevent naturally occurring cases of EHM. While a preliminary experimental challenge study indicated some benefit associated with vaccination with a modified live vaccine,¹⁹ no specific recommendation can be made in terms of vaccination for the prevention of EHM at this time. However, based on the presumed similar pathogenic mechanism between EHV-1 abortion and neurologic disease,^{45,46} some likely parallels exist in terms of the requirement for immunological protection. The control of cell-associated viremia is thought to be critical for the prevention of EHV-1 abortion⁴⁷ and, presumably, neurologic disease. Therefore, the goal of any vaccination program aimed at the prevention of EHV-1 abortion or neurologic disease is to stimulate those immune responses that can reduce or eliminate cell-associated viremia. The identification of these protective immune responses to EHV-1 has been the focus of much research for the past 40 years.¹ From this work, it is clear that mucosal antibodies can play a role in preventing infection of the respiratory tract and in limiting virus shedding.⁴⁸ Although short-lived, mucosal antibody is protective and its secretion can be increased through intramuscular vaccination.⁴⁸ Thus, vaccination can be expected to reduce nasopharyngeal virus shedding during an outbreak and thereby limit the spread of infection. While vaccination also increases serum antibody titer, this fails to alter the duration of cell-associated viremia or the outcome of pregnancy following challenge infection with EHV-1.⁴⁷ By contrast, CTL that lyse virus-infected cells appear to be required to prevent abortion.^{1,47} While recent efforts have focused on IFN- γ expression as a surrogate for CTL activity, its relationship with disease susceptibility has not yet been established.^{21,49} These results support the general contention that protection against EHV-1 will likely require both neutralizing antibody and CTL responses.¹

Vaccines currently available for EHV-1 include both modified live and inactivated products.⁵⁰ The modified live vaccine currently available in the United States is marketed as Rhinomune,^d and performs well in controlling respiratory infection and shedding.^{e,19} While early studies of this vaccine demonstrated that it is safe to administer to pregnant mares,^{51,52} it does not carry the claim that it aids in protection against EHV-1 abortion. That claim has been registered with the USDA for 2 inactivated, ad-

juvanted vaccines (Pneumabort K-Fort Dodge and Prodigy-Intervet). Pneumabort K decreased the incidence of abortion in vaccinated mares in 1 study.⁵³ However, in subsequent studies there were no differences in the occurrence of abortion with use of this vaccine.^{54,55} In the case of EHV-1 abortion, therefore, there are methodological differences and limitations in the experimental design of published studies that preclude definitive conclusions regarding vaccine efficacy.⁴⁷ Nevertheless, the widespread use of intramuscular vaccines, improved methods of managing breeding stock, or both, appear to have reduced the incidence of EHV-1-related abortions in the United States.⁵⁶ Less is known regarding the use of vaccines to prevent neurologic disease. While a single study indicated the possibility of vaccinal protection against EHM,¹⁹ the numbers of animals were small and the model was limited in its ability to reproduce neurologic disease. For current vaccination recommendations for products available in North America, the reader is referred to the web-based AAEP vaccination recommendations.⁵⁷

The induction of protective immunity against EHV-1 therefore remains a substantial challenge. One reason for this is our lack of understanding of EHV-1's ability to interfere with the immune system. Similar to what has been found for other herpesviruses, EHV-1 has evolved multiple immune evasion strategies limiting antibody and CTL-mediated immune responses.^{58,59} This could explain the short-lived duration of immunity that follows natural infection. The future of EHV-1 vaccination will be critically dependent on our understanding of EHV-1 viral immune evasion strategies.

Disease control and prevention. What are the key factors to consider in controlling disease caused by EHV-1?

This question addresses how to prevent EHV-1 disease or, failing that, how to prospectively limit its severity, impact, and spread. For many other diseases this might be achieved by eradication of the infectious agent, but this is impossible for EHV-1. For this reason clinicians must plan for how to respond to outbreaks.

Control measures can be divided into measures designed to prevent or reduce the likelihood of outbreaks, and measures designed to limit the spread of disease when an outbreak occurs. The impact of abortion outbreaks in broodmare operations has prompted the development of guidelines for the prevention of such outbreaks. The British Horserace Betting Levy Board publishes Codes of Practice on Equine Diseases, which includes an excellent section on EHV-1, is updated annually.⁴⁰ Allen et al³ describes the underlying rationale for prevention of abortion or neurologic disease in pregnant mares as depending on procedures described by the acronym "SISS":

- Segregation of pregnant mares from all other horses on the premises.
- Isolation for a period of not <3 weeks of all mares entering the stud farm, including those that are returning after leaving the premises.
- Subdivision of pregnant mares into small physically separated groups for the duration of gestation.

- Stress reduction by avoiding physiological stress: maintain social structures, avoid prolonged transport, relocation, poor nutrition, parasitism, environmental exposure, and *en masse* weaning of juveniles.

These principles are comprehensively explained in the source material,³ and they can also be adapted and expanded for populations other than pregnant mares as explained by Allen, 2002.⁶⁰ The 3 key principles for control of spread of EHV-1 are to:

1. Subdivide horses into the small epidemiologically isolated closed groups.
2. Minimize risks of exogenous and endogenous (stress-induced viral reactivation) introduction of EHV-1.
3. Maximize herd immunity through vaccination.

Outbreak response. What are the key things I need to know as I plan for, and respond to, an outbreak of clinical EHV-1 infection?

The priorities for management of an outbreak of EHV-1 are⁶⁰:

1. Early diagnosis.
2. Prevention of further spread.
3. Management of clinical cases.

The priorities for preparing for an outbreak should support these 3 objectives. For early diagnosis, it is vital to have established a diagnostic plan for responding to syndromic diagnoses, and for EHV-1 this means respiratory, neurological, or abortigenic disease.⁴⁰ The diagnostic techniques best suited to detecting EHV-1 infection are described above, and the clinician needs to identify a laboratory that can provide these test in advance of needing these services. A good understanding of what samples to take, how and when they can be transported to a laboratory, and how and when results will be available is vital for outbreak management. It is also very important to have suitable sampling materials available, which for both virus isolation and PCR-based diagnosis will mean synthetic swabs (eg, polyester or nylon) of approximately 6 in. in length, and viral transport media.⁴⁰ If viral transport medium is not available, then a dry swab in a sterile tube can be submitted for PCR analysis. In addition, the clinician will need suitable clothing to allow for contact with potentially infected horses, and prevention of transmission to other horses. The importance of early diagnosis of EHV-1 infection is vital, as several specific interventions may be implemented as a result of this diagnosis. In situations where the syndromic diagnosis means that EHV-1 is suspected, the clinician should proceed with measures designed to contain EHV-1 spread until a specific diagnosis is achieved, or EHV-1 infection is excluded.

In the case of an EHV-1 outbreak, Allen et al³ describes the measures for containing the spread of EHV-1 using the acronym "DISH":

- Disinfection of areas contaminated by virus from the aborted fetus and placental membranes.
- Isolation of affected horses.

- Submission of clinical samples to a diagnostic laboratory.
- Implementation of hygienic procedures to prevent spread of infection (biosecurity).

The AAEP guidelines for Infectious Disease Outbreak Control⁴⁰ provide a comprehensive plan for implementation of both syndromic and EHV-1-specific control measures. Our understanding of the duration and intensity of EHV-1 shedding by infected horses has changed in recent years, consequent to experiences gained during major outbreaks of EHM at the University of Findlay and the Ohio State University,³⁵ and at Colorado State University (L. Goehring and P. Morley, personal communication, 2008). It is apparent that horses affected by EHM, and perhaps by other clinical manifestations of EHV-1, can shed infectious amounts of virus for 21 days or even longer after initial infection. For these reasons, it is vital to house affected horses in isolation facilities whenever practicable. Our ability to effectively prevent spread of infection from EHM cases when they are managed in the same building as other horses, even with extensive barrier precautions, are ineffective. Both aerosol and fomite transmission are important modes of transmission. Similarly, aborted fetuses and fetal membranes, and infected foals, are important sources of infectious virus.

In the face of an EHV-1 outbreak, vaccination can be used in horses at increased risk of exposure. There is some controversy associated with this practice because of the concern that EHM may be associated with a history of frequent vaccination.³⁵ However, there are no reports of vaccination in this circumstance precipitating or exacerbating the occurrence of EHM cases. In previously vaccinated horses, a booster EHV-1 vaccine can lead to a rapid anamnestic response and contribute to reducing spread of infectious virus.

At the end of an outbreak of EHV-1 (when no new cases are occurring), there are a number of challenges, including when to lift the quarantine, how to disperse previously infected horses, and how to decontaminate the facility.⁶⁰ Previously, a period of 21 days after the occurrence of any new cases of EHV-1 infection was recommended for the lifting of quarantine, as this was 3 times the typical 7 day period of nasal shedding.⁶⁰ This recommendation has been extended to 28 days in the recent AAEP guidelines⁴⁰ because of evidence of more protracted shedding in clinical cases of EHM in particular. In determining this period for lifting of quarantine, it is vital to understand that while the EHV-1 continues to be transmitted among a group of horses, you cannot start the countdown to the release of the quarantine; ie, the 21–28 day period can only be counted from the time that new infections are prevented by biosecurity and quarantine procedures. Alternative strategies for lifting quarantine can be used, such as a reduced quarantine period of 14 days, followed by testing all horses by real-time PCR analysis of nasal swabs for 2–4 consecutive days. This approach can be further augmented by twice daily monitoring of rectal temperatures, so that a period of 14

days without pyrexia can constitute the quarantine, followed by PCR testing. The expense of such extensive testing can also be greater than the cost of a more prolonged and effective quarantine. Even after lifting of the quarantine, horses that are dispersed to other stables should be quarantined on arrival, and their health monitored. No special measures for the long-term husbandry are currently recommended for horses infected or exposed to the neuropathogenic forms of EHV-1. Given that this form of EHV-1 normally affects 5–20% of latently infected horses, the risks associated with horses exposed to these strains during outbreaks are likely no greater than for the normal horse population. Decontamination of facilities may be accomplished using extensive cleaning followed by application of a number of disinfectants (such as quaternary ammonium compounds, accelerated peroxide and peroxygen compounds, or iodophor disinfectants), although phenolic disinfectants offer the best solution in the presence of organic materials. Alternately, virus in the environment is very unlikely to survive in an infectious form 21 days after depopulation of horses.

Treatment. What therapeutic modalities are useful for treating EHM, beyond supportive and symptomatic care?

The treatment of horses with myeloencephalopathy involves empiric supportive care, including nursing and supportive care in cases of recumbency, maintenance of hydration and nutrition, and frequent bladder and rectal evacuation.⁶¹ Nonsteroidal anti-inflammatory therapy is frequently used as an adjunctive therapy, although their capacity to affect the development of the lesions of EHM is unknown. Similarly, corticosteroids and more recently immunomodulators are both used in EHM treatment, although the justification is theoretical, as no evidence-based study has demonstrated efficacy of either drug class for EHM. Similarly, antiviral drugs are also unproven in terms of their value to treat EHM, although their theoretical appeal has led to their increasing use against a background of an improved understanding of their pharmacodynamics.

Corticosteroids are immunosuppressive drugs and could aid in the control or prevention of the cellular response adjacent to infection of CNS endothelial cells,⁶¹ thereby potentially reducing vasculitis, thrombosis and the resultant neural injury. This theoretical benefit of corticosteroid treatment of EHM has never been demonstrated in a clinical setting. Possible outcomes could include a positive effect through reduction of hypersensitivity disease associated with infection, or a deleterious effect due to a reduced immunological control of EHV-1 infection. Given our poor understanding of their efficacy in treating EHM, the use of corticosteroids is currently reserved for EHM cases presenting in recumbency or with severe ataxia, in which the prognosis is guarded for survival.

The value of administrations of immunostimulants for prevention of EHV-1 infection is similarly hard to assess. Immunostimulants could be administered to a horse before exposure to a potential stressor, such as transportation, performance, changes in the environ-

ment, or exposure to new horses. In this instance activation of the immune system could theoretically prevent viral reactivation or replication. One study evaluated the efficacy of inactivated *Parapox ovis* virus in young horses subjected to the stress of weaning, transport, and commingling with yearlings, and determined their susceptibility to clinical respiratory disease during natural EHV-1 exposure. There was some evidence of a reduction in clinical signs of respiratory disease.⁶² No other published studies are available describing the use of immunomodulators for treatment or prevention of EHV-1 infection, and consequently, it is impossible to determine whether they have any value. Overall, our understanding of the value of immunomodulators for EHV-1 treatment remains rudimentary, be they either immunostimulants or immunosuppressive agents.

Antiviral drugs, and specifically virustatics, are of theoretic value for the treatment of EHV-1 and have demonstrated in vitro efficacy against EHV-1.⁶³ The thymidine kinase inhibitor acyclovir (9-[(2-hydroxyethoxy)methyl]-guanine) is a synthetic purine nucleoside analog that selectively inhibits the replication of herpesviruses.⁶⁴ The drug is phosphorylated initially by herpesvirus viral thymidine kinase, followed by 2 other phosphorylations by host cell kinases. The triphosphate acyclovir compound binds to and inhibits the viral DNA polymerase for the formation of viral DNA. Pharmacokinetics of acyclovir after single oral administration (10 and 20 mg/kg) to adult horses has been associated with high variability in serum acyclovir-time profiles and poor bioavailability below the concentrations required for viral inhibition.⁶⁵ A single 10 mg/kg IV infusion results in a greater peak serum concentration. Controlled clinical studies of IV administration of acyclovir in EHV-1-infected horses have yet to be performed, and it seems likely that there are better choices than acyclovir for EHV-1 treatment.

Another nucleoside analog, valacyclovir shows greater promise based on pharmacokinetic data, although the lack of generic formulations makes it expensive in some countries. The bioavailability of the prodrug valacyclovir at 30 mg/kg PO twice a day is in the order of 35–40%. The recommended dose of valacyclovir is 30 mg/kg PO q8h for the 1st 48 hours, decreased to 20 mg/kg PO q12h.^{66,67} Currently, the effects of timing of valacyclovir administration relative to the onset of EHV-1 infection or EHM development on treatment outcome are unknown.

Additional compounds have been described that have in vitro activity against EHV-1 but there have been no reports of their use in horses. These include nitazoxanide compounds,^f and interfering (silencing) RNA.^g

In summary, there is currently limited scientific rationale for the use of immunomodulators, and no evidence-based studies of the value of antiviral drugs in the prevention and treatment of EHV-1 infection. Of greatest importance, studies of antiviral drugs are needed to evaluate their value in the control of EHV-1 infection in both early and late phases of natural disease,

and for the reduction of shedding of virus during outbreaks.

Summary

Our understanding of the features of EHV-1 is increasing, but there is more to learn before we can best address the challenges that this virus presents. In almost every area of this paper we repeatedly encounter limitations of our understanding, that depend principally on our lack of understanding of the pathogenesis of the diseases EHV-1 causes. The clear message is that future progress will be dependent on research into viral pathogenesis and epidemiology.

Footnotes

^a Perkins GA, Goodman LB, Tusjimura K, et al. Investigation of neurologic equine herpesvirus 1 epidemiology from 1984–2007: Abstract #385, 26th Annual American College of Veterinary Internal Medicine Forum, San Antonio, TX. J Vet Intern Med 2008;22:819 (abstract)

^b Carr EA, Pusterla N, Schott HC, II, et al. Equine herpesvirus-1 (EHV-1) recrudescence and viremia in hospitalized critically ill horses: Abstract #123, 26th Annual American College of Veterinary Internal Medicine Forum, San Antonio, TX. J Vet Intern Med 2008;22:737 (abstract)

^c Pusterla N, Mapes S. Detection and characterization of equine herpesvirus-1 strains in submandibular lymph nodes and trigeminal ganglia from horses using real-time PCR: Abstract #381, 26th Annual American College of Veterinary Internal Medicine Forum, San Antonio, TX. J Vet Intern Med 2008;22:818 (abstract)

^d Pfizer Animal Health, New York, NY

^e Goehring L, Hussey SB, Rao S, et al. Control of EHV-1 viremia & nasal shedding by current commercial vaccines: Abstract #124, 26th Annual American College of Veterinary Internal Medicine Forum, San Antonio, TX. J Vet Intern Med 2008;22:737 (abstract)

^f Callan RJ, Ashton LV, Goehring L. Itazoxanide and tizoxanide inhibit EHV-1 and influenza type A virus replication in vitro: Abstract #126, 26th Annual American College of Veterinary Internal Medicine Forum, San Antonio, TX. J Vet Intern Med 2008;22:738 (abstract)

^g Fulton A, Perkins GA, Peters S, et al. Treatment of EHV-1 infection using RNA interference: Abstract #125, 26th Annual American College of Veterinary Internal Medicine Forum, San Antonio, TX. J Vet Intern Med 2008;22:738 (abstract)

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