Nasal Shedding of Equine Herpesvirus-1 from Horses in an Outbreak of Equine Herpes Myeloencephalopathy in Western Canada

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Background: There is little information on the duration of nasal shedding of EHV-1 from horses with naturally occurring equine herpesvirus myeloencephalopathy (EHM).

Objectives: To evaluate the duration of nasal shedding of EHV-1 in horses affected by EHM.

Animals: One hundred and four horses naturally exposed to EHV-1, 20 of which had clinical signs of EHM.

Methods: All horses on affected premises were monitored. Those horses developing EHM were sampled in a longitudinal outbreak investigation. Nasal swabs were collected daily from 16 of 20 horses affected by EHM. A qPCR was performed on 98 of 246 nasal swab samples to determine nasal shedding duration. Historical and clinical information was analyzed to evaluate potential risk factors for developing EHM and duration of shedding during this outbreak.

Results: The last day shedding was detected in any horse was Disease Day 9. EHV-1 was detected in two-thirds of horses tested on Disease Days 0–3. The amount of EHV-1 DNA found in nasal swabs varied markedly and was not associated with disease severity or age. The odds of developing EHM were greater for febrile horses (OR = 20.3; 95% CI 3.4–390.3; P = .01) as well as for horses attending the riding clinic (OR = 4.1; 95% CI 0.84–21.65; P = .08).

Conclusions and Clinical Importance: Biosecurity measures should be implemented for a minimum of 14 days beyond the onset of clinical signs of EHM. Animal managers cannot rely on the severity of clinical signs to predict the duration of EHV-1 shedding.

Key words: EHV-1; Myeloencephalitis; Neurologic; PCR.

Equine herpesvirus type 1 (EHV-1) is an alphaherpesvirus that is ubiquitous in the horse population and most horses have been infected by 1 year of age.1,2 In horses, EHV-1 typically causes mild respiratory disease, and is also associated with late-term abortion, neonatal foal death, and neurologic disease (equine herpes myeloencephalopathy or EHM). It has been speculated that recently recognized outbreaks of EHM are associated with infections by strains of EHV-1 that have an increased likelihood for causing neurologic disease and that these strains can be typified by genotypes resulting from a single nucleotide dimorphism within the DNA polymerase gene (ORF30).3–7

One of the major challenges in controlling spread of EHV-1 is in recognizing which horses are likely to be shedding virus and therefore would represent a contagious disease risk. There is little information regarding the duration of nasal shedding of EHV-1 that can be expected in naturally infected horses with EHM. Despite the previously held belief that horses exhibiting signs of EHM were no longer shedding virus, findings from a large, well-publicized outbreak clearly demonstrated the threat of nosocomial infection that can be associated with hospitalization and care of horses affected by EHM.8 Studies have described nasal shedding in experimentally infected horses, but it is not clear how this information relates to naturally occurring infections, especially as it has not been possible to reliably induce neurologic disease with these experimental models.9–12 There is little consensus regarding the recommended duration of quarantine in affected populations. All of these recommendations are empirically derived using anecdotal evidence and information from experimental infection trials. Some operations have used a 21- to 28-day quarantine (beyond disease cessation) with or without testing for EHV-1 via PCR.13 However, in the absence of testing, the American Association of Equine Practitioners has recommended a 28-day quarantine subsequent to the cessation of disease in the last case.14 Alternatively, as discussed in the American College of Veterinary

Abbreviations:
EHM = equine herpes myeloencephalopathy
PCR = polymerase chain reaction
qPCR = quantitative polymerase chain reaction (real time PCR)
Outbreak Day = days since EHM was recognized in the index cases
Disease Day = days since EHM was recognized in each individual horse

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Internal Medicine Consensus Statement, quarantine time might be reduced to 14 days with subsequent nasal swab testing via real time PCR for 2–4 consecutive days after disease cessation in the last case. The absence of information regarding virus shedding in horses with naturally occurring disease and the knowledge that latent carriage complicates management of this disease make it difficult to develop objective quarantine recommendations for those managing outbreaks of EHM.

The objectives of this report were to evaluate the duration of nasal shedding of EHV-1 in horses affected by EHM and to describe the characteristics of an outbreak of EHM in western Canada during the spring of 2008.

Materials and Methods

This outbreak of EHM involved horses at 3 facilities in Saskatchewan, Canada (a boarding facility, a referral veterinary hospital, and a private farm). Veterinarians responsible for the care of these horses collaborated to organize this investigation and to manage the outbreak so as to minimize spread to other horse populations. At the time the outbreak was recognized, plans were initiated to collect nasal swabs daily from as many horses with EHM as possible to evaluate the duration of nasal shedding of EHV-1. For this report, the day that neurologic disease was diagnosed in the first 2 cases of EHM is designated as Outbreak Day 0. The day of onset of EHM was designated as Disease Day 0 for each affected horse.

Neurologic Examination, Grading, and Case Definition

Daily observations of the horses housed at the boarding facility were performed by the horse owners, as directed by the managing veterinarians (NT, KP, SM, and BB), and were collected using a secure database that was available online from the boarding facility’s website. Information collected included horse breed, sex, age, use, presence of fever (rectal temperature >101.3°F or 38.5°C), limb edema, and nasal discharge. The owners of horses without information in the database received a follow-up phone survey to acquire this information once the outbreak was resolved. In addition, housing and exercise locations at the facility (including time spent outdoors, time spent in the arena, and time spent in the tack-up stall) were obtained from facility managers and horse owners after the outbreak had resolved. When indicated, horses were examined by a veterinarian (NT, KP, BB, or SM) for signs of neurologic deficits including one or more of ataxia, paresis, deficits of conscious proprioception, and cranial nerve dysfunction. The severity of ataxia and proprioceptive deficits of each case were classified.

Briefly, clinical scores ranged from 0 to 5, with grade 0 indicating an absence of neurologic signs, grade 1 indicating an absence of neurologic abnormalities at normal gaits but apparent with manipulation, and grade 5 indicating that horses were recumbent and unable to rise. For this investigation, a case of EHM was defined as those horses with ataxia or proprioceptive deficits (≥grade 1) where onset was linked to a common exposure identified during the course of this investigation (a riding clinic held at the boarding facility prior to the outbreak). Neurologic assessment was performed by 2 or more study veterinarians with the final neurologic grade being a consensus opinion. Observations regarding inpatients at the referral veterinary hospital were performed daily by the managing veterinarians. Clinical observations that were collected included the occurrence of fever, limb edema, nasal discharge, or neurologic dysfunction. In addition, 12 horses that developed EHM underwent follow-up neurologic examination by a veterinarian (NT or BB) 7 months after the initial onset of signs.

Nasal Swab and Tissue Sample Collection

Sample collection was planned to occur daily on all boarding facility horses with EHM for up to 30 days after detection of neurologic signs. Because investigation plans had not been established prior to the onset of the outbreak, daily sampling did not begin until Outbreak Day 7. In addition, it was not possible to collect all samples from all horses because of their temperament or due to the owners’ wishes regarding management of the horses during the outbreak. Horses were restrained for sample collection and a single polyester fiber tipped swab with a 10-cm handle was inserted into the nasal passage and rubbed against the mucosa for at least 3 seconds. Swabs were placed into 1 mL of virus transport media and stored at −80°C until processed. Tissue samples from the euthanized horses were collected from the central nervous system (CNS) and processed at the time of post-mortem evaluations. Informed owner consent was obtained for all procedures including exams and nasal swab collection.

Sample Processing

Three PCR assays were used to evaluate nasal swabs for the presence of EHV-1. Samples from 4 horses were evaluated at 2 different laboratories using real time PCR, which provided initial confirmation of EHV-1 infection in the index cases in addition to characterization of the ORF30 genotype. Samples from 4 horses were evaluated at 2 different laboratories using real time PCR of nasal swab samples prior to being discharged from the facility (quarantine release). The majority of samples were evaluated after the end of the outbreak using quantitative PCR, and results were categorized by threshold cycle (Ct) number: Negative = Ct >41 or no recovery of viral DNA, Very Low Positive = Ct 39-41, Low Positive = Ct 36-38, Medium Positive = Ct 33-35, High Positive = Ct 30-32, and Very High Positive = Ct 27-29. All qPCR assays were conducted in duplicate in 96-well plates. Internal control samples were included in each plate, with a range of standards from 10⁴ to 10⁶ copies of the gB gene in a plasma as previously described. Our quality control standards required PCR efficiencies in the range of 95–105%, and that standards containing 10 copies gave a Ct ≤41. All samples that were collected from horses ≤9 days after onset of EHM were evaluated with qPCR; additional samples were analyzed if more were available, and at least 1 positive result had been obtained on samples collected on day 8 or 9 after disease onset. The authors’ experience using this qPCR assay in previous experimental infection studies suggested that samples with Very High, High, and Medium Ct numbers likely represent horses with a high infectious risk to other horses, whereas samples with Low and Very Low Ct numbers likely represent horses with a very low infectious risk. Serum antibody to EHV-1 was evaluated using a whole virus EHV-1 ELISA, which has been previously described.

Data Analysis

Data entered in the online database were combined with information collected in other records. Data entries were validated and explored using frequency distributions and descriptive statistics. Multivariable logistic regression was used to evaluate factors that might be associated with the development of EHM. The dependent variable for this analysis was clinical diagnosis of
EHM (y/n), and the independent variables that were evaluated included participation in the riding clinic held 3 weeks prior to the outbreak (y/n) [ATTENDED RIDING CLINIC], fever (y/n) [FEVER], limb edema or swelling (y/n) [LIMB EDema], mucopurulent nasal discharge (y/n) [NASAL DISCHARGE], gender (female or male) [SEX], use of common tack-up stalls ≥ 2 times per week (y/n) [TACK-UPSTALLS], exercising ≥ 2 times per week in the common riding arena (y/n) [ARENA], stabling location at the boarding facility (housed outside, wing A, wing B, or wing C) [HOUSING LOCATION], housing (outside ≤ 8 hours per day or outside 24 hours per day) [HOUSING], and age (≤ 5, 6–10, ≥ 10 years) [AGE]. Univariable analysis was performed to assess individual exposure variables with a critical α of 0.25 to be included in the multivariable model building process. The final model was identified using backwards selection with a critical α of 0.05 for retention in the model. Confounding was identified by ≥ 20% change in parameter estimates when variables were individually removed from the multivariable models. When identified, confounding variables were forced into the multivariable models regardless of P-values. First-order interaction terms for main effects variables included in final models were also evaluated. Odds ratios (OR) and profile likelihood 95% confidence intervals (95% CI) were calculated using the results of logistic regression models. A Cox proportional hazards model was used to estimate the predicted average probability of EHV-1 nasal shedding after neurologic disease onset for 16 of the affected horses. The baseline survivor function of the null model, using right and left censoring, was determined. Univariable models for age (≤ 10 years versus >10 years), fever (yes, no, unknown), and neurologic grade (≤ 3 versus >3) were evaluated. For this part of the analysis, an event was the cessation of nasal shedding defined as 2 consecutive negative results on nasal swab PCR.

Results

Overview of Disease Occurrence and EHV-1 Shedding

This outbreak of EHM occurred in March and April 2008, with 20 of 104 horses that were housed at three different facilities in Saskatchewan (a boarding facility, a referral veterinary hospital, and a private farm) developing signs of neurologic disease. In total, 6 of the 20 affected horses (30%) were severely affected (maximum neurologic grade ≥ grade 4), and 3 of these horses were euthanized; 2 were euthanized because of the severity of disease (both had grade 5 neurologic deficits) and 1 was euthanized after the outbreak due to residual neurologic deficits.

Of the 20 affected horses in this outbreak, 45% (9) were Thoroughbred and Thoroughbred crosses, 25% (5) were Quarter Horse-type horses, and 10% each were Arabian-type horses (2), Pony of the Americas (2), and unknown (2). The mean age of horses affected by EHM in this study was 10 years (range: 4–17 years) and the mean age of those horses unaffected by EHM was 12 years (range: 2–30 years). There was no statistically significant association between age and the occurrence of EHM.

Tissue samples for EHV-1 testing were collected from the CNS at postmortem evaluation of the 2 horses euthanized during the outbreak. Daily nasal swab collection was initiated on Day 7 of the outbreak, and ≥ 1 nasal swab was collected from 16 horses with EHM. EHV-1 was detected in nasal secretions, tissues, or both of 12 of these 16 horses (75%, Fig 1). EHV-1 was also detected in nasal secretions from 1 additional horse that was febrile, but did not develop neurologic disease.

Disease (EHM) was first recognized on the same day (Outbreak Day 0) in 2 horses stabled at the boarding facility. Because of concerns regarding the ability to provide adequate veterinary care if the horses became recumbent, they were transported to a referral veterinary hospital and managed with barrier nursing precautions. Disease occurred on the 3rd premises after personnel and 2 horses from that farm participated in a riding clinic that was held just prior to the outbreak at the boarding facility. EHV-1 is not a reportable disease in Saskatchewan, but at the recommendation of the veterinarians managing this
outbreak, the boarding facility instituted a voluntary quarantine on Outbreak Day 2, as did the referral veterinary hospital on Outbreak Day 4 (horses were placed in isolation at the time of admission). The voluntary quarantine at the referral veterinary hospital and boarding facility continued for 29 and 45 days, respectively. Approximately 7 months after the outbreak, 12 of the 20 horses that developed EHM were re-evaluated and 5 (42%) were found to have residual neurologic deficits with 10 (83%) improving 1–2 grades. All had returned to their intended use and previous level of performance.

**Disease at the Boarding Facility**

The boarding and training facility where diseased horses were first identified was utilized not only by people with horses stabled at the facility but also by others visiting with their horses for riding lessons, training events, and competitions. This facility was cleaned and maintained by full-time managers and veterinary care was provided by local veterinarians including staff employed by the referral hospital. Eighty-nine horses that were owned by 60 different people were resident at the facility on Outbreak Day 0; 37 were housed outside 24 hours per day in open-air, dirt-floor paddocks and 52 were housed inside in 8–9 ft (2.4–2.4 m) dirt-floor stalls for up to 18 hours per day and outside in open-air dirt-floor paddocks for the remaining time. Of the 89 horses, 63 were geldings, 25 were mares (including 2 pregnant mares), and 1 was an intact male. Breeds represented included Thoroughbred and Thoroughbred crosses (n = 33), Foreign Warmblood (16), Quarter Horse-type horses (22), Arabian-type horses (9), Pony of the Americas (2), Morgan (2), Peruvian Paso (1), Welsh pony (1), and Andalusian (1). Horses were used by clubs and individuals for various types of activities including pleasure riding, eventing and jumping, reining, dressage, and polo. The boarding facility consisted of a barn with 4 wings (A, B, C, and D) around a common indoor riding arena (Fig 2). Wing C contained several “tacking-up” stalls utilized by many of the horses at the facility as well as visiting horses. At the time of the outbreak, there were no formal biosecurity protocols in place that applied to all horses brought into the facility.

Approximately 3 weeks prior to the onset of disease in the index cases (Outbreak Day-19), a riding clinic was held at the facility which included riders and horses from the surrounding area (n = 9) in addition to riders with resident horses (n = 13). Owners of the resident horses reported that 1 week prior to the onset of neurologic disease in the index case (Outbreak Days-7–0), they had noticed nonspecific signs of illness in several horses, including 2 cases of mild colic (Horse 1 and another resident horse which did not develop EHM but was receiving corticosteroid therapy for an unrelated medical condition; Fig 1), fevers, distal limb edema or swelling, lethargy, inappetence, nasal discharge, and ocular discharge. Complete blood counts and serum biochemical examination were performed on 3 horses affected by this vague illness and results were considered unremarkable. On Outbreak Day 0, the first 2 cases of neurologic disease were diagnosed by a veterinarian (NT) and transported to the referral veterinary hospital for further evaluation and treatment (Horses 3 and 7; Fig 1). At this time, the presumptive diagnosis was EHM.

On Outbreak Day 2, a voluntary quarantine was instituted by the boarding facility at the recommendation of the veterinarians treating the index cases because of the presumptive diagnosis of EHM and the contagious nature of this disease. Biosecurity measures initiated at that time included the use of barrier nursing precautions (waterproof boot covers, gloves, and coveralls) when contacting all horses, designation of an isolation area for housing affected horses (wing D of the boarding facility), and utilization of footbaths containing an accelerated hydrogen peroxide disinfectant at the entrance of the isolation wing as well as at all entrances to the boarding facility. The same coveralls were used when caring for multiple affected horses, but were not used outside of the boarding facility. In total, 16 horses at the boarding facility developed clinical signs consistent with EHM. The voluntary quarantine

![Fig. 2. Map of boarding facility indicating stabling locations of affected (1–16; in order of EHM occurrence) and unaffected horses.](image-url)
of the boarding facility was released on Outbreak Day 45, 28 days after the onset of clinical signs of Horse 20, which was the last case recognized at this facility.

**Disease at the Referral Veterinary Hospital**

Upon admission to the referral veterinary hospital, the Index Cases (Case 3 and 7, Fig 1) were assigned a presumptive diagnosis of EHM based upon the history of mild respiratory disease among in-contact horses and the characteristic neurologic signs in the affected horses including hind limb ataxia and urinary incontinence. They were isolated in the food animal ward in stalls equipped with hoists for better management should they become recumbent. The food animal ward was adjacent to, but spatially segregated from, the equine ward. All entrances to the food animal ward, with the exception of one, were closed to personnel and animal traffic. Footbaths containing an accelerated hydrogen peroxide disinfectant were placed at the only entrance point as well as at every stall. In addition, barrier nursing precautions including boot covers, gloves, coveralls, and cloth barrier gowns dedicated to use with individual patients were instituted for all animals. These same biosecurity measures were also implemented in the equine ward.

Findings from analysis of the cerebrospinal fluid samples obtained from the Index Cases on Outbreak Day 1 included xanthochromia, elevated protein concentration (1.67 g/L and 0.89 g/L; ref. 0.2–0.8 g/L), and normal nucleated cell counts (1 × 10^6/L for both; ref. 0–8 × 10^6/L). Increases in antibody concentrations identified in paired serum samples (obtained on Outbreak Days 0 and 7) were also supportive of the EHM diagnosis in the Index Cases. On Outbreak Day 4, the referral hospital was voluntarily closed to non-emergency admission of equine and camelid cases and all equine inpatients were quarantined. Analysis of nasal swabs and spinal cord of horses that were euthanized later confirmed EHV-1 infection, and test results for 2 samples indicated that these contained D variant of the ORF30 dimorphism.

At the time of admission of the 2 Index Cases (Outbreak Day 0), there were 3 other horses hospitalized at the referral veterinary hospital (including 1 pregnant mare, which was hospitalized due to laminitis, Horse 2 Fig 1). Three additional horses were admitted with emergent conditions on Outbreak Days 11, 17, and 18, respectively. All owners were advised of EHV-1 infections among hospitalized horses at the time of admission. On Outbreak Day 11, despite increased biosecurity precautions including the use of barrier nursing precautions in the hospital, the pregnant mare (Horse 2) developed neurologic deficits. Despite treatment, this horse became recumbent and was unable to stand (neurologic grade 5), aborted, and was euthanized on Disease Day 3 due to its poor clinical status and prognosis. A postmortem examination performed on this horse revealed lesions within the brain and spinal cord consistent with EHV-1 infection. In addition, real-time qPCR performed on postmortem tissue samples from this horse were positive for EHV-1 with D variant of the ORF30 dimorphism. Because of the timing and circumstances of disease occurrence, veterinarians caring for this horse concluded that this mare’s infection likely represented a nosocomial infection of EHV-1 within the referral hospital. Real-time PCR performed on the aborted fetus were positive for EHV-1 with N variant of the ORF30 dimorphism. No other EHV-1 infections were detected among inpatients.

As Horses 3 and 7 (Index Cases) were improving clinically, they were transported back to the isolation ward of the boarding facility on Outbreak Day 13. After 7 days without identifying signs of EHV-1 infection in any inpatients, nasal swab samples were obtained twice daily from all inpatients for 4 days (Outbreak Days 22, 23, 24, and 25) and were tested using real-time PCR. EHV-1 was not identified in any of these samples, and all horses were discharged to their home facilities on Outbreak Day 29. Prior to admission of any other horses, both the equine and food animal wards were thoroughly cleaned and disinfected.

**Disease at the Private Farm**

Owners who had moved their horses to or from the affected boarding facility between Outbreak Day 19 through Outbreak Day 1 (including participants in the riding clinic) were contacted by the managing veterinarians. They were asked to monitor their horses closely for clinical signs consistent with EHV-1 infection and to institute their own voluntary quarantine. Neurologic disease was subsequently identified in another horse on Outbreak Day 8 on a farm that housed 2 horses that had participated in the riding clinic. In total, 3 of the 9 horses that were resident on this farm developed disease that was diagnosed as EHM. One of these horses subsequently became recumbent and was euthanized on Disease Day 2 (Outbreak Day 10) because of its worsening condition. A postmortem examination revealed pathologic changes consistent with EHM, and tissue samples were positive for EHV-1 with D variant of the ORF30 dimorphism. It was not possible to collect samples from the other horses on this farm.

**Timing and Duration of EHV-1 Nasal Shedding**

Of the 20 horses diagnosed with EHM, >10 nasal swab samples were collected from 12 horses, 1–3 samples were collected from 4 horses, and it was not possible to obtain any samples from the remaining 4 horses (Fig 1). A total of 246 nasal swab samples were collected from these 16 horses of which 98 were tested by qPCR for EHV-1; other samples had been collected >9 days after onset of EHM. Of the samples tested, 66% (65/98) had negative results (Ct>41), 15% (15/98) were positive, but likely represented a low or very low infectious risk to other horses (Low or Very Low virus quantities), and results for 18% (18/98) of samples
were indicative of shedding high amounts of virus, which likely indicate these horses to be a high infectious risk to other horses (8 had Medium virus quantities, 4 had High virus quantities, and 6 had Very High virus quantities; Fig 1). Among horses that were sampled on Disease Day \( \geq 5 \), 64% (9/14) were found to shed detectable quantities of virus Day 5 or later after onset of disease (3 with Medium or High virus quantities), and 43% (6/14) were found to shed detectable quantities on Disease Day 7 or later (2 with Medium and 4 with Low or Very Low virus quantities). EHV-1 was detected in nasal secretions of at least half of horses tested on Disease Days 1–3 (Fig 1). The last day of shedding that was detected in any horse was Disease Day 9.

Seven of 11 (64%) horses that were tested at least once \( \leq 3 \) days after onset of EHM were positive for EHV-1 on at least 1 sample. However, because plans for daily sampling of horses were not initiated until Outbreak Day 8, sampling was less complete for several of the affected horses during the early course of their disease. As such, it is not possible to comment on the proportion of all affected horses that likely posed an infectious risk to other horses in the early stages of clinical disease.

Based on a Cox proportional hazards model of data collected from 16 horses, the average expected probability of EHV-1 nasal shedding was 66.7% on Disease Day 1 (1 day after the onset of neurologic signs), 45.7% on Disease Day 4, 24.3% on Disease Day 7 and 4.8% on Disease Day 10 (Fig 3). The duration of nasal shedding was not statistically significantly associated with age \((P = .25)\), fever \((P = .49)\), or maximum neurologic grade \((P = .55)\).

**Risk Factors for Clinical Disease**

Of horses in the outbreak population on which data were collected (86%; 89/104), fever was noted at some point during the outbreak in 62% (8/13) of horses that subsequently developed EHM and 32% (21/65) of horses that did not develop EHM. Limb edema was noted in 57% (8/14) of horses that developed EHM and 55% (36/66) of horses that did not develop EHM. Nasal discharge was noted in 43% (6/14) of horses that developed EHM and 27% (18/66) of horses that did not develop EHM.

Variables for Fever, Attended Riding Clinic, Limb Edema, Nasal Discharge, Arena, and Housing Location passed screening and were included in multivariable model building (Table 1). The final multivariable model included Fever and Attended Riding Clinic, the latter variable being forced into the model as it was the main risk factor of interest. Results suggested that the odds of developing EHM was 20.3 times greater for those who were febrile compared with those who were afebrile during the outbreak (95% CI 3.4, 390.3; \( P = .01 \)), and the odds of developing EHM was 4.1 times greater for those who attended the riding clinic compared with those who did not attend (95% CI 0.84, 21.65; \( P = .08 \)).

**Discussion**

Results of this investigation suggest that nasal shedding of EHV-1 can occur for at least 9 days from onset of neurologic signs in horses with naturally occurring EHM and that a high proportion of horses are probably shedding virus at the time of onset of neurologic disease (Figs 1 and 3). Subjectively, given the relatively small number of horses evaluated, it would seem prudent to more generally assume that horses with EHM can occasionally shed virus even longer than shown here. Thus, in the absence of rigorous testing, it would be sensible to assume that horses with EHM can, in extreme circumstances, shed virus, and likely pose an infectious risk for at least 2 weeks, which is in agreement with previous reports. These data also suggest that serial testing can aid in drawing conclusions about contagious disease risk for individual horses. However, the duration of nasal shedding in horses affected by EHM appears to be variable and not apparently predictable based on the maximum severity of neurologic signs (ie, mildly affected horses can sometimes shed longer than severely affected horses, and vice versa). This outbreak of EHM included 3 distinct sites, all of which could be linked to a boarding facility through in-contact horse movement with 1 case of EHM likely the result of a nosocomial infection, despite awareness of the contagious nature of EHV-1 and the use of rigorous biosecurity measures.

Univariable analysis was performed on a number of factors related to the epidemiology of exposure as well as intrinsic factors of the host. Only two of these factors were significantly associated with EHM upon univariable analysis, fever \((P = .003)\), and attending the riding clinic \((P = .02)\). Fever remained significantly associated with EHM \((P = .01)\) in the final model, which is similar to other studies. This would suggest monitoring for fevers to be an important part of
managing an outbreak. Although attending the riding clinic was not statistically significant in the final model, the association was very strong and likely relevant to the epidemiology of this outbreak. Interestingly, age was not associated with EHM in this outbreak, despite previous reports indicating otherwise.\(^{15,16,20,21,23}\)

Based on a Cox proportional hazards model, two-thirds of horses in this study were expected, on average, to be shedding EHV-1 on Disease Day 1 and approximately half on Disease Day 4. By Disease Day 10, the likelihood of shedding had decreased to 4.9%, which suggests that a minimum 2-week recommendation for the duration of quarantines would be prudent. However, it should be noted that this recommendation is based on observations in a limited number of horses and as such longer durations of shedding might have been seen if more horses were available for study.

Thus, in the absence of serial testing to more explicitly investigate shedding risk, managers should consider a more extended quarantine period to maintain a more risk-averse management strategy. While we did not find an association between age, fever or neurologic grade with the average probability of shedding, it is important to note that these findings may also have been influenced by the limited number of horses that could be included in our analyses.

It is important to consider uncertainty that is inherent in management decisions regarding contagious disease risk for populations when based upon qPCR results for nasal swabs. Collecting nasal swab samples from horses can be quite difficult as some horses do not tolerate the procedure very well, which undoubtedly affects variability in the quality of samples (ie, the amount of nasal secretion that can be obtained on a swab). Also, although it has not been investigated in published research, the amount of virus contained in equal volumes of nasal secretion probably varies throughout any given day. As such, there are limitations to the certainty that we can have about how representative results are for a sample obtained from 1 horse at 1 time on 1 day. From a practical population management perspective, we are probably more concerned about false-negative results than we are about uncertainty in quantification of virus in test-positive samples. The results of this study show that quantities of virus detected in nasal swabs can go up or down over time, and we can be more confident that shedding has truly ceased when obtaining negative results serially over multiple days.

As previously stated, we were unable to sample all horses with clinical EHM and those that were sampled were not all sampled beginning on Disease Day 0. Because we sampled from only a few horses in the early part of disease, we are not as confident about what proportion of horses shed virus early in disease (i.e., on Disease Day 0–2). However, the majority of affected horses (at least 64%) that were tested were found to shed virus 5–7 days after onset of disease. We were unable to sample horses without clinical EHM and as such cannot comment on shedding in horses with subclinical infections. However, we still recommend quarantine of all in-contact horses from

### Table 1. Results of univariable analysis of associations with the occurrence of neurologic disease (EHM) among horses stabled at a boarding facility.

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<td>0.78–8.42</td>
<td>.12</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal Discharge</td>
<td>Yes</td>
<td>24</td>
<td>0.636–7.15</td>
<td>.29</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attended Riding Clinic</td>
<td>Yes</td>
<td>13</td>
<td>1.24–19.92</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arena</td>
<td>≥ 2 times per week</td>
<td>55</td>
<td>0.88–94.50</td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td>&lt;2 times per week</td>
<td>21</td>
<td></td>
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<tr>
<td>Housing</td>
<td>Outside ≤ 8 hours per day</td>
<td>51</td>
<td>0.59–6.24</td>
<td>.31</td>
</tr>
<tr>
<td></td>
<td>Outside 24 hours per day</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housing Location</td>
<td>Wing A</td>
<td>19</td>
<td>0.57–9.78</td>
<td>.27</td>
</tr>
<tr>
<td></td>
<td>Wing B</td>
<td>15</td>
<td>0.02–3.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wing C</td>
<td>17</td>
<td>0.66–11.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outside</td>
<td>38</td>
<td></td>
<td></td>
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<tr>
<td>Tack-up Stalls</td>
<td>≥ 2 times per week</td>
<td>17</td>
<td>0.24–4.64</td>
<td>.81</td>
</tr>
<tr>
<td></td>
<td>&lt;2 times per week</td>
<td>59</td>
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</table>

95% CI = 95% confidence interval; OR = odds ratio.
affected premises and isolation of all clinically affected horses.

The qPCR used in this study employs primers and probes specific for the gene sequence of glycoprotein B of EHV-1. This system is an effective method of EHV-1 detection from nasal swabs of experimentally infected horses with mild respiratory signs.9–12 The minimum level of detection is not known precisely, but in the authors’ experience, it can detect as few as 10 genomic copies, and a sample which is positive on 1 run will consistently have positive results when repeatedly tested, although there is some variability at lower copy numbers (especially below 100 copies).9 To account for inherent variability in laboratory results while maintaining the ordinal characteristic of the data, categories were assigned to each sample based on the threshold cycle (Ct) number. Results that we categorized as Low or Very Low likely indicated shedding of less than 100 viral copies per sample,9 and in the authors’ opinion, likely represent a very low infectious risk to other horses. On the basis of previous experience, we are confident that horses categorized as shedding Medium virus quantities pose a risk to other horses given sufficient contact, and horses with High and Very High amounts of virus detected in samples represent horses that pose a high infectious risk to other horses even with minimal contact. These conclusions are based partially on correlation between results of virus culture and those of qPCR assays,9 and experiences testing samples from clinically affected horses during outbreaks. In general, samples are variably culture-positive if they contain 100 to 1,000 genomic copies, and are reliably culture-positive when they contain ≥1,000 copies; these cutoffs correspond approximately with the breakpoints used to define Medium and High categories for this investigation. Any horse which is shedding viable virus may pose an infectious risk to another horse, including those shedding Low or Very Low virus quantities, but susceptibility to infection and disease will be variable among horses even when exposed to higher virus quantities. In addition, as noted previously, quantification of virus in a single sample on a single day may not perfectly characterize that horses’ nasal shedding or its infectious risk to other horses.

Shedding was detected intermittently in some horses, but horses that had Negative results after the onset of clinical signs did not subsequently have High or Very High test results. This suggests that a single negative result after onset of clinical signs may be strongly predictive that a horse is no longer contagious. In other words, once a clinically affected horse tests negative it is less likely to shed high numbers of EHV-1 and therefore likely represent a very low contagious disease risk. However, this observation needs to be confirmed in investigations of other outbreaks.

The finding in this report of a positive mare for D variant strain of EHV-1 and its aborted fetus positive for N variant strain of EHV-1, to the authors’ knowledge, has not been previously reported. It would suggest that this mare had, at some point, been infected by both variants of EHV-1. We are unable to speculate any further on its importance.

The source of the virus in this outbreak was not determined; however, it seems likely that the virus had been introduced during a riding clinic held at the boarding facility prior to this outbreak, which is supported by the increased odds of developing EHM in horses attending the riding clinic. It has been previously reported that outbreaks are often seen after the introduction of a new horse, in a facility with high horse traffic, or within a stressful environment.9

Studies such as this one need to be repeated in multiple outbreaks to establish evidence-based recommendations for management of this disease. As such, we would suggest that veterinarians plan for this contingency in future outbreaks.13 This study further documents the contagious threat posed by horses clinically affected by EHM, and suggests that disease severity is not a good indicator of contagiousness. On the basis of findings of this study, in the absence of laboratory testing of animals, we recommend that biosecurity measures be utilized when managing EHM cases a minimum of 9 days beyond the onset of clinical signs and perhaps more prudently for 2 weeks. Managers can be most confident that risks related to quarantine release have been minimized if they base their timing on obtaining multiple negative PCR results after this initial period.15 This report also illustrates the potential for EHV-1 to spread between farms and within a referral veterinary hospital, reinforcing the need for biosecurity measures to be a normal part of daily operations at equine boarding and medical facilities.

**Footnotes**

9 Dacron tipped swabs, Fisher Scientific, Pittsburgh, PA
10 Transport media –D-MEM + Glutamax-1, penicillin, streptomycin, gentamicin, fungizone, HEPES buffer and fetal calf serum
11 Colorado State University, Fort Collins, CO
12 Livestock Disease Diagnostic Center, University of Kentucky, Lexington, KY
13 Animal Health Laboratory, University of Guelph, Guelph, ON
14 PROC GENMOD, SAS v. 9.2, SAS Inc, Carey, NC
15 PROC PHREG, SAS v. 9.2, SAS Inc
16 Peraxigard, Bayer Healthcare, Animal Health Division, Bayer, Inc, Toronto, ON
17 Penbritz 228M, West Penitone, Inc, Anjou, Quebec

**Acknowledgment**

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**References**


