

Outbreak of Neurologic Disease Caused by Equine Herpesvirus-1 at a University Equestrian Center

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Background: Equine herpesvirus type 1 (EHV-1) infection causes neurologic disease in horses. However, risk factors for the disease and long-term prognosis are poorly characterized.

Hypothesis: There are identifiable risk factors for equine herpes-1 myeloencephalopathy.

Animals: The entire population of 135 horses housed within the equestrian facility.

Methods: A descriptive study investigated the clinical, serologic, virologic, and management aspects of an outbreak of EHV-1 myeloencephalopathy.

Results: Out of 135 horses at the facility, 117 displayed signs of EHV-1 infection. Forty-six horses developed neurologic deficits characterized by symmetrical hind limb ataxia and weakness. Twelve horses that developed neurologic deficits became recumbent and did not survive. The development of severe neurologic deficits during the outbreak was associated with the presence of residual deficits at the 6-month examination. Within 1 year of the outbreak onset, all horses that survived had returned to an exercise level comparable to that experienced before the outbreak. Factors associated with the development of neurologic disease included age of >5 years, location in the south or arena stall areas, and highest rectal temperature on day 3 or later of the febrile period.

Conclusions and clinical importance: Being >5 years of age, having had a rectal temperature of >103.5°F, and highest rectal temperature occurring on or after the 3rd day of the febrile period were the factors most predictive of the development of neurologic disease and death. Data obtained during this outbreak substantiate previous findings relating to clinical aspects and diagnosis of EHV-1 myeloencephalopathy. The prophylactic and therapeutic use of acyclovir during this outbreak is described.

Key words: Acyclovir; Epidemic; Equine myeloencephalopathy.

Equine herpesvirus type-1 (EHV-1) infection can result in several clinical syndromes in horses: upper respiratory disease, acute pulmonary vasculitis and respiratory distress, neonatal infection, abortion, and neurologic disease.^{1,2} The manifestations of EHV-1 infection are dictated by the virus strain and several factors relating to the horse, including age, immunologic status, and reproductive or pregnancy status. Infection may involve a single animal or be associated with large outbreaks of disease. In susceptible horse populations, infection with a neurovirulent strain of EHV-1 often results in high rates of morbidity and mortality with resultant devastating economic, welfare, and emotional consequences. In recent years, numerous outbreaks of EHV-1 myeloencephalopathy have been reported (G. Allen, personal communication).

This article describes the sequence of events associated with an outbreak of disease caused by a neuro-pathogenic strain of EHV-1 at a large equestrian riding facility in January 2003. Clinical, serologic, and virologic data obtained throughout the outbreak are

reported. Data related to the prophylactic and therapeutic use of acyclovir are presented.

Materials and Methods

Horses and Premises

The University of Findlay's English riding complex is a high traffic facility that consists of 3 stall barns connected by 2 large indoor riding arenas. All barns share a common airspace.

The riding school herd consisted of 135 horses at the time of the EHV-1 outbreak on January 11, 2003. One hundred of these horses were resident horses at the university, and 35 horses were new additions to the riding program. The resident horses consisted of both privately owned and school horses and all had resided at the facility for at least 5 months before the onset of the outbreak. Breeds represented included 64 Thoroughbreds, 40 Warmbloods, 13 Quarter Horses, and 18 horses of various other breeds. There were 80 (59%) geldings and 55 (41%) mares. There was 1 pregnant mare in her 9th month of gestation. The north, south, and arena stall areas housed 39, 71, and 25 horses, respectively. The 35 new horses entered the complex between 1 to 7 days before the outbreak. Twenty-seven of these new additions were privately owned horses sent to the facility for breaking and training. The mean age of these horses was 2.3 years, and all were stalled in the north stall barn along with 12 resident horses. Eight of the new horses were student-owned horses. The mean age of these horses was 7.5 years, and these horses were stalled among the resident horses in the south and arena stall areas. The resident horses had a mean age of 10.8 years and occupied stalls in all 3 barn areas.

Resident horses were vaccinated every 3 months with an inactivated EHV-1/EHV-4 vaccine product.^a The last vaccine had been administered 2 months before the outbreak. The pregnant mare had also received 2 vaccinations with an inactivated vaccine product containing EHV-1 abortigenic strains (EHV-1p and EHV-1b)^b at 5 and 7 months of gestation. New horses entering the facility for the semester were required to be vaccinated against influenza virus and herpesvirus 10–45 days before arrival. The type of vaccine product administered to the new arrivals was not mandated.

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Rectal temperatures were measured daily for all horses from the onset of the outbreak. Fever was defined as a rectal temperature above 101°F. In the present outbreak, the diagnosis of EHV-1 was confirmed initially by polymerase chain reaction (PCR) and virus isolation. From that time on, all horses that developed a fever or ataxia were considered to be infected with EHV-1. In the absence of clinical signs of EHV-1, infection was confirmed by virus isolation or a 4-fold or greater increase in antibody titer between acute and convalescent samples.

All neurologic examinations were conducted by or in conjunction with the first author. The examinations were performed outside of the stall and consisted of cranial nerve evaluation, gait analysis at the walk, and assessment of tail and anal tone. A 5-point grading system was used to classify the degree of neurologic deficits.³ Grade 0 indicates the absence of neurologic deficits; grade 1 indicates the absence of deficits at normal gaits but detectable with manipulation; grade 2 indicates deficits at normal gaits that are worsened with manipulation; grade 3 indicates more severe deficits at normal gaits and the horse may stumble or fall with manipulation; grade 4 is assigned to horses that fall or may fall at normal gaits; and grade 5 is assigned to recumbent horses that are unable to rise on their own.

Serologic and Virologic Testing

Nasopharyngeal swabs and blood samples were collected from 5 febrile horses early in the course of the outbreak (January 14). The swabs were placed in viral transport media and submitted for PCR detection and virus isolation (grown in cell culture) for EHV-1, EHV-4, and influenza. At the same time, serum samples were submitted for initial virus neutralizing antibody (VNA) titers for these same viruses. Twelve days later, convalescent antibody titers were measured for EHV-1 only.

On January 22, heparinized whole blood samples were collected from 12 additional horses for EHV-1 virus isolation. Serum samples were also submitted from 16 horses for initial EHV-1 VNA titers on January 22. Heparinized blood and serum samples were submitted for virus isolation and initial VNA titers on the 1st day of the febrile period (January 28) from the first 5 horses during the 2nd wave of fevers.

Nasopharyngeal swabs and heparinized blood samples were collected and submitted for virus isolation from a sample group of 20 horses after the outbreak on March 10. This time period was 3 weeks after the return to a normal temperature of the last clinically affected horse.

Blood samples were obtained from 5 horses during the 3rd day of treatment with acyclovir for plasma-acyclovir assay by high-pressure liquid chromatography (HPLC). Samples were obtained at 30, 60, 180, and 300 minutes after the PO administration of acyclovir (10 mg/kg).

Vaccination Data and Neurologic Reassessment

Vaccination records for the year before the outbreak were obtained for all horses. Information regarding the frequency of vaccination against EHV-1, vaccine type, and vaccine manufacturer was recorded. A 6-month follow-up neurologic examination was conducted on 26 of the 32 horses that had survived the neurologic manifestations of EHV-1.

Statistical Methods

All variables in the data set were examined, and continuous variables were converted to categorical variables to facilitate the analysis. The categorical variables were determined based on the distribution of the continuous variables. In some variables where there were only 2 categories, they were separated at the median value. In some of the variables that was not possible. Because of the

low numbers in some cells, the categories were collapsed. Crude associations were examined for the outcomes of survival, the development of neurologic disease, and neurologic deficits (if any) at a 6-month follow-up neurologic examination. Horses that did not survive were defined as EHV-1-affected horses that died or became recumbent and deteriorated despite therapy and were euthanized for humane reasons. Variables examined included age, breed, sex, whether the horse received acyclovir, barn location, duration of the febrile period, highest temperature during the illness and on which day it occurred, whether they received an immune stimulant^c before development of neurologic signs, number of vaccines received in the year before the outbreak, whether they developed neurologic signs, and the grade of the neurologic deficits during the illness. All variables were examined using logistic regression.^d Variables associated with a *P* value ≤ .05 were considered significant. Multivariate analysis was not performed because of the small cell numbers in many of the variables, which resulted in a lack of convergence.

Results

Case History of Outbreak

On January 11, fever accompanied by mild depression and inappetence were recognized in 2 resident horses. One horse was stalled in the south barn, and the other was stalled in the arena. Approximately 97 additional horses developed a fever during the next 2 days. Horses in all areas of the facility were affected, and there was no apparent pattern of infection identified with respect to barn location or resident status. Peak recorded temperatures, in individual horses, ranged from 101.6°F to 106.0°F. The duration of the febrile period ranged from 1 to 7 days (mean 4 days). The additional initial signs displayed by the horses in the outbreak varied with age. The group of 27 young horses that had recently entered the facility before the outbreak onset displayed signs of upper respiratory disease, fever, and mild inappetence, whereas in the older resident horses, clinical evidence of respiratory disease was absent. In addition, nonpruritic wheals and lower limb edema developed in some horses during the febrile period. The limb edema was warm and painful to the touch, and affected horses were stiff when walking. The nature of the edema appeared to differ from the edema that may result from stall confinement and inactivity. Limb edema seemed to be present more often in horses that subsequently developed neurologic disease.

The initial case of neurologic disease was diagnosed on January 16 in the only pregnant mare at the facility. EHV-1 was suspected at that time, and quarantine of the facility was instituted. Horses were confined to their stalls, movement of horses on and off the premises was halted, and strict measures regarding employee and student traffic were instituted. Owners of horses that had left the facility in the previous 2 weeks were contacted and informed of the potential exposure to EHV-1 and of the potential risks that their horses presented to other in-contact horses. An additional 41 horses developed neurologic signs within the next 7 days (Fig 1). The time interval between the development of a fever and the onset of neurologic signs ranged from 4 to 8 days (mean 6 days). The time interval from the end of the febrile period to the onset of neurologic signs

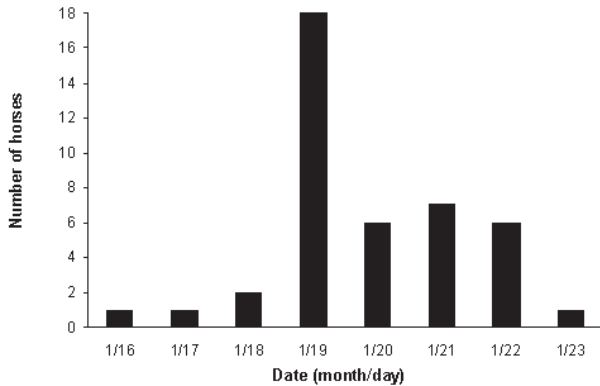


Fig 1. Onset of neurologic disease in 42 horses affected in the 1st wave of equine herpesvirus type-1 infection.

ranged from 1 to 4 days (mean 2 days). All horses were afebrile at the onset of neurologic disease.

The predominant signs in the initial 42 neurologically affected horses were hindlimb ataxia and paresis with decreased tail and anal tone. Toe dragging in the hind limbs, knuckling of the rear fetlocks, and an overall reluctance to move were frequently observed. The deficits in the hindlimbs were symmetric or only mildly asymmetric. Many of the more severely affected horses displayed mild neurologic deficits in the forelimbs, but cranial nerve deficits were observed in only 2 recumbent horses. Additionally, 7 horses developed urinary incontinence, and 1 horse developed penile paresis. Of the first 42 cases of neurologic disease, there were 12 horses with grade 5, 2 horses with grade 4, 12 with grade 3, 7 horses with grade 2, and 9 horses with grade 1 neurologic deficits. None of the horses with grade 5 deficits survived.

By January 20, fever had been detected in 107 out of 135 (80%) horses. No additional cases of EHV-1 (fever) were detected until a 2nd wave of fevers began on January 28. Eleven resident horses developed a fever from this date through February 13. Four horses of this group developed neurologic disease. The first 2 horses had grade 1 deficits, and the last 2 horses displayed grade 3 deficits. One horse, affected during the 2nd wave, that did not develop apparent ataxia or paresis had a seizure 14 days after the development of the fever.

The quarantine of the facility was lifted on March 10, which was 3 weeks after the return to a normal temperature of the last identified case of EHV-1.

Serologic and Virologic Analyses

At the onset of the outbreak, the earliest confirmation of EHV-1 infection came from positive PCR detection of EHV-1 DNA from 2 out of 5 nasopharyngeal swabs obtained on January 14. EHV-1 was isolated from the nasopharyngeal swabs in 4 of these same 5 horses. Initial EHV-1 VNA titers were 1/40 or less in 4 horses, with a 1/160 titer in the pregnant mare. The pregnant mare was euthanized before blood could be collected for measurement of convalescent titers. Therefore, 4 horses had convalescent titers measured, and all seroconverted.

Heparinized whole blood samples were collected on January 22 from 12 horses with neurologic disease of 1–2 days duration (approximately 6–7 days after the development of fever). EHV-1 was isolated from 2 of the 12 horses in this group.

Blood was also collected from 16 horses for measurement of initial EHV-1 serum antibody titers on January 22. Nine of these 16 horses had neurologic disease of 1–2 days duration. Four of these 9 horses with neurologic disease had SN titers $\geq 1/160$. The neurologic grades in these horses were 1, 1, 2, and 5. The other 5 of 9 horses had titers $\leq 1/80$. The neurologic grades in these horses were 3, 3, 5, 5, and 5. The remaining 7 of 16 horses that had serum submitted for initial EHV-1 titers on January 22 were 6–7 days past their febrile period, but did not develop neurologic disease. All of these 7 horses had titers $\geq 1/160$.

Heparinized blood samples and serum samples were submitted on the 1st day of fever (January 28) from the initial 5 horses in the 2nd wave. EHV-1 was isolated from all horses. In contrast to the low titers obtained from the horses at the onset of the 1st wave of fevers, the initial SN titers were 1/160 or greater in these horses. Additionally, only 1 of these horses seroconverted, and convalescent titers were lower in 2 of the horses.

After cessation of the outbreak (March 10), virus isolation was unsuccessful from nasal swabs and blood from 20 previously affected horses, indicating the absence of prolonged viremia or viral shedding in these horses. Additionally, on March 10, the introduction of 15 naive horses into the facility for use in the riding program did not result in new clinical cases of EHV-1.

Management of Affected Horses

Stall confinement was initiated during the 1st week of the outbreak and each horse's rectal temperature was taken daily. Flunixin meglumine^c (1.1 mg/kg IV q 24h), or phenylbutazone^f (4.4 mg/kg PO q 24h) was administered as indicated for high temperatures. Nine student-owned resident horses received an immune stimulant during the 1st week of the outbreak at their owner's or regular veterinarian's request. After the onset of neurologic disease at the facility, horses were examined in their stalls at least 4 times a day, and a neurologic examination was performed for all horses suspected of having neurologic deficits. After veterinary examination, horses with greater than grade 2 deficits were moved to the indoor sand arena to ensure adequate monitoring and treatment. An intravenous catheter was placed in these horses, and they received dimethyl sulfoxide (DMSO)^g (1 gm/kg IV q24h for 3 days), flunixin meglumine (1.1 mg/kg IV q 24h for 3 days), and dexamethasone^h (0.2mg/kg IV q24h for 3 days). Acyclovirⁱ (20 mg/kg PO q8h for 5 days) was administered to all neurologically affected horses when it became available on January 20. After 3 days of therapy, the dosage of dexamethasone was tapered to 0.1 mg/kg for an additional 3 days if the horse's neurologic status remained stable. Horses with a neurologic grade of 2 or

less were treated in a similar manner, with the exception that they did not receive DMSO.

The first 6 neurologically affected horses became recumbent. One horse was recumbent at the time of initial diagnosis, whereas the remaining horses became recumbent within approximately 4–24 hours of treatment. Additionally, 5 of the next 16 neurologic cases became recumbent. Horses that became recumbent were managed at the facility for 24–48 hours. Therapy included intravenous fluids, tranquilization as indicated, frequent repositioning, and bladder catheterization. The recumbent horses that deteriorated within this time period were either referred for continued intensive care and sling support or euthanized, depending on the owners' desires.

The 11 horses involved in the 2nd wave of fevers were treated in a different manner. These horses received flunixin meglumine (1.1 mg/kg IV q24h) after the development of a fever, and this treatment was continued for 10 days. Because the mean time from the development of a fever to the onset of neurologic disease was 6 days in the 1st wave of affected horses, the decision was made to start acyclovir (10 mg/kg PO 5 times daily for 5 days) and dexamethasone (0.1 mg/kg IV q24h for 5 days) prophylactically on day 5 after the development of the fever. The 1st horse from this group to develop neurologic deficits showed grade 1 deficits on day 6. The signs did not progress. The 2nd horse developed grade 1 deficits on day 5 and remained stable. The 3rd horse developed grade 3 deficits on day 4, before dexamethasone and acyclovir therapy. Treatment with acyclovir and dexamethasone was instituted, and the deficits in this horse also remained stable. Acyclovir and dexamethasone therapy was started on the 2nd day of fever for the 4th horse, which still developed grade 3 deficits on day 4. All horses involved in the 2nd wave of disease survived.

Acyclovir Treatment and Prophylaxis

Acyclovir was administered to 99 horses during the initial wave of the outbreak. Seventy-three horses were treated with acyclovir (20 mg/kg PO q8h for 5 days) starting on January 20. Another 26 horses were started on the same acyclovir regime on January 23 when additional acyclovir became available. At the time of acyclovir treatment initiation, 11 horses had become recumbent and had either been euthanized or referred for further treatment. Therefore, there were 25 of the remaining 124 horses that did not receive acyclovir. Twenty-two of these horses were 3 years of age or younger and the owners declined treatment of the 3 remaining horses. Treatment of the 22 young horses was not instigated because of financial reasons and the fact that it was evident, by the time acyclovir became available, that the incidence of neurologic disease in the young horses was low and deficits were mild. The 11 horses affected during the 2nd wave of fevers received a prophylactic 5-day course of acyclovir during the 1st wave of infection and again after the onset of their fevers.

Nineteen of the 99 horses that initially received acyclovir had neurologic disease of 1–2 days duration by the time treatment was initiated. Acyclovir was administered as prophylaxis in the remaining 80 horses. Fifty-six of the 80 horses had been febrile by the time treatment was initiated, and therefore acyclovir was used for potential prophylaxis against the neurologic manifestations of EHV-1 in this group of horses. Acyclovir was used as potential prophylaxis against EHV-1 infection in the 24 horses that had not had a fever before the initiation of treatment.

Of the 19 horses in which acyclovir was used as a treatment for neurologic disease, only 1 horse became recumbent. Eleven out of the 56 horses in which acyclovir was used for neurologic prophylaxis developed neurologic deficits within 1–3 days after receiving acyclovir. There were 3 horses with grade 1 deficits, 2 with grade 2 deficits, 4 with grade 3 deficits, and 2 with grade 4 deficits. The neurologic signs stabilized within 24–48 hours in all 11 horses, and no horses became recumbent. Four of the 24 horses that had not shown signs of EHV-1 infection developed a fever on the 4th or 5th day of acyclovir prophylaxis. One of these 4 horses developed grade 1 neurologic deficits. Two additional horses developed a fever within 2 days of completion of acyclovir prophylaxis, but neither horse developed neurologic deficits.

Serum urea nitrogen and creatinine concentrations were within normal limits in the 7 horses tested while receiving acyclovir. The mean plasma concentration of acyclovir achieved in the group of 5 horses tested by HPLC was 0.3ug/mL (range 0.07–0.87 ug/mL).

Vaccination History

There were 129 (96%) horses that were vaccinated against EHV-1 and 6 (4%) horses that had not been vaccinated against EHV-1 in the year preceding the outbreak. Four horses had been vaccinated with a modified live EHV-1 vaccine,^j and 125 horses had received an inactivated EHV-1 vaccine containing EHV-1 and EHV-4 antigens. Thirteen of the 125 horses were vaccinated with an inactivated vaccine produced by one manufacturer,^k and 112 horses were vaccinated with an inactivated vaccine manufactured by another.^a Twenty-five horses (19%) had been vaccinated once, 20 horses (15%) twice, 30 horses (22%) three times, and 54 horses (40%) four times in the year preceding the outbreak.

Neurologic Reassessment

Twenty-six of 32 horses that recovered from the neurologic manifestations of EHV-1 were available for follow-up at 6 months after the outbreak. There were 12 horses that had grade 3 of 5 deficits during the outbreak. At the 6-month examination, 6 of the 12 horses had grade 2 neurologic deficits, 4 horses had grade 1 deficits, and 2 horses had no deficits. Six of the 12 horses were being ridden at a level comparable to the level they were at before the outbreak. The neurologic grades of these horses were 0, 0, 1, 1, 2, and 2. The grades of the horses

Table 1. Table of variables potentially associated with survival.

Variable	Category	No.	Survived (%)	P Value
Acyclovir	No	36	69.4	<.0001
	Yes	99	97.0	
Age	1–5 years	40	100	.005
	6–10 years	40	87.5	
	>10 years	55	83.6	
Barn	Arena	25	92.0	.003
	North barn	39	100	
	South barn	71	83.1	
Duration of fever	≤3 days	52	92.3	.27
	>3 days	64	85.9	
Day of highest temperature	≤2	75	96.0	.0009
	>2	40	75.0	
No. of vaccines	≤2	51	92.2	.44
	>2	84	88.1	
Immune stimulant before central nervous system signs	No	126	89.7	.94
	Yes	9	88.9	
Neurologic disease	No	89	100	<.0001
	Yes	46	69.6	
Neurologic grade	≤2	19	100	<.0001
	>2	27	48.2	
Breed	Thoroughbred	64	93.8	.31
	Warmbloods	40	85.0	
	Others	31	87.1	
Sex	Geldings	80	90.0	.87
	Mares	55	89.1	
Highest temperature category	≤103.5°F	60	95.0	.02
	>103.5°F	55	81.8	

not back in full work were 1, 1, 2, 2, and 2. Six of the horses had grade 2 deficits during the outbreak, and 5 of these horses were neurologically normal by 6 months. One horse had grade 1 deficits. All 6 horses were back in full work. Eight horses that initially had grade 1 deficits during the outbreak were neurologically normal by 6 months and all of these horses were back in full work.

All horses that suffered from urinary incontinence made a full recovery, and the 1 horse that seized had no recurrences. Within 1 year of the onset of the outbreak, all surviving horses returned to an exercise level comparable to that experienced before the outbreak.

EHV-1 Morbidity and Mortality

The overall EHV-1 morbidity, based on the presence of a fever or virus isolation, was 88% (119 horses) of the 135 horses. There was no apparent difference in morbidity with respect to age, sex, breed, or location in the barn in this outbreak. Forty-six horses (34%) developed neurologic disease during the outbreak. The incidence of neurologic disease was 8%, 46%, and 40% in the north, south, and arena stall areas, respectively. The incidence of neurologic disease in febrile horses was similar between the initial and 2nd waves of the outbreak, whereas 29% of the neurologically affected horses in the initial wave became recumbent as compared to none in the 2nd wave. The overall mortality rate due to neurologic disease was 12% (14/

119), and the neurologic case-fatality rate was 30% (14/46). All deaths were related to the development of neurologic disease. Overall, 71 horses developed fever but did not develop neurologic deficits, and all cases of neurologic disease were preceded by a fever. Eighteen horses did not develop any clinical signs of EHV-1 infection, although EHV-1 was isolated from blood in 2 of these horses.

Results of Data Analyses

There were 3 outcomes that were examined to determine factors associated with survival, development of neurologic disease, and 6-month follow-up neurologic status. Variables related to the outcome survival are in Table 1; variables related to the outcome of neurologic deficits (yes/no) are in Table 2; and variables related to residual neurologic deficits at 6-month follow-up are in Table 3. The prophylactic, therapeutic, or both prophylactic and therapeutic use of acyclovir; age; location in barn; date of highest temperature; neurologic deficits; highest neurologic grade; and temperature >103.5°F were associated with survival (Table 1). Age, barn location, highest temperature on day 3 or later, number of vaccines, and use of an immune stimulant were associated with development of neurologic deficits (Table 2). Horses whose temperature was highest in first 2 days of the febrile period and horses whose deficits were grade 3 or greater out of 5 were associated with residual neurologic deficits at the 6-month follow-up (Table 3).

Table 2. Table of variables potentially associated with neurologic disease.

Variable	Category	No. of Horses	Neurologic Disease (%)	<i>P</i> Value
Acyclovir	No	36	41.7	.27
	Yes	99	31.3	
Age	1–5 years	40	15.0	.007
	6–10 years	40	42.5	
	>10 years	55	41.8	
Barn	Arena	25	40.0	<.0001
	North barn	39	7.69	
	South barn	71	46.5	
Duration of fever	≤3 days	52	34.6	.40
	>3 days	64	42.2	
Day of highest temperature	≤2	75	29.3	.007
	>2	40	55.0	
No. of vaccines	≤2	51	23.5	.04
	>2	84	40.5	
Immune stimulant before central nervous system signs	No	126	31.8	.04
	Yes	9	66.7	
Breed	Thoroughbred	64	28.1	.35
	Warmbloods	40	37.5	
	Others	31	41.9	
Sex	Geldings	80	33.8	.92
	Mares	55	34.6	
Highest temperature category	≤103.5°F	60	31.7	.13
	>103.5°F	55	45.5	

Discussion

The entry of a large number of new horses into the facility before the outbreak and the rapid dissemination of infection precluded identification of the source of EHV-1 infection. The stress associated with the change in the horse population, the unrestricted movement of

horses within the facility, and reduced ventilation in the barns during the winter likely contributed to the rapid spread of infection. The initial signs displayed by the young horses during this outbreak were similar to those seen with other viral respiratory infections, whereas the older horses were febrile but had no signs of respiratory disease. This age-related difference in initial signs of

Table 3. Table of variables potentially associated with presence of neurologic disease at 6 months.

Variable	Category	No. of Horses	Neurologic Disease (%)	<i>P</i> Value
Acyclovir	No	36	0.00	ND
	Yes	99	100	
Age	1–5 years	40	50.0	.95
	6–10 years	40	40.0	
	>10 years	55	38.5	
Barn	Arena	25	33.3	.70
	North barn	39	0.00	
	South barn	71	42.1	
Duration of fever	≤3 days	52	41.7	.87
	>3 days	64	38.5	
Day of highest temperature	≤2	75	53.3	.03
	>2	40	11.1	
No. of vaccines	≤2	51	0.00	.31
	>2	84	41.7	
Immune stimulant before central nervous system signs	No	126	42.9	.49
	Yes	9	25.0	
Neurologic grade	≤ 2	19	7.14	<.0001
	>2	27	81.8	
Breed	Thoroughbred	64	41.7	.30
	Warmbloods	40	57.1	
	Others	31	16.7	
Sex	Geldings	80	31.2	.24
	Mares	55	55.6	
Highest Temp Category	≤103.5°F	60	42.9	.52
	>103.5°F	55	30.0	

EHV-1 has been observed in other outbreaks and is likely a result of previous repeated exposures to EHV-1 experienced by older horses.^{1,3,4-8}

There was an apparent association in the present study between age and the risk of development of neurologic disease. Neurologic deficits developed more frequently in horses that were older than 5 years, and severe deficits (\geq grade 3) were only observed in this group of older horses. The absence of neurologic deficits in foals and young horses has been noted in other outbreaks of EHV-1.^{5,6,9,10} The reason for this apparent difference in the effect of age on the development of neurologic disease is unknown, but can be explained by the role that the horse's immune system plays in the extent and severity of the vasculitis, as well as the development of the vascular thrombosis.¹¹ This potential effect of age on the immune response may reflect the horse's previous exposure to EHV-1 and EHV vaccination history.^{9,11}

The incidence of neurologic disease in the present outbreak varied between the different stall areas, despite a similar incidence of EHV-1 infection in these areas. This finding could also be explained by the age difference of horses in these stall areas. The average age of horses in the north stall area was 4.2 years, as compared to 10.5 years for horses in the south stall area and 12.1 years for horses in the arena stalls. Additionally, experimental infection of horses with neurovirulent strains of EHV-1 failed to demonstrate a dose-dependent relationship between the dose of virus encountered at the respiratory mucosa and the development of neurologic disease.¹² These findings would suggest that age, rather than barn location (viral load), was associated with the development of EHV-1 myeloencephalopathy during this outbreak. However, these data are potentially confounded by other factors.

The neurologic signs displayed by the horses in this outbreak and disease progression were similar to that described in various other reports.⁷ In the present outbreak, the development of neurologic signs and severity of neurologic deficits were negatively associated with survival. As reported previously, the prognosis for horses that became recumbent was poor.^{7,13,14}

The monitoring of rectal temperatures was invaluable in the surveillance and management of horses involved in this outbreak. The duration of the febrile period and the time from the end of the febrile period to the onset of neurologic signs were relatively consistent throughout the outbreak. Temperature profiles were not found to be significant predictors of neurologic disease or survival in other reports of EHV-1 infection.^{15,16} In the present outbreak, the duration of the febrile period was not associated with the development of neurologic disease or survival. In contrast, horses that developed a temperature $>103.5^{\circ}\text{F}$ were more likely to die. Additionally, those horses whose highest temperature occurred on or after the 3rd day of the febrile period were more likely to develop neurologic signs and die. These findings related to the predictive value of temperature are similar to those reported from experimental infection of horses with a neurovirulent strain of EHV-1. In these

experimental studies, horses challenged with EHV-1 often displayed a biphasic temperature response or a peak temperature toward the end of the febrile period.^{12,17} It is generally believed that a greater magnitude of viremia occurs in horses affected with a paralytic strain of EHV-1 and that the 2nd or later temperature peak coincides with the viremia.^{1,12,18,19} Therefore, it appears reasonable to conclude that these higher-peak and later-peak temperatures may reflect a greater magnitude of viremia and thus greater potential for the development of neurologic disease.

The antiviral drug acyclovir has selective inhibitory activity against herpes simplex in people by virtue of its inhibition of herpesvirus DNA polymerase and virus replication.²⁰ Acyclovir has been used in the treatment of EHV-1-related myeloencephalopathy and in neonatal respiratory disease, but its efficacy is undetermined.^{15,21} A recent study indicated that the bioavailability of acyclovir is low after PO administration to horses.²² In this study, a mean maximal serum concentration of 0.2 ug/mL was obtained after a single 10 mg/kg PO dose of acyclovir. However, the testing of 5 horses involved in the outbreak reported here revealed a higher mean serum concentration of acyclovir (0.3ug/mL) after multiple 10 mg/kg doses of acyclovir. Additionally, viral sensitivity testing indicated that an acyclovir concentration of 0.3 ug/mL was sufficient to inhibit the in vitro replication of the EHV-1 strain obtained from this outbreak and 3 additional strains of EHV-1 obtained from other outbreaks.²³

During this outbreak, it was difficult to assess the clinical efficacy of acyclovir administration after the onset of neurologic disease because of the normally rapid stabilization of neurologic deficits observed in horses affected with EHV-1. However, 11 of 22 neurologically affected horses that either did not receive acyclovir or received it at least 24 hours after the onset of neurologic deficits became recumbent and did not survive. In contrast, only 1 of 24 neurologically affected horses became recumbent when treatment was initiated prophylactically or within 24 hours of the onset of neurologic disease. The prophylactic use, early therapeutic use, or both prophylactic and therapeutic use of acyclovir appeared to be associated with survival; however, other confounding factors potentially existed to explain this effect. Surveillance was enhanced after the initial cases of neurologic disease. This afforded earlier detection of neurologically affected horses and earlier therapeutic intervention, potentially resulting in an improvement in survival. Additionally, although there were no apparent differences in age, breed, sex, or vaccination status between horses affected at various stages of the outbreak; it is plausible that horses affected early in the course of the outbreak, before acyclovir use, may have been more innately susceptible to the development of viremia and spinal cord infection. However, in this and other outbreaks, severe cases of neurologic disease developed at all stages of the outbreak.^{14, 24-26}

The immune mechanisms responsible for protection against EHV-1 infection and the development of viremia

are still not completely understood.¹¹ Initial VNA titers obtained from 5 horses in the 1st wave of the outbreak, on the 1st or 2nd day of their febrile periods, were low (≤ 40) in 4 out of the 5 horses. There was no apparent correlation between these initial or convalescent titers and the development or severity of neurologic disease in the 1st wave of infection. In apparent contrast to these findings were initial serologic data obtained from 16 horses approximately 7 days after the onset of their febrile periods. This sample period coincided with the time frame of neurologic disease onset in those horses that suffered from this manifestation of EHV-1. Eleven of these 16 horses involved in the initial wave had VNA titers of 1/160 or greater. Four horses from this group developed neurologic disease, and 1 horse developed severe deficits. Ten of the 11 horses in this group survived. In contrast, the remaining 5 of the 16 horses had VNA titers of ≤ 80 . All of these horses developed severe neurologic deficits, and 3 horses did not survive. Additionally, all of the 5 horses sampled from the 2nd wave of fevers had high (≥ 160) VNA titers on the 1st day of their febrile periods. Only 1 of these 5 horses developed neurologic deficits, which were grade 1 in severity, and all horses survived. The limited serologic data obtained during this outbreak would suggest that an early antibody response to EHV-1 infection may be associated with a reduced risk for the development or severity of neurologic disease. As serum VNA is only one measure of the immune response, it would be more likely that early VNA production reflects an overall heightened response of other adaptive immune factors. If an early heightened response were to exist in some horses, then it potentially would act to reduce the quantity of virus-infected cells entering the circulation from the respiratory tract, in turn reducing the extent of the viremia and the risk of neurologic disease development.

There is conflicting information regarding the efficacy of current vaccines and vaccination protocols advocated for the prevention of EHV-1.²⁷ It has previously been suggested that frequent EHV-1 vaccination or vaccination of exposed horses during an outbreak increases the risk of EHV-1-associated neurologic disease, although the reason for this potential association is unclear.^{7,8} In the present outbreak, there was no apparent difference in the effect of vaccine type or manufacturer on the incidence of EHV-1 infection or development of neurologic deficits, although only 4 horses had received a modified-live vaccine before the outbreak. However, there appeared to be an association between horses that were vaccinated 3 to 4 times in the year preceding the outbreak and the development of neurologic manifestations of EHV-1, despite the fact that the frequency of vaccination had no effect on the incidence of EHV-1 infection. This apparent association between more frequent vaccination and development of neurologic disease should be interpreted with caution. The majority of horses that were vaccinated 3 to 4 times annually were older (>5 years) resident horses, whereas many of the young horses had been vaccinated for the first time before entering the riding facility. Since age was also

associated with the development of neurologic disease, it is difficult to assess the impact of vaccination alone. Additional factors, including previous exposure to EHV-1 and individual responses to vaccination, may have also influenced the immune response and susceptibility to EHV-1 myeloencephalopathy.

Footnotes

- ^a Flu Vac Innovator, Fort Dodge Animal Health, Fort Dodge, IA
^b Equine Rhinopneumonitis Vaccine (Pneumobort- k + 1b), Fort Dodge Animal Health, Fort Dodge, IA
^c Equistim, ImmunoVet Inc, Tampa, FL
^d Proc Genmod, SAS, Inc, Cary, NC
^e FluMeglumine, Phoenix Pharmaceutical Inc, St. Joseph, MO
^f Phenylbutate tablets, Phoenix Pharmaceutical Inc, St. Joseph, MO
^g DMSO, Solvent Sales Inc, Bensenville, IL
^h Dexamethasone solution, Phoenix Pharmaceutical Inc, St. Joseph, MO
ⁱ Acyclovir, Ivax Pharmaceuticals Inc, Miami, FL
^j Rhinomune, Pfizer Animal Health, Exton, PA
^k Prestige II, Intervet Inc, Millsboro, DE
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