Prevalence of equine herpesvirus-1 infection among Thoroughbreds residing on a farm on which the virus was endemic

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Objective—To determine the incidence of equine herpesvirus-1 (EHV-1) infection among Thoroughbreds residing on a farm on which the virus was known to be endemic.

Design—Prospective cohort study.

Animals—10 nonpregnant mares, 8 stallions, 16 weanlings, 11 racehorses, and 30 pregnant mares and their foals born during the 2006 foaling season.

Procedures—Blood and nasopharygeal swab samples were collected every 3 to 5 weeks for 9 months, and placenta and colostrum samples were collected at foaling. All samples were submitted for testing for EHV-1 DNA with a PCR assay. A type-specific EHV-1 ELISA was used to determine antibody titers in mares and foals at birth, 12 to 24 hours after birth, and every 3 to 5 weeks thereafter.

Results—Results of the PCR assay were positive for only 4 of the 1,330 samples collected (590 blood samples, 590 nasopharyngeal swab samples, 30 placentas, and 30 colostrum samples), with EHV-1 DNA detected in nasal secretions from 3 horses (pregnant mare, stallion, and racehorse) and in the placenta from 1 mare. Seroconversion was detected in 3 of 27 foals during the first month of life.

Conclusions and Clinical Relevance—Results suggested that there was a low prevalence of EHV-1 infection among this population of Thoroughbreds even though the virus was known to be endemic on the farm and that pregnant mares could become infected without aborting. Analysis of nasopharyngeal swab samples appeared to be more sensitive than analysis of blood samples for detection of EHV-1 DNA. (*J Am Vet Med Asoc* 2007;231: 577–580)

Equine herpesvirus-1 is a major infectious cause of abortion in mares and is associated with substantial financial costs to breeding farms as a result of losses caused by abortion and the cost of prevention programs. Despite the widespread use of EHV-1 vaccines, abortion secondary to EHV-1 infection is still a concern for mares residing on commercial stud farms.[1] The virus can cause lifelong latent infection, with periodic reactivation and shedding important in maintaining EHV-1 in the horse population.[2] It is estimated that > 50% of the horse population is latently infected with EHV-1, and it is hypothesized that reactivation in latently infected horses is the major biological source of infective virus.[3]

Recently, type-specific ELISAs and PCR assays have been used to study the epidemiology of EHV-1 infection in vaccinated and unvaccinated mares and their foals residing on commercial Thoroughbred breeding farms.[4-7]

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Abbreviation

EHV-1 Equine herpesvirus-1

Collectively, these studies[4,5] have provided direct (PCR assays) and indirect (ELISAs) evidence for infection of foals during the first 5 weeks after birth, with subsequent horizontal spread up to and even after the time of weaning. However, these studies were limited to mares and their foals, included only a random sample of all horses on the farm, followed up a group of horses for a relatively short period by means of serologic testing only, did not include mares in the late stages of pregnancy or other groups of horses residing on breeding farms, or were conducted in the southern hemisphere. As a result, the incidence of viral reactivation and shedding among mares, foals, and other horses residing at Thoroughbred farms in North America on which EHV-1 is endemic is largely unknown. Given the high incidence of latently infected horses and the potential for reactivation, we hypothesized that transmission of EHV-1 within and between resident horses on breeding farms would occur frequently, despite vaccination, and that transmission would be facilitated by less-than-optimal segregation of horse groups. The purposes of the study reported here were to determine the incidence of EHV-1 infection among vaccinated Thoroughbreds and

unvaccinated foals residing at a farm on which the virus was known to be endemic and to identify potential temporal associations between individual horses and groups of horses in regard to EHV-1 infection.

Materials and Methods

Sample population and farm management—The study was designed as a prospective cohort study and was performed on a single farm located in Northern California that bred and trained racing Thoroughbreds. The farm had experienced several outbreaks of EHV-1–related abortion along with isolated abortions attributed to EHV-1 infection in the years prior to the study. Ten nonpregnant mares, 8 stallions, 16 weanlings, and 11 racehorses randomly selected from the available population were included in the present study, along with 30 pregnant mares and their foals born during the 2006 foaling season. All study procedures were approved by the Institutional Animal Care and Use Committee of the University of California.

As a matter of routine farm management, all resident mares on the property were vaccinated at 3, 5, 7, and 9 months of gestation with a commercial inactivated EHV-1 vaccine.[a] In addition, all pregnant mares on the farm were vaccinated at 10 months of gestation with a commercial combination vaccine containing killed EHV-1 virus.[b] All foals were vaccinated with the same combination vaccine at 3, 4, and 6 months of age, and all nonpregnant mares and stallions were vaccinated with the combination vaccine in March each year. All resident racehorses were vaccinated with the combination vaccine every 60 days while in training.

All pregnant and nonpregnant mares included in the study were commingled with other resident mares on the farm in pasture lots containing 8 to 12 mares or were housed in stalls in close contact with other mares. Pregnant and nonpregnant mares were housed in close proximity to racehorses trained on the property. After foaling, mares and their foals were housed in individual stalls until the foals were 4 weeks of age, after which they were turned out to pasture in groups of 6 to 10 mares with their foals. All stallions were housed individually in a barn separate from all other animals on the farm. All foals were box weaned at 6 to 7 months of age and were then kept together in a single pasture separated from other horses on the farm by a distance of approximately 50 m. Mean age at weaning for the 16 weanlings included in the study was 7 months (range, 6 to 9 months); all 16 had been weaned during September or October 2005.

Sample collection—Nasopharyngeal and blood samples were obtained from all available animals included in the study every 3 to 5 weeks between September 2005 and May 2006. In the weanlings and adult horses, a 16-inch nasopharyngeal swab[c] was inserted into the left or right nostril via the ventral meatus to the level of the pharynx until swallowing was induced to maximize collection of nasopharyngeal secretions. Owing to the relatively smaller size of the ventral nasal meatus, a sterile culture swab[d] was used to collect nasopharyngeal samples in the foals. Swab tips were placed in conical centrifuge tubes containing 5 mL of viral transport medium (minimum essential medium with 0.125% gentamicin and 0.1% amphotericin B). Blood samples were collected by means of jugular venipuncture with a 1-inch, 20-gauge needle[e] into sterile plain evacuated tubes and tubes containing EDTA.[e]

For foals born to the 30 pregnant mares during the 2006 foaling season, samples were collected prior to suckling and at 12 to 24 hours of age. In addition, a colostrum sample was collected from the mare prior to nursing and placed in a sterile, plain evacuated tube, and nasopharyngeal and blood samples were also obtained. A physical examination of the foal was performed 12 to 24 hours after birth, and any abnormalities were recorded. An aliquot of anticoagulated blood from the foal was used to determine plasma IgG concentration with a commercial stall-side ELISA.[f]

Small sections (2 × 2 cm) of allantochorion were obtained from the cervical star, the uterine body, and the bases of both uterine horns within 6 hours of expulsion of fetal membranes. Tissue specimens were placed in sterile 3-mL screw-top tubes[g] and refrigerated. Samples that were not analyzed the same day were frozen at –80°C until analyzed.

All samples collected on the farm were transported to the laboratory in a timely fashion and refrigerated until processing the same day or the next day. Blood samples collected into plain evacuated tubes were allowed to clot at room temperature and then centrifuged; serum was collected and stored at –20°C until analyzed.

PCR assay for EHV-1 DNA—A commercial DNA kit[h] was used in accordance with the manufacturer's directions to extract DNA from blood samples, nasopharyngeal swab samples, and colostrum. Small portions (5 × 5 mm) of the tissue specimens were obtained with sterile scissors, mixed in lysis buffer,[i] and incubated with proteinase K at 56°C for 14 hours to allow tissue digestion; DNA was extracted from tissue digests and eluted in PCR-grade water.

Real-time PCR assays targeting the glycoprotein B gene of EHV-1 were performed; assays for the housekeeping gene glyceraldehyde-3–phosphate dehydrogenase were performed as quality-control samples. The real-time PCR assay was based on detection of a specific, 90-base pair product of the glycoprotein B gene of EHV-1 (ORF33; GenBank accession No. NC 001491). Amplification efficiency was calculated from the slope of a standard curve generated from dilution of a sample positive for EHV-1 DNA, and amplification efficiency for the target gene was calculated to be 95%. Analytical specificity was verified by sequencing PCR assay products for the target gene. The assay mixture contained 400nM of each primer (forward primer, TATACTCGCTGAGGATGGAGACTTT; reverse primer, TTGGGGCAAGTTCTAGGTGGTT), 80nM of the probe (ACACCTGCCCACCGCCTACCG) and mastermix,[j] and 1 mL of the DNA sample in a final volume of 12 mL. Samples were amplified in a combined thermocycler-fluorometer[k]; the thermocycling protocol consisted of 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C.

ELISA for anti–EHV-1 antibodies—A commercially available type-specific ELISA[l] was used to measure EHV-1 antibody titers in mares at the time of foaling and in foals

before and 12 to 24 hours after suckling and every 3 to 5 weeks until 90 days of age. This ELISA can differentiate between antibodies against EHV-1 and antibodies against EHV-4 on the basis of structural differences in the glycoprotein G of the 2 viruses and has been validated in field studies.[7-9] On the basis of the manufacturer's recommendations, results were considered negative, doubtful, or positive if optical densities of the test wells were < 0.1, 0.1 to 0.2, and > 0.2, respectively. Seroconversion in foals was defined as an increase in optical density of > 0.129, compared with the previous optical density, as described.[7]

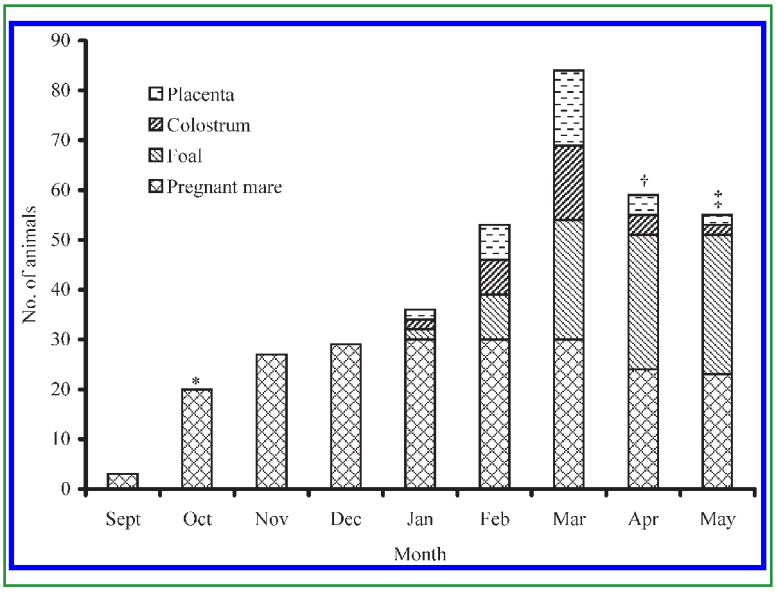

Figure 1—Bar graph illustrating collection of samples (blood and nasopharyngeal swab samples) from 30 pregnant mares and their foals each month during a study of EHV-1 infection among horses residing on a Thoroughbred farm on which the virus was known to be endemic. Placenta and colostrum samples were obtained at the time of foaling. All samples were submitted for testing for EHV-1 DNA with a PCR assay, and serum samples were submitted for determination of anti–EHV-1 antibodies with a type-specific ELISA. *Results of the PCR assay were positive for a nasopharyngeal sample from 1 pregnant mare. †Three unvaccinated foals seroconverted. ‡Results of the PCR assay were positive for placenta samples from 1 mare.

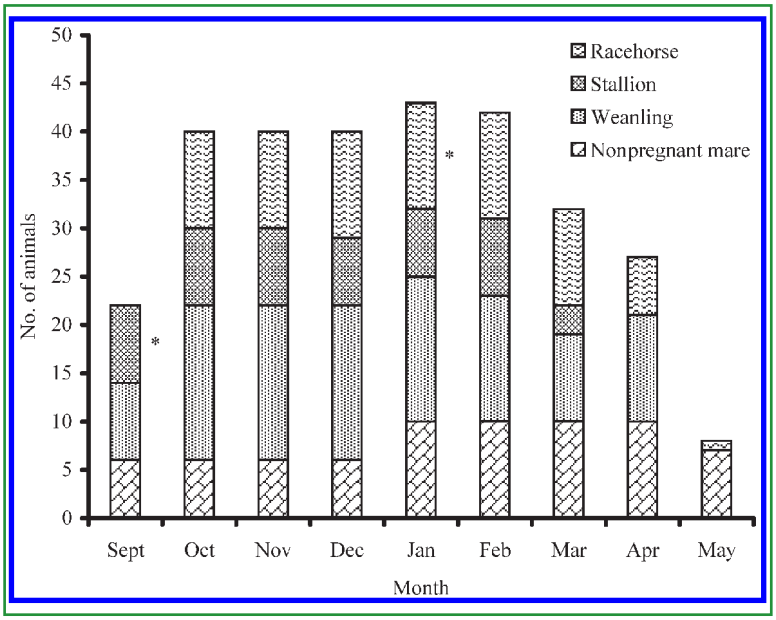

Figure 2—Bar graph illustrating collection of samples (blood and nasopharyngeal swab samples) from 10 nonpregnant mares, 8 stallions, 16 weanlings, and 11 racehorses each month during a study of EHV-1 infection among horses residing on a Thoroughbred farm on which the virus was known to be endemic. All samples were submitted for testing for EHV-1 DNA with a PCR assay. *Results of the PCR assay were positive for a nasopharyngeal sample from 1 stallion (September) and 1 racehorse (January).

Results

A total of 1,330 samples were collected and tested for EHV-1 DNA with the PCR assay. This included 590 blood samples, 590 nasopharyngeal swabs, 30 placentas (4 tissue samples/placenta), and 30 colostrum samples. Each of the extracted samples passed quality-control testing, as determined by low cycle threshold values for the housekeeping gene glyceraldehyde-3–phosphate dehydrogenase.

Results of the PCR assay were negative for all 590 blood samples. Similarly, results of the PCR assay were negative for all but 3 of the 590 nasopharyngeal swab samples. Results were positive for a single nasopharyngeal swab sample collected from a pregnant mare at 159 days of gestation in October 2005 (**Figure 1**). Results of the PCR assay were also positive for 2 of the 4 placenta samples collected from this mare following parturition in May 2006; results were positive for samples obtained from the uterine body and base of the gravid horn, but were negative for samples obtained from the cervical star and base of the nongravid horn. Results of the PCR assay were positive for a single nasopharyngeal swab sample collected from a stallion in September 2005 and for a single nasopharyngeal swab sample collected from a racehorse in January 2006 (**Figure 2**).

Serum samples obtained from 26, 22, and 11 foals at 1, 2, and 3 months of age, respectively, were available for serologic testing for anti–EHV-1 antibodies. Most foals had a progressive decline in optical density values during this period, compatible with colostral antibody decay. In 3 foals, however, an increase in optical density values compatible with seroconversion (ie, an increase in optical density of > 0.129) was observed at 1 month of age; all 3 foals had been born in March 2006.

Discussion

Results of the present study suggested that there was a low prevalence of EHV-1 infection among this population of Thoroughbreds. Equine herpesvirus-1 DNA was detected in nasal secretions from only 3 of the 75 horses, despite collection of multiple samples during a 9-month period. Prior to the study, we had expected a high prevalence of EHV-1 infection on this farm given the high horse population density, minimal segregation of pregnant mares from other horses, and transient population of race-

horses visiting the farm, but this was not the case. The low prevalence we found may have been biased by increased EHV-1 immunity among resident horses as a result of a recent abortion epidemic.

An important finding of the present study was the failure to detect EHV-1 DNA in whole blood samples from any of the horses. This finding supports recent work that revealed poor sensitivity of a real-time PCR assay in detecting EHV-1 DNA in buffy coat leukocytes.[10] Much greater sensitivity was achieved when peripheral blood mononuclear cells from the same experimentally infected horses were assayed. Nevertheless, the detection of EHV-1 DNA in nasal secretions of horses shedding the virus in the present study demonstrated the reliability of the PCR assay. On the basis of our findings, therefore, we conclude that PCR assay of nasal secretions is more sensitive than assay of buffy coat leukocytes for detection of EHV-1 infection.

In the present study, we were able to obtain serologic evidence of EHV-1 infection during the first month after birth in foals born to vaccinated mares. The peak incidence of infection for these foals occurred in April, which was similar to the time period reported elsewhere for foals in the northern hemisphere.[11] The detection of early infection of foals supports results of a previous epidemiologic study[4] performed on vaccinated Thoroughbred mares and their foals in the southern hemisphere. Vaccination of horses in the other groups included in the present study prevented meaningful interpretation of serologic data. Therefore, no temporal association between mare and foal seroconversion could be identified.

We did not detect EHV-1 DNA in nasal secretions or blood samples collected from foals and mares before or at the time that seroconversion was detected in 3 foals. In contrast, other epidemiologic studies[5,11] involving mares and their foals have detected EHV-1 DNA in nasal secretions or viremia early after birth by use of a nested PCR assay. This discrepancy may be related, in part, to differences in the sensitivities of the PCR assays used in these studies and to the high risk of carryover contamination associated with open-tube assays. The sampling frequency used in the current study may have failed to detect nasal shedding or viremia, which is known to be of short duration in experimentally infected animals.[12]

The finding in the present study that resident horses were infected with EHV-1 may have important implications for the breeding farm. Tissue samples for which results of the PCR assay were positive were obtained from the same mare that was found to have viral DNA in its nasal secretions at 159 days of gestation. A live healthy foal with no detectable EHV-1 antibodies in its blood prior to suckling was born after 387 days of gestation. To the authors' knowledge, the presence of EHV-1 DNA in placental samples in the absence of abortion has not been reported previously, and we hypothesize that in this mare, a latent infection became reactivated during gestation, resulting in placental infection but without causing sufficient lesions to result in abortion. This hypothesis is supported by the absence of a serologic response in the foal at birth and the lack of gross or histologic evidence of inflammation in placental tissues. The stallion found to have EHV-1 DNA in its nasal secretions could potentially have infected mares through nasal shedding and semen. Equine herpesvirus-1 DNA has been isolated from semen collected from clinically normal stallions that had been experimentally infected, although venereal spread resulting in infection of susceptible mares has not yet been proven.[13,14] Evidence of EHV-1 DNA in a racehorse supports the current practice on the farm of segregating pregnant mares from racehorses.

a. Pneumobort-K, Fort Dodge Animal Health, Fort Dodge, Iowa.
b. Prestige with Havlogen, Intervet Inc, Millsboro, Del.
c. Procto Swab, Fox Converting Inc, Green Bay, Wis.
d. Transporter, Healthlink, Hardy Diagnostics, Santa Maria, Calif.
e. Kendall Monoject, Tyco Healthcare Group LP, Mansfield, Mass.
f. SNAP foal IgG test, IDEXX Laboratories Inc, Westbrook, Me.
g. Cryovial, Phenix Research Products, Hayward, Calif.
h. DNeasy blood kit, Qiagen, Valencia, Calif.
i. DNeasy tissue kit, Qiagen, Valencia, Calif.
j. Universal TaqMan Mastermix with AmpErase UNG, Applied Biosystems, Foster City, Calif.
k. 7900 HTA, Applied Biosystems, Foster City, Calif.
l. Svanovir, Svanova Biotech AB, Uppsala, Sweden.

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