A multicenter case-control study of risk factors for equine protozoal myeloencephalitis

Noah D. Cohen, VMD, MPH, PhD, DACVIM; Robert J. MacKay, BVSc, PhD, DACVIM; Ellen Toby, PhD; Frank M. Andrews, DVM, MS, DACVIM; Bonnie S. Barr, VMD, DACVIM; Jill Beech, VMD, DACVIM; William V. Bernard, DVM, DACVIM; Carol K. Clark, DVM, MS, DACVIM; Thomas J. Divers, DVM, DACVIM, DACVECC; Martin O. Furr, DVM, PhD, DACVIM; Catherine W. Kohn, VMD, DACVIM; Michel Levy, DVM, DACVIM; Stephen M. Reed, DVM, DACVIM; Thomas L. Seahorn, DVM, MS, DACVIM, DACVECC; Nathan M. Slovis, DVM, DACVIM

Objective—To identify risk factors for equine protozoal myeloencephalitis (EPM) among horses examined at 11 equine referral hospitals.

Design—Case-control study.

Animals—183 horses with EPM, 297 horses with neurologic disease other than EPM (neurologic controls), and 168 horses with non-neurologic diseases (non-neurologic controls) examined at 11 equine referral hospitals in the United States.

Procedures—A study data form was completed for all horses. Data were compared between the case group and each of the control groups by means of bivariate and multivariate polytomous logistic regression.

Results—Relative to neurologic control horses, case horses were more likely to be ≥ 2 years old and to have a history of cats residing on the premises. Relative to non-neurologic control horses, case horses were more likely to be used for racing or Western performance.

Conclusions and Clinical Relevance—Results indicated that cats may play a role in the natural epidemiology of EPM, that the disease is less common among horses < 2 years of age relative to other neurologic diseases, and that horses used for particular types of competition may have an increased risk of developing EPM. (*J Am Vet Med Assoc* 2007;231:1857–1863)

Equine protozoal myeloencephalitis is a progressive neurologic disease of horses most often attributed to infection with the apicomplexan parasite *Sarcocystis neurona*, although infection with *Neospora hughesi*, another apicomplexan parasite, may also cause the disease.[1,2] Treatment of the condition is not uniformly effective, is expensive, and may be associated with adverse effects.[1] Consequently, there is great need to address methods for preventing this disease. Serologic surveys indicate that, on average, approximately half of horses in the United States have been exposed to the organism; however, only a small fraction of these horses ultimately develop EPM.[1,3–5] A recent survey[6] that collected data from > 2,900 horse farms in 28 states regarding various health disorders, including EPM, found that the owner-reported incidence of cases of EPM among horses ≥ 6 months old was 14 ± 6 (mean ± SEM) cases per 10,000 horses per year. Collectively, these data indicate that many exposed horses are resistant to infection, whereas a fraction of exposed horses are prone to develop disease. Identification of factors that predispose some horses to develop EPM could provide important clues for preventing this disease. To the authors' knowledge, only 1 study[7] of risk factors for EPM in the United States has been reported. Thus,

Abbreviations

EPM	Equine protozoal myeloencephalitis
IQR	Interquartile range

From the Department of Large Animal Clinical Sciences (Cohen), College of Veterinary Medicine, and Department of Statistics (Toby), College of Science, Texas A&M University, College Station, TX 77843-4475; Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 100136 (MacKay); Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 (Andrews); Rood & Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580 (Barr, Bernard); Department of Clinical Sciences, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348 (Beech); Peterson & Smith Equine Hospital, 4747 SW 60th Ave, Ocala, FL 34474 (Clark); Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (Divers); Marion duPont Equine Medical Center, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Leesburg, VA 22075 (Furr); Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 (Kohn, Reed); Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 (Levy); and McGee Medicine Center, Hagyard Equine Medical Institute, 4250 Iron Works Pike, Lexington, KY 40511 (Seahorn, Slovis). Dr. Bernard's present address is 4901 Mt Horeb Pike, Lexington, KY 40511. Dr. Reed's present address is Rood & Riddle Equine Hospital, PO Box 12070, Lexington, KY 40511. Dr. Seahorn's present address is 629 Craig Ln, Georgetown, KY 40324.

Supported by Fort Dodge Animal Health, with supplemental support from the Link Equine Research Fund, Texas A&M University.
The authors thank Ms. Heather Quiram and Ms. Lindsay German for technical assistance.
Address correspondence to Dr. Cohen.

the purpose of the study reported here was to identify risk factors for EPM among horses examined at equine referral hospitals in the United States. The study was designed as a case-control study.

Materials and Methods

Study population—Horses were recruited from 11 equine referral hospitals in the United States, including 8 veterinary teaching hospitals (Cornell University College of Veterinary Medicine, University of Florida College of Veterinary Medicine, Ohio State University College of Veterinary Medicine, University of Pennsylvania School of Veterinary Medicine, Purdue University School of Veterinary Medicine, University of Tennessee College of Veterinary Medicine, Texas A&M University College of Veterinary Medicine and Biomedical Sciences, and Virginia-Maryland Regional College of Veterinary Medicine) and 3 private practice hospitals (Hagyard Equine Medical Institute, Lexington, Ky; Peterson & Smith Equine Associates, Ocala, Fla; and Rood & Riddle Equine Hospital, Lexington, Ky).

The study used an incident case-control design. Each participating hospital was asked to provide data for at least 10 horses with EPM (cases), 10 horses with non-neurologic disease (non-neurologic controls), and 6 horses with neurologic diseases other than EPM (neurologic controls) each year for 3 years (September 1, 2001, to August 31, 2003); hospitals were permitted to contribute data for additional cases and controls if available. Horses were considered to have EPM if the diagnosis had been made by a diplomate of the American College of Veterinary Internal Medicine (specialty of Large Animal Internal Medicine or Neurology) at one of the participating institutions. Non-neurologic controls were defined as the next horse > 6 months of age that was admitted to the hospital after a case of EPM. Neurologic controls were defined as horses > 6 months of age admitted for neurologic problems other than EPM.

Miniature horses, donkeys, and mules were specifically excluded from the study. In addition, horses that had seizures or head shaking as their only neurologic abnormality, horses ≤ 6 months old, horses with only mild neurologic abnormalities, horses for which the chief complaint was poor performance or altered behavior, and horses with a relapse of EPM were excluded from enrollment as cases in the study. These exclusion criteria were established to exclude horses for which the probability of EPM was considered low on the basis of breed or clinical signs, or for which the disease was not a new occurrence.[1] Horses with a previous history of EPM and horses that were ≤ 6 months old were excluded from enrollment as controls in the study. The rationale for excluding horses ≤ 6 months old was that EPM is rare in this age group.[1,7,8]

Data collection—The following data, when available, were collected for horses included in the study: referral hospital where the horse was examined; age; breed (Arabian, Quarter Horse, Standardbred, or Thoroughbred); sex; month and year of admission; diagnosis or primary medical problem; duration of clinical signs prior to admission; activity or use of horse (breeding, English performance, pleasure riding, racing, Western performance, or other); whether hay and concentrate fed to the horse was stored in a secured manner; whether sweet feed was fed exclusively to the horse; whether pelleted or extruded feed was fed exclusively as concentrate to the horse; duration of residence on the current premises; whether opossums were reported within a 1-mile radius of where the horse currently resided; whether there were cats present on the premises where the horse currently resided; category of medical problem for which the horse was admitted (eg, musculoskeletal disease or colic); whether there had been any adverse health event or other stressful event (eg, transportation for > 150 miles or parturition) during the past 60 days, other than the episode for which the horse was admitted; history of vaccination against EPM (including number of vaccinations, date of initial vaccination against EPM, and number of times vaccinated against EPM); history of horses with EPM at the farm or stable of current residence; whether pyrimethamine, trimethoprim, sulfonamides, toltrazuril, ponazuril, diclazuril, nitazoxanide, nonsteroidal anti-inflammatory drugs, or corticosteroids (intra-articular or parenteral administration) had been administered within 90 days of admission; duration of treatment for EPM and whether owners had observed a response to treatment (for horses that had been treated for EPM); results of immunoblot testing of serum or CSF for antibodies against *S neurona*; results of CSF analysis, including cytologic examination; results of radiography of the cervical region; results of cervical myelography; results of serologic testing for West Nile virus infection; discharge status (alive, euthanized, or died); and results of a necropsy.

For horses with signs of neurologic disease, whether the following clinical signs were observed during hospitalization was recorded: signs of multifocal neurologic disease, asymmetric neurologic disease, upper motor neuron disease, or lower motor neuron disease; spinal ataxia localized by examination to a lesion affecting the anatomic area from C1 through C6, from C7 through T2, from T3 through L4, or caudal to L4; evidence of cerebral disease; evidence of disease of the brainstem; and recumbency.

Data were recorded on forms at each participating hospital; horse owners or their representatives were interviewed when necessary to obtain information. Not all data were available for all horses. Data forms were transmitted to Texas A&M University for data entry. Prior to data entry, forms were examined for nonsensical values, and any values in question were resolved by communication with the participating investigator at the referral hospital. All data were entered twice, and any discrepancies between entries were resolved.

Data analysis—Data were analyzed by use of bivariate polytomous logistic regression to identify associations between disease status (case, neurologic control, or non-neurologic control) and individual independent variables (eg, age or breed). Whereas standard logistic regression modeling is based on analysis of data for which the outcome is dichotomous (eg, yes vs no, or live vs dead), polytomous logistic regression permits

analysis of data for which the outcome is polytomous (ie, consisting of > 2 categories). The polytomous logistic regression strategy enabled us to simultaneously examine associations between cases and each of the 2 control groups, rather than fitting 2 separate logistic regression models (ie, 1 model for comparison of the case group with the neurologic control group and a second model for comparison of the case group with the non-neurologic control group). Odds ratios were estimated from logistic regression models, and their 95% confidence intervals were calculated by means of maximum-likelihood methods. Models were constructed such that the case group was the reference category. Variables for which the *P* value for screening bivariate analyses was < 0.10 were included in the multivariate model. The multivariate model was a forward stepwise model, fit purposefully on the basis of the magnitude of the observed *P* values from bivariate analyses, whereby variables were sequentially added from the smallest to the largest significant *P* value. Continuous data were summarized as median and IQR (25th to 75th percentile). Categoric data were summarized as proportions. The χ^2 test was used to test for associations between disease status (case vs neurologic control) and individual categoric variables; values of $P < 0.05$ were considered significant.

Results

A total of 648 horses were enrolled in the study, including 183 cases, 297 neurologic controls, and 168 non-neurologic controls. This distribution reflected the fact that some participating hospitals contributed a disproportionate number of neurologic controls (**Table 1**). There were no significant differences between the case group and either control group with respect to year or month of admission; however, more horses were enrolled during the first 12 months of the study (322 horses) than during the second 12 months (208) or third 12 months (118) of the study.

Eighty-eight of the 183 (48%) case horses were described as having multifocal neurologic signs at the time of admission. This was significantly ($P = 0.014$) higher than the proportion of neurologic control horses reported as having multifocal neurologic signs at admission (108/294 [37%]; information was not available for all horses). The proportion of case horses reported as having asymmetric neurologic signs (120/183 [66%]) was significantly ($P < 0.001$) higher than the proportion of neurologic control horses with asymmetric neurologic signs (114/294 [39%]). Proportions of case and control horses with upper motor neuron signs were similar (140/183 [77%] and 210/294 [71%], respectively); however, lower motor neuron signs were significantly ($P = 0.040$) more frequently reported for case horses (79/183 [43%]) than for neurologic control horses (99/294 [34%]). Most case (122/183 [67%]) and neurologic control (178/294 [60%]) horses had neurologic signs localized to a lesion affecting the anatomic area from C1 through C6; however, case horses were significantly ($P = 0.003$) more likely to have lesions localized to the T3 through L4 region (39/183 [21%]) than were neurologic control horses (33/294 [11%]). The proportion of case horses that were recumbent at admission (10/173 [6%]) was significantly ($P = 0.005$) less than the proportion of neurologic control horses recumbent at admission (40/297 [13%]).

Table 1—Distribution of case horses (ie, horses with EPM), neurologic control horses (ie, horses with neurologic diseases other than EPM), and non-neurologic control horses (ie, horses with non-neurologic disease) enrolled in a multicenter case-control study of risk factors for EPM among horses examined at 11 referral hospitals in the United States.

	No. (%) of horses		
Study site	Cases	Neurologic controls	Non-neurologic controls
1	16 (34)	17 (36)	14 (30)
2	21 (31)	26 (39)	20 (30)
3	13 (30)	20 (45)	11 (25)
4	18 (24)	48 (64)	9 (12)
5	9 (35)	8 (31)	9 (35)
6	10 (29)	15 (43)	10 (29)
7	19 (37)	11 (22)	21 (41)
8	14 (27)	24 (47)	13 (25)
9	19 (31)	23 (38)	19 (31)
10	18 (22)	44 (54)	19 (23)
11	26 (24)	61 (55)	23 (21)
Total	**183 (28)**	**297 (46)**	**168 (26)**

Results of immunoblot testing performed prior to admission for serum antibodies against *S neurona* were reported for 24 case and 44 neurologic control horses; results were positive for 19 of 24 (79%) case and 30 of 44 (68%) neurologic control horses. Results of immunoblot testing performed prior to admission for CSF antibodies against *S neurona* were reported for 10 case horses (7 with positive results) and 22 neurologic control horses (11 with positive results). Ninety-three of 95 (98%) case horses were positive for serum antibodies against *S neurona* when tested while hospitalized, compared with 82 of 118 (40%) neurologic control horses. One hundred thirty-five of 143 (94%) case horses were positive for CSF antibodies against *S neurona* when tested while hospitalized, compared with 61 of 143 (43%) neurologic control horses. Cervical radiography was performed on 111 of the 183 (61%) case horses and 201 of the 297 (68%) neurologic control horses. Thirty-three (18%) case and 94 (32%) neurologic control horses underwent myelography, and 64 of these horses (all neurologic control horses) were reported as having myelographic evidence of a stenotic or compressive myelopathy.

For non-neurologic control horses, the most common categories of medical problem for which the horse was admitted were alimentary tract disease, including colic (n = 33), musculoskeletal disease (32), and respiratory tract disease (18).

Age, breed, sex, and horse use—Age was not known for 14 horses enrolled in the study. Median age of the case horses was 6 years (IQR, 3 to 12 years), median age of the neurologic control horses was 4 years (IQR, 2 to 10 years), and median age of the non-neurologic control horses was 7 years (IQR, 3 to 13 years). Exploratory data analysis revealed that the proportion of case horses < 2 years old (10/181 [6%]) was lower than proportions of neurologic control (66/290 [23%]) and non-neurologic control (18/163 [11%]) horses < 2 years old and that the relationship of age with disease status did not appear lin-

ear in the logit for either control group. Thus, age was considered as a categoric variable (< 2 years vs ≥ 2 years). In bivariate analyses, neurologic control and non-neurologic control horses were more likely than case horses to be < 2 years old (**Table 2**); however, neither breed nor sex was significantly associated with disease status. Sixty of 183 (33%) case horses, 109 of 297 (37%) neurologic control horses, and 71 of 168 (42%) non-neurologic control horses were mares. The most common breed was the Thoroughbred, and proportions of horses of this breed were similar for the case (64/183 [35%]), neurologic control (106/297 [36%]), and non-neurologic control (58/168 [35%]) groups. When breeding was used as the reference category for horse use, non-neurologic control horses were less likely to be used for racing or Western performance than were case horses.

Farm characteristics and management practices—There was no evidence that storing hay or concentrate in a secured manner was significantly associated with disease status. The proportions of horses fed hay that was stored in a secured manner were similar for the case (62/158 [39%]), neurologic control (109/254 [43%]), and non-neurologic control (67/155 [43%]) groups. Similarly, the proportions of horses fed concentrate that was stored in a secured manner were similar for the case (137/158 [87%]), neurologic control (208/252 [83%]), and non-neurologic control (132/155 [85%]) groups. Neither feeding sweet feed nor feeding pelleted or extruded feed exclusively as concentrate was associated with disease status. For horses for which this information was reported, proportions of case, neurologic control, and non-neurologic control horses fed sweet feed exclusively as concentrate were 25%, 26%, and 25%, respectively. Similarly, proportions of case, neurologic control, and non-neurologic control horses fed pelleted or extruded feed exclusively as concentrate were 13%, 13%, and 12%, respectively.

Duration of residence on the current premises was greater for non-neurologic control horses than for case horses, but was not different between neurologic control horses and case horses (Table 2). Median duration of residence on the current premises was 12 months (IQR, 6 to 36 months) for case horses, 12 months (IQR, 6 to 30 months) for neurologic control horses, and 24 months (IQR, 6 to 60 months) for non-neurologic control horses.

Reported observation of opossums within a 1-mile radius of the current residence was not significantly associated with disease status. Proportions of horses for which it was reported that opossums were seen within a 1-mile radius of the current residence were 70% (107/153) for case horses, 73% (180/246) for neurologic control horses, and 78% (118/151) for non-neurologic control horses. Presence of cats on the premises was associated with disease status (Table 2). Proportions of horses for which it was reported that cats resided on the premises were 95% (151/159) for case horses, 88% (222/251) for neurologic control horses, and 89% (137/154) for neurologic control horses.

Other historical data—Neurologic control horses were less likely to have a history of an adverse health event or other stressful event during the past 60 days than were case horses (Table 2), but the likelihood of an adverse or stressful event was not significantly different between non-neurologic control horses and case horses. Proportions of horses with a history of an adverse or stressful event during the preceding 60 days were 22% (40/183) for case horses, 15% (44/296) for neurologic control horses, and 21% (36/168) for non-neurologic control horses. History of vaccination against EPM was not significantly associated with disease status. Proportions of horses with a history of being vaccinated against EPM were 7% (12/177) for case horses, 6% (17/289) for neurologic control horses, and 7% (12/162) for non-neurologic control horses. Of the 41 horses vaccinated against EPM, 3 (2 case horses and 1 neurologic control horse) had been vaccinated by the referring veterinarian as an adjunct to treatment for presumed EPM. Among horses for which data regarding a history of horses with EPM at the farm or stable were available, proportions of horses with a farm history of EPM

Table 2—Results of bivariate polytomous logistic regression analysis of risk factors potentially associated with EPM among horses examined at 11 referral hospitals in the United States.

	Neurologic controls		Non-neurologic controls	
Variable	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
Age < 2 y	5.0 (2.5 to 10.1)	< 0.001	2.1 (0.9 to 4.8)	0.067
Horse use				
English performance	1.1 (0.6 to 2.2)	0.793	0.6 (0.3 to 1.2)	0.165
Other	1.9 (0.8 to 4.6)	0.169	1.1 (0.4 to 3.0)	0.834
Pleasure	1.3 (0.7 to 2.5)	0.428	1.0 (0.5 to 2.0)	0.978
Racing	1.2 (0.6 to 2.2)	0.640	0.5 (0.3 to 1.1)	0.071
Western performance	0.5 (0.2 to 1.2)	0.145	0.4 (0.2 to 0.9)	0.036
Duration of residence (mo)	1.0 (< 1.0 to > 1.0)	0.272	> 1.0 (< 1.0 to > 1.0)	0.069
Presence of cats at farm	0.4 (0.2 to 0.9)	0.029	0.4 (0.2 to 1.0)	0.056
Adverse event within past 60 days	0.6 (0.4 to 1.0)	0.052	1.0 (0.6 to 1.6)	0.928
Previous treatment for EPM	0.7 (0.5 to 1.2)	0.192	0.1 (< 0.1 to 0.3)	< 0.001

OR = Odds ratio. CI = Confidence interval.

For all factors, the reference category was horses with EPM. For both control groups, an OR > 1 indicates the factor was less likely to be observed among horses with EPM and an OR < 1 indicates the factor was more likely to be observed among horses with EPM.

Table 3—Results of multivariate polytomous logistic regression of risk factors potentially associated with EPM among horses examined at 11 referral hospitals in the United States.

	Neurologic controls		Non-neurologic controls	
Variable	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
Age < 2 y	3.9 (1.8 to 8.2)	< 0.001	1.5 (0.6 to 3.9)	0.365
Presence of cats at farm	0.4 (0.2 to 0.9)	0.027	0.3 (0.1 to 0.8)	0.016
Previous treatment for EPM	0.7 (0.4 to 1.1)	0.109	0.1 (< 0.1 to 0.3)	< 0.001

See Table 2 for key.

Table 4—Results of multivariate polytomous logistic regression of risk factors potentially associated with EPM among horses examined at 11 referral hospitals in the United States, after exclusion of previous treatment for EPM as a potential risk factor.

	Neurologic controls		Non-neurologic controls	
Variable	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
Age < 2 y	4.8 (2.2 to 10.7)	< 0.001	2.3 (0.9 to 5.8)	0.072
Horse use				
English performance	1.0 (0.5 to 2.2)	0.986	0.4 (0.2 to 1.0)	0.056
Other	0.9 (0.3 to 2.6)	0.883	0.7 (0.2 to 2.0)	0.493
Pleasure	1.3 (0.6 to 2.7)	0.488	1.0 (0.4 to 2.0)	0.897
Racing	0.8 (0.4 to 1.7)	0.539	0.4 (0.2 to 0.9)	0.026
Western performance	0.5 (0.2 to 1.2)	0.143	0.3 (0.1 to 0.8)	0.013
Presence of cats at farm	0.4 (0.2 to < 1.0)	0.049	0.4 (0.2 to 1.0)	0.053

See Table 2 for key.

were 12% (2/17) for case horses, 16% (14/88) for neurologic control horses, and 21% (12/57) for non-neurologic control horses. The proportions of horses with a history of previous treatment differed significantly among groups. Proportions of horses with a history of previous treatment for EPM were 27% (48/175) for case horses, 23% (61/265) for neurologic control horses, and 4% (5/120) for non-neurologic control horses.

Risk factors for EPM—The final multivariate logistic regression model included terms for age (< 2 years vs ≥ 2 years), history of cats residing on the premises, and previous treatment for EPM (**Table 3**). Of these variables, only a history of cats residing on the premises was significantly associated with whether a horse would be designated a case relative to both control groups. Because previous treatment for EPM was not deemed to be potentially causally associated with development of EPM, a multivariate model that excluded this term was also fit (**Table 4**). Variables retained in this model included age, history of cats residing on the premises, and use of the horse for racing or Western performance.

Discussion

In the present study, risk factors for EPM identified by use of multivariate polytomous logistic regression included age ≥ 2 years, a history of cats on the premises where the horse resided, and use of the horse for racing or Western performance. After adjusting for other factors in the model, the association between age ≥ 2 years and EPM was only significant when case horses were compared with neurologic control horses. Although details of specific diagnoses for neurologic control horses were not obtained, it is likely that many of these horses had cervical stenotic myelopathy, a neurologic condition that might be expected to be relatively more commonly diagnosed among horses < 2 years. This hypothesis is supported by the fact that 33 of 66 (50%) neurologic control horses < 2 years old underwent myelography, whereas only 26% (61/231) of neurologic control horses ≥ 2 years old underwent myelography. Our finding of an association between age and EPM is in contrast with findings of a previous study[7] that failed to detect a significant difference in age between horses with EPM and neurologic control horses. The reason for this discrepancy is unknown, but may reflect differences between the 2 studies in regard to case definitions, the manner in which age was considered in analysis, and study populations. Consistent with our finding, exposure to *S neurona* and *N hughesi* is reportedly uncommon among foals, and EPM is uncommon among horses < 2.5 years old.[8]

When horses with EPM were compared with non-neurologic control horses, the odds of EPM were significantly greater for horses used for racing or Western performance, relative to horses used for breeding. This observation is consistent with previous findings that horses with EPM were significantly more likely than non-neurologic control horses to have been involved in racing or showing[7] and that estimated annual incidence of EPM in the United States was highest among horses used for racing, showing, or competition.[6] There are a number of explanations for this association. Horses used for more strenuous activities may be more likely to be identified as having neurologic deficits. Alternatively, stresses associated with transport and competition might predispose to development of clinical disease. It is also possible that the economic value or potential of such horses might result in these horses being more likely to be evaluated by a veterinarian. In this study, this association did not appear to be confounded by age. The reasons racing and

Western performance were not significantly associated with EPM when case horses were compared with neurologic control horses are unknown. However, many of the horses with cervical stenotic myelopathy that were < 2 years old were Thoroughbreds intended for racing, and this could have masked an association between racing and EPM. It is also unclear why Western, but not English, performance horses were significantly more likely to have EPM. One possible explanation is a lack of adequate study power, in that the *P* value for English performance horses to be more likely to have EPM, relative to non-neurologic control horses, was close to our cutoff for significance ($P = 0.056$).

A history of cats on the premises was significantly associated with EPM when case horses were compared with neurologic control horses. Domestic cats were the first intermediate host for *S neurona* that was identified,[1] and seroprevalence of *S neurona* exposure was significantly higher among cats from farms where horses with EPM were identified than among cats examined at spay-neuter clinics in the same region.[9] Thus, results of the present study provide further evidence that cats may play a role in the epidemiology of EPM. This finding should be interpreted with caution, however, because cats were commonly reported to reside at farms and stables where horses in both control groups were housed. Thus, the presence of cats alone does not explain the occurrence of EPM. Moreover, there are other intermediate hosts for *S neurona*.[1] Thus, our finding should be considered important with respect to understanding the role of cats in the epidemiology of EPM, but should not be interpreted as evidence that cats in barns cause EPM.

Previous treatment for EPM was common for both case and neurologic control horses in the present study. This finding was not surprising because EPM is one of the few equine neurologic diseases for which specific, effective treatments exist. Although this factor was retained in our initial multivariate model, it was excluded from the subsequent model because it was considered to be an effect of having neurologic disease rather than a potential cause of EPM.

Although not significantly associated with EPM in multivariate modeling, a history of an adverse or stressful event within the preceding 60 days, such as long-distance transport, parturition, surgery, injury, or illness, was more common among case than neurologic control horses in the present study, but, in contrast to findings of a previous report,[7] was not more common in case than in non-neurologic control horses. The present study also failed to demonstrate a significant association between secured storage of either concentrate or hay and reduced risk of EPM. The reasons for these discrepancies between findings of the present study and a previous report[7] are unknown but may relate to aforementioned differences in study methods and designs. The proportions of horses fed hay that was stored securely were similar between studies, but the proportions of horses fed concentrate that was stored securely could not be directly compared between studies. As observed in the present study, however, maintaining concentrate in secure containers is a relatively common practice.

Opossums (*Didelphis virginiana*) are considered the definitive host for *S neurona*, but we did not find a significant association between EPM and a history of opossums within a 1-mile radius of the farm in the present study. The proportion of horses residing at a farm or stable with opossums within a 1-mile radius was high (> 70%) in the present study, indicating that horse farms were generally located in areas where opossums might be observed. Use of a 1-mile radius may not have provided adequate resolution of true exposure, and determining whether opossums were seen at the farm of interest might have provided better exposure data. Given that opossums are nocturnal, however, the fact that horse owners who are primarily with their horses during daytime or early evening did not observe opossums may not accurately reflect true exposure.

The present study had a number of limitations. Antemortem diagnosis of EPM remains problematic, primarily because of the potential for false-positive results.[1,10,11] Thus, misclassification of cases and controls with respect to this disease was probable in the present study. There did appear to be significant differences between cases and neurologic controls with regard to several variables pertaining to clinical signs and diagnosis, such as evidence of multifocal neurologic signs at admission and results of immunoblot testing for antibodies against *S neurona* and myelography. The case definition for EPM used in the study was determined a priori by the investigators. Because of problems with accurate antemortem diagnosis of EPM,[1,10,11] results of immunoblot testing were not required for making a diagnosis of EPM. The probability of misclassification of cases following evaluation by a clinical expert was not considered to be greater than that associated with using results of immunoblot testing. Nevertheless, all case horses in the present study had positive results for immunoblot testing of serum, CSF, or both for antibodies against *S neurona*. The low percentage of horses that had been vaccinated with the conditionally licensed EPM vaccine during the time of the study was unlikely to have had a major influence on misclassification of cases. It should be noted that a large proportion of neurologic control horses had positive results for immunoblot testing of both serum and CSF. Although some of these horses may have been EPM cases that were misclassified or might have been horses simultaneously affected by EPM and another neurologic disease, these findings illustrate the potential for false-positive results associated with immunoblot testing of CSF. The potential for horses to have simultaneously been affected by EPM and another neurologic disease (eg, cervical compressive stenotic myelopathy) represents another source of misclassification.

Although case and control horses were enrolled prospectively in the present study, there were problems with data collection and quality inherent to case-control studies. First, data were generally reported by owners, and there were no efforts to validate historical data. Second, there was often a lag between admission and diagnosis, resulting in some missing data when follow-up was not performed or not possible. Third, data were contributed by multiple centers, but the analysis reported here ignores center effects. Results of bivariate analysis involving mixed-effects logistic regression modeling in which center was fit as a random effect yielded similar results to the bivariate results of our polytomous logistic regres-

sion model, and stratified analysis by center did not reveal evidence of either confounding or effect modification by center. Nevertheless, development of methods for mixed-effects polytomous logistic regression would have improved the modeling strategy used in this report.

In summary, risk factors for EPM identified in the present study included age ≥ 2 years, cats at the premises of residence, and horse use. The odds ratios were not particularly large, and although our observed findings corroborated previous results, none of these factors sheds new light on methods for preventing what remains an important neurologic disease of horses.

References

1. Dubey JP, Lindsay DS, Saville WJA, et al. A review of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM). *Vet Parasitol* 2001;95:89–131.
2. Marsh AE, Barr BC, Madigan J, et al. Neosporosis as a cause of equine protozoal myeloencephalitis. *J Am Vet Med Assoc* 1996;209:1907–1913.
3. Bentz BG, Granstrom DE, Stamper S. Seroprevalence of antibodies to *Sarcocystis neurona* in horses residing in a county of southeastern Pennsylvania. *J Am Vet Med Assoc* 1997;210:517–518.
4. Saville WJ, Reed SM, Granstrom DE, et al. Seroprevalence of antibodies to *Sarcocystis neurona* in horses residing in Ohio. *J Am Vet Med Assoc* 1997;210:519–524.
5. Blythe LL, Granstrom DE, Hansen DE, et al. Seroprevalence of antibodies to *Sarcocystis neurona* in horses residing in Oregon. *J Am Vet Med Assoc* 1997;210:525–527.
6. USDA. APHIS, Veterinary Services Web site. EPM: equine protozoal myeloencephalitis in the US National Animal Health Monitoring System. May 2001. Available at: www.aphis.usda.gov/vs/ceah/ncahs/nahms/equine/equine98/eq98EPM.PDF. Accessed Mar 7, 2007.
7. Saville WJ, Reed SM, Morley PS, et al. Analysis of risk factors for the development of equine protozoal myeloencephalitis in horses. *J Am Vet Med Assoc* 2000;217:1174–1180.
8. Duarte PC, Conrad PA, Wilson WD, et al. Risk of postnatal exposure to *Sarcocystis neurona* and *Neospora hughesi* in horses. *Am J Vet Res* 2004;65:1047–1052.
9. Stanek JF, Stich RW, Dubey JP, et al. Epidemiology of *Sarcocystis neurona* infections in domestic cats (*Felis domesticus*) and its association with equine protozoal myeloencephalitis (EPM) case farms and feral cats from a mobile spay and neuter clinic. *Vet Parasitol* 2003;117:239–249.
10. Daft BM, Barr BC, Gardner IA, et al. Sensitivity and specificity of western blot testing of cerebrospinal fluid and serum for diagnosis of equine protozoal myeloencephalitis in horses with and without neurologic abnormalities. *J Am Vet Med Assoc* 2002;221:1007–1013.
11. Duarte PC, Daft BM, Conrad PA, et al. Evaluation and comparison of an indirect fluorescent antibody test for detection of antibodies to *Sarcocystis neurona*, using serum and cerebrospinal fluid of naturally and experimentally infected, and vaccinated horses. *J Parasitol* 2004;90:379–386.