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

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Objective—To determine sensitivity and specificity of western blot testing (WBT) of CSF and serum for diagnosis of equine protozoal myeloencephalitis (EPM) in horses with and without neurologic abnormalities.

Design—Prospective investigation.

Animals—65 horses with and 169 horses without neurologic abnormalities.

Procedure—CSF and serum from horses submitted for necropsy were tested for *Sarcocystis neurona*-specific antibody with a WBT. Results of postmortem examination were used as the gold standard against which results of the WBT were compared.

Results—Sensitivity of WBT of CSF was 87% for horses with and 88% for horses without neurologic abnormalities. Specificity of WBT of CSF was 44% for horses with and 60% for horses without neurologic abnormalities. Regardless of whether horses did or did not have neurologic abnormalities, sensitivity and specificity of WBT of serum were not significantly different from values for WBT of CSF. Ninety-four horses without EPM had histologic evidence of slight CNS inflammation.

Conclusions and Clinical Relevance—The low specificity of WBT of CSF indicated that it is inappropriate to diagnose EPM on the basis of a positive test result alone because of the possibility of false-positive test results. The high sensitivity, however, means that a negative result is useful in ruling out EPM. There was no advantage in testing CSF versus serum in horses without neurologic abnormalities. Slight CNS inflammation was common in horses with and without *S neurona*-specific antibodies in the CSF and should not be considered an indication of CNS infection with *S neurona*. (*J Am Vet Med Assoc* 2002;221:1007–1013)

Equine protozoal myeloencephalitis (EPM) is a debilitating protozoal disease of the CNS that is typically caused by infection with *Sarcocystis neurona*,[1] although recent reports[2-5] have suggested that it may less commonly be caused by infection with *Neospora* spp. Antemortem diagnosis of EPM is problematic, because clinical signs can be variable, and the disease can be differentiated clinically from other neurologic and musculoskeletal diseases only with difficulty. On the other hand, postmortem demonstration of compatible histopathologic CNS lesions and immunostaining for *S neurona* is a highly accurate method of diagnosing EPM.[6]

The **western blot test (WBT)**, an immunoblot test that detects IgG specific for *S neurona*, became available in 1993[7] and has gained wide acceptance as an antemortem test for EPM in horses. Either serum or CSF can be used, and a positive WBT result for a sample of CSF from a horse with clinical signs suggestive of EPM has been interpreted as indicative of CNS infection and in situ antibody production. However, an increase in the number of horses in which EPM has been diagnosed antemortem on the basis of positive CSF test results and a lack of confirmation of the diagnosis at necropsy in many cases[a,b] has led to uncertainty about the meaning of a positive WBT result.

False-positive results for WBT of CSF may be a result of iatrogenic contamination of the CSF with blood in seropositive horses,[8] an increase in permeability of the blood-brain barrier in horses with neurologic disease other than EPM,[9] cross-reactivity in horses infected with *Neospora* spp[2] or some species of *Sarcocystis* other than *S neurona*,[10] or subclinical *S neurona* infection of the CNS concurrent with another neurologic disease.[10] Beyond this, the usefulness of WBT of CSF as an accurate indicator of latent CNS infection in horses without neurologic abnormalities has not been explored, although positive results were reported for CSF from 31% of 254 such horses in 1 study.[11]

Notwithstanding these apparent limitations, WBT

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of CSF has been used as a guide for treatment decisions in horses without neurologic abnormalities, the assumption being that a positive result is indicative of subclinical CNS infection or incipient disease. However, concerns have arisen that reliance on the assumption that a positive WBT result for a CSF sample is indicative of infection with *S neurona* may lead to inappropriate diagnosis of EPM in horses with other types of neurologic disease and over-diagnosis of subclinical infection or incipient EPM in healthy horses.

Collection of CSF is difficult, often expensive, and not without risk. For this reason, the WBT is often performed only on serum. However, positive WBT results for serum samples are generally interpreted only as an indication of exposure to *S neurona*. For instance, although clinical disease is uncommon in horses, in previous studies[12-15] in which the WBT was used to test serum samples from clinically normal horses in the United States, between 22 and 65% of horses were considered to have been exposed to the agent. The sensitivity and specificity of using the WBT on serum for diagnosis of EPM have not been established. Thus, there is no good evidence that a positive test result is indicative of CNS disease. However, it is generally accepted that if results of the WBT performed on a serum sample are negative, there is a high probability that the horse is not infected with *S neurona*.[16]

The purpose of the study reported here was to determine sensitivity and specificity of the WBT performed on CSF and serum samples for diagnosis of EPM in horses with neurologic abnormalities and for diagnosis of subclinical CNS infection in horses without neurologic disease. Results of postmortem examination were used as the gold standard against which results of the WBT were compared.

Materials and Methods

Procedures—Horses submitted for necropsy to the California Animal Health and Food Safety Laboratory System between mid-1996 and 1999 were examined. This included horses submitted under the California Animal Health and Food Safety Laboratory System and California Horse Racing Board postmortem program and horses from the general California horse population. Many racehorses were euthanatized for humane reasons because of musculoskeletal injuries; others were euthanatized because of irreversible medical conditions.

During the first half of the study, most horses submitted for necropsy underwent a postmortem CNS examination and were included in the study, provided that CSF was collected within 3.5 hours after death or euthanasia, CSF from horses with positive WBT results contained < 100 RBC/µL, and tissues were generally well preserved. Beginning in mid-1998, horses for which results of the WBT of CSF were negative were included in the study only rarely, as the desired number of horses with negative CSF test results had been reached. In addition, during that time fewer horses from which CSF had been collected after death (as opposed to immediately after euthanasia) were included. We also began soliciting submission of horses with neurologic abnormalities to increase the chance of obtaining horses with positive CSF test results.

Horses were classified on the basis of clinical signs as having or not having neurologic abnormalities. For horses with neurologic abnormalities, information on clinical signs was obtained from the necropsy request form, frequently supplemented by conversation with the submitting veterinarian or owner. All horses with a history of neurologic abnormalities were examined by a veterinarian in the field. However, the extent and thoroughness of the examination were not recorded. Horses with chronic neurologic disease may not have had a recent neurologic examination because of the long-standing nature of the disorder. For horses without neurologic abnormalities, a neurologic examination was not performed.

Samples of blood and CSF were collected in the field by practitioners or technicians before or shortly after (30 to 45 minutes, depending on the racetrack) death or euthanasia. In some instances, samples were collected by laboratory personnel after arrival of the carcass at the laboratory, always within 3.5 hours after death. For these horses, time of death was recorded from submission records or was obtained from the individual submitting the horse for necropsy. Cerebrospinal fluid samples were collected from the cisterna magna with 12.5-cm, 20-gauge needles into tubes containing EDTA. Blood and CSF samples were packed with ice for transport to the laboratory. A questionnaire was included with supplies for collecting blood and CSF samples. If the attending veterinarians did not complete the questionnaire, a technician or author attempted to contact the veterinarian, trainer, or owner by telephone. Among other things, the questionnaire asked for information regarding treatment for EPM during the preceding 3 months and any time prior to the preceding 3 months.

Laboratory testing—Red blood cell count of CSF samples was done with a hemocytometer as soon as possible after arrival at the laboratory, although in a few instances, CSF RBC count was not determined until as long as 96 hours after death. All WBT was performed by a single laboratory,[c] which used a procedure similar to 1 described previously.[7] Nonspecific reactions were inhibited by diluting samples with blocking reagent. Immunoreactivity of test samples was compared with that of diluted positive control samples. Results were reported as negative (*S neurona*-specific IgG not detected), positive with weak reactivity (interpreted as equivocal or borderline), positive (*S neurona*-specific IgG detected), and positive with high reactivity (interpreted as an exceptionally high concentration of *S neurona*-specific IgG). Horses with positive WBT results for CSF were excluded from the study if CSF RBC count was > 100 RBC/µL of CSF. For horses with negative WBT results for CSF, CSF RBC count was irrelevant.

Albumin concentration of the CSF was determined by concentrating samples and electrophoretic quantitation. Cerebrospinal fluid IgG concentration was determined by means of low-level radial immunodiffusion.[d]

Necropsy procedures—Necropsy procedures were performed as necessary to identify the cause of the underlying complaint; in some instances, this included removal of portions of the brain. The brain and spinal cord were fixed in neutral-buffered 10% formalin and routinely processed for histologic examination of H&E-stained sections. Transverse sections of the thalamus (unilateral), cerebrum (unilateral); cerebellum; brain stem at the level of the rostral colliculus, cerebellar peduncles, mid-medulla, and obex; cervical segments 1, 3, 4, 5, 6, and 7; thoracic segments 1 and 3 and the middle and distal portions; lumbar segments 3, 4, 5, and 6; and 2 sacral sections were examined. Following microscopic examination, fixed brain and spinal cord were transversely sliced at 3- to 5-mm intervals for gross examination, at which time additional sections were selected if lesions were suspected. More detailed examinations were carried out in many horses with neurologic abnormalities in an attempt to identify the underlying disease.

Immunostaining for *S neurona* was performed on CNS

sections having microscopic lesions compatible with EPM. Two immunostaining methods similar to those previously described were used.[17,18] The first method[e] was later modified.[f] Both methods used *S neurona* polyclonal antiserum produced in rabbits and diluted 1:1,600. Cross-reactivity of this polyclonal antiserum has been studied with immunohistochemical methods.[18-21] The antiserum was shown to specifically react with *S neurona* merozoites in equine and murine CNS.[19-21] It failed to cross react with *Sarcocystis falcatula* in avian and opossum tissue,[19] but showed cross-reactivity to *S falcatula* cyst wall in another study.[18] The antiserum has been tested with negative results against *Sarcocystis cruzi* in calf tissue, *Hammondia hammondi* in mouse tissue, and unknown *Sarcocystis* spp in liver from a bear, a chinchilla, a sea lion, a dog,[a,g] and a horse.[22] The serum was shown to not react with *Toxoplasma gondii* and sometimes reacted weakly with *Neospora caninum*.[23]

Data analysis—Horses were considered to have neurologic abnormalities if they had a history of ataxia, incoordination, paresis, paralysis, cranial nerve deficit, muscle atrophy, loss of proprioception, seizures, or convulsions or if they were recumbent because of neurologic disease or had a performance problem or lameness that was suspected to be secondary to EPM. Horses without any neurologic signs were considered to not have neurologic abnormalities. This included horses euthanatized because of acute musculoskeletal injury sustained during racing or training, horses that died suddenly during racing or training, horses that died during anesthesia or after recovery from anesthesia for surgical repair of acute musculoskeletal injuries, and horses euthanatized because of some irreversible condition, such as pleuropneumonia, colitis, laminitis, or chronic musculoskeletal disease.

Horses were classified as positive for EPM if histologic examination revealed CNS lesions compatible with *S neurona* infection and *S neurona* was demonstrated in lesions by means of immunostaining. Horses were classified as suspect for EPM if histologic examination revealed CNS lesions compatible with *S neurona* infection, but results of immunostaining were negative. The mildest degree of inflammation compatible with classification of horses as suspect for EPM was perivascular lymphocytic cuffing of 3 to 4 vessels with > 10 perivascular leukocytes and inflammation of neuropil in at least 1 section (gliosis; infiltrates of mononuclear leukocytes or multinucleated giant cells). For analysis, horses suspect for EPM and horses positive for EPM were combined into the positive for EPM category. Horses were classified as negative for EPM if they did not have consistent histologic lesions or had lesions insufficient to allow them to be classified as suspect. Horses with lesions that would otherwise have caused them to be classified as suspect for EPM were classified as negative for EPM if protozoal parasites other than *Sarcocystis* spp were identified in CNS sections.

Statistical analyses—Sensitivity and specificity were calculated with standard formulas.[24] **Confidence intervals (CI)** for estimates of test sensitivity and specificity were calculated by use of exact binomial methods. Sensitivity and specificity of WBT of CSF were compared between horses with and without neurologic abnormalities with Fisher exact or χ^2 tests. Sensitivity and specificity of WBT of CSF versus serum were compared with McNemar tests. Cerebrospinal fluid IgG concentrations were compared among groups by use of Kruskal-Wallis 1-way ANOVA. Multiple comparisons with a Mann-Whitney test were performed if the overall *P* value was < 0.05. Albumin and IgG concentrations in CSF samples collected at the time of death were compared with values for samples collected after death with the Mann-Whitney *U* test for ranked data. For all analyses, a value of $P < 0.05$ was considered significant.

Results

During the study period, 376 horses were submitted for postmortem examination, all of which had test results for CSF and 373 of which had test results for serum. Of the 373 horses with serum test results, 160 (42.9%) had positive WBT results. Of the 376 horses submitted, 142 did not undergo a postmortem examination. One hundred thirty-three of these had negative WBT results for CSF and 9 had positive results. The remaining 234 horses were included in the study.

Of the 234 horses included in the study, 154 (66%) were Thoroughbreds and 35 (15%) were Quarter Horses. The remainder represented a variety of breeds. One hundred fifty-two (65%) horses were from California race tracks, and 82 (35%) were from the general California horse population.

Results of WBT of CSF were positive for 112 horses, of which 12 were classified as positive for EPM, 8 were classified as suspect for EPM, and 92 were classified as negative for EPM. Results of WBT of CSF were negative for the remaining 122 horses, of which 3 were classified as suspect for EPM and 119 were classified as negative for EPM.

Of the 12 horses classified as positive for EPM, 9 had neurologic abnormalities and 3 did not. For all 12, results of WBT of CSF were positive with high reactivity or positive. The 9 horses with neurologic abnormalities all had a history of clinical signs for ≤ 3 months.

Of the 112 horses for which results of WBT of CSF were positive, 41 had neurologic abnormalities, including 13 classified as positive for or suspect for EPM. Of the remaining 28, 7 had compressive myelopathy, 9 had degenerative myelopathy of undetermined etiology, 5 had other neurologic disease, and 2 had a nonneurologic disease. In the remaining 5, no diagnosis was made. Of the 122 horses for which results of WBT of CSF were negative, 24 had neurologic abnormalities.

Overall, 65 horses had neurologic abnormalities and 169 did not. Sensitivity of WBT of CSF was 87% for horses with neurologic abnormalities and 88% for horses without neurologic abnormalities (**Table 1**); however, CIs were wide. Specificity was 44% for horses with neurologic abnormalities and 60% for horses without. Specificity of WBT of CSF was significantly ($P = 0.04$) higher for horses without neurologic abnormalities than for horses with. Interpretation of positive with weak reactivity as a negative result increased specificity to 72% (95% CI, 58 to 84%) and 85% (95% CI, 79 to 90%) for horses with and without neurologic abnormalities, respectively, but decreased sensitivity to 80% (95% CI, 52 to 96%) and 75% (95% CI, 35 to 97%), respectively.

Sensitivity and specificity of WBT of serum were not significantly different from sensitivity and specificity of WBT of CSF (Table 1), except when all horses were combined. When all horses were combined, specificity of WBT of CSF was significantly ($P = 0.046$) higher than specificity of WBT of serum. Specificity of WBT of serum was significantly ($P = 0.02$) higher for horses without neurologic abnormalities than for horses with.

Questions regarding treatment for EPM were

Table 1—Sensitivity and specificity of western blot testing (WBT) of CSF and serum for diagnosis of equine protozoal myeloencephalitis (EPM) in horses with and without neurologic abnormalities

Sample and group	WBT result*	Postmortem diagnosis of EPM: Positive†	Postmortem diagnosis of EPM: Negative	Sensitivity (%)	Specificity (%)
CSF					
Horses with neurologic abnormalities (n = 65)				87 (60–98)	44 (30–59)
	Positive	13[a]	28		
	Negative	2[b]	22		
Horses without neurologic abnormalities (n = 169)				88 (47–100)	60 (52–68)‡
	Positive	7[c]	64		
	Negative	1[d]	97		
Serum					
Horses with neurologic abnormalities (n = 63)				80 (52–96)	38 (24–53)
	Positive	12[e]	30		
	Negative	3[f]	18		
Horses without neurologic abnormalities (n = 168)				88 (47–100)	56 (48–64)‡
	Positive	7[c]	71		
	Negative	1[d]	89		

For sensitivity and specificity, values in parentheses are 95% confidence intervals.

*All WBT positive result categories combined. †Includes horses positive for EPM and horses suspect for EPM. ‡Specificity for horses without neurologic abnormalities was significantly ($P = 0.04$ for CSF and 0.02 for serum) higher than specificity for horses with neurologic abnormalities.

[a]Includes 9 horses positive for EPM and 4 horses suspect for EPM. [b]Includes 2 horses suspect for EPM. [c]Includes 3 horses positive for EPM and 4 horses suspect for EPM. [d]Includes 1 horse suspect for EPM. [e]Includes 8 horses positive for EPM and 4 horses suspect for EPM. [f]Includes 1 horse positive for EPM and 2 horses suspect for EPM.

answered for 39 horses with neurologic abnormalities and 83 horses without. Twenty-two of 39 horses with neurologic abnormalities had been treated for EPM at some time in their lives. For 17 of these, results of WBT of CSF were positive, and 9 of these 17 had been treated within the 3 months prior to euthanasia. Three of the 9 were positive for EPM. Of the 83 horses without neurologic abnormalities, only 3 had a history of being treated for EPM.

Many horses had slight lymphocytic perivascular cuffing not severe enough to warrant classification as suspect for EPM. For the 189 horses classified as negative for EPM (horses for which a definitive cause of neurologic abnormalities was identified were excluded), those that had CNS inflammation were no more likely to have *S neurona*-specific antibody in CSF than were horses without CNS inflammation (odds ratio, 1.44; $P = 0.22$; **Table 2**).

Eight horses classified as negative for EPM had rare, difficult to detect, intracytoplasmic protozoa in CNS capillary endothelium or foci of gliosis, but results of immunohistochemical stains for *S neurona* were negative. Thin-section electron micrography of 1 specimen revealed merozoites in a parasitophorous vacuole. For 3 of these 8 horses, WBT of CSF yielded positive results with weak reactivity.

Concentration of IgG in CSF was determined for 108 horses for which results of WBT of CSF were positive (19 classified as positive or suspect for EPM, 27 with neurologic abnormalities classified as negative for EPM, and 62 without neurologic abnormalities classified as negative for EPM) and 34 horses for which results of WBT of CSF were negative (all 34 were classified as negative for EPM). Median IgG concentration was close to the upper reference limit for all 4 groups (reference range, 3 to 9 mg/dL). Median CSF IgG concentration for horses negative for EPM for which results of WBT of CSF were also negative (7 mg/dL; range, 3 to 26 mg/dL) was significantly ($P < 0.05$) lower than median concentration for horses with neurologic abnormalities that were negative for EPM and for which results of WBT of CSF were positive (10 mg/dL; range, 4 to 56 mg/dL). However, values for horses with EPM (9 mg/dL; range, 4 to 64 mg/dL) and for horses without neurologic abnormalities that were negative for EPM and for which results of WBT of CSF were positive (9 mg/dL; range, 4 to 31 mg/dL) were not significantly different from values for the other groups.

Table 2—Cross-tabulation of results of WBT of CSF for *Sarcocystis neurona*-specific antibodies and histologic examination of the CNS for perivascular cuffing in 189 horses negative for EPM

CNS Perivascular cuffing	Results of WBT of CSF: Positive	Results of WBT of CSF: Negative	Total
Present*	46	48	94
Absent	38	57	95
Total	84	105	189

*Perivascular cuffing was identified but was not severe enough to warrant classification of the horse as suspect for EPM.

Horses for which a definitive cause of neurologic abnormalities was identified were excluded; horses with protozoal CNS infection by organisms other than *Sarcocystis* spp and horses with degenerative myelopathy were included.

Odds ratio = 1.44; $P = 0.22$.

Discussion

Results of the present study suggest that sensitivity of WBT of CSF from horses as a test for *S neurona* infection was high, regardless of whether horses did or did not have neurologic abnormalities. This suggests that the test is useful in ruling out EPM when the prevalence of infection is low or moderate. On the other hand, specificity of WBT of CSF, the ability to identify horses truly free from EPM, was low, although

it improved when positive with weak reactivity results were interpreted as negative. Nevertheless, these findings indicate that WBT should be used only as an aid in the diagnosis of neurologic disease in horses and should be performed in conjunction with a thorough diagnostic evaluation. In addition, test results should be interpreted in light of knowledge of disease prevalence and the test's limitations. The value of a negative test result far outweighs the usefulness of a positive result. It is likely that the high percentage of horses free from EPM with positive WBT results for CSF is a result of high analytic sensitivity of the WBT, possibly in combination with in situ production of *S neurona*-specific antibodies in exposed horses.

In the present study, results of postmortem examination were used as the gold standard against which results of the WBT were compared. Unfortunately, the true sensitivity of postmortem examination for detection of CNS infection with *S neurona* is unknown, because it is impractical to examine the entire CNS. However, the probability of detecting inflammation histologically is high, because distribution of EPM lesions is generally multifocal. Thus, small foci of inflammation are likely to be detected, even though a major focus may be missed. In the present study, such an animal would very likely have been classified as suspect for EPM. Detailed gross examination of the CNS did not identify any cases of EPM in which the diagnosis had not already been made histologically.

In previous studies[25,26] of horses with neurologic abnormalities in which the diagnosis was confirmed at postmortem examination, sensitivity of WBT of CSF was reported to be ≥ 89%, which is similar to the 87% sensitivity found in the present study. However, CIs for sensitivity estimates were wide in the present study because of the small number of horses with EPM. Inclusion of horses suspect for EPM, which in fact may not have had EPM, with horses positive for EPM introduced the possibility of misclassification bias, suggesting the sensitivity could be higher than 87%. Sensitivity was similar for horses with and without neurologic abnormalities, indicating that whether the WBT would correctly identify horses with EPM did not differ between horses with neurologic disease and horses that were clinically normal. However, the lack of neurologic examination of horses classified as not having any neurologic abnormalities may have introduced a small bias in this comparison. No horse with a WBT result of positive with weak reactivity or negative was found to have, by immunohistochemical staining, *S neurona* in the CNS.

Specificity of WBT of CSF in the present study was 44% for horses with neurologic abnormalities and 60% for horses without. These values indicated that 56% of horses negative for EPM that had neurologic abnormalities and 40% of horses negative for EPM that did not have neurologic abnormalities had *S neurona*-specific antibody in the CSF. Specificity estimates in the present study were lower than those reported in previous studies (> 90%[25] and 77%[26]) of horses with neurologic abnormalities in which postmortem examination was used as the gold standard. Some of this difference may be related to different interpretations of weak-positive test results, as the previous studies did not indicate how a weak-positive test result was interpreted. The sampling schemes also likely differed, but the previous studies, which were retrospective studies, did not provide information on the sample selection process.

Specificity was substantially improved in the present study by interpreting a positive with weak reactivity test result as a negative result, although this slightly reduced the sensitivity. With this test interpretation, the likelihood of a false-positive result for horses with neurologic abnormalities was reduced by more than a third, and the loss of sensitivity did not result in misidentification of any horses positive for EPM. This suggests that positive with weak reactivity results for WBT of CSF are better interpreted as negative or as inconclusive (ie, equivocal or borderline), as suggested by others.[8,27,c] It is possible that positive with weak reactivity results for WBT of CSF are normal in seropositive horses.

The lower specificity for horses with neurologic abnormalities, compared with horses without neurologic abnormalities, in the present study was probably, at least in part, a result of increased permeability of the blood-brain barrier in some horses with neurologic diseases other than EPM, although this was not reflected by differences in CSF IgG concentration. Alternately, there may have been continued in situ antibody production following resolution of EPM in some horses with chronic neurologic abnormalities that were classified as negative for EPM. The question of whether some horses have antibodies in the CNS because they had EPM at 1 time in their lives is very difficult or impossible to answer by pathologic examination. Therefore, the possibility that *S neurona* infection in the past was the cause of neurologic disease in 14 horses negative for EPM that had positive WBT results for CSF and in which another cause could not be identified cannot be totally excluded.

Responses to questionnaires in the present study indicated that approximately half the horses with neurologic abnormalities that had positive WBT results for CSF, including horses with positive with weak reactivity results, had been treated for EPM. It is known that treatment makes it more difficult to detect *S neurona* in tissues. Nevertheless, if these horses in fact had EPM, at the time of necropsy one would expect detectable CNS inflammation sufficient to classify them as suspect for EPM.

Other workers have reported a high percentage (31%) of horses without neurologic abnormalities that had positive WBT results for CSF.[11] In the present study, 40% of horses negative for EPM that did not have any neurologic abnormalities had positive CSF test results. Positive test results in these horses may have been caused by previous *S neurona* exposure. Eighty-seven percent of horses without neurologic abnormalities that had positive serum test results also had positive CSF test results. This could indicate subclinical infection of the CNS in most exposed horses, which would agree with the mild elevation in CSF IgG concentration in such horses. Alternately, even with an intact blood-brain barrier, antibody may be found in the CSF, as evidenced by a low concentration of IgG in

normal CSF. It is possible that WBT, because it is so sensitive, is able to detect this low concentration of *S neurona*-specific antibody in CSF from healthy *S neurona*-exposed horses. The high sensitivity of the WBT was demonstrated by Miller et al,[8] who reported that for CSF that yielded negative WBT results, contamination with seropositive blood at a concentration of 1 to 10 ppm resulted in detectable antibody in the CSF. Finally, it is possible that although precautions were taken to prevent blood contamination of CSF in the present study, these precautions were not sufficiently stringent.

For antemortem diagnosis of neurologic disease, clinicians need to know the probability that an animal with a positive test result truly has the disease and the probability that an animal with a negative test result is truly disease free. These probabilities are the predictive values, which are calculated from sensitivity, specificity, and disease prevalence.[24] For this reason, it is imperative to consider disease prevalence in the population under consideration when interpreting test results. In the present study, prevalence of EPM among horses with neurologic abnormalities was 23% (15/65), resulting in a positive predictive value of 32% (13/41). The prevalence of EPM is lower in horses without neurologic abnormalities; therefore, a much lower positive predictive value would be expected for this population.

In the present study, sensitivity of WBT of serum was 80% for horses with neurologic abnormalities and 88% for horses without, indicating that testing of serum also had a low false-negative rate. This makes sense biologically, as initiation of a humoral immune response is expected before *S neurona* enters the CNS. Thus, testing serum may be useful in ruling out EPM in horses, regardless of whether they do or do not have neurologic abnormalities. However, in areas of high disease prevalence, testing of CSF from horses with neurologic abnormalities, even horses that are seronegative, may be indicated, especially if the disease is acute and other causes of neurologic disease have been ruled out.

Infection with *S neurona* was subclinical in 3 of the 12 horses in the present study positive for EPM. This is not surprising, because recognition of neurologic abnormalities varies greatly with the observer and day-to-day handling of the animal. In addition, a neurologic examination was not performed in any of the horses that did not have a history of neurologic abnormalities; thus, some may in fact have had unrecognized neurologic signs. However, there is no doubt subclinical infection occurs, and expression of clinical signs depends on location and progression of lesions. Two of these 3 horses had locally extensive EPM lesions, and all 3 horses were in race training.

Determination of CSF IgG concentration was not helpful in interpreting a positive WBT result in the present study, as shown by the lack of significant differences among CSF IgG concentrations for horses positive for EPM, horses negative for EPM that had neurologic abnormalities, and horses negative for EPM that did not have neurologic abnormalities. On the other hand, horses without EPM that did not have neurologic abnormalities and had negative WBT results for CSF had significantly lower CSF IgG concentrations than did horses without EPM that did have neurologic abnormalities and had positive WBT results for CSF. One would have expected all horses with positive WBT results for CSF to have significantly higher CSF IgG concentrations than horses with negative results, but this was not the case in the present study. It is probable that damage to the blood-brain barrier, immune response to infection with other agents, or test variability resulted in a wide range of CSF IgG concentrations and reduced the usefulness of measuring IgG concentration.

Collection of CSF was delayed in 19 of the 112 horses (17%) in which results of WBT of CSF were positive. Leakage of plasma into CSF is expected as the blood-brain barrier deteriorates after death. However, in all of these horses, CSF was collected within 3.5 hours after death, and we found little evidence of leakage associated with this delay. In particular, sensitivity was slightly higher and specificity was markedly higher for the 65 horses from which samples were collected after death, compared with values for horses from which samples were collected at the time of euthanasia. In addition, CSF albumin and IgG concentrations were not significantly different between 12 horses without EPM that had no neurologic abnormalities and negative CSF WBT results from which samples were collected at the time of death and 8 similar horses from which samples were collected after death, although numbers of animals may have been too low for reliable statistical evaluation.

Median CSF RBC count for horses in the present study with positive WBT results was 6.5/μL (range, 0 to 100/μL). Miller et al[8] suggested that the RBC count is the most accurate indicator of iatrogenic CSF contamination and demonstrated that contamination of CSF with blood resulting in as few as 8 RBC/μL of CSF may give a positive WBT result in horses with moderately strong seropositivity. However, such slight contamination with blood does not consistently result in false-positive results. In the present study, 14 horses that were moderately or strongly seropositive and had a median CSF RBC count of 20/μL (range, 0 to 450/μL) had negative WBT results for CSF. Further, for horses in which results of WBT of serum and CSF were positive, a CSF RBC count < 8/μL (n = 50) was not associated with fewer false-positive CSF WBT results than a count ≥ 8/μL (51). Overall, we consider a cutoff of 100 RBC/μL of CSF adequate to maintain a low number of false-positive results for WBT of CSF.

Many horses not classified as positive or suspect for EPM in the present study had slight lymphocytic perivascular cuffing that was statistically unrelated to whether antibody was detected in the CSF (Table 2). It is possible that in some of these horses, this inflammation was caused by infection with *S neurona*. However, infection with protozoa other than *Sarcocystis* spp or other CNS infection was the likely cause of the inflammation in others. Eight horses negative for EPM that had slight multifocal nonsuppurative perivascular cuffing had intracytoplasmic protozoa that were determined to not be *S neurona* on the basis of negative immunostaining results and location of merozoites in a parasitophorous vacuole. These 8 horses were classi-

fied as negative for EPM even though the type of inflammation could not with certainty be distinguished from that caused by *S neurona*. By extension, some horses in the present study that were classified as suspect for EPM may have been falsely classified, in that the parasite responsible for CNS inflammation was not detected.

[a]Barr BC, California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California, Davis, Calif: Personal communication, 1997 (lack of confirmation of EPM at necropsy) and 2002 (antiserum cross reactivity).

[b]Sielman ES, Sweeney CR, Habecker P. Correlation of antemortem *Sarcocystis neurona* testing with postmortem findings (abstr). *J Vet Int Med* 1997;11:105.

[c]Equine Biodiagnostics Inc, Lexington, Ky.

[d]VMRD Inc, Pullman, Wash.

[e]ABC method,Vector, Burlingame, Calif.

[f]Envision method, Dako Corp, Carpinteria, Calif.

[g]Specimens provided by JP Dubey, Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Sciences Institute, USDA-ARS, BARC-East, Beltsville, Md.

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