

Utility of 2 Immunological Tests for Antemortem Diagnosis of Equine Protozoal Myeloencephalitis (*Sarcocystis neurona* Infection) in Naturally Occurring Cases

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Background: Antemortem diagnosis of equine protozoal myeloencephalitis (EPM) is challenging. Limited information is available regarding a commercial test (surface antigen 1 [SAG-1] ELISA). Performance of another commercial test (indirect fluorescent antibody test [IFAT]) using samples from an independent group has not been well described.

Hypothesis/Objectives: The primary goal was to evaluate the SAG-1 ELISA and IFAT using naturally occurring EPM cases. A secondary goal was to obtain more information regarding clinical presentation.

Animals: Hospital cases were admitted over 20 months and classified into 4 groups. Confirmed positive cases (n = 9) had asymmetric or multifocal neurologic deficits or both and postmortem lesions consistent with EPM. Confirmed negative cases (n = 17) had variable clinical signs and postmortem lesions consistent with another neurologic disease (or no lesions). Suspected positive cases (n = 10) had asymmetric or multifocal deficits or both, marked improvement after treatment for EPM, and other likely diseases excluded. Suspected negative cases (n = 29) had orthopedic disease and no neurologic deficits.

Methods: Results of immunological testing (SAG-1 ELISA and IFAT on serum or cerebrospinal fluid [CSF] or both), neurologic examinations, CSF analyses, and postmortem examinations were analyzed retrospectively.

Results: SAG-1 ELISA sensitivity was 12.5% (95% CI, 1.6–38.4) and specificity was 97.1% (95% CI, 84.7–99.9) using serum. IFAT sensitivity was 94.4% (95% CI, 72.7–99.9) and specificity was 85.2% (95% CI, 66.3–95.8) using serum; sensitivity was 92.3% (95% CI, 64.0–99.8) and specificity was 89.7% (95% CI, 72.7–97.8) using CSF.

Conclusions and Clinical Importance: Low sensitivity of the SAG-1 ELISA limited its usefulness for antemortem diagnosis of EPM in this patient population.

Key words: ELISA; Indirect fluorescent antibody test; Neurologic; Western blot.

Equine protozoal myeloencephalitis (EPM) is a frequently diagnosed neurologic disease of horses in North America. The most common etiology is *Sarcocystis neurona* infection of the central nervous system, although infection with other protozoal species, such as *Neospora hughesi*, also has been documented.^{1,2} Antemortem diagnosis of EPM is always presumptive, because definitive diagnosis requires postmortem confirmation of *S. neurona* infection by microscopic identification, immunohistochemistry, culture, or polymerase chain reaction (PCR).³ Antemortem diagnosis is most accurate if 3 criteria are fulfilled: presence of compatible clinical signs that are referable to the central nervous system, exclusion of other common neurologic or musculoskeletal diseases, and confirmation of exposure to *S. neurona* by immunological testing.³

Several immunological tests have been developed for use in the diagnosis of EPM. The oldest and most well-established test is the Western blot (WB), which is a semi-quantitative test for antibodies against merozoite lysate.⁴ Initial estimates of its sensitivity and specificity were high

Abbreviations:

| | |
|-------|------------------------------------|
| AUC | area under the curve |
| CSF | cerebrospinal fluid |
| EHV-1 | equine herpesvirus 1 |
| EPM | equine protozoal myeloencephalitis |
| IFAT | indirect fluorescent antibody test |
| PCR | polymerase chain reaction |
| ROC | receiver operating characteristic |
| SAG-1 | surface antigen 1 |
| WB | western blot |
| WNV | West Nile virus |

(sensitivity 89% and specificity 71% on serum; sensitivity 89% and specificity 89% on cerebrospinal fluid [CSF]).⁵ Other data suggested that WB specificity was lower because of anti-*S. neurona* antibody production in clinically normal horses.⁶ A later study supported this suggestion, estimating WB specificity to be 38% for serum and 44% for CSF from horses with neurologic disease.⁷ Subsequently, an indirect fluorescent antibody test (IFAT) was developed and evaluated by Duarte et al,^{8,9} with estimated sensitivities of 83% (serum) and 100% (CSF) and specificities of 97% (serum) and 99% (CSF).⁹ When compared directly with the WB, the IFAT sensitivity was equivalent but its specificity was higher.⁸ The IFAT is a quantitative test that yields a titer result. The aforementioned estimates were obtained after test optimization with cut-off values of 1:80 (serum) and 1:5 (CSF). Also, the surface antigen 1 (SAG-1) ELISA, which is based on an immunodominant SAG of *S. neurona*, has been commercially introduced. The initial description of this test in the literature did not provide sensitivity and specificity estimates.¹⁰ A different group

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evaluated the use of 4 independently developed ELISAs based on the 4 major SAGs of *S. neurona* (SAGs 1–4) and found that the SAG-1 ELISA had the lowest sensitivity (68%) and specificity (71.4%) of all the SAG-1 ELISAs.¹¹

The primary objective of this study was to evaluate further the more recently developed immunological tests for EPM in a clinically relevant manner. Test results on samples from horses that were presented to a referral hospital in the mid-Atlantic region were utilized to assess performance of the SAG-1 ELISA and IFAT in the diagnosis of EPM. Additionally, descriptive information regarding the clinical presentation of these cases was collected to improve understanding of this complicated disease.

Materials and Methods

A retrospective study was performed using horses that were presented to the internal medicine or sports medicine sections of the George D. Widener Hospital for Large Animals for evaluation of gait abnormalities or possible neurologic disease from August 2007 to April 2009. For initial inclusion in the study, horses had to be considered EPM suspects, have neurologic and lameness examinations, and be tested for antibodies against *S. neurona* using serum, CSF or both. Final inclusion depended on the availability of sufficient information to classify the horse into 1 of 4 groups: confirmed positive for EPM, suspected positive for EPM, confirmed negative for EPM, and suspected negative for EPM.

Confirmed positive cases were defined as nonsurviving horses that had neurologic signs consistent with asymmetric or multifocal central nervous system lesions or both and had postmortem lesions consistent with EPM. Neurologic necropsies were performed in standard fashion by university pathologists, who determined cases to be consistent with EPM based on the presence of multifocal or focally extensive lymphocytic, lymphohistiocytic, or lymphoplasmacytic myelitis, encephalitis, or both. Immunohistochemistry, PCR testing or both for *S. neurona* and other neurologic diseases were utilized at the discretion of the pathologists to confirm a diagnosis but were not performed in every case.

Suspected positive cases were defined as surviving horses that had neurologic signs consistent with asymmetric or multifocal central nervous system lesions or both for which other likely differential diagnoses were excluded. Other neurologic diseases in the mid-Atlantic region for which recovery is possible include central nervous system trauma, cervical vertebral malformation or stenotic myelopathy, temporohyoid osteoarthropathy, West Nile virus (WNV) infection, equine herpesvirus (EHV-1) myeloencephalopathy, neosporosis, and neuroborreliosis. Therefore, diagnostic tests to exclude these diseases included skull or spinal imaging or both (radiography, myelography, nuclear scintigraphy, ultrasonography, endoscopy, magnetic resonance imaging), immunological testing (for WNV, neosporosis, and neuroborreliosis), and PCR (for EHV-1). The attending clinician for each case determined a neuroanatomical diagnosis, likely differential diagnoses, and appropriate ancillary diagnostic tests. Consequently, not all diseases and diagnostic tests were considered and performed for each case. Finally, suspected positive cases also were required to display marked improvement after specific treatment for EPM as assessed by a veterinarian. All horses received an FDA-approved treatment for EPM (ponazuril),^a although in some cases extra-label dosing was used for part or all of the treatment course, and in some cases supplemental treatment was administered.

Confirmed negative cases were defined as nonsurviving horses that had signs of either central nervous system or neuromuscular

disease. These cases either had postmortem lesions consistent with a disease other than EPM or had no central nervous system lesions despite thorough examination.

Suspected negative cases were defined as surviving horses that could be subcategorized into 1 of 2 groups. The first subgroup consisted of horses with no neurologic deficits detected during a full neurologic examination that were diagnosed with orthopedic disease. The second subgroup consisted of horses exhibiting neurologic deficits consistent with a focal or symmetrical central nervous system lesion that were presumptively diagnosed with a disease other than EPM based on ancillary diagnostic testing. One last criterion utilized only for this second subgroup of suspected negative cases was that these horses with neurologic disease were required to have a negative EPM WB test on CSF.

As part of the diagnostic testing for every case, serum, CSF, or both were collected routinely for commercial EPM testing at 2 different laboratories.^{b,c} Results from both laboratories were returned as titers. For SAG-1 ELISAs on serum, the minimum reported titer was 1:16, with results <1:16 considered negative by the laboratory. However, at the time of testing, the laboratory recommended using a cut-off titer of 1:32, such that horses with titers \geq 1:32 should be considered positive, and horses with titers <1:32 should be considered negative. For IFATs on serum, the minimum reported titer was 1:10, with results <1:10 considered negative by the laboratory. At the time of testing, results were reported as post-test probabilities, but previous work by the laboratory had determined 1:80 to be the optimal cut-off titer, such that horses with titers <1:80 should be considered negative.⁹ IFATs on CSF were performed at 1 dilution (1:5) with results <1:5 considered negative and results \geq 1:5 considered positive.

Receiver operating characteristic (ROC) curves were constructed by plotting sensitivity versus 1-specificity (false-positive rate) for various potential cut-off points for the antibody tests. For the purposes of this analysis, confirmed and suspected positive cases were grouped together as were confirmed and suspected negative cases. The area under the curve (AUC) was calculated using the trapezoidal rule, and the standard error of the AUC and 95% confidence limits were calculated.¹² The difference in the AUC for the ROC curve generated using the IFAT results and the AUC for the ROC curve generated using the SAG-1 results was compared by calculating a *z* statistic adjusted for correlation because of the use of the same patients for both tests.¹³

For the purposes of this study and determination of sensitivities and specificities, results were dichotomized into positive and negative results. Using ROC analysis, cut-off points that optimized sensitivity and specificity for each test were selected. Descriptive statistics (sensitivity, specificity, and 95% exact binomial confidence intervals) were performed by standard methods and statistical software.^d Finally, Fisher's exact test was used to assess the association between neurolocalization and survival.

Results

Initially, 107 horses met the criteria for inclusion. However, insufficient information was available to categorize 42 horses, therefore, 65 horses were included in final analysis and were classified as follows: 9 confirmed positive, 10 suspected positive, 17 confirmed negative, and 29 suspected negative (of which 19 horses were lame and 10 horses were neurologic).

Information obtained during clinical evaluation is summarized in Tables 1 and 2. Table 1 presents the main neuroanatomical diagnosis for each horse that displayed neurologic signs. When a horse with neurologic signs displayed signs consistent with lesions in multiple

Table 1. Main neuroanatomical diagnosis.

| EPM Classification | Cranial Fossa (Forebrain) | Caudal Fossa (Cerebellum, Brainstem) | C1-T2 | T3-L3 | L4-S5 |
|--------------------|---------------------------|--------------------------------------|-------|-------|-------|
| Confirmed positive | 2 | 5 | 1 | 0 | 1 |
| Suspected positive | 0 | 2 | 3 | 3 | 2 |
| Total positive | 2 | 7 | 4 | 3 | 3 |
| Confirmed negative | 4 | 2 | 5 | 0 | 3 |
| Suspected negative | 4 | 0 | 4 | 0 | 2 |
| Total negative | 8 | 2 | 9 | 0 | 5 |

anatomical regions or had postmortem confirmation of lesions in multiple anatomical regions, the region associated with the most severe signs or lesions was selected as the main neuroanatomical diagnosis. Caudal fossa or brainstem localization was most common for positive horses, whereas cervical and cranial fossa or forebrain localizations were most common for negative horses. Intracranial signs were more common in nonsurviving (7/9) than in surviving (2/10) positive horses. The association between survival and lesion localization was significant ($P = .023$). Table 2 presents results obtained from horses that had full CSF cytology performed. For both positive and negative horses, measured variables (including nucleated cell count, total protein, and microscopic evaluation with nucleated cell differential analysis) most often were within normal limits. However, microscopic evaluation in 4 positive cases revealed reactive lymphocytes, which were not noted in any of the negative cases.

For the IFAT, the AUC of the ROC curve was 0.90 (95% confidence limits, 0.81–1.00), which indicates a high degree of discrimination. For the SAG-1 ELISA, the AUC of the ROC curve was 0.46 (95% confidence limits, 0.27–0.63), suggesting a nondiscriminatory test. The difference in AUC for the 2 tests was significant ($P < .01$) (Fig 1). Using ROC analysis, optimal IFAT sensitivity and specificity were obtained at a cut-off titer of 1 : 80, and optimal SAG-1 sensitivity and specificity were obtained at a cut-off titer of 1 : 32.

Results of immunological testing are summarized in Table 3. Estimated sensitivities and specificities were similar regardless of whether both confirmed and suspected cases were used in analysis (top half of table) or whether only confirmed cases were included (bottom half of

Table 2. CSF analysis.

| EPM Classification | WNL | Increased NCC | Increased TP | Reactive Lymphs |
|--------------------|-----|---------------|--------------|-----------------|
| Confirmed positive | 6 | 0 | 1 | 2 |
| Suspected positive | 3 | 1 | 2 | 2 |
| Total positive | 9 | 1 | 3 | 4 |
| Confirmed negative | 11 | 2 | 2 | 0 |
| Suspected negative | 10 | 0 | 0 | 0 |
| Total negative | 21 | 2 | 2 | 0 |

WNL, CSF analysis was within normal limits; Increased NCC, increased nucleated cell count (>5 nucleated cells/ μ L CSF); Increased TP, increased total protein (>90 mg/dL CSF); Reactive lymphs, cytologist commented that reactive lymphocytes were present.

table). Using the cut-off titers of 1 : 32 for the SAG-1 ELISA on serum and 1 : 80 for IFAT on serum, sensitivity of the SAG-1 ELISA was low at 12.5%, whereas sensitivity of the IFAT was high (94.4% on serum and 92.3% on CSF). Specificity of the SAG-1 ELISA on serum (97.1%) was higher than the IFAT on serum (85.2%). IFAT specificity improved only slightly when CSF rather than serum was tested (89.7% versus 85.2%).

Discussion

EPM is 1 of the few equine neurologic diseases for which medical treatments are available and recoveries (partial to complete) are frequently observed. Therefore, diagnostic tests with high sensitivities are desirable to avoid misdiagnosing cases as negative and missing treatment opportunities. We found the sensitivity of the SAG-1 ELISA to be unacceptably low at 12.5%. There are no published estimates of sensitivity and specificity for this test, although previously it was reported to be positive for 85% of horses with a presumptive diagnosis of EPM using 1 : 100 as a positive titer and also to be positive for 24% of presumptively normal horses.¹⁰ An rSnSAG1 ELISA created and evaluated in a study by different investigators yielded a sensitivity of 68% and

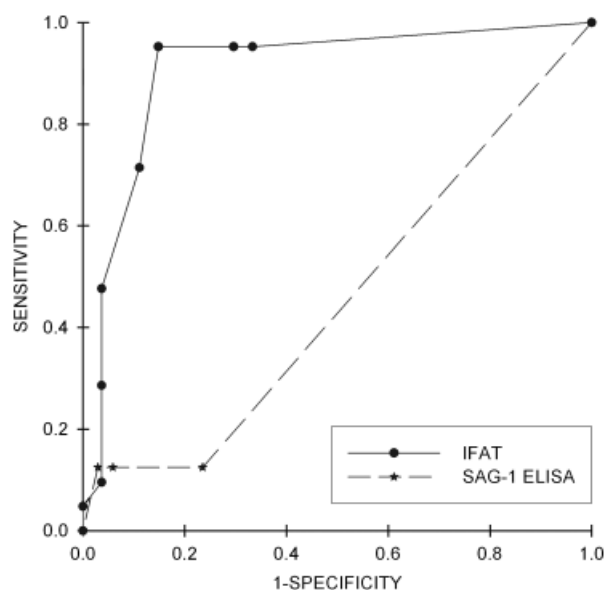


Fig 1. Receiver operating characteristic curves for indirect fluorescent antibody test (IFAT) and surface antigen 1 (SAG-1) ELISA tests on serum for diagnosis of equine protozoal myeloencephalitis.

Table 3. EPM test accuracy.

| Test | Sensitivity ^a (%) | 95% CI Lower Upper | | Specificity ^a (%) | 95% CI Lower Upper | |
|--------------------------------------|------------------------------|--------------------|------|------------------------------|--------------------|------|
| IFAT on serum (n = 45) ^b | 94.4 | 72.7 | 99.9 | 85.2 | 66.3 | 95.8 |
| SAG-1 on serum (n = 50) ^b | 12.5 | 1.6 | 38.4 | 97.1 | 84.7 | 99.9 |
| IFAT on CSF (n = 42) ^b | 92.3 | 64.0 | 99.8 | 89.7 | 72.7 | 97.8 |
| IFAT on serum (n = 20) ^c | 87.5 | 47.4 | 99.7 | 83.3 | 51.6 | 97.9 |
| SAG-1 on serum (n = 22) ^c | 12.5 | 0.3 | 52.7 | 100 | 80.7 | 100 |
| IFAT on CSF (n = 19) ^c | 100 | 60.7 | 100 | 84.6 | 54.6 | 98.1 |

^aNote that sensitivities and specificities were calculated using cut-off values of 1 : 80 for IFAT on serum, 1 : 32 for SAG-1 ELISA on serum, and 1 : 5 for IFAT on CSF.

^bValues for top half of table were calculated using results from both confirmed and suspected cases.

^cValues for bottom half of table were calculated using results from confirmed cases only.

specificity of 71%.¹¹ The 12.5% sensitivity documented here is markedly lower than indicated in both other reports and could result from several factors, including parasite strain variation, variable equine immune response, and test error. The first explanation appears to be the most probable. Recent work has shown that *S. neurona* merozoites express a gene family of immunogenic SAGs, designated SnSAGs 1–5, and that some *S. neurona* isolates do not express SnSAG1, often expressing the mutually exclusive SnSAG5.^{14,15} The prevalence of various *S. neurona* strains has not yet been determined in relationship to different geographic regions of North America. However, the likely reason for the very low sensitivity in our case population is that *S. neurona* strains lacking SnSAG1 predominate in the mid-Atlantic region. Until the geography of strain diversity is well-known, use of a test that only measures antibodies against SnSAG1 or SnSAG5 cannot be recommended, as false-negative results would be likely.

In contrast, the IFAT had a high sensitivity of 94.4% on serum and 92.3% on CSF. Although it is difficult to compare results from different investigators because of varying study methodologies, these sensitivities are very similar to those previously published for both the IFAT and WB.^{4–9} Notably, in the present study only 2 horses with EPM had negative IFAT results, and both horses had been presented with peracute signs. One horse, a confirmed case, was initially seronegative but positive on CSF. Repeated serology 1 week later showed seroconversion (from <1 : 10 to 1 : 160). Conversely, another horse (presumptive case) was initially seropositive but negative on CSF. Repeated CSF analysis approximately 2 weeks later was positive. These cases highlight the importance of retesting horses with acute clinical signs compatible with EPM if initial results are negative, regardless of whether serum or CSF was tested. If sero- or CSF conversion occurs, it offers strong support for a diagnosis of EPM. Although none of these clinical cases were initially negative on both serum and CSF, this scenario is theoretically possible as well.

One of the main challenges in the diagnosis of EPM is seroconversion against *S. neurona* in clinically normal horses, which lowers the specificity of all current antibody-based diagnostic tests. Although initial estimates of WB specificity were moderately high (71% on serum and 89% on CSF), more recent estimates were lower (38% on

serum and 44% on CSF for horses with neurologic signs).^{4,7} Likewise, our estimates of IFAT specificity (85.2% on serum and 89.7% on CSF) are lower than those first reported (97% on serum and 99% on CSF using naturally infected horses).⁹ Our estimates were obtained using cut-off titers identical to those used previously and therefore the lower estimated specificities may be because of our patient population, with potentially more clinically normal horses demonstrating sero- and CSF positivity.

One frequently discussed issue in EPM diagnostic tests is whether serial serum and CSF testing is beneficial, or whether serum testing alone is sufficient. When paired serum and CSF samples were tested using WB, moderate agreement was present, with a κ statistic of 0.47.¹⁶ In that study, 29% (33/112) of CSF samples from seropositive horses were negative, leading the authors to support CSF testing of seropositive horses. When the usefulness of CSF testing was assessed using IFAT and a computer model, investigators concluded that testing CSF alone was more useful than testing serum alone, but that testing CSF after a serum test had limited usefulness and usually did not change the posttest probability (of the horse having EPM) by very much.¹⁷ In the current study, only 3/34 (9%) clinical cases had disparate serum and CSF IFAT results when a serum cut-off value of 1 : 80 was utilized, with 2/19 (10%) seropositive horses having negative results on CSF. Two of the 3 horses have already been discussed (both peracute cases of EPM, 1 of which was initially seronegative but positive on CSF and the second of which was initially seropositive but negative on CSF). The other case was a presumptive negative case that had a negative CSF result with a positive serum titer of 1 : 160. These findings appear to agree with the aforementioned conclusions regarding the IFAT that CSF testing alone is more accurate than serum testing alone, but that testing CSF after testing serum has limited usefulness overall if a cut-off serum titer of 1 : 80 is utilized. However, when dealing with clinical cases, particularly those horses demonstrating acute signs, testing both serum and CSF will result in the fewest misclassifications. Furthermore, CSF analysis is an important diagnostic adjunct and may support or exclude the presence of other central nervous system diseases such as EHV-1 myeloencephalopathy or bacterial meningitis, which could manifest as sole or comorbid conditions.

Another frequently discussed issue is the effect of blood contamination on CSF tests. For the WB, CSF contamination with a small amount of moderately or strongly immunoreactive blood, corresponding to as few as 8 RBCs/ μ L CSF, may cause false-positive CSF results.¹⁸ There was no effect on IFAT results when CSF was contaminated with moderately or strongly immunoreactive blood at up to 10,000 RBCs/ μ L.¹⁹ Although ideally all CSF samples included in this study would have been free of blood contamination, this scenario was not possible in a teaching hospital where clinicians of varying experience were collecting CSF samples, usually via the lumbosacral space in standing, sedated horses. Therefore, none of the CSF samples were excluded for blood contamination. The median number of RBCs/ μ L CSF in this study was 23, with a range of 0–150,000. Only 2 samples, however, had RBC numbers $>10,000/\mu$ L and were likely to have enough contamination to affect CSF IFAT results. One of these samples, from a confirmed positive horse, was positive despite the fact that a serum sample collected on the same day was negative. Therefore, blood contamination was unlikely to cause a false-positive CSF result. The other sample, from a confirmed negative horse, was positive as was a serum sample collected on the same day. If this patient's sample is excluded, the specificity of the IFAT on CSF would increase slightly, from 89.7% to 92.9% (all cases), or from 84.6% to 91.7% (confirmed cases only).

Aside from assessments of test accuracy, several other interesting results were observed during this study. Although EPM is by its nature usually a multifocal disease, the most predominant neurologic signs in the majority of cases were localized to the brainstem. These signs included dysphagia, changes in vocalization (laryngeal or pharyngeal dysfunction), vestibular dysfunction, facial paralysis, and changes in mentation (depression to obtundation to stupor). Although signs of brainstem disease have been attributed to EPM in the past,³ they were more frequent in this case population than previously reported. This difference could result from the referral nature of our practice, in that horses with brainstem disease tend to have more severe or debilitating disease which prompts or necessitates referral, or could potentially be related to *S. neurona* strain variation. Although strain-related pathogenicity and neuroanatomic predilections have not yet been described, they may exist. The positive horses with intracranial signs were substantially more likely to be euthanized than the positive horses with spinal cord signs, which may indicate either that the presence of intracranial signs and subsequent debilitation often leads to euthanasia or that horses develop intracranial signs when they are unable to successfully clear the central nervous system protozoal infection (possibly because of variations in immune response), and that these horses are unlikely to recover from EPM. The negative horses in this study often showed signs of cervical spinal cord or forebrain (prosencephalic) disease. Horses with cervical spinal cord disease were most often diagnosed with vertebral stenosis or malformation, and horses with forebrain disease were most often diagnosed with acquired epilepsy or head trauma.

CSF frequently was analyzed cytologically during diagnostic evaluation of these horses, and results were often normal, in keeping with previous findings.³ However, in cytological analysis of 4 samples (all from EPM positive horses) moderate to large numbers of reactive lymphocytes containing azurophilic granules were seen, despite a normal nucleated cell count. Although no immunostaining was performed, these reactive lymphocytes were considered to be most consistent with CD8+ T cells, likely indicating a cell-mediated immune response. Although reactive lymphocytes are more commonly observed with viral or rickettsial infections, they appear in these cases to be stimulated by *S. neurona* infection. Therefore, the presence of reactive lymphocytes should not lead clinicians away from EPM and in fact may be suggestive of the disease.

Historically, EPM studies have been difficult to perform because of the absence of a reliable infection model and the lack of an antemortem diagnostic gold standard. Previous studies evaluating diagnostic tests often have utilized a combination of samples from both naturally and experimentally infected horses, and many of these samples are used repeatedly in assessments.^{7–9} The current study is unique in that it utilized a new sample set from clinical cases originating from the mid-Atlantic region. As these samples were not used in the development or optimization of either analyzed test, they allow for a more objective assessment of test performance in a clinical setting. Intuitively, a study that evaluates test performance is only as good as its sample classification (into positive and negative cases). Definitive diagnosis of EPM requires postmortem examination, and only 26 horses (9 positive and 17 negative) were euthanized during the study period. To increase sample size, presumptive cases were included in this study (10 positive and 29 negative). Definitions for suspected positive and negative cases were rigorous to avoid misclassification. Despite these efforts, a potential criticism of including the suspected cases would be the possibility of misclassification. Therefore, data are presented in 2 ways: with all positive and negative cases included and with only necropsy-confirmed cases included. The results were surprisingly similar regardless of whether or not presumptive cases were included. Four of the 6 estimates of sensitivity or specificity differed by $\leq 5\%$, and the biggest difference that occurred when presumptive cases were excluded was $< 8\%$. This finding supports accurate classification of suspected cases without necropsy confirmation. Another potential criticism is overestimation of specificity based on the fact that neurologic horses were excluded from the suspected negative group if they had positive CSF WB results. However, this criterion only led to the exclusion of 1 horse, and this horse did not have positive serum or CSF results on either IFAT or SAG-1 ELISA tests. Therefore, this criterion did not falsely increase test specificity.

The main objective of this study was to evaluate the 2 most recently commercially marketed tests for EPM using samples from clinical cases that had not been utilized in test optimization. Before this study, unpublished data suggested that false-negative results may be a problem

with the SAG-1 ELISA, but only 12 clinical cases (4 thought to have EPM) from North America were tested, and only 1 horse had postmortem confirmation of diagnosis.²⁰ The current study confirms those concerns with a much larger sample set. The low sensitivity of the SAG-1 ELISA precluded its usefulness in our patient population, as it misclassified most EPM-positive horses as negative. The IFAT demonstrated a high sensitivity and specificity when cut-off values of 1 : 80 (serum) and 1 : 5 (CSF) were used. Although these results may vary when other patient populations are assessed, they highlight the importance of validating diagnostic tests with clinical cases.

Footnotes

- ^a Marquis (15% w/w ponazuril), Bayer, Shawnee Mission, KS
^b SAG-1 ELISAs were performed by Antech Diagnostics (USA)
^c IFATs were performed by the Immunology/Virology Laboratory at the University of California—Davis, Davis, CA
^d Statistix 9.0, Analytical Software, Tallahassee, FL
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