ACVIM Consensus Statement

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Clinical Diagnosis of Equine Protozoal Myeloencephalitis (EPM)*

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Equine protozoal myeloencephalitis (EPM) has been widely described in the veterinary literature. Even in appropriately treated horses it can be a progressive, debilitating neurological disease. Either of the known causative agents, *Sarcocystis neurona* (common) and *Neospora hughesi* (rare), can produce signs of focal or multifocal central nervous system disease. Although spinal ataxia and weakness appear to be the most common presentation of EPM, signs are variable among affected horses and can mimic any other equine neurological disease. As a result, EPM is inherently a difficult diagnosis to establish definitively, and the diagnosis must always be considered tentative in the living horse. It is not surprising, therefore, that confusion exists among veterinarians attempting to diagnose this disease and interpret ancillary test results. The following consensus opinion is intended to serve as an aid to equine clinicians attempting to establish a diagnosis of EPM in horses presented for evaluation of neurological disease.

Clinical Signs

First, thorough physical and neurological examinations are the primary and most important diagnostic procedures for evaluation of horses suspected of having EPM. Conclusive evidence of neurological abnormalities must be present and musculoskeletal disorders must be eliminated as the primary cause of lameness. We recognize that neurological abnormalities can be accompanied by lameness of musculoskeletal origin in performance horses; thus, thorough lameness evaluation might also be required in horses with a primary complaint of abnormal gait. Neurological examination findings that support a diagnosis of EPM include evidence of multifocal disease, evidence of lesions affecting both upper and lower motor neurons, muscle atrophy, or asymmetric signs. Although most recent publications describe these classic signs, EPM has also been diagnosed in horses with symmetric signs referable to a single focus of central nervous system (CNS) disease. Less commonly, presenting complaints can also include signs referable to brain or brain stem disease. These include head tilt and circling, facial paralysis, atrophy of muscles of mastication, atrophy of the tongue, central blindness, seizures, and behavioral abnormalities. In almost all cases, these signs are asymmetric. These signs can also be found with other equine neurological diseases, and a complete diagnostic work-up must be performed to exclude other potential causes. In addition to establishing evidence of neurological disease, a thorough neurological examination might allow neuroanatomic localization of the lesion(s). Localization of the lesions(s) is important in deciding which additional tests are to be pursued.

Ancillary Tests

Cervical Radiography and Myelography

If cervical spinal cord disease is suspected, based on appropriate neurological examination and neuroanatomic localization, standing lateral cervical radiographs should be performed to screen for possible cervical vertebral abnormalities. It is not unusual to find radiographic abnormalities in horses in which cervical orthopedic disease was not suspected. Many horses with arthritis and remodeling of the cervical facets might not demonstrate signs of pain such as neck splinting, abnormal head carriage, or resistance to flexion. Although finding arthritis of the cervical facets does not confirm that cervical spinal cord compression is the cause of abnormal neurological signs, such findings would support performing myelography to investigate spinal cord compression. In addition, sagittal ratios of the cervical vertebrae can be determined and can be a valuable aid in the interpretation of cervical radiographs.[1] Compression of the cervical spinal cord is highly suggested by a sagittal ratio below 0.5 (at spinal cord levels C_3 through C_6), and such a result would support performing a myelogram.[1] If cervical radiographs and the sagittal ratio are normal, a compressive myelopathy is unlikely; however, in an occasional horse with signs of cervical cord disease and normal scout cervical radiographs, myelography could still be rewarding to demonstrate compression (eg, by an extradural tumor) or focal swelling of the spinal cord and narrowing of the adjacent dye columns. Thus, it is worthwhile to consider myelography in all horses with signs of cervical spinal cord disease, and it is recommended in horses for which the procedure is covered by medical insurance.

We recognize that financial constraints limit the use of myelography in many uninsured horses. Thus, in patients with clinical signs of cervical cord disease and abnormalities on scout cervical radiographs, a tentative diagnosis of EPM can only be supported by confirming the presence of

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specific anti-*S. neurona* IgG antibody by immunoblot of cerebrospinal fluid (CSF). We recognize that the result of the immunoblot of CSF in this setting is compromised by low sensitivity and specificity for diagnosis of EPM. However, it is worthwhile to perform this test because a negative immunoblot result would make EPM an unlikely diagnosis. It also warrants mention that horses with signs of cervical spinal cord disease can have both a compressive myelopathy and EPM. The presence of both diseases can only be truly confirmed at postmortem examination.

Cerebrospinal Fluid Analysis

Cerebrospinal fluid analysis is indicated in all horses with neurological disease. As with myelography, financial constraints might lead some clients to spend limited resources on treatment rather than on a complete diagnostic evaluation. Thus, when clinical signs are compelling, CSF collection and evaluation might not always be pursued. This approach remains reasonable as long as the client has made a fully informed decision regarding other diagnostic considerations. However, when there are signs of brain disease, CSF collection and analysis should be pursued more aggressively because results are often helpful in ruling out other causes of neurological diseases.

In general, results of cytological and biochemical analysis of CSF of horses with neurological disease are of limited diagnostic value because there are few changes that are either sensitive or specific for a particular diagnosis. Nevertheless, when CSF is collected in the evaluation of patients suspected to have EPM, cytological analysis should be performed for two reasons. First, a red blood cell (RBC) count should be determined to validate that the sample is not dramatically contaminated with peripheral blood. Ideally, the sample should have <5 RBCs/μL for immunoblot testing to be of value. Unfortunately, many samples, especially those collected from the lumbosacral space, can have a higher RBC count. Clearly, samples that are grossly discolored pink to red are highly contaminated and should not be submitted for immunoblot testing. At present, we suggest that samples have no more than 50 RBCs/μL if they are to be submitted for immunoblot testing. If such a sample were analyzed, however, a negative immunoblot would indicate that EPM is very unlikely. We recognize that high serum anti-*S. neurona* antibody titers in some horses can produce false positive CSF immunoblot results even at this low concentration of blood contamination, however. Second, cytological analysis should be performed because it could provide results that assist in supporting or refuting the diagnosis of EPM. The importance of cytological evaluation of CSF has been brought to light by the recent emergence of West Nile viral encephalomyelitis (WNVE) in horses in North America. Preliminary experience indicates that it can be difficult to distinguish EPM from WNVE on the basis of clinical signs. However, in contrast to horses with EPM, most horses with WNVE appear to have abnormal CSF cytological findings, which include a moderate mononuclear pleocytosis with increased protein concentration.

Immunodiagnosis

Before the last decade, the diagnosis of EPM was based on clinical signs, elimination of other neurological disorders, and response to treatment. Introduction of the immunoblot test for detection of anti-*S. neurona* IgG was a major advance in the diagnosis of EPM.[2] This test was subsequently refined and is the method currently used by Equine Biodiagnostics, Inc. (EBI; http://www.ebiky.com/). Immunoblots of CSF by this method have been reported to have a sensitivity and specificity of 89%, based on postmortem evaluation of 295 cases of neurological disease of which approximately 40% were histologically confirmed cases of EPM.[3]

Neogen Laboratories subsequently developed a similar immunoblot method. This laboratory reports semiquantitative results for CSF samples based on the intensity of reactivity to the 17-kd protein band on the immunoblot. A single, unitless value (0–100) is reported as the relative quantity (RQ).[4] Higher RQ values are suggestive of greater amounts of antibody against the 17-kd antigen, and this value is expected to decline during successful treatment. The clinical relevance of the RQ is unclear. In a study of the clinical efficacy of ponazuril (a new treatment for EPM), it was found that RQ values tended to decrease during treatment, but the change did not achieve statistical significance.[5] RQ values have also been shown to increase after experimental challenge of horses with *S. neurona*.[4] At present, however, there are no published data to suggest that this semiquantitative immunoblot is of any greater value than results obtained by other immunoblot methods for diagnosis of EPM.

Another modification of the original immunoblot technique was described by Rossano and coworkers from Michigan State University (MSU).[6] In this technique, the immunoblots are pretreated with pooled, purified bovine IgG collected from animals with high titers against *S. cruzi*. In theory, antigens common to *S. cruzi* and *S. neurona* merozoites are recognized and blocked by bovine IgG. When the test serum sample is subsequently added to the immunoblot, only proteins that are not common to these *Sarcocystis* spp. should be recognized. This modified immunoblot was reported to have a sensitivity and specificity approaching 100% when serum samples from 6 EPM horses (confirmed by culture of *S. neurona* from neural tissue) and from 57 horses from the Eastern hemisphere were tested.[6] Controversy exists about this modified immunoblot (MSU test) technique, and not all EPM investigators and parasitologists agree with the premise of the modification.[3] Further investigation is warranted to resolve the various controversies involved in immunoblot interpretation.

Immunoblot results from all three major (ie, commercial) laboratories have not been directly compared; however, comparative results from Neogen and EBI have been reported. These results indicate a high degree of concordance for both serum (82%) and CSF (85%), and there is no identifiable trend among the disparate results.[4] Thus, at present it is not possible to recommend use of one laboratory over another for immunoblot testing.

Although development of immunoblot testing has been a major advance for the diagnosis of EPM, it has become

clear that there are limitations to its use. First, because EPM will not develop unless the parasite enters the CNS, seropositivity alone is not adequate to confirm EPM as the cause of neurological disease. However, when the parasite invades the CNS, antibodies can also be detected in CSF and a positive CSF immunoblot result provides support for the conclusion that EPM is the cause of the neurological disease. It has been shown in other species, however, that limited antibody movement from serum to CSF can occur in the absence of CNS infection.[7] A second limitation became apparent when it was demonstrated that contamination of CSF with small amounts of peripheral blood during collection could lead to a false positive test result (if the horse was seropositive). Blood contamination of CSF is best assessed by manually counting RBCs with a hemacytometer. Historically, samples with fewer than 300–500 RBCs/μL CSF were considered to be ''clean'' for the purposes of analysis. However, recent work by Miller and colleagues has found that this threshold was much too liberal because in vitro contamination of CSF with blood from a horse with a very high serum concentration of anti-*S. neurona* antibodies led to positive immunoblot results with counts as low as 8 RBCs/μL CSF.[8] As described above, we do not recommend that CSF with >50 RBCs/μL be submitted for immunoblot testing. Interpretation of immunoblot results on CSF with counts >10 RBCs/μL must always be interpreted with caution.

An additional issue that clinicians might find confusing is the presentation of results as ''very weak positive'' or ''weak positive.'' All commercial laboratories have presented such results to alert the clinician to the presence of reactivity that could be consistent with the presence of anti-*S. neurona* IgG. Because some horses might not develop a vigorous antibody response to *S. neurona*, these results could be consistent with a diagnosis of EPM in some horses. Similar results have been reported from samples collected shortly after experimental exposure.[4] ''Weak'' or ''very weak positive'' reactions are borderline and should be interpreted as preliminary positives then confirmed by repeat testing in 3–4 weeks. A ''low positive'' (from EBI, at least) represents what is believed to be a truly positive reaction that is simply not as strong as a simple ''positive.''

Polymerase chain reaction

A polymerase chain reaction (PCR) test for detection of *S. neurona* DNA in CSF was also developed, and it subsequently became commercially available through EBI. This test detects minute amounts of parasite-specific DNA. Although a powerful and highly specific test, it has not been found to be clinically useful because of the many false negative results.[9] The reasons for this have never been clearly established, but it may be because of the rapid destruction of parasite DNA in the CSF environment or the possibility that parasite DNA is rarely present in the CSF. Thus, it is our opinion that PCR testing of CSF is of little value, and we do not recommend it for routine diagnosis of EPM. In contrast, PCR testing of neural tissue could be a useful postmortem test. Although not advertised, EBI accepts tissue samples for analysis by means of the commercial PCR assay.

Albumin quotient and IgG index

Because accurate RBC quantification must be performed within a few hours after sample collection, it is not always a practical test when CSF samples are collected under field conditions. To resolve this, the albumin quotient (AQ) was validated for use in the horse by Andrews et al.[10] The AQ compares the concentration of albumin in CSF to that in serum with the following formula.

$$AQ = (ALB_{csf}/ALB_{serum})100$$

Normal values in horses have been reported to be less than 2.2,[10] and values greater than 2.2 are reported to suggest either ''leakage'' of protein through the blood-brain barrier or blood contamination of the sample during collection. Unfortunately, because a high AQ can be caused by either of these problems, the test is not specific for blood contamination. Consequently, we do not recommend use of the AQ as a test of blood contamination of CSF and find results of little value in the overall approach to diagnosis of EPM.

The IgG_{index} is an additional ancillary test that is intended to determine whether CSF IgG concentration exceeds that which is normally present from diffusion. It is determined from the following formula.

$$IgG_{index} = IgG_{csf}/IgG_{serum} \times ALB_{serum}/ALB_{csf}$$

In theory, a high IgG_{index} is supportive of IgG production in the CNS and thereby might provide further support for a diagnosis of EPM in a horse with a positive CSF immunoblot result.

Normal horses were reported to have an IgG_{index} of less than 0.30.[10] In an early study of a small number of horses with EPM, IgG_{index} was reported to be increased at the time of initial diagnosis and decreased during treatment.[11] However, in a subsequent report, no difference was found between IgG_{index} values in normal and EPM-affected horses.[9] In another study, IgG_{index} was found to decrease during treatment for EPM, although the value at the beginning of treatment had no predictive value for outcome.[5] It seems, therefore, that the IgG_{index} provides limited diagnostic information regarding diagnosis of EPM, and we do not recommend its routine use. It may provide some information regarding response to treatment, however.

Interpretation of Immunoblot Results

Although a high sensitivity and specificity have been reported for the CSF immunoblot test for diagnosis of EPM, these values were determined with horses that had neurological disease and were suspected of having EPM. In these situations, incidence of disease in the population (ie, pretest probability of having the disease) is high, leading to skewed results. A more clinically relevant question is, ''What is the probability that a positive test result indicates that the horse truly has the disease?'' This is referred to as the positive predictive value (PPV), with an obvious corollary, the negative predictive value (NPV). The PPV and NPV are influenced by the sensitivity and specificity, as well as the prev-

alence of the disease in the population. Thus, the ''diagnostic efficiency'' of a test depends on the population of animals being tested. In a population of horses with a high likelihood of having EPM, such as horses referred to an equine internist with clinical signs of EPM, the PPV can be very high (ie, >90%). In populations of horses with a low incidence of disease, such as clinically normal horses, the PPV drops rapidly, whereas the NPV increases. Cohen and MacKay presented a series of calculations that demonstrate that at a true prevalence of 1% of the population, the PPV drops to 8%—a profoundly poor diagnostic efficiency.[12] The NPV in the same population is 99%.[12] The obvious conclusion is that immunoblot testing of CSF should not be performed in normal horses as a screening test for EPM because of its poor predictive value. In particular, the use of a CSF immunoblot for *S. neurona* before purchase has no justification.

In conclusion, a clinical diagnosis of EPM is currently best established in horses that have neurological abnormalities consistent with EPM and that have a positive immunoblot test on an uncontaminated CSF sample, in which lameness and other causes of neurological disease can be excluded. A definitive diagnosis can only be made during postmortem examination, and these can often remain inconclusive unless the parasite is detected on routine histological sections or is identified by immunohistochemistry, PCR, or culture of neural tissue. Finally, a favorable response to treatment, especially when subsequently followed by a relapse of similar clinical signs, is also supportive of a diagnosis of EPM in the living horse.

References

1. Rush-Moore B, Granstrom D, Reed S. Diagnosis of equine protozoal myeloencephalitis and cervical stenotic myelopathy. Compend Contin Educ Pract Vet 1995;17:419–426.

2. Granstrom D, Dubey J, Davis S, et al. Equine protozoal myeloencephalitis: Antigen analysis of cultured *Sarcocystis neurona* merozoites. J Vet Diag Invest 1993;5:88–90.

3. Dubey J, Lindsay D, Saville W, et al. A review of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM). Vet Parasitol 2001;95:89–131.

4. Cutler T, MacKay R, Ginn P, et al. Immunoconversion against *Sarcocystis neurona* in normal and dexamethasone-treated horses challenged with *S. neurona* sporocysts. Vet Parasitol 2001;95:197–210.

5. Furr M, Kennedy T, MacKay R, et al. Efficacy of ponazuril 15% oral paste as a treatment for equine protozoal myeloencephalitis. Vet Ther 2001;2:215–222.

6. Rossano M., Mansfield L, Kaneene J, et al. Improvement of western blot test specificity for detecting equine serum antibodies to *Sarcocystis neurona.* J Vet Diag Invest 2000;12:28–32.

7. Lappin R, Chavkin M, Munana K, Cooper C. Feline ocular and cerebrospinal fluid *Toxoplasma gondii*-specific humoral immune responses following specific and non-specific immune stimulation. Vet Immunol Immunopathol 1996;55:23–31.

8. Miller M, Sweeney C, Russell G, et al. Effects of blood contamination of cerebrospinal fluid on western blot analysis for detection of antibodies against *Sarcocystis neurona* and on albumen quotient and immunoglobulin G index. J Am Vet Med Assoc 1999;215:67–71.

9. Miller M, Bernard W. Usefulness of cerebrospinal fluid indices and the polymerase chain reaction test for *Sarcocystis neurona* in diagnosing equine protozoal myeloencephalitis. AAEP Proc 1996;42: 82–84.

10. Andrews F, Maddux J, Faulk B. Total protein, albumin quotient, IgG and IgG index determinations for horse cerebrospinal fluid. Prog Vet Neur 1990;1:197–204.

11. Andrews F, Latimer F, Grubbs S, Abraha T. CSF indices after repeated spinal taps in horses diagnosed with equine protozoal myeloencephalitis. Proc 43rd Meet AAEP 1997;43:10–12.

12. Cohen N, MacKay R. Interpreting immunoblot testing of cerebrospinal fluid for equine protozoal myeloencephalitis. Comp Cont Educ Pract Vet 1997;19:1176–1181.