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. Compression of the cervical spinal cord is highly suggested by a sagittal ratio below 0.5 (at spinal cord levels C3 through C6), and such a result would support performing a myelogram. 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...
specific anti-\textit{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.

\textbf{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 \(< 5 \text{ RBCs/\muL} \) 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/\muL 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-\textit{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.

\textbf{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-\textit{S. neurona} IgG was a major advance in the diagnosis of EPM. 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.

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). 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. RQ values have also been shown to increase after experimental challenge of horses with \textit{S. neurona}. 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). In this technique, the immunoblots are pretreated with pooled, purified bovine IgG collected from animals with high titers against \textit{S. cruzi}. In theory, antigens common to \textit{S. cruzi} and \textit{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 \textit{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 \textit{S. neurona} from neural tissue) and from 57 horses from the Eastern hemisphere were tested. Controversy exists about this modified immunoblot (MSU test) technique, and not all EPM investigators and parasitologists agree with the premise of the modification. 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. 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, sero-
positivity 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. A second limitation be-
came apparent when it was demonstrated that contamina-
tion 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 hemacy-
tometer. Historically, samples with fewer than 300–500
RBCs/µL CSF were considered to be "clean" for the pur-
poses of analysis. However, recent work by Miller and col-
leagues has found that this threshold was too liberal
because in vitro contamination of CSF with blood from a
horse with a very high serum concentration of anti-S. neu-
rona antibodies led to positive immunoblot results with
counts as low as 8 RBCs/µL CSF. As described above, we
do not recommend that CSF with >50 RBCs/µL be sub-
mitted for immunoblot testing. Interpretation of immuno-
blot 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 "weak positive" or "very weak positive." All commercial laboratories have
presented such results to alert the clinician to the pres-
ence of reactivity that could be consistent with the pres-
ence 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 border-
line 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 sub-
sequently 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 neg-
ative 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 tis-
sue 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.

\[
AQ = \frac{(ALB_{serum}/ALB_{csf})}{100}
\]

Normal values in horses have been reported to be less
than 2.2,10 and values greater than 2.2 are reported to sug-
gest either "leakage" of protein through the blood-brain
barrier or blood contamination of the sample during collec-
tion. 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
diagnosis of EPM in a horse with a positive CSF immu-
noblot result.

Normal horses were reported to have an IgG index of less
than 0.3.10 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 How-
ever, in a subsequent report, no difference was found be-
 tween 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 in-
formation regarding diagnosis of EPM, and we do not rec-
ommend 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 re-
ported for the CSF immunoblot test for diagnosis of EPM,
these values were determined with horses that had neuro-
logical 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 pre-
vective value (PPV), with an obvious corollary, the nega-
tive predictive value (NPV). The PPV and NPV are influ-
enced by the sensitivity and specificity, as well as the prev-
Diagnosis of EPM


