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Equine Protozoal Myeloencephalitis: An Updated Consensus Statement with a Focus on Parasite Biology, Diagnosis, Treatment, and Prevention

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Equine protozoal myeloencephalitis (EPM) remains an important neurologic disease of horses. There are no pathognomonic clinical signs for the disease. Affected horses can have focal or multifocal central nervous system (CNS) disease. EPM can be difficult to diagnose antemortem. It is caused by either of 2 parasites, *Sarcocystis neurona* and *Neospora hughesi*, with much less known about *N. hughesi*. Although risk factors such as transport stress and breed and age correlations have been identified, biologic factors such as genetic predispositions of individual animals, and parasite-specific factors such as strain differences in virulence, remain largely undetermined. This consensus statement update presents current published knowledge of the parasite biology, host immune response, disease pathogenesis, epidemiology, and risk factors. Importantly, the statement provides recommendations for EPM diagnosis, treatment, and prevention.

Key words: Encephalitis; Equine myeloencephalopathy; Equine neurologic; Equine protozoal disease; Myelitis; *Neospora hughesi*; *Sarcocystis neurona*.

Parasite Biology and Disease Pathogenesis

EPM was initially called "segmental myelitis" by Rooney in Kentucky in 1970.[1] The syndrome was renamed "focal encephalitis-myelitis" because of brain involvement. Prickett, Rooney, and others described 44 cases of the disease in 1968[2] and 52 cases of the disease in 1970[1] at the annual meeting of the American Association of Equine Practitioners (AAEP). Protozoa were first observed in association with characteristic lesions in 1974,[3,4] and the disease was given its current name, equine protozoal myeloencephalitis by Mayhew et al., who reported on 45 cases at the AAEP meeting in

Abbreviations:

BBB	blood–brain barrier
CNS	central nervous system
CSF	cerebrospinal fluid
CVSM	cervical vertebral stenotic myelopathy
ELISA	enzyme-linked immunosorbent assay
EMND	equine motor neuron disease
EPM	equine protozoal myeloencephalitis
FDA	Food and Drug Administration
IFAT	indirect fluorescent antibody test
PYR	pyrimethamine
SAG	surface antigen
SDZ	sulfadiazine
Se	sensitivity (of a diagnostic test)
SIG	special interest group
Sp	specificity (of a diagnostic test)
WB	Western blot

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1976.[5] It is now well established that EPM can be caused by either *Sarcocystis neurona*[6] or *Neospora hughesi,*[7–11] although the majority of cases are because of infection with *S. neurona*.

Sarcocystis neurona has a 2-host life cycle that alternates between the definitive host, and any of multiple mammal intermediate hosts. The opossum *Didelphis virginiana* is the definitive host for *S. neurona* in North America.[12] As well, South American opossums can act as definitive hosts for *S. neurona* in the southern hemisphere.[13] Sexual reproduction by the parasite in the intestinal epithelium of the infected opossum results in the production of sporozoite-containing sporocysts that are passed in the feces. The sporozoites are infectious for the intermediate hosts, which include skunks,[14] raccoons,[15] armadillos,[16] and cats.[17] *S. neurona* forms latent sarcocysts in the muscle tissue of the intermediate host; sarcocyst-laden muscle is the source of infection for the opossum. Opossums are commonly infected with *S. neurona*[18] and can generate significant contamination of the environment in locations which they frequent.

Horses are infected with *S. neurona* by ingesting food or water that has been contaminated with feces from an infected opossum. Although *S. neurona* sarcocysts were described in 1 case of a 4-month-old foal with clinical signs of EPM,[19] it is unlikely that horses are normal intermediate hosts that contribute to the parasite's life cycle as *S. neurona* sarcocysts are not found typically in tissues of these animals and equine carcasses are seldom accessible to opossums. Importantly, *S. neurona* is not transmitted horizontally between horses, nor can it be transmitted to horses from nonequine intermediate hosts. Antibodies against *S. neurona* in foals before suckling have been reported,[20,21] but vertical transmission of this parasite in horses is probably uncommon. Thus, opossums are the major source of *S. neurona* infection for horses. The exact mechanisms by which *S. neurona* enters the CNS are not known, but are thought to involve either infection of endothelial cells or leukocytes.[22–25]

The complete life cycle of *N. hughesi* is unknown, so all mode(s) of transmission of this parasite to horses remain poorly understood. Canids are a definitive host for the related species *Neospora caninum,*[26] but it has not been established that dogs or wild canids are a definitive host for *N. hughesi*. Vertical transmission of *N. caninum* is very efficient in cattle, and several recent studies indicate that *N. hughesi* can be transmitted transplacentally in horses.[27,28]

All horses are believed to be susceptible to EPM, but it is clear that not all horses that are infected with *S. neurona* or *N. hughesi* will develop disease. Studies in both mice and horses experimentally infected with *S. neurona* have demonstrated a critical role for the immune response in preventing disease.[29–32] Additionally, some EPM-affected horses have demonstrated altered immune responses, some of which are antigen-specific.[25,33–35] As is clear from the finding that not all horses have demonstrated decreased immune responses with the methodology employed, the mechanisms involving the development of disease remain poorly understood.

It is unclear what influences the progression to severe neurologic disease. Factors such as variations in protozoal inoculum and stress-induced immune suppression have been implicated in the occurrence of EPM.[36–38] However, efforts to increase stress (ie, by additional transport of infected horses) and treatment with immunosuppressive steroids did not cause a concomitant increase in disease severity.[39,40] Genetic variation has been observed among the strains of *S. neurona* that have been analyzed,[41–43] and there is some evidence that specific parasite genotypes may be particularly virulent in marine mammals.[44] However, such an association was not apparent in isolates from horses suffering from EPM.

Epidemiology and Risk Factors

A survey using postmortem data from 10 diagnostic centers throughout the United States and Canada found that a majority of EPM cases (61.8%) occurred in horses that were 4 years old or less, whereas only 19.8% of the EPM cases reviewed were in horses 8 years or older.[45] Thoroughbreds, Standardbreds, and Quarter Horses were most commonly observed, but no sex or seasonal bias could be established. A smaller retrospective study of 82 horses with histologic lesions compatible with EPM suggested that EPM risk was highest among male Standardbreds.[46] The mean age of affected horses was 3.6 $\pm$ 2.8 years, similar to that found by Fayer et al.[45]

The seroprevalence of *S. neurona* in horses from the United States has varied widely, ranging from as low as 15% to a high of 89%, depending on geographic location.[47–51] Seroprevalences of 35.6% and 35.5% have been observed in horses in Brazil and Argentina, respectively,[52,53] thus indicating that this parasite commonly infects horses in South America.

In general, the seroprevalence of *N. hughesi* is low in horses. Serum antibodies against *N. hughesi* have been reported in more than 10% of horses in some geographic regions,[7,54–58] whereas other studies found antibodies against *N. hughesi* in much lower proportions of horses (ie, <3%).[52,53,59–62] Some of the variation may be because of geographic differences, but studies that used Western blot to confirm serologic results have suggested that seroprevalence to *N. hughesi* is commonly overestimated.[57,59,62]

A survey reported in 2001 by the National Animal Health Monitoring System (NAHMS) estimated that the annual incidence of EPM in horses 6 months of age or older was 14 $\pm$ 6 cases per 10,000 horses.[63] While it is now known that *N. hughesi* can cause neurologic disease in horses,[7–11] the proportion of EPM cases attributable to this parasite species remains uncertain.

EPM usually occurs sporadically and seldom involves more than 1 horse on a farm,[5,64] although clusters of cases can occur.[65,66] A retrospective study found that young horses (1–5 years) and older horses (>13 years) had a higher risk of developing EPM,[67] as observed previously. EPM occurred the least in the winter, with

the risk 3 times higher in spring and summer and 6 times higher in the fall. On a given premise, the presence of opossums (2.5-fold), previous diagnosis of EPM (2.5-fold), and the presence of wooded areas (2-fold) were also associated with increased risk of EPM. The likelihood of EPM was reduced by one third when wildlife was prevented access to feed and by one-half when a creek or river was present as a water source.

Immune suppression because of stress or advanced age might predispose a horse to development of EPM.[36] Stressful events such as heavy exercise, transport, injury, surgery, or parturition have all been found to increase the risk of EPM.[67] Racehorses and show horses had a higher risk of developing EPM compared to breeding and pleasure horses. Not surprisingly, horses with EPM that were treated with an anticoccidial drug were 10 times more likely to improve than untreated horses.[36]

Clinical Signs

Clinical signs of EPM vary from acute to chronic with insidious onset of focal or multifocal signs of neurologic disease involving the brain, brainstem, or spinal cord.[64] Initial signs might include dysphagia, evidence of abnormal upper airway function, unusual or atypical lameness, or even seizures.[68] Severely affected horses might have difficulty standing, walking, or swallowing and the disease can progress very rapidly. Occasionally, the clinical signs stabilize, only to relapse days or weeks later.

The variability of clinical signs is because of infection of both white and gray matter at multiple sites in the CNS. Signs of gray matter involvement include focal muscle atrophy and severe muscle weakness, whereas damage to white matter frequently results in ataxia and weakness in limbs caudal to the site of infection. Early signs of EPM such as stumbling and frequent interference between limbs can be confused with lameness. Horses affected with EPM commonly exhibit a gradual progression in severity and range of clinical signs. In some cases, however, a gradual onset can give way to a sudden exacerbation in the severity of clinical illness, resulting in recumbency.

The vital signs in affected horses are usually normal and animals appear bright and alert. Some horses with EPM appear thin and mildly obtunded. Neurologic examination often reveals asymmetric ataxia, weakness, and spasticity involving all 4 limbs. Areas of hyporeflexia, hypalgesia, or complete sensory loss are occasionally present. The most common signs of brain/brainstem disease include obtundation, head tilt, facial nerve paralysis, and difficulty in swallowing, although signs are not necessarily limited to these areas.[69]

Recommendations for EPM Diagnosis

Definitive diagnosis of EPM requires postmortem confirmation of protozoal infection of the CNS (see below). For highest accuracy in antemortem diagnosis, the following steps are recommended. (1) The presence of clinical signs consistent with EPM should be confirmed by conducting a thorough neurologic examination. (2) Other potential causes should be ruled out using available tools (eg, cervical radiography). (3) Immunodiagnostic testing of serum and CSF should be conducted to confirm intrathecal antibody production against *S. neurona* or *N. hughesi*. The ratio of antibody in serum to CSF will reveal intrathecal antibodies in most cases of EPM. The Goldman-Witmer coefficient (C-value) or the antigen-specific antibody index (AI) should be applied for cases that have ELISA titer results that are equivocal (ie, the serum:CSF ratio equals the cut-off) or when a condition that compromises the blood–brain barrier is suspected. The SnSAG2, 4/3 ELISA serum:CSF titer ratio and NhSAG1 ELISA serum:CSF titer ratio are the only tests currently offered commercially that provide information regarding intrathecal antibody production based on serum and CSF titers. The commercially available *S. neurona* and *N. hughesi* IFATs do determine antibody titers in both the serum and CSF, but the laboratory does not calculate ratios at this time.

Basis for Recommendations

In horses with clinical signs consistent with CNS disease, EPM should be considered as a differential. Affected horses should initially have a thorough neurologic examination to identify abnormalities, and localize the lesion(s), which will allow one to further refine the differentials. This, combined with the use of appropriate diagnostic tests, will assist in diagnosing EPM and ruling out other causes. Some of the most consistent/classic clinical signs include asymmetric gait and focal muscle atrophy. When these signs are present, EPM should be considered as a top differential diagnosis. EPM-affected horses are not painful, and rarely febrile, unless comorbidities exist.

Differential Diagnoses

Almost all neurologic diseases in horses can have clinical signs that are also present in EPM-affected horses. A thorough neurologic examination and diagnostic tests are needed to distinguish between EPM and other differentials. Some diseases have other more consistent/classic signs that allow one to rule them in or out. With cervical vertebral stenotic myelopathy (CVSM), signs usually are symmetric and, typically, the pelvic limbs are more severely affected than the thoracic limbs. Focal muscle atrophy is not common. Trauma should also be considered as a differential cause of spinal cord damage at any level, potentially causing abnormal neurologic signs in 1 to all limbs.

In horses where there is a history of respiratory disease or an outbreak of abortion, EHV-1-associated neurologic disease should be considered as a more likely differential. EHV-1-affected horses may be febrile shortly before or at the onset of neurologic signs. In the EHV-1-affected horses, neurologic signs typically manifest as symmetric, with primary pelvic limb weakness and ataxia, bladder distention, usually without incontinence, and, more rarely, perineal hypalgesia, tail paraly-

sis, fecal retention and in some cases incontinence as well. Some affected horses show rapidly progressing signs of ataxia and can sometimes have cranial nerve deficits, often involving cranial nerves VII to XII. In other cases, cerebral signs occur.

Another disease which should be considered as a differential diagnosis is equine motor neuron disease (EMND). Affected horses with early stages of disease typically have severe limb weakness with muscle fasciculations and tremors. Horses with chronic EMND can have widespread, profound, muscle atrophy.

Other differentials of spinal cord disease that can result in similar clinical signs include extradural and spinal cord tumors, epidural abscess, migrating metazoan parasites, rabies, West Nile viral encephalomyelitis, equine degenerative myeloencephalopathy/neuroaxonal dystrophy, lead poisoning, creeping indigo toxicity, Lyme neuro-borreliosis, vascular malformations, and discospondylopathies. If affected horses have signs of cranial nerve or brain involvement, EPM should be considered as a differential. Other rule outs include viral encephalomyelitides, neoplasia, head trauma, brain abscess, migrating parasites, temporohyoid osteoarthropathy, polyneuritis equi, cholesterol granuloma, metabolic derangement, and hepatoencephalopathy.

Postmortem Diagnosis

Confirmation of EPM on postmortem examination is based on demonstration of protozoa in CNS lesions, although the diagnosis frequently is made presumptively even when parasites are not detected if the characteristic inflammatory changes are found. In 2 reported series, organisms were seen in H&E sections of CNS tissue in 10 to 36% of suspected cases.[46,70] Sensitivity was increased from 20 to 51% by immunohistochemical staining with antibody against *S. neurona*.[70] Although it has not been demonstrated experimentally, the use of PCR to detect parasites in CNS tissues might aid postmortem diagnosis of EPM. There is decreased likelihood of finding parasites histologically in tissues from affected EPM horses that have been treated with antiprotozoal drugs.[46]

Immunodiagnostic Testing

Overview

There are several immunodiagnostic tests currently in use for EPM diagnosis. Importantly, these tests are an adjunct to diagnosis and not the mainstay. Performing serology as part of a general health screen or prepurchase examination is discouraged because of the very low positive predictive value when a nonneurologic horse is tested. In horses showing gait deficits, EPM serology should not be used to distinguish whether the deficits are caused by CNS or musculoskeletal disease. Presence or absence of neurologic disease is determined by the clinical examination, and serology can then help refine the differential diagnoses list for a neurologic horse.

All commonly used tests are based on detection of antiprotozoal antibodies in serum, CSF, or both. As EPM occurs only in a small proportion of horses infected with *S. neurona,*[63] testing for serum antibodies against *S. neurona* has minimal diagnostic value unless the serologic results are negative (low positive predictive value but high negative predictive value).[71,72] However, detection of serum antibodies against *N. hughesi* in a neurologic horse has a higher positive predictive value because of a much lower seroprevalence. A negative serum test usually indicates that the horse has not been infected and alternative diagnoses should be pursued or that the EPM-suspect horse resides in a geographic area of low exposure to the infecting parasite. However, a recently infected horse might display clinical signs before seroconversion, and repeated serologic testing in 10–14 days is indicated for horses with recent development of compatible clinical signs. Detection of antibodies in the CSF is more informative, but alone is not a definitive indicator of EPM as there is passive transfer of antibody across a healthy blood–brain barrier (BBB).[73] Additionally, blood contamination of CSF samples can cause false-positive results.[74–76] Logically, horses with higher serum titers are more likely to have detectable antibody levels in CSF in both of these circumstances.

Use of quantitative assays to detect intrathecal antibody production, indicating active parasite infection in the CNS, provides an accurate approach for EPM diagnosis. The Goldman-Witmer coefficient (C-value) and the antigen-specific antibody index (AI) are tests of proportionality that assess whether the amount of pathogen-specific antibody in the CSF is greater than should be present from normal passive transfer across the BBB. These methods have been used in human medicine to diagnose CNS infections caused by a variety of pathogens,[77–79] including the apicomplexan *T. gondii*.[80,81] The value of these tests for EPM diagnosis was demonstrated initially with a sample set of 29 clinical cases.[75] This study also showed that minor blood contamination of the CSF sample (ie, up to 10,000 red cells per μL) will not confound the assay results. Subsequently, 2 additional studies examining a more extensive collection of horses with neurologic disease showed that a simple serum:CSF antibody titer ratio was sufficient in many cases for an accurate diagnosis of EPM caused by *S. neurona*.[71,72] Although use of a serum:CSF titer ratio should be equally effective for diagnosis of EPM caused by *N. hughesi*, an optimal serum:CSF titer ratio cut-off needs to be established.

Available tests for EPM caused by S. neurona

Numerous serologic tests have become available during the past 2 decades to aid in the diagnosis of EPM caused by *S. neurona*, including Western blot (WB), indirect fluorescent antibody test (IFAT), and surface antigen (SAG) enzyme-linked immunosorbent assays (ELISAs). Descriptions of testing options and reported test performance are shown in Table 1.[71,72,82–93] All tests can be performed on serum or CSF, and none is

Table 1. Performance of commercially available immunologic tests for antibodies against *Sarcocystis neurona*.

			Reported performance		
Test	Laboratory	Interpretation	Sample	Sensitivity (%)	Specificity (%)
WB[87]	EDS	Band pattern read and interpreted visually (subjective)	Serum	89[93], 80[82], 89[83]	71[93], 38[82], 87[83]
	UC Davis IDEXX	Results usually reported as negative, weak positive, low positive, or positive	CSF	89[93], 87[82]	89[93], 44[82]
mWB[90]	Michigan State	Similar to standard WB (above)	Serum	100[90], 89[83]	98[90a], 69[83] ([a]*n.b., negative cases not from North America*)
IFAT[83]	UC Davis	Serum positive at ≥1:80 has ≥55% probability[a] of EPM	Serum	89[83], 83[84], 94[89], 59[71]	100[83], 97[84], 85[89], 71[71]
		Serum negative at ≤1:40 has ≤33% probability[a] of EPM	CSF	100[84], 92[89], 65[71]	99[84], 90[89], 98[71]
		CSF positive at ≥1:5 has 92% probability[a] of EPM	Serum:CSF titer ratio	65[71]	98[71]
SAG1 ELISA[86]	Antech	Serum positive at ≥1:16 but recommended cutoff ≥1:32	Serum	68[88], 13[89]	71[88], 97[89]
SAG2, 4/3 ELISA[91]	EDS	Serum positive for exposure at ≥1:250	Serum	30–86 (depending on cutoff)[72], 71[71]	37–88 (depending on cutoff)[72], 50[71]
		CSF correlates well with EPM if ≥1:40	CSF	77–96 (depending on cutoff)[72], 88[71]	58–96 (depending on cutoff)[72], 86[71]
		Serum:CSF titer ratio very predictive of EPM if ≤100	Serum:CSF titer ratio	86 (cutoff ≤50) or 93 (cutoff ≤100)[72], 88[71]	96 (cutoff ≤50) or 83 (cutoff ≤100)[72], 100[71]
SAG1, 5, 6 ELISA[92]	Pathogenes	Serum positive at ≥1:8, indicating infection	Serum	N/A	N/A

WB, Western blot; mWB, modified Western blot; IFAT, indirect fluorescent antibody test; SAG, surface antigen; ELISA, enzyme-linked immunosorbent assay; EDS, Equine Diagnostic Solutions (Lexington, KY); UC Davis, University of California at Davis; EPM, equine protozoal myeloencephalitis; CSF, cerebrospinal fluid.

[a]Based on pretest probability of 10%; see reference 85.

considered a gold standard. The WB, the first immunodiagnostic test described for EPM, is a qualitative test for antibodies against merozoite lysate. Its use has largely been supplanted by more quantitative tests, and positive WB results have limited diagnostic utility. However, negative WB results retain a high negative predictive value. The IFAT is a quantitative (end-point titer) test for antibodies against culture-derived whole merozoites. Although serum titers obtained with the IFAT have been used to predict the likelihood of EPM, with higher titers suggesting greater probability of disease, studies that have used diverse collections of neurologic disease cases have shown that a serum titer alone is a poor predictor of EPM.[71,72] As a quantitative test, the IFAT can be used to calculate a serum:CSF titer ratio. However, this information is not routinely provided by the laboratory.

Most recent research has focused on the SAG ELISAs, quantitative (end-point titer) tests based on *S. neurona* surface antigens. These molecules have proven to be good serologic targets in the assays because of their high level of expression in the parasite and their immunogenicity in infected horses.[94–96] The SnSAG2 ELISA and the SnSAG4/3 ELISA accurately detect antibodies against *S. neurona* in equine serum and CSF samples[88,91] and were used to demonstrate the value of detecting intrathecal antibody production for EPM diagnosis.[71,72] An ELISA based on the SnSAG1 surface protein has been described.[86] However, this antigen is not expressed by all strains of *S. neurona*,[43] thereby reducing its utility for serologic detection[88] and EPM diagnosis.[89] An ELISA combining SnSAG1 with 2 additional SnSAGs (SnSAG5 and SnSAG6) is currently offered. However, no published reports describe validation of this assay, so it is unclear whether the test reliably detects antibodies to *S. neurona*.

Several studies have directly compared different tests for EPM (caused by *S. neurona* infection);[71,83,89] these publications and 3 unpublished studies presented at ACVIM EPM Society SIGs[97–99] are detailed in Table 2.[71,83,89,97–99] Although none of the studies examined all of the currently available tests, and the types of samples utilized were variable, some general conclusions are evident. Testing serum alone yielded less accurate results than testing CSF alone or a serum:CSF titer ratio, generally because of low specificity. One notable exception was the SAG1 ELISA, which showed poor sensitivity. Poor to fair test agreement was observed; samples that were split and submitted to multiple labs often had discrepant results. Three of the 6 comparison studies evaluated the SAG2, 4/3 ELISA serum:CSF titer ratio; in all 3 studies this test demonstrated the highest

Table 2. Test comparisons related to EPM caused by *Sarcocystis neurona*

References	Tests (and samples) compared	Sample origin	Results	Author conclusions
Duarte et al. (2003)[83]	• WB (serum) • mWB (serum) • IFAT (serum)	• Necropsy cases (9 positive, 39 negative)	• Similar Se (89%) for all 3 • Variable Sp (IFAT 100%, WB 87%, mWB 69%)	IFAT accuracy was better than WB tests.
Saville (2007)[99]	• WB (serum) • mWB (serum) • IFAT (serum) • SAG1 ELISA (serum)	• Experimental cases (1 *Sarcocystis neurona* positive, 1 *S. fayeri* positive, 2 negative) • Clinical cases (3 positive, 10 negative) • Necropsy case (1 positive)	• Variable for each case; limited agreement between tests	WB and IFAT were most accurate, though IFAT was cross-reactive with *S. fayeri*. mWB tended to have false-positive results, whereas SAG1 ELISA tended to have false-negative results.
Johnson et al. (2010)[89]	• IFAT (serum, CSF) • SAG1 ELISA (serum)	• Necropsy cases (9 positive, 17 negative) • Clinical cases (10 positive, 29 negative)	• Marked difference in Se (IFAT serum 94%, IFAT CSF 92%, SAG1 ELISA serum 13%) • Comparable Sp (IFAT serum 85%, IFAT CSF 90%, SAG1 ELISA serum 97%)	Low Se limited the usefulness of the SAG1 ELISA.
Reed et al. (2010)[97]	• WB (CSF) • IFAT (serum) • SAG1 ELISA (serum) • SAG2, 4/3 ELISA (serum:CSF ratio)	• Necropsy cases (7 positive, 5 negative) • Clinical cases (6 positive, 2 negative)	• Variable Se (SAG2, 4/3 ELISA 90%, WB 90%, IFAT 70%, SAG1 ELISA 55%) • Variable Sp (SAG2, 4/3 ELISA 100%, WB 95%, SAG1 ELISA 90%, IFAT 85%)	SAG2, 4/3 ELISA serum:CSF ratio was the most accurate.
Renier et al. (2012)[98]	• IFAT (CSF) • SAG2, 4/3 ELISA (serum:CSF ratio)	• Necropsy cases (6 positive, 17 negative) *(*n.b., 1 positive case because of *Neospora hughesi* not *S. neurona)*	• IFAT Se (100%) higher than SAG2, 4/3 ELISA Se (83%) • SAG2, 4/3 ELISA Sp (100%) higher than IFAT Sp (82%)	IFAT advantages include testing for *N. hughesi* and use as serum stand-alone test. *(n.b., SAG2, 4/3 ELISA serum:CSF ratio had higher overall accuracy.)*
Johnson et al. (2013)[71]	• IFAT (serum, CSF, serum:CSF ratio) • SAG2, 4/3 ELISA (serum, CSF, serum:CSF ratio)	• Necropsy cases (11 positive, 28 negative) • Clinical cases (6 positive, 14 negative)	• SAG2, 4/3 ELISA serum:CSF ratio was most accurate (97%) • IFAT CSF and serum:CSF ratio also had high accuracy (88%)	Serum testing alone was least accurate; more accurate methods should be used. SAG2, 4/3 ELISA serum:CSF ratio was most accurate.

ACVIM, American College of Veterinary Internal Medicine; EPM, equine protozoal myeloencephalitis; SIG, special interest group; WB, Western blot; mWB, modified Western blot; IFAT, indirect fluorescent antibody test; SAG, surface antigen; ELISA, enzyme-linked immunosorbent assay; Se, test sensitivity; Sp, test specificity; CSF, cerebrospinal fluid.

overall accuracy as compared to the WB, IFAT, and SAG1 ELISA. However, the SAG1, 5, 6 ELISA has not yet been evaluated in any comparison study, so its performance is currently unknown.

Available tests for EPM caused by N. hughesi

Two serologic assays are currently offered for measuring antibodies against *N. hughesi* in equine samples

Table 3. Commercially available immunologic tests for antibodies against *Neospora hughesi*.

Test	Laboratory	Interpretation	Reported performance
IFAT[100]	UC Davis	• Serum positive at ≥1:320; negative at <1:40 • CSF positive at ≥1:5	• Serum Se 100%, Sp 100% at cutoff of 1:640 • Serum Se 100%, Sp 71% at cutoff of 1:320 • Se and Sp estimates calculated using samples from experimentally infected horses, not EPM cases
ELISA[62]	EDS	• Serum positive at ≥1:500 • CSF positive at ≥1:5 • Serum:CSF titer ratio provides most accurate EPM diagnosis	• Serum Se 94%, Sp 95% compared to WB detection antibodies (not EPM cases)

IFAT, indirect fluorescent antibody test; ELISA, enzyme-linked immunosorbent assay; UC Davis, University of California at Davis; EDS, Equine Diagnostic Solutions (Lexington, KY); CSF, cerebrospinal fluid; EPM, equine protozoal myeloencephalitis; Se, sensitivity; Sp, specificity; WB, Western blot.

(Table 3). An ELISA based on the major parasite surface antigen NhSAG1[62] is available from Equine Diagnostic Solutions, LLC, whereas an IFAT using whole *N. hughesi* tachyzoites is offered by the School of Veterinary Medicine, University of California-Davis, Veterinary Immunology Laboratory. Based on analysis of 1006 random equine samples, the NhSAG1 ELISA provides an estimated 94% sensitivity and 95% specificity for detecting antibodies against *N. hughesi* when compared to Western blot results. The *N. hughesi* IFAT sensitivity and specificity for detecting antibodies against *N. hughesi* was reported to be 100% and 71.4%, respectively, at a cut-off of 1:320.[100] These values were based on samples from 3 naturally infected, 7 experimentally infected, and 7 naïve horses. Of note, neither the *N. hughesi* IFAT nor the NhSAG1 ELISA have been fully validated for EPM diagnosis because of an inadequate number of samples from EPM cases caused by this parasite.

Recommendations for EPM Treatment and Prevention

For treatment of EPM, it is recommended that 1 of the FDA-approved anticoccidial drugs should be used to control infection. The current FDA-approved drugs are: a) Ponazuril (Marquis®; Merial, Inc., Duluth, Georgia, 30096, USA); b) Diclazuril (Protazil®; Merck Animal Health, Madison, NJ, 07940, USA); and c) Sulfadiazine/Pyrimethamine (eg, ReBalance®; PRN Pharmacal, Pensacola, Florida, 32514, USA). Additional medical and supportive treatment should be provided based on the severity of neurologic deficits and complications arising from them.

Basis for Recommendations

Folate-Inhibiting Drugs

A combination of sulfadiazine and pyrimethamine (SDZ/PYR) was 1 of the initial treatment for EPM. Sulfonamides and pyrimethamine act synergistically by interfering with folic acid metabolism and biosynthesis of purine and pyrimidine nucleotides necessary for the parasite's survival.

A dosage regimen of PYR, 1 mg/kg PO q24 h, and SDZ, 20 mg/kg PO q24 h for up to 6 months was the earliest treatment for EPM. As dietary folate can interfere with the uptake of diaminopyrimidine drugs like PYR,[101] hay should not be fed for 2 hours before or after treatment. PYR given PO to horses at 1 mg/kg/d achieves a concentration of approximately 0.02 to 0.10 μg/mL in the CSF 4–6 h after administration.[102] These experimental horses were allowed free access to prairie hay, potentially reducing the bioavailability of the drug.[101] One of the PK characteristics is that steady-state CSF concentrations of PYR can be obtained after 4–6 hours after a single PO administered dose at 1 mg/kg/d. Further, short half-lives of these compounds suggest that there will be large fluctuations between peak and trough concentrations in the CSF after single daily administration. Additionally, as PYR is concentrated in CNS tissue relative to plasma,[103] the concentration at the desired site of action might be >0.1 μg/mL. Mean peak CSF concentrations of sulfonamide after single or multiple dosing (22–44 mg/kg) have been reported to be approximately 2–8 μg/mL.[104] These drugs are available as an FDA-approved product (ReBalance®; PRN Pharmacal). Treatment efficacy determined by clinical improvement (2 or more improvement grades in the overall neurologic dysfunction) or reversion to a CSF negative status for *S. neurona* by immunoblot after 90 days of treatment showed success in 60–70% of treated horses.[105]

The toxic effects of these drugs relate to the inhibition of folate synthesis and include bone marrow suppression, anorexia, urticaria, and self-limiting diarrhea.[102,106] Typically, there is progressive mild anemia (PCV in the low 20s) over a 6-month treatment period; neutropenia and thrombocytopenia can be seen in some cases as well. Pyrimethamine is teratogenic, causing abortions in rats and congenital defects in

pups.[107] In addition, mares treated with pyrimethamine in late pregnancy had a fatal syndrome observed in the foals.[108] Of the 4 mares, 3 had been supplemented with folic acid. In other species, folic acid supplementation will not prevent PYR-induced toxicosis[109] or can even exacerbate it.[107] Therefore, the use of folic acid in EPM-affected horses treated with PYR cannot be justified.

Benzeneacetonitrile Drugs

Diclazuril and ponazuril, 2 members of the benzeneacetonitrile group of compounds, have been approved by the FDA for treatment of EPM (US FDA, Protazil® antiprotozoal oral pellets. 1.56% diclazuril. Freedom of Information Summary; US FDA, Marquis™ antiprotozoal oral paste. 15% w/w ponazuril. Freedom of Information Summary). With demonstrated broad-spectrum anticoccidial activity in many avian and mammalian species, these drugs are related to the herbicide atrazine and are thought to target the parasite's apicoplast organelle.[110] The activity of benzeneacetonitrile compounds against *S. neurona* and *N. caninum* was initially shown *in vitro*.[111–113] In horses, pharmacokinetic studies have established that therapeutic steady-state concentrations of both diclazuril and ponazuril are achieved by day 7 using labeled doses.[114–116] Moreover, use of a loading dose of ponazuril at 15 mg/kg resulted in steady-state concentrations in blood and CSF by day 2.[117] Furthermore, the concurrent administration of vegetable oil (1/2 cup) has shown to increase the bioavailability of the FDA-approved ponazuril product up to 15% (M. Furr, unpublished observations). A loading dose for the FDA-approved diclazuril product is not required and use of vegetable oil does not increase its bioavailability (Hunyadi, unpublished observations). The FDA-approved benzeneacetonitrile compounds exhibited efficacy ranging from 62 to 67% based on a neurologic examination improvement of 1 grade or becoming negative to antibodies against *S. neurona* in serum and CSF.[118] Because ponazuril and diclazuril are highly selective against apicomplexan parasites, little to no toxicity is to be expected at therapeutic doses.[119]

Duration of treatment will mainly depend on response to antiprotozoal administration. While the FDA-approved products are labeled for a treatment course of 28 days, the majority of horses with EPM are treated for a longer period of time, generally 6–8 weeks or longer if clinical improvement is still apparent under treatment. Discontinuation of antiprotozoal treatment should be based on neurologic improvement. At this time, antibody retesting in blood, CSF, or both is not recommended to determine discontinuation of antiprotozoal drug administration.

Supportive Medical Treatment

Nonsteroidal anti-inflammatory drugs such as flunixin meglumine are frequently given to moderately or severely affected horses during the first 3–7 days of antiprotozoal treatment and in an attempt to prevent worsening of neurologic deficits during the early antiprotozoal treatment. In the case of horses which are in danger of falling down or exhibit signs of brain involvement, the additional use of a short course of corticosteroids (0.1 mg/kg of dexamethasone once or twice daily) and dimethyl sulfoxide (1 g/kg as a 10% solution IV or by nasogastric tube once or twice daily) may control the inflammatory response and associated clinical signs. Because the damaged CNS is susceptible to oxidant injury, vitamin E (eg, 20 IU/kg daily per os) is often used as an adjunct antioxidant treatment; it remains to be determined experimentally whether this practice is beneficial.

Biologic Response Modifiers

Based on the assumption that horses that develop EPM may be immune compromised, immunomodulators have anecdotally been included by some in treatment of the disease. The drugs used include levamisole (1 mg/kg PO q12h for the first 2 weeks of antiprotozoal treatment and for the first week of each month thereafter), killed *Propionibacterium acnes* (Eqstim™; Neogen, Lansing, MI), mycobacterial wall extract (Equimune® IV; Bioniche Animal Health Vetoquinol, Belville, ON, Canada), inactivated parapox ovis virus (Zylexis, Zoetis, Florham Park, NJ), and transfer factor (4Life® Transfer Factor, 4LifeResearch, Sandy, UT). Because no studies have been conducted to evaluate their efficacy in EPM horses, no recommendations can be made.

Prevention of EPM

Preventative approaches to EPM can be achieved by decreasing stress along with reducing exposure to scat from opossums. Practical approaches such not feeding off the ground, providing separate sources of fresh water for horses and preventing wildlife access to horse pastures, paddocks, and stalls may also help reduce the incidence of protozoal infections in horses.

Intermittent use of coccidiostatic and coccidiocidal drugs is another approach used to prevent EPM. Two prophylactic studies have looked at the use of ponazuril after an experimental challenge.[120,121] Treatment at either 2.5 or 5.0 mg/kg PO q24h of ponazuril was administered beginning 7 days before experimental challenge and continued for 28 days.[120] In that study, administration of ponazuril reduced clinical signs and delayed seroconversion. Intermittent ponazuril paste administration at 20 mg/kg PO every 7 days was associated with a significantly decreased intrathecal anti-*S. neurona* antibody response in horses experimentally inoculated with *S. neurona* sporocysts.[121] Collectively, these 2 studies showed that daily or intermittent treatment with ponazuril minimized but did not eliminate infection in horses experimentally infected with *S. neurona*. Recently, pharmacokinetics of daily low-dose diclazuril (0.5 mg/kg PO q24h) given to adult healthy horses were investigated.[116] Diclazuril pellets, given at a

low-dose, attained plasma and CSF concentrations known to inhibit *S. neurona* and *N. caninum* in cell culture. The daily administration of a low-dose diclazuril pellet topdressing to healthy foals from a farm with a high exposure rate to *S. neurona* significantly reduced the monthly seroprevalence to *S. neurona* when compared to untreated foals.[122] The authors of that study suggested that the reported difference in temporal seroprevalence between treated and untreated foals was likely because of the successful reduction of *S. neurona* infection in foals receiving a daily low-dose diclazuril. This preventive strategy has the potential to be used in high-risk horses in an attempt to reduce the incidence of EPM, although, future longitudinal studies will be required before establishing a standard protocol.

Future directions

While considerable progress has been made as the original EPM consensus statement in 2002, many questions remain unanswered. The highest priority areas identified by the EPM organizing committee include: (a) identifying whether *S. neurona* can establish a persistent but inapparent infection in the horse, (b) elucidating the nature of the immune response in protection and disease, (c) determining how *S. neurona* causes disease and whether organisms need to be present to cause pathologic changes and clinical signs, (d) elucidating whether *S. neurona* parasite genotype influences infection and severity of signs, (e) identifying whether co-infection with other pathogens can be a contributing factor in EPM cases, (f) expanding the fundamental knowledge of *N. hughesi* as a cause of EPM, including identifying the definitive host, determining all modes of transmission and investigating the host-pathogen relationship, including the protective immune response. The Committee urges support for the aforementioned projects as the knowledge gained from these studies will lead to earlier and more accurate diagnosis, preventive approaches and more efficacious treatments.

Summary

Based on the currently published information, it is recommended that horses with neurologic signs consistent with EPM, because of *S. neurona* or *N. hughesi*, have a thorough neurologic examination performed. With this information, neurologic deficits can be identified and the lesion(s) localized. Differentials can be developed and appropriate diagnostic testing can be performed to rule in EPM and rule out other diseases. Current recommendations are for serum and CSF testing for *S. neurona*, *N. hughesi*, or both to identify whether intrathecal antibody production is present. Treatment recommendations for EPM include an FDA-approved treatment, as well as supportive care. Duration of treatment is based on resolution of clinical signs. Horses that develop recurrent signs should be reassessed. As more knowledge is elucidated on the virulence of *S. neurona* and *N. hughesi* and the immune phenotype is elicited, more accurate diagnose, more efficacious treatments, and better preventative approaches will be identified.

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