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Evidence-Based Review of Diagnosis and Treatment of *Sarcocystis neurona* Infection (Equine Protozoal Myeloencephalitis)

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Equine protozoal myeloencephalitis (EPM) should only be diagnosed when compatible clinical signs and seropositivity are present and other likely diseases have been excluded. Available evidence suggests that the Western blot test is highly sensitive but not as specific as the indirect fluorescent antibody test, which is also highly sensitive. New data indicate that the surface antigen-1 (SAG-1) enzyme-linked immunosorbent assay (ELISA) may be insensitive, likely because of the *Sarcocystis neurona* strain variation. None of the available/approved treatments are obviously superior; all have shown an ~60% success rate in clinical trials. Author’s address: New Bolton Center, 382 West Street Road, Kennett Square, Pennsylvania 19348; e-mail: aljdvm03@gmail.com. © 2009 AAEP.

1. Introduction

*Sarcocystis neurona* infection is the most common cause of EPM (equine protozoal myeloencephalitis). This neurologic disease presents a diagnostic challenge to practitioners, because many horses are exposed to the protozoa and clinical signs can mimic many other conditions. Treatment is also challenging, because several medications are available and response to treatment is not consistent among horses. This review summarizes current commercially available diagnostic and therapeutic options and the evidence supporting each option.

2. Diagnosis

A complete neurologic exam should always be the mainstay of diagnosis. If clinical signs cannot be attributed to lesions in one or more regions of the central nervous system (CNS), EPM should not be considered. However, EPM can mimic almost any neurologic disease and cause signs ranging from a single cranial or peripheral neuropathy to diffuse CNS dysfunction. Certain patterns, such as multifocal signs, mixed lower motor neuron and upper motor neuron signs (e.g., both muscle atrophy and spinal proprioceptive ataxia), and asymmetric signs are more commonly observed with EPM than other neurologic diseases.

Additional support for a diagnosis of EPM should include exclusion of other common neurologic diseases and confirmation of exposure to *S. neurona* through serology and/or cerebrospinal fluid (CSF) analysis. For example, negative results of survey cervical radiography and/or myelography can exclude cervical vertebral malformation or cervical stenotic myelopathy. Absence of fever and respiratory disease, as well as negative buffy coat and nasal swab polymerase chain reaction (PCR), can exclude Equine Herpes Virus-1 myeloencephalopathy. Negative IgM capture enzyme-linked immunosorbent assay (ELISA) or absence of mosquito vectors can exclude West Nile virus from consideration.
3. Diagnostic Tests

The three most commonly used ante-mortem diagnostic tests (Western blot [WB], indirect fluorescent antibody test [IFAT], and surface antigen-1 (SAG-1) ELISA) are all based on anti- \textit{S. neurona} antibodies and can be performed on serum or CSF. These tests provide supportive evidence only; none is considered a gold standard. Necropsy remains the only definitive test. In a previous review, the evidence and sensitivity/specificity estimates previously discussed as well as summarizes new data available regarding the SAG-1 ELISA.

**WB**

Initial estimates of the sensitivity (Se) and specificity (Sp) of the WB test were high (serum Se = 89%; serum Sp = 71%; CSF Se = 89%; CSF Sp = 89%).

Shortly thereafter, other data indicated that the WB Sp likely was lower for detecting EPM, because similar proportions of normal horses and abnormal horses (with neurologic signs, gait abnormalities, or performance problems) were positive on CSF WB.

A more recent prospective study estimated WB Se/Sp for both neurologic and normal horses; results on CSF yielded Se = 87% and Sp = 44% for neurologic horses and Se = 88% and Sp = 60% for normal horses (serum results were similar). If weak positive results were considered negative, Sp improved but Se declined. Limitations of this study included questionable accuracy of classification of horses, a small number of confirmed positive cases, and blood contamination of CSF samples. A modified WB (with a blocking step) reported improved Se/Sp (100% and 98%, respectively); however, it was only tested on a small number of confirmed positive horses and was never tested on horses that had potentially been exposed to \textit{S. neurona} but did not have clinical disease.

One of the difficulties in interpreting WB results is the high number of seropositive horses. For this reason, testing CSF has been advocated over serum and will likely result in a reduction in false-positive results. When a large number of paired serum and CSF results were analyzed, 29% of CSF samples from seropositive horses were negative. Unfortunately, only a small amount of blood contamination may affect CSF results; if the blood is moderately or strongly reactive, as few as 8 red blood cells (RBCs)/μl in the CSF may cause false-positive results.

**IFAT**

Initial comparative analysis of the IFAT using a small subset of serum samples from the Daft et al. study yielded comparable Se values (88.9% for IFAT, WB, and modified WB) but differing Sp values (IFAT = 100%; WB = 87.2%; and modified WB = 69.2%). The IFAT was subsequently analyzed with a larger sample set of naturally infected horses (including samples used in previous studies), yielding a Se value of 83.3% and a Sp value of 96.9% on serum samples and a Se value of 100% and a Sp value of 99% on CSF samples. As before, limitations included the small number of positive cases and questionable classification of horses.

One caveat to the IFAT is that crossreaction with \textit{Sarcocystis fayeri} could lead to false-positive results; it is currently unclear whether or not this is a clinically relevant problem. One benefit of the IFAT compared with the WB is that CSF blood contamination has a far less significant effect; there was no effect on IFAT results at any serological titer when the CSF was contaminated with ≤10^4 RBCs/μl.

**SAG-1 ELISA**

The newest commercially available test is the SAG-1 ELISA, which is based on an immunodominant surface antigen of \textit{S. neurona} (SAG-1). Its first evaluation in the literature using naturally occurring cases lacks detail and does not include Se and Sp estimates. Three hundred thirty samples (serum and CSF) from horses with “a presumptive diagnosis of EPM” were tested, and 85.4% were positive. Unfortunately, no inclusion criteria were specified. One hundred forty-seven samples from presumptively normal horses were also analyzed, and 24% were positive. Again, these cases were not defined.

Data from the author of this review indicate that the SAG-1 ELISA shows poor Se and fair Sp for naturally occurring cases in the mid-Atlantic region. In this patient population, preliminary results showed that the overall Se of the SAG-1 ELISA was 17% and Sp was 79% with a cutoff value of 1:16. The low sensitivity severely limited the usefulness of this test in this population.

Hoane et al. independently evaluated the use of ELISAs for EPM diagnosis. They determined that Se (90.9–95.5%) and Sp (78.6–92.9%) values were high for ELISAs based on other surface antigens (SAGs 2, 3, and 4), but their SAG-1 ELISA had a Se of only 68.2% and Sp of 71.4%. They proposed that the reduced sensitivity of the SAG-1 ELISA may be explained by the fact that this surface antigen may be absent in certain \textit{S. neurona} isolates in nature. Later work supported \textit{S. neurona} surface antigen diversity and showed that SAG-5 is an alternative surface antigen of \textit{S. neurona} strains, which is mutually exclusive to SAG-1. Therefore, the SAG-1 ELISA is only likely to be useful in areas where SAG-1 expressing strains of \textit{S. neurona} predominate; in areas where SAG-5 expressing strains predominate, the test will fail to identify infected horses. \textit{S. neurona} strains lacking SAG-1 have been identified in a variety of geographic locations.
4. Treatment

There are four treatments currently approved by the Food and Drug Administration (FDA) for EPM, but only three have been commercially available: sulfadiazine and pyrimethamine combination, ponazuril, and nitazoxanide. Diclazuril has been approved but not yet marketed. Recently (spring of 2009), the commercially available form of nitazoxanide has been discontinued. Although multiple investigators have examined in vitro activity of various drugs, their pharmacokinetics, and their use in prevention of experimental infection, this review will focus only on the efficacy and side effects of the four approved treatments as documented in clinical cases.

Sulfadiazine and Pyrimethamine

The FDA-approved combination is sulfadiazine and pyrimethamine dosed at 20 mg/kg sulfadiazine and 1 mg/kg pyrimethamine daily for a minimum of 90 days. If the FDA-approved combination is unavailable, some practitioners opt to use trimethoprim-sulfa tablets (20–30 mg/kg, q 12–24 h, PO) with pyrimethamine tablets (1 mg/kg, q 24 h, PO) because of availability or ease of administration. However, these drugs are not FDA-approved for EPM treatment, and therefore, this regimen constitutes extra-label/unapproved use. All three of the drugs (trimethoprim, sulfadiazine, and pyrimethamine) inhibit enzymes in the folic acid pathway and thereby, inhibit thymidine synthesis; trimethoprim and pyrimethamine inhibit dihydrofolate reductase, whereas sulfadiazine blocks the conversion of para-aminobenzoic acid to dihydrofolic acid.

A field study was performed during the approval process at multiple sites to evaluate two dose levels of sulfadiazine and pyrimethamine (1 x and 2 x the aforementioned dose for 90–270 days). Inclusion criteria included neurologic signs and positive CSF WB; treatment success was defined by negative CSF WB and/or marked clinical improvement (two or more grades of improvement). Of the horses completing the study, 16 of 26 (61.5%) treated at the 1 x dose had successful outcomes. The most common adverse reaction was bone marrow suppression (anemia, leukopenia, neutropenia, and/or thrombocytopenia) in 12% of the 1 x group and 21% of the 2 x group.

An epizootic of EPM at a farm was described during which 12 horses were diagnosed with EPM based on neurologic signs and positive CSF WB. All horses were treated with pyrimethamine and trimethoprim-sulfamethoxazole until they were negative on CSF WB (45–211 days). Eleven of twelve horses became negative on CSF WB in this time period. Adverse effects attributed to treatment included fever, leukopenia, anorexia, depression, acute worsening of ataxia, mild anemia, and abortions.

In addition to blood dyscrasias, folic acid deficiency may lead to gastrointestinal disturbances such as glossitis. Stallions treated for 90 days with trimethoprim-sulfamethoxazole and pyrimethamine may have changes in copulatory form and agility along with altered pattern and strength of ejaculation. Three mares receiving sulfonamides, pyrimethamine (with or without trimethoprim), and folic acid delivered foals with congenital defects that died or were euthanized.

Ponazuril

Ponazuril is a triazinetrione antiprotozoal drug that targets the “apicoplast” organelle and inhibits energy metabolism (respiratory chains). The label dosage regimen for ponazuril is 5 mg/kg PO daily for 28 days.

A multi-center field study was performed during the approval process for ponazuril and subsequently published. Inclusion criteria mandated that horses have neurologic gait abnormalities, normal cervical radiographs, and positive CSF WB results. Horses were divided into two treatment groups: 5 mg/kg daily for 28 days or 10 mg/kg daily for 28 days. One hundred one horses were included, and 63 (62%) had a favorable outcome (improving by at least one neurologic grade 90 days after stopping treatment or becoming negative on CSF WB). Sixty percent (28 of 47) in the low-dose group improved and 65% (35 of 54) in the high-dose group improved. No adverse effects were noted in either group as reported in the efficacy study. However, information provided by the manufacturer reports “unusual daily observations” in eight animals that may have been related to treatment including blisters on nose and mouth, skin rash or hives, loose stools, mild colic, and a seizure. This information also mentioned slightly different results for the high-dose group in that 32 of 55 (58%) horses improved.

Nitazoxanide

As previously mentioned, nitazoxanide paste is no longer commercially available. This drug is a 5-nitrothiazole antiparasitic drug that inhibits the pyruvate:ferredoxin oxidoreductase (PFOR) enzyme-dependent electron transfer reaction essential for anaerobic energy metabolism.

Before the research provided by the manufacturer of nitazoxanide paste, there were two case series that described the use of nitazoxanide. McClure and Palma described two horses diagnosed with EPM on the basis of neurologic signs and positive CSF WB results that were treated with nitazoxanide tablets and/or paste at 50 mg/kg daily for either 28 or 42 days; both horses improved, and the one with longer follow-up information had no residual neurologic abnormalities. Vatistas et al. described their initial experiences using nitazoxanide (50 or 75 mg/kg daily for 28 days) in seven horses diagnosed with EPM using the same criteria; five horses became neurologically normal, one horse improved,
and one was unchanged. Reported side effects in these studies included anappetence and depression.

Two field studies for efficacy and safety were conducted during the approval process for nitazoxanide. When the efficacy data were analyzed, 49 horses from the first study and 250 from the second study were included. Inclusion criteria were more stringent for the first study; horses were required to have asymmetric spinal ataxia or multifocal neurologic signs along with positive CSF WB results. In the second study, neither a videotape of the neurologic exam nor CSF analysis was required. Also, some horses in the second study had been previously treated for EPM with other drugs. In the first study, 57% of horses improved by one grade and/or became negative on CSF WB as assessed by clinical investigators. In the second, less stringent study, 81% were considered treatment successes. Side effects were seen in 22 of 81 (27%) horses in the first study and 129 of 416 (31%) horses in the second study. The most common adverse reactions were fever, anorexia/reduced appetite, and lethargy/depression. Twenty-eight horses died or were euthanized in the second study, and five of these cases were potentially caused by nitazoxanide use, prompting the following warning: “administration of nitazoxanide can disrupt the normal microbial flora of the gastrointestinal tract leading to enterocolitis. Deaths due to enterocolitis have been observed while administering the recommended dose in field studies.”

Diclazuril

Diclazuril is a triazinetrione antiprotozoal agent similar to ponazuril with an unknown (but possibly similar) mechanism of action.

A multi-center clinical field study was performed using diclazuril pellets at a dose of 1 mg/kg daily for 28 days. Criteria for inclusion were similar to other field trials and consisted of neurologic signs and positive CSF WB tests. Horses were also tested for other neurologic diseases and excluded if abnormal results were obtained. Forty-two horses completed the study; 67% were considered successes (negative CSF WB and/or improvement by one grade) by clinical investigators. Reported adverse reactions were not clearly linked to drug administration and included worsening neurologic status and laminitis.

5. Conclusions

Ante mortem diagnosis of EPM is always presumptive and should be based on the presence of compatible neurologic signs, exclusion of other likely diseases, and positive serology and/or CSF analysis for S. neurona infection. Testing CSF samples (alone or in addition to serum samples) improves specificity. Three types of serologic tests are commercially available to aid in diagnosis. Available evidence indicates that both the WB and IFAT have similar sensitivities (~90%). The estimated IFAT specificity is higher than the estimated WB specificity, and the IFAT CSF results are much less affected by blood contamination than WB CSF results. The SAG-1 ELISA has the least amount of evidence to support its use, and preliminary data yield a low sensitivity that will likely limit its use in some or most geographic regions.

Results of efficacy studies were surprisingly similar for each of the four approved therapies when similar methodologies and means of assessing improvement were used. Regardless of drug, ~60% of treated horses improved by at least one neurologic grade or became negative on CSF WB. Prospective, randomized, blinded clinical trials would aid in assessing if one drug shows superior efficacy. Based on reported side effects, ponazuril and diclazuril seem to have the fewest reported adverse effects.

References and Footnotes

1. Johnson AL. Which is the most sensitive and specific commercial test to diagnose Sarcocystis neurona infection (equine protozoal myeloencephalitis) in horses? Equine Vet Ed 2008;20:166–168.


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