EPM Diagnosis

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Could EPM be causing your horse's performance to be just a little off?

Equine protozoal myeloencephalitis (EPM) continues to be an elusive disease to prevent, diagnose, and treat. In this special collection of three articles this month, we will delve into the often-contentious world of EPM research to share what practitioners and those studying the disease have discovered.

What is EPM?

Equine protozoal myeloencephalitis describes an infection of the central nervous system with an intracellular (they cannot reproduce outside their host cell) protozoan parasite known as *Sarcocystis neurona*. To complete its life cycle, this organism requires a definitive host (the opossum), which feeds on the muscles of a dead intermediate host (such as a raccoon, skunk, cat, or armadillo). *S. neurona* is contained in the muscles in the form of sarcocysts, which once ingested by the opossum will mature and pass in their infective stage (sporocysts) in opossum feces.

Horse feed, hay, or pasture that is contaminated by opossum feces is then unintentionally consumed by a horse; the ingested sporocysts penetrate and infect a horse's leukocytes (white blood cells, predominantly monocytes) and endothelial cells that line the blood vessels. It is speculated that, like a "Trojan horse," the parasites cross the horse's blood-brain barrier by hiding in the leukocytes, then they are released within the central nervous system (CNS) tissues, where they inflict their damage.

Clinical Presentation

A typical presentation of EPM is that of neurologic signs, specifically asymmetric atrophy (muscle wasting) and ataxia (incoordination), with the atrophy occurring in focal muscle areas. A horse will appear to have neurologic signs in one side of his body, such as one hind limb that drags, or an eyelid or lip that droops on one side. In more subtle cases, it is sometimes difficult to determine if a horse is lame or is exhibiting neurologic signs such as ataxia. Any number of neurological signs can be present with EPM. Those signs can mimic other neurological diseases or musculoskeletal diseases, particularly of the pelvic, sacroiliac, or lumbosacral regions, or conditions due to cervical vertebrae stenosis (narrowing of the vertebral canal) or instability, also known as wobbler's syndrome. A horse affected by EPM might demonstrate signs consistent with brain disease, cranial nerve damage, peripheral nerve problems, obscure lameness, behavioral changes, or any combination of these.

A physical exam helps identify the type and extent of a horse's incoordination and neurologic signs, and it helps localize the lesions to specific areas of the central nervous system. While a thorough clinical and neurologic exam corroborates the presence of a neurologic problem, without testing it is often difficult to know for certain whether EPM is the only cause of disease, or whether other problems are occurring concurrently (such as wobbler's). *S. neurona* can affect any portion of the central nervous system and can, therefore, mimic other diseases, making generalizations during diagnosis impractical.

One must also consider the likelihood of active infection relative to the geographical residence of the horse and how prevalent the disease occurs in that location, or in locations the horse has visited. Certain parts of the country have a higher incidence of EPM occurrence than others. Granstrom, one of the original pioneers who studied the emergence of EPM, has a wealth of knowledge about the disease. Granstrom remarks, "Exposure, as measured by prevalence based on positive results on blood testing, varies widely across the U.S. It ranges from zero in desert regions of the West to 65% and higher in some temperate areas where large numbers of opossums live and are able to spread the infective stage. Fortunately, the incidence of EPM is well below 1% of the equine population in the U.S. The percentage of horses that develop clinical disease following exposure is difficult to estimate, but apparently it is very low. Other factors, such as stress, appear to have the greatest influence on which horses actually develop clinical disease."

Testing for EPM

One of the major areas of controversy surrounding EPM is testing. Testing requires identification of markers of infection, such as antibodies specific for *S. neurona*. Such antibodies might be found in the blood of a horse that has been exposed, but not necessarily infected (no overt disease resulted from exposure). Antibodies found in the cerebrospinal fluid (CSF) cast a higher suspicion that the neurologic signs exhibited by a horse might be linked to infection and subsequent parasitic damage of spinal cord tissues.

Jennifer Morrow, PhD, of IDEXX Laboratories, emphasizes that testing for the antibody IgG (immunoglobulin G, the protein with antibody activity that's found in highest concentration in serum) has never been promoted as a "screening" test, but rather these tests are intended to be used as an adjunct to clinical assessment when diagnosing a horse showing neurologic signs. She notes that existing test procedures are similar in that they detect specific IgG raised in response to infection by *S. neurona*.

Granstrom concurs, "None of the current assays should be used as a screening test for horses that don't exhibit clinical signs, for example, for prepurchase examination. Current EPM tests are useful adjuncts to the clinical diagnosis of active disease."

PCR Testing

PCR (polymerase chain reaction) testing identifies minute particles of DNA specific to *S. neurona* when protozoa are present in the spinal fluid. The parasite might not remain following its damage to the central nervous system, so a negative PCR test does not entirely eliminate *S. neurona* from the list of suspicion.

A big problem in tracking the presence of antibodies in the CSF is that any blood contamination in a CSF sample can yield a positive result even if a horse has only been exposed (there could be antibodies in the blood). Morrow notes, "The PCR test on CSF only for the presence of *S. neurona* parasites, while a very specific test, has poor clinical sensitivity; due to the transient appearance of parasite DNA in the CSF, it often tests as a 'false negative.'

Western Blot or Immunoblot Assay

Granstrom reports that although a number of diagnostic tests are commercially available, he feels the first and best test is the Western blot (WB) or immunoblot assay developed at the University of Kentucky in 1991 and now run by IDEXX. A Western blot test gives a reading of "positive" or "negative" as to the presence of antibodies in the serum or CSF fluid, but it does not quantify the extent. Nor does it determine if a horse is only showing antibodies from a previous exposure and is not actively infected with EPM (this can lead to false positives, or saying a horse is actively infected when it is not in danger of developing disease).

Granstrom comments, "The assay has undergone a number of enhancements and has withstood the test of time. It remains the gold standard to which all other tests are compared. Many claims have been made about the development of other assays with greater sensitivity and/or specificity, but these assays have not performed as well as predicted in actual practice. A set of standard samples was submitted to various commercial labs and the results were reported by Dr. William Saville (of The Ohio State University veterinary school) at the 2007 annual meeting of the American College of Veterinary Internal Medicine (ACVIM). Based on the findings (see below), the original EPM Western blot test continues to outperform other assays."

Morrow notes, "A positive result (on serum or CSF) by the standard Western blot supports a diagnosis of EPM in the presence of accompanying clinical signs. A negative standard Western blot result (serum or CSF) essentially rules it out, except for acute onset cases or in immune-compromised horses for which there might not be a detectable IgG response. While the Western blot test format is not intended to be a quantitative one and doesn't provide a number, it gives a semiquantitative assessment of antibody level, so samples collected at different times can be directly compared to each other, with observable changes in levels."

She is a believer in testing CSF with Western blot to corroborate a diagnosis, noting, "For vets experienced in doing a spinal tap, the CSF Western blot test gives further support to a positive diagnosis, while a negative result is generally a good rule-out."

Immunofluorescent Antibody Test

An immunofluorescent antibody test (IFAT) identifies surface antibodies in a blood sample. This means the test is specific for antibodies against an entire protozoon rather than portions or protein components of the organism. Granstrom remarks, "The IFAT relies on antigens (substances foreign to the body that evoke an immune response) at the surface of the parasite to detect antibodies, which are shared, in part, with *S. fayeri*, another common equine parasite. Intracellular protein antigens are not exposed for detection. In theory, surface antigens should be available throughout active infection as parasites are continually released from dying cells and infecting new ones."

The IFAT reads out a quantifying number, or titer, that expresses the concentration of antibodies circulating in the horse's blood. In theory, a high concentration of antibodies in the blood indicates the horse is, or has been recently, infected. Comparative titers taken a few weeks apart indicate if a horse's

antibody level is rising, and, if so, this corroborates an active infection that could be linked with clinical signs of neurologic disease.

Proponents of the IFAT suggest it might be used as a screening test to determine if it is necessary to pursue a spinal tap for CSF testing on those horses with borderline titers.

Paulo Duarte, DVM, MPVM, PhD, a veterinary epidemiologist currently based in Kenya, used the IFAT as a diagnostic test while researching EPM at Colorado State University. He explains the testing process, "Parasites cultured in the laboratory are fixed into small wells in microscope slides. Antibodies, commercially produced in the laboratory against the horse's natural antibodies and linked with fluorescent material, are added to these wells, followed by the blood serum of the horse being tested for EPM. If antibodies against EPM parasites are present in the blood of the horse being tested, they will adhere around the cells of the parasites in the slides. In a chain reaction, the anti-horse antibodies with the fluorescent material will stick to the horse's natural antibodies adhered around the parasite, making it glow. This is considered to be a positive reaction. If there are no natural antibodies against the EPM parasites in the blood of the horse being tested, then this ‘chain reaction' will not occur and the parasite will not glow, reflecting a negative result."

Duarte suggests that the IFAT is an alternative diagnostic tool and offers some advantages over the most common test used for EPM, the Western blot. He comments, "The main advantage is that you can quantify the amount of antibodies in serum and interpret the test differently based on the antibody concentrations. A high antibody concentration would be more indicative of infection compared to a low antibody concentration, even though both could be above the threshold concentration for a positive result on the Western blot."

Duarte is enthusiastic about the sensitivity of this test, noting, "In our studies, approximately 83% of the horses confirmed infected with *S. neurona* were correctly diagnosed by the IFAT as infected. Similarly, 97% of the horses that were confirmed not to be infected with *S. neurona* were correctly classified by the IFAT as noninfected."

Granstrom comments, "In general, rising titers can be a useful diagnostic tool for many diseases. However, it's important to remember that horses may be exposed to *S. neurona* repeatedly without causing disease. An *S. neurona* titer may or may not be related to the current clinical picture. In my opinion, it's most important to know if the horse is positive or negative, i.e., has it been exposed or not. The overwhelming majority of negative horses (on serum or CSF) don't have EPM. Positive serum samples with a wide range of antibody concentration are extremely common with or without clinical disease. Positive CSF warrants concern."

Duarte discusses the confusion of a positive test result obtained with cerebrospinal fluid obtained directly from the spinal canal: "Because there is natural passage of antibodies from blood to the CSF, a positive CSF test might indicate presence of antibodies from blood as opposed to antibodies produced in the central nervous system, which would indicate infection. Using the IFAT, the concentration of antibodies in the blood can be determined and interpreted accordingly to increase the confidence in the

serum test results alone. Likely, the best use of a CSF test is by itself, when a spinal tap is required to rule out other causes of neurological disease."

ELISA

An enzyme-linked immunosorbent assay (ELISA) is a test for detecting antibodies to surface proteins of the protozoa, and it is currently only available through Antech Diagnostics. The results are given as a quantitative titer as with the IFAT. Antibodies it detects are very specific, and some strains will not be detected, hence, leading to a negative titer in spite of a horse having an active infection. Granstrom explains the limitations: "The ELISA test relies on recombinant SAG1 (a specific protein) antigen to detect antibodies. However, it has been demonstrated that many *S. neurona* isolates do not produce SAG1. Horses infected with those strains will test falsely negative. Antibodies produced against *S. fayeri*, another common equine parasite, cross react with SAG1."

Gene Expression Identification

Martin Furr, DVM, Dipl. ACVIM, a professor and Adelaide C. Riggs Chair in Equine Medicine at the Virginia-Maryland Regional College of Veterinary Medicine's Marion duPont Scott Equine Medical Center, is excited about the potential for gene expression identification of EPM horses. He explains that different diseases express themselves in a DNA "signature." Using this technology on a horse's blood sample should allow positive identification of a diseased horse based on six gene markers that have a high sensitivity and specificity for EPM.

Furr reports, "The test we describe is a gene expression assay; that is, when infected with a particular agent, a characteristic set or combination of genes is expressed or suppressed as compared to normal background values. The genes that are either expressed or suppressed are compared to a standard for horses known infected with *S. neurona*, and if the ‘pattern' of upregulated and downregulated genes matches, then the diagnosis is confirmed. Essentially, it is looking for a specific ‘pattern' of gene expression in the test horse and comparing it to a known positive case."

He reports, "Experimentation found the test to be greater than 90% sensitive and specific for *S. neurona* infection during the first month of infection."

At the present time, this test is not yet ready for field testing, which is required before it can be made commercially available.

Granstrom acknowledges the growing field of genetic testing. He says, "Recent completion of the equine genome sequence and ongoing efforts to create a roadmap of various functional areas of the genome should allow researchers to answer questions related to genetic predisposition for many diseases. I doubt that we will find a straightforward genetic defect related to *S. neurona* susceptibility, similar to what was found with hyperkalemic periodic paralysis (HYPP, a muscle disorder in Quarter Horses). It seems more likely that a complex relationship between genetic makeup and environmental factors will cloud the picture. I suspect that we'll find a broad array of complex genetic patterns associated with susceptibility to clinical disease, which may or may not have significant diagnostic relevance."

Vaccine Interference with Testing

A horse that has been vaccinated against EPM (using the original Fort Dodge Animal Health product) will have developed antibodies to the protozoa and the component proteins of the protozoa, even though the horse hasn't actually been infected with EPM. This poses a problem for screening or testing since a blood or CSF test result will come up positive regardless of whether or not a vaccinated horse is truly neurologic due to EPM. This vaccine is no longer available, although new vaccine research is in progress (see section on EPM Prevention on page 48).

Sharon Witonsky, DVM, PhD, Dipl. ACVIM, an associate professor of large animal clinical sciences at the Virginia-Maryland Regional College of Veterinary Medicine, is actively involved in EPM research. She reports, "In our study (2004), 89% of horses that were initially seronegative became seropositive at Day 14 following the second EPM vaccine (Fort Dodge) dose. Of those horses which were initially negative for antibodies in the CSF prior to vaccination, 57% became positive for *S. neurona*-specific antibodies in the CSF at Day 14 following the second vaccine dose. There were no detectable differences in the Western blot banding patterns between horses that became positive due to vaccination versus natural exposure." This means the "positive" test for EPM was reading the vaccine rather than naturally occurring infection.

Morrow concurs based on the study, which was done after the vaccine was first released: "Vaccinated horses developed an IgG response in serum and CSF that could not be distinguished by Western blot from natural infection. Unfortunately, we did not have access to those horses to follow duration of persistence, so it's unknown how long it lasts."

The Final Tally

Morrow notes a pertinent point: "Although it's known that response to treatment is used by some vets to corroborate a diagnosis of EPM, it's really not the ‘best practice.' For a number of years (and maybe still), there was a misperception that ‘all samples tested positive,' so many vets chose to stop testing. Morrow explains that recent analysis of many thousands of clinical samples (from the United States)--submitted to and tested by standard Western blot--indicated that on average 58% of sera and 38% of CSF tested Western blot positive, with distinctive geographic differences. She continues, "So, many samples do test negative and a negative result is usually a very good rule-out. A test is a much smaller initial investment than a round of treatment that may or may not be effective, or necessary."

William Saville, DVM, PhD, Dipl. ACVIM, of Ohio State's veterinary school, has been instrumental in comparing different testing methods for EPM. He reports, "The Western blot (IDEXX/EBI) and IFA (University of California, Davis) results were in the best agreement with each other and the histories of the horses in the study set. Since none of the 21 sera tested positive by the SAG1 ELISA, it's difficult to draw any conclusions about its ability to detect horses infected with *S. neurona*."

Granstrom concurs, "In my opinion, none of the tests are capable of validating the presence of clinical disease alone, despite claims to the contrary. A positive PCR test probably comes the closest, but parasite DNA is rarely present in sufficient quantities to detect. The rest of the assays detect the

presence of antibody--some with more specificity and/or greater sensitivity than others. The best of these is nothing more than an adjunct to a good clinical history and a thorough neurologic exam."

Granstrom concludes by noting, "The combination of serum and CSF analysis provides the greatest amount of information and affords the clinician the best opportunity to make an informed decision and accurate diagnosis."

In the future, he has hopes that another ELISA test will become available: "The recombinant SAG2 ELISA being developed by Dan Howe, PhD, at the University of Kentucky looks promising. It will be interesting to see how it performs under clinical conditions. In actual practice, it may require additional antigens to compensate for the inability of the immune system to recognize every antigen it encounters."

Another Protozoon

Granstrom reports, "Although far less common, *Neospora hughesi* infection produces essentially the same clinical disease as EPM. A similar array of *N. hughesi*-based EPM tests have been developed that simply substitute *N. hughesi* for *S. neurona* to detect antibody in serum or cerebrospinal fluid. The apparent incidence of EPM due to *N. hughesi* infection is so low that the demand for testing is extremely low. Laboratory testing has shown that *Neospora* spp is susceptible to many of the same drugs used to treat *S. neurona* infection. I am unaware of any work done to establish a unique treatment regimen for equine neosporosis."

Take-Home Message

EPM is a disease that can mimic many other neurological or musculoskeletal problems. Coupled with a thorough physical and neurologic exam, testing on blood and/or CSF is valuable for confirming a diagnosis of EPM so appropriate treatment can be implemented.