



# EQUINE REPRODUCTION SVC

\*\*\* CLICK HERE \*\*\*

Current Articles &  
Research

Reproduction  
Foal Disorders  
Lameness

Check back often for  
new additions

## Testing for EPM, Lyme, and Other Equine Neurologic Diseases

A suddenly clumsy gelding has become aggressive toward a favorite pasture buddy, is salivating profusely, and wants to cuddle with his handlers, which for him is unusual. It's July in the Northeast, and the mosquitoes are out. It's clear he is neurologic, but what tests should you run? This is the kind of diagnostic puzzle that frustrates equine veterinarians. All they and the horse's owners want is a clear diagnosis, so that potentially life-saving treatment can begin, but the truth is that many equine neurologic diseases can only be definitively diagnosed on post-mortem examination.

Amy L. Johnson, DVM, Dipl. ACVIM, in her role as an assistant professor of Large Animal Medicine and Neurology at University of Pennsylvania School of Veterinary Medicine's New Bolton Center, admits that much of what she has learned about distinguishing one neurologic disease from the next has been from examining severe cases that have succumbed to neurologic illness, and she's applying what she's learned in studying these horses to help keep other horses out of necropsy. To help veterinarians navigate the confusing landscape of laboratory diagnostics for neurologic disease in the live horse, she presented guidelines for diagnosis at the 2015 American Association of Equine Practitioners Convention, held Dec. 5-9 in Las Vegas.

"If you know the best sample to collect, as well as the best test to request, it should improve your ability to diagnose neurologic diseases in horses," said Johnson, who focused primarily on equine protozoal myeloencephalitis (EPM) and Lyme disease in her presentation, but also touched on other infectious neurologic diseases as well. "Not only is choosing the test important, but you need to know how to interpret the test, and that's not necessarily as easy as it sounds.

"These tests require you to use all of your clinical skills ... you really need to be methodical," she said. "A neurologic workup should never start by pulling blood and sending it off to a lab. You need to start with your clinical examination, figure out if the horse really does have signs of neurologic disease."

If he does, localize the lesion and come up with a list of rule-outs that are appropriate for the signalment (age, breed, sex, and other identifying characteristics) of the animal, your location, and the history; all of these are factors that you need to ascertain by talking to the owner and thinking about the case.

"Only then do you figure out which ancillary lab tests are appropriate to submit," she said. "And when you get those results back, you do need to interpret them a little bit cautiously and understand all of the ambiguous results you can get and how you should interpret the information in light of the patient's clinical status."

Johnson also showed videos of several neurologic cases during her presentation, including the gelding described, providing signalment, case history, clinical exam findings, and lab test findings, and polling veterinarians in attendance via a smartphone app on what their next case steps would be.

### EPM

"We all know that EPM diagnosis is challenging, because there is extensive equine exposure to the protozoa (*Sarcocystis neurona*), and it can cause very variable signs of clinical disease ... it can mimic anything," she said. Though *Neospora hughesi* can also cause EPM, she detailed *S. neurona* in her presentation because it's the most common EPM cause and what she sees the most of in the Mid-Atlantic region.

She bases EPM diagnosis in the antemortem (live) horse on three principles:

1. **Presence of neurologic disease:** "Compatible clinical signs that are referable to the central nervous system," she said.
2. **Rule outs of other likely causes:** "That's why cervical radiographs might be so important, make sure there's no evidence of neck trauma, or some sort of stenotic or compressive myelopathy."
3. **Confirmation of *S. neurona* (or *N. hughesi*)-specific antibodies in CSF, serum, or both:** "Only then do we look for proof of exposure to the causative organism, and usually that's through immunodiagnosics, by documentation of antibody production in the horse."

"Your index of suspicion should be very, very high if you have a combination of ataxia, atrophy, and asymmetry," she added. "Also remember that the diagnosis of EPM is always presumptive without a post-mortem exam," she said.

Commonly used antemortem tests, which can be run on blood, cerebrospinal fluid (CSF), or both, and none of which are considered a gold standard, include:

- Western Blot (Standard, sWB, and Modified mWB)
- Indirect fluorescent antibody test (IFAT)
- Surface antigen (SAG) ELISAS (enzyme-linked immunoassays, SAG 2, 4/3; and SAG 1, 5, 6)

She explained that *S. neurona* expresses multiple surface antigens, and all of the SAG ELISAs are named by which antigens they detect antibody production against.

Polled veterinarians listening to Johnson's presentation reported they were using IFAT and SAG 2, 4/3 most in their practices. So, with all those tests available, what's so tricky about confirming EPM in the horse? Well, a positive blood test simply indicates that the horse has been exposed to *S. neurona*, but it doesn't necessarily mean he is currently infected and has central nervous system disease. As for looking at titers, Johnson doesn't find their magnitude to be helpful in her region in indicating likelihood of active infection vs. exposure. However, a negative blood test generally means no *S. neurona* exposure and, therefore, no EPM.

Of course, there's an exception to every rule, and in this case it is a horse that's been recently infected. He might not show up positive for more than a week.

"If the horse 'smells' like an EPM case," she said, "and you get a negative blood result back, and you still think the horse has EPM, test again in 10 to 14 days, and if that horse seroconverts during that time period—goes from negative to positive—that's really strong evidence, actually, that EPM is causing the disease." The same thing can happen with cerebrospinal fluid—testing before antibodies against *S. neurona* are measurable.

The rub with CSF is that false positives are common because of blood contamination and because antibodies diffuse over into the CSF in normal horses. With imperfect tests on both sides and no gold standard, what's a veterinarian to do? "I always encourage people, when it is possible, to test both blood and spinal fluid," she said. "And I realize that's tough to do in the field." Johnson said that a diagnostic test based on the titer ratio between serum and CSF antibodies, which authors describe in the recently available American College of Veterinary Internal Medicine's updated EPM consensus statement, provides the best diagnostic answers.

### Lyme Disease

Then there's Lyme neuroborreliosis, which is neurologic disease caused by *Borrelia burgdorferi* infection, carried by Ixodes ticks. It causes variable clinical signs.

"We think infection and seroconversion are very common, but true Lyme neuroborreliosis is likely rare," she said. In the polled veterinary audience, 51% reported they had never seen a case of Lyme neuroborreliosis and 40% weren't sure. "Maybe my definition of Lyme neuroborreliosis is too stringent, and that's why I think I only see a few cases. But I do think this is even more challenging than EPM," she said, noting that while she began studying Lyme nine years ago, she's not much further in understanding it than she was when she started.

For Lyme, equine diagnostic testing potential criteria include:

1. Possible exposure;
2. As with EPM, neurologic signs;
3. Abnormal CSF;
4. Evidence of nervous system infection by polymerase chain reaction testing (PCR, a type of test for pathogen DNA) or intrathecal (within the spinal cord) antibody production; and
5. It might also help to have a positive blood test or isolation of *B. burgdorferi* from other sites.

Antemortem tests available for Lyme include:

- Western blot;
- ELISA;
- IFAT;
- Stall-side SNAP tests; and
- The Multiplex assay, which 79% of the poll respondents in the audience said they were using. The Multiplex assay shows if the horse has antibody production against three different outer surface proteins: OspA, OspC (thought to indicate early infection), and OspF (thought to indicate chronic infection), she explained. Unlike other species, horses produce antibodies against OspA with natural infection; they don't need to be vaccinated to develop antibodies.

"I don't know, quite frankly, how important it is to know whether you have early infection or chronic infection," she said. "I'm not sure whether it makes a difference in terms of diagnosis and treatment, and also, it's complicated because a lot of horses have more than one exposure. What if you have a horse that has 12 ticks on it that are infected with *Borrelia* over the course of a year, and it's getting reinfected or re-exposed, what do its antibodies do, and how does that correlate with what you see on the Multiplex? I don't think anybody knows how to use all the information that is provided by the test."

She can say with confidence, however, that interpreting Lyme test results is similar to EPM. Positive blood result? The horse has been infected at some point with *B. burgdorferi*, and it could have disease, but that might not be true at test time. There's also the issue of vaccination; if the horse has been vaccinated against Lyme, the veterinarian needs to know what antigens the vaccine contained before interpreting test results.

"If you get a negative blood result, I think that it usually means no exposure or no infection, no disease, but not always," she said. "There are horses that have local antibody production such that they truly have infections with *Borrelia* but are negative on blood tests, and those horses are frustrating. As with EPM, is it better to use paired blood and CSF? I think it is, but this is not even as straightforward as EPM."

A few other things (yes, more) can make diagnosing Lyme disease complicated. Horses with Lyme neuroborreliosis have a leaky blood-brain barrier, so it's expected that there will be more antibodies in the CSF than would be usual. But the practitioner needs to remember that the Multiplex test is run on an undiluted CSF sample, whereas blood testing is done on a diluted sample.

For this reason, she said, the CSF titers will be about twice what you see in the blood of an exposed but uninfected horse, while they'll be at least four times what you see in blood in a sample that has evidence of intrathecal *B. burgdorferi* antibody production. However, she cautions that this approach to analyzing results is unvalidated. Also, some horses that are negative on blood are positive on CSF, and vice versa, so a paired test (blood and CSF) isn't a bad idea with suspect cases.

Lastly, Johnson mentioned a few take-homes about other viral diseases:

- If a veterinarian is concerned about EHV-1 (myeloencephalopathy) infection, "for any individual neurologic case we recommend that you submit both blood and nasal swabs for PCR testing ... you can have a horse that is infected with EHV-1 that is positive on blood and not nasal swabs, or vice versa, even though a lot of horses are positive on both.
- There is no advantage to testing CSF for West Nile virus.
- Always pay close attention to vaccination history in light of the signs. Unfortunately, said Johnson, vaccination was the clue for the gelding described in the opening of this article, who ended up with a rabies diagnosis post-mortem. "The horse (a recent adoption) came with no vaccination history and is a good example of how these horses can just slip through the cracks," said Johnson. "It's important to talk about vaccination history for these adopted horses and obtain a written record of what the horse has actually received."

In summary, Johnson said, "Lab tests are helpful in the diagnosis of neurologic disease but are an adjunct, not the be-all and end-all! If it were as simple as taking a blood test and seeing what the results say, anyone could do it, and there would be no confusion. Instead, appropriate diagnosis requires a lot of careful attention and thought regarding the history, clinical exam, and interpretation of test results. Vets need to stay up-to-date on what tests are available and how to interpret the results, as two different patients with the same lab test results could end up having two very different diseases."