By Heather Smith-Thomas
Photos by Anne M. Eberhardt

Looking at EPM

Parasite affects nerves that control the horse's movements

Equine protozoal myeloencephalitis (EPM) is one of the most common causes of neurological problems in horses. The parasite that causes this disease is a one-celled protozoan that invades the brain and spinal cord and affects nerves that control the horse's movements.

THE DISEASE

The first description of EPM in horses was published in 1970. It took several more years before research began to unravel some of the mysteries of this debilitating disease, and we are still learning about it. Most EPM cases in horses are caused by the protozoan *Sarcocystis neurona*, though some are caused by a protozoan called *Neospora hughesi*. *S. neurona* is found in North, Central, and South America and parasitizes several hosts including the cowbird, raccoon, cat, skunk, armadillo, sea otter, and opossum. The horse is an accidental host that picks up the parasite when eating feed contaminated with feces from an animal shedding oocysts. The most common mode of infection in Thoroughbreds is consumption of feed contaminated with opossum feces.

If the parasite gets past the horse's natural defenses and invades the brain or spinal cord, the horse can show signs of EPM. If the parasite attacks the spinal cord, primary signs are ataxia (inability to control muscle movements), stumbling and incoordination, weak and wobbly gait, multi-limb lameness, and muscle atrophy. If the parasite attacks the brain, which happens less commonly, signs may include depression, blindness, behavioral changes, seizures, and cranial nerve paralysis (affecting facial muscles, hindering ability to chew or swallow, with head-tilting and atrophy of various head muscles).

Dr. Nicola Pusterla, associate professor at the University of California-Davis, says about 10% of the neurological case load at their large animal hospital is diagnosed as EPM. "We also see a second organism that causes it. *N. hughesi* was once thought to be just a disease of the western part of the U.S., but we know now it is more widespread," said Pusterla.

DIAGNOSTIC TESTS

Diagnosis is often based on clinical signs but can be difficult because other neurologic disorders such as wobbler syndrome can show similar signs. After ruling out other nervous system diseases, laboratory tests are generally used to determine if the horse has EPM.

Currently, several tests are available, including the Western blot, ELISA, and most recently the IFAT test. The Western blot test has been available the longest.

Horses become accidental hosts of the *Sarcocystis neurona* parasite that causes EPM

"It is qualitative and gives a positive or negative result, checking for antibodies," Pusterla said. It can show that a horse has been exposed to *S. neurona*. A positive result does not mean the horse has an active infection, however. Many horses come into contact with this parasite, develop antibodies, and never display symptoms of EPM.

The ELISA (enzyme-linked immunosorbent assay) test is quantitative, measuring the level of antibodies in the horse. Titers above a certain level indicate not only exposure to *S. neurona* but possible active infection, if the horse is also showing clinical signs.

Dr. Dan Howe of the Gluck Equine Research Center at the University of Kentucky helped develop the ELISA tests in 2005, based on specific *S. neurona* proteins (antigens).

"We have also done this for *Neospora hughesi*. These assays seem to be very accurate in terms of sensitivity and specificity for detecting antibodies against these two parasites in a horse, whether in serum or cerebrospinal fluid (CSF)," Howe said. "By comparing the amount of antibody present in the serum and the amount present in the spinal fluid, you can assess with some confidence whether the horse has an active infection in the central nervous system."

He is presently working on a new ELISA test that uses a blend of surface antigens.

The most recently developed IFAT (indirect immunofluorescent antibody tests) can check for both organisms that cause EPM. "These two tests were developed and validated at UC-Davis," said Pusterla. "With a mathematical model the researchers have also determined probability of the disease based on titer (antibody level in serum and in cerebrospinal fluid). If the horse is showing neurologic signs, the higher the antibody titer is, the more likely you are dealing with EPM," he said.

Spinal taps to test the cerebral spinal fluid are still useful. "If we find high titers in a neurologic horse, indicating a high probability of disease (80-90% probability), there is not much benefit in also doing a spinal tap. But if we get low titers, with probability of disease between 30-60%, then it is worthwhile to do the spinal tap," explained Pusterla.

"We've had a few instances in which a sample had undetectable antibodies in blood but high levels of antibodies in the cerebrospinal fluid. We would have missed them if we hadn't tested the CSF. If diagnostic work doesn't match the clinical impression, you need to pursue it further rather than just ruling out EPM because the test was negative. If you still suspect EPM, it's worthwhile to do a spinal tap, which mainly helps rule out other diseases that can be tested via the CSF," he said.

No foolproof test exists for EPM as any of the existing tests may sometimes show a false positive. The lab at UC-Davis offers both the Western blot and IFAT tests at the same time, which can help, but there is still a need for better tests. Dr. Sharon Witonsky, an associate prrofessor at Virginia-Maryland Regional College of Veterinary Medicine is part of a group doing research on the equine immune system's response to infection, trying to define better what is happening with immune responses in horses that develop EPM.

Another diagnostic test being developed uses new technology involving a gene chip that has many bacteria, protozoa, and viruses on it. According to Dr. Steve Reed of Rood & Riddle Equine Hospital in Lexington, this test might be useful to diagnose neurological conditions caused by herpes viruses, encephalitides, etc., and some researchers would like to develop this test for EPM. This may open a new diagnostic testing model for all neurologic diseases, according to Reed.

"At this point, however, the best foundation for diagnosis is still a very thorough neurological exam," said Dr. Amy Johnson of the New Bolton Center at the University of Pennsylvania. "EPM gets blamed for many things, in terms of poor performance, lameness, or other diseases that have other explanations. I encourage horse owners to get their veterinarian involved early in the process before deciding that the horse has EPM and starting treatment," she said.

TREATMENT

Some horse owners and veterinarians, frustrated with diagnostic challenges, simply treat a suspicious horse for EPM and monitor the horse's response. If the horse improves, they figure it had EPM.

Several medications are now available to treat this disease. For many years there were no FDA-approved drugs for EPM, and veterinarians used compounded drugs that were effective in killing or inhibiting protozoa. These drugs included ponazuril, diclazuril, nitazoxanide, and pyrimethamine-sulfadiazine. Ponazuril was the first approved drug, marketed as Marquis. The pyrimethamine-sulfadiazine combination also gained FDA approval (marketed as Rebalance), but the company that made it went out of business. Currently it's used as a compounded medication.

Johnson said there are new developments in treatment options.

"A research group in Illinois recently looked at giving a loading dose of ponazuril (the active ingredient in Marquis) at the beginning of treatment, to increase spinal fluid levels of the drug more quickly," Johnson said. "Veterinarians have been doing this in the field, and, anecdotally, it seems to help. There hasn't been any published clinical research on this; the project in Illinois did not treat clinically affected horses. They were just administering the

drug to normal horses to measure the blood and spinal fluid levels obtained. They found that cerebrospinal fluid levels increase to steady levels after just one day with the loading dose, whereas a typical maintenance dose took 10 or 11 days to reach the same steady-state levels in the CSF."

"Another thing this group experimented with was mixing DMSO with ponazuril. They found absorption was much better than when ponazuril was given alone. DMSO is very good at carrying many drugs through physiologic barriers," she said.

Dr. Rob MacKay of the University of Florida published an article in 2006 suggesting that treating EPM horses earlier in the disease process results in a better overall prognosis for full recovery. If a horse owner is suspicious that a lameness or incoordination problem might be EPM, he/she should have the horse assessed and tested.

Many horses exposed to the parasite never show any clinical signs

Reed says many horses respond seemingly well to treatment but still have muscle loss.

"A Thoroughbred racehorse, for instance, even if he is healthy again, if he's lost half his gluteal muscles, he won't be able to compete again at top level," he said.

PREVENTION

At present no vaccine protects against *S. neurona* or *N. hughesi*. An earlier vaccine against *S. neurona*, made by Fort Dodge under a temporary conditional license, failed to pass subsequent tests for efficacy and was withdrawn. "They were not able to prove efficacy because they had a hard time establishing an animal model," said Pusterla. Researchers must have a way to reproduce the disease consistently in a test group of horses in order to test a vaccine's effectiveness. Standard procedure is to vaccinate one group of horses and leave a control group unvaccinated, then challenge both groups with the disease.

There will be another formulation of diclazuril (similar to ponazuril) available soon, in pelleted form that could be mixed with the horse's feed. This anti-protozoal drug could be used for prevention of EPM (fed at a low dose for

How The Parasite Invades Horses

There are many types of sarcocysts in the environment, including specific types that infect cattle, sheep, dogs, etc. This parasite takes advantage of a predator-prey relationship. The organism is passed in feces of the carnivore (canine or feline) and eaten by a grazing animal, where it continues development in the gut and passes into other parts of the body to form cysts in muscles. When the herbivore dies or is killed and eaten by a predator or scavenger that serves as the other host, cysts come to life in the gut of that carnivore and produce oocysts to be passed out with feces and eaten by a herbivore, to start the cycle again.

The protozoan parasites cannot be passed from horse to horse and are only picked up when contaminated feed is consumed. After entering the horse's gut, oocysts go through one round of replication. Then the parasite wants to enter the blood stream and be disseminated throughout the body, to create cysts. It does this by entering the white blood cells.

In most horses the immune response takes care of the infection. But in a few instances, especially in stressed horses, the parasite overcomes the immune system and travels through the body, attacking the spinal cord or brain. Dr. Siobhan Ellison says the surface of these parasites is covered with molecules called surface antigens.

"These allow the parasite to enter cells of the host and modify its immune system, making the horse more likely to get EPM," Ellison said.

If the parasite can get into the white blood cells, it can be carried around the body, hidden from the immune system.

"One theory is that horses with an inflamed gut have more white cells than normal in the gut," Ellison said. "The parasite can then be picked up and carried into the bloodstream. Most of the time the horse will get rid of that little infection, but about 1% of the time—which is about the incidence of this disease in horses—if it's the proper strain of *Sarcocystis*—it is carried to the central nervous system and the horse gets EPM."

The route of transmission for *Neospora hughesi* is different and some horses become infected without eating contaminated feed. Dr. Nicola Pusterla at the University of California-Davis has been researching how this organism is spread.

"A closely related organism called *Neospora caninum* causes abortion in cattle and has a huge economic impact on the livestock industry," Pusterla said. "In cattle there are two routes of transmission. One is horizontal—going to cattle from the definitive host (dogs or wild canines, who pass oocysts in their feces, and defecate on pasture or hay). But the most efficient way the organism is transmitted in cattle is vertically, from an infected dam to the offspring during gestation."

In the cow's gut, the parasite reproduces and penetrates the lining of the small intestine, to enter various body tissues, including the fetus (via the placenta) in pregnant animals.

"Infection of the fetus can cause abortion, or birth of a persistently infected animal, depending on the immune stage of the fetus when infection occurs," Pusterla said. Infected female calves can in turn transmit the parasite to their offspring.

He thinks this mode of transmission also occurs in horses—from dam to fetus. "I've been working with a herd in which two mares tested serologically positive to *Neospora hughesi*. Every one of their offspring we've tested shows evidence of vertical transmission. It definitely happens in horses as well as in cattle. Once the animals are congenitally infected, they can pass the disease to their progeny. If it's a filly and she's bred after she grows up, she can transmit EPM to her foals," he said. *By Heather Smith-Thomas*

TRADE ZONE

long periods) as well as for treatment. The formulation already has FDA approval, but hasn't been put into a commercial product yet.

"I haven't seen any studies on this, but it is definitely something people will consider," Pusterla said. "It may not be labeled for prevention, but EPM is always easier to prevent than to treat, so some people may use it for prevention, especially in animals at risk—such as young animals in stressful situations or high levels of competition."

Good management practices—such as keeping feed in closed containers and keeping wildlife out of barns and feed areas—is the best prevention at this point. Minimizing stress is another way to help protect horses, because stress may inhibit the immune system.

WORKING TOWARD A VACCINE

Protozoal diseases are a challenge for creating effective vaccines.

"There are a lot of technical issues. Also, we have to think about where we want to halt the organism—at the gut, or while they are still moving around in the body (because we still don't know how they get into the central nervous system)," said Pusterla.

The problem with a vaccine is that our present diagnostic tests rely on indirect assessment of exposure, measuring antibody titers. Most vaccines use whole or parts of organisms, and the tests don't differentiate between an animal that's vaccinated and one that has been naturally exposed.

"I'm not sure how successful a vaccine would be," Johnson said. "Many horses are exposed to the parasite and never show any clinical sign. They are able to fight it off on their own, without being vaccinated. In horses that don't have an immune system strong enough to fight this infection, I don't know that a vaccine would enable them to be successful

in clearing the parasite, or whether their immune system would still be less able to build an adequate response."

A vaccine may only protect horses that are already protected by their own immune systems and not help horses that have trouble. Only a very small percent actually develop EPM; it has been estimated that less than 1% of horses exposed to the parasite actually show signs of clinical disease and have problems with their brain or spinal cord.

Because there are several strains and at least two protozoa that can cause EPM, effectiveness of a vaccine might depend on whether it mixed different strains or was just targeting one.

The picture is complicated by the fact several strains of *Sarcocystis* do not cause disease but share some of the surface antigens. Dr. Siobhan Ellison, who has done research for many years on EPM, says statements saying 60-70% of horses have been exposed to *S. neurona* are incorrect. "They have been exposed to *Sarcosystis*, yes, but not necessarily the strain that causes EPM," she explained.

Opossum feces may contain multiple species of *Sarcocystis*.

Ellison, president of Pathogenes Inc., a company working to develop an EPM vaccine, has come up with a model of infection in which she tested a new vaccine using one of the surface proteins of *S. neurona*, which appears to be effective. She has plans for a larger efficacy study. This vaccine would not protect against *Neospora*, however.

"We still don't understand enough about the disease," said Howe. "A lot of horses get infected with *Sarcocystis neurona*. You could test blood on 100 horses here in the Lexington area and probably half of them would show positive antibodies. They have encountered it, probably several times, during their lives," he said.

"We don't understand why most horses can encounter the infection and have no problems. Others, when they get infected, develop EPM, but we don't know why. This is the key point for understanding EPM and being able to control it better, especially from the standpoint of an effective vaccine," he said.

"Thankfully, we have drugs for treating horses, but the drawback is that you are treating after the individual has already become infected. This is a neurologic disease, and damage done to the central nervous system may not heal like other tissues do," said Howe. There may be residual impairments after recovery. If we could prevent the infection, this would be better.