
EPM



Newsletter

Michigan State University, College of Veterinary Medicine

March 2001

Volume 3

New Information on the Lifecycle of *Sarcocystis neurona*

Sarcocystis neurona is a protozoan parasite that causes equine protozoal myeloencephalitis (EPM). EPM is a neurological disease that can attack both the brain and spinal cord of horses and often mimics other neurological or lameness problems. The course of disease with EPM can be acute (come on very quickly) or cause less severe, more chronic problems. Many horses have a mild lameness as the first sign of disease, which may be difficult to distinguish from other orthopedic problems resulting in lameness.

The parasite multiplies in the intestines of opossums. It is shed as a sporocyst in the feces of the opossum and can contaminate feed sources, such as grain, hay or grass, depending on where the opossum defecates. Recently, it has been shown in the laboratory that cats can act as an intermediate host for the parasite. That is, when cats were given high numbers of sporocysts from opossums orally, they developed *Sarcocystis neurona* sarcocysts in their muscles. These sarcocysts can infect opossums when the opossums eat the sarcocysts in cat muscles. It is unlikely that cats commonly act as an intermediate host in the natural setting. Scientists are examining what other animals may fulfill this role.

Horses become infected by ingesting sporocysts in feed that is contaminated with opossum feces. It is presumed that the parasite must penetrate the gastrointestinal tract to travel through the blood stream to the brain and spinal cord. The mechanism by which the organism enters the central nervous system is unknown at this time. It is also unknown how long the process takes from ingestion until disease is recognized in the horse. However, a horse may be exposed to *S. neurona* without developing disease. Horses are dead-end hosts for the parasite; they cannot transmit the organism to other horses or to opossums. *Submitted*

by Linda S. Mansfield, M.S., V.M.D., Ph.D.,
Departments of Large Animal Clinical Sciences and
Microbiology, College of Veterinary Medicine,
Michigan State University, East Lansing, MI. □

Prevalence of *Sarcocystis neurona* Antibodies in Michigan Horses

Sixty percent of the horses in Michigan have antibodies to *Sarcocystis neurona*, according to a study recently completed by the EPM Research Group at Michigan State University's Population Medicine Center. The purpose of the study was to determine what proportion of Michigan horses have been infected with the parasite and to identify horse management factors that may put a horse at greater or reduced risk of testing positive.

The study sample was designed using a map of the Michigan opossum population. The state was divided into three "opossum districts." The northernmost district (#1) had the fewest opossums, the southernmost district (#3) had the most opossums, and the central district (#2) was intermediate compared to the other two.

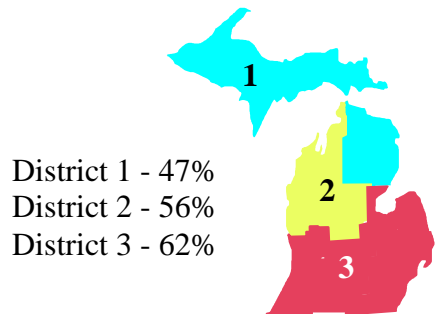
Over 1,100 horses from 98 farms were tested for antibodies in their blood (serum). The results were obtained using the new MSU Western blot test, which has improved accuracy over pre-existing Western blot tests. A positive blood test indicates that a horse has been infected by the parasite at some point in its life, but cannot be used to determine whether the parasite has gained access to the central nervous system (the brain and spinal cord).

In addition to collecting blood from every horse in the study, information was obtained from the farm owners about how their horses were housed, what feeds they were given and how feeds were provided (such as outdoors versus indoors, etc.). Information about the individual horses was also collected, such as breed, age, travel history and

whether the horse had ever been diagnosed with EPM.

The results of the blood testing showed that antibody prevalence (seroprevalence) increased along with the opossum population. (See map.)

Overall Seroprevalence - 60%



Nearly half (48%) of the horses aged 1 year or less tested positive, indicating that the parasite is very common on Michigan horse farms. Horses aged 11 or more were twice as likely to test positive, compared to horses aged 1 year or less. In a separate analysis of a subset of the data (comprised of horses that had lived their whole lives on one farm), there was a slight increase in risk of testing positive among horses exposed to pasture.

There were no management factors (such as horse housing, how feeds were provided, etc.) that were strongly associated with the likelihood of testing positive. This suggests to the researchers that in areas with opossums, horses can encounter the parasite in many feeds and situations, and there isn't one simple change in management that horse owners can make to prevent infection. The association between opossum abundance and seroprevalence does provide a rationale for making an effort to prevent opossums from getting into barns and feed bins, and to relocate or cull opossums to keep them away from horse farms.

One encouraging finding of the study was that of the 1,121 horses tested, only 10 had been diagnosed with EPM within 1 year. This means that even though many horses will test positive for serum antibodies to *Sarcocystis neurona*, very few will go on to develop disease. This reinforces the importance of getting a proper diagnosis of a neurological disease and not relying on a blood test to determine whether a horse has EPM. So although a blood test can be used for initial screening, further testing and clinical examination is needed to confirm a diagnosis of EPM. *Submitted by Mary Rossano M.S., Department of Large*

Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI. □

Treatments for EPM: What's New for 2001?

There has been a lot of research activity since the last edition of this newsletter on medications to treat horses with EPM. Several drug trials have been completed and the results are currently under review by the FDA. These products are expected to hit the market later this year.

However, before we talk about new medications for treating EPM, there are a few points about treating horses with EPM that we should discuss. First, diseases that affect the nervous system are **bad diseases** because nervous tissue, in comparison to other body tissues, has a poor ability to recover once it has been damaged. Thus, when EPM damages the central nervous system, it is unrealistic to expect full recovery in all affected horses. Thus, treatment results can be frustrating and are unpredictable. Second, the protozoa responsible for EPM, *Sarcocystis neurona* and *Neospora hughesii* (in an occasional case), hide out inside neuronal cells in the central nervous system. As a result, they can effectively evade many arms of the immune system and long-term treatment is usually required. Third, as anyone who has treated a horse for EPM can attest, treatment is expensive. Thus, an **accurate diagnosis must be established** before treatment is pursued! Unfortunately, despite development of the western blot test to detect antibodies to *Sarcocystis neurona* in blood and spinal fluid, a definitive diagnosis still cannot be made in the living horse. Thus, the diagnosis is best made by presence of signs consistent with neurological disease, exclusion of other known neurological diseases, and positive western blot test results in both blood and spinal fluid.

Once daily oral administration of a **sulfadiazine/pyrimethamine** suspension remains the most commonly recommended (and most economical) treatment for new cases of EPM. Both drugs inhibit production of folic acid by the protozoa and thereby result in inhibition of growth (termed static drugs) and eventual slow death of the parasite. The latter drug (pyrimethamine) is also referred to as the anti-malarial drug and may be better known by its trade name Daraprim®. The

treatment ranges from 2-6 months in duration and the drug combination is most economically acquired from compounding pharmacies and sold at costs of \$100 for a month's supply for a full-sized horse. (Licensed compounding pharmacies in your area can be found by having your veterinarian contact the International Academy of Compounding Pharmacies at 1-800-927-4227 or via the internet at www.iacprx.org.) In general, we expect about two-thirds of treated horses to improve with this treatment but only about 50% (of all affected horses) are likely to return to performance at their prior level of competition. Treatment should continue for 30 days beyond resolution of neurological deficits or stabilization of clinical signs (in the case of incomplete recovery). To date, there is no information to support any benefits of prolonged treatment for stable cases of EPM or prophylactic use of these medications in an attempt to prevent EPM. Once medication is stopped, horses have a 10-20% chance of relapse. Although unproved, clinical signs similar to the initial disease support incomplete killing of the protozoa during the first round of treatment, rather than a new infection, as the cause of relapse. Initially, dietary supplementation with folic acid was widely recommended for horses being treated with sulfadiazine/pyrimethamine suspensions. However, horses actually absorb folic acid in a slightly different chemical form, tetrahydrofolate, which is plentiful in pasture and good quality hay. Supplementation with folic acid may actually inhibit intestinal absorption of tetrahydrofolate and has been incriminated as a cause of birth defects in a few foals whose dams were receiving sulfadiazine/ pyrimethamine suspensions and folic acid during pregnancy. The occasional horse shows signs of folic acid deficiency (mild depression and anemia due to bone marrow suppression) while being treated with these anti-folate drugs. The best treatment is pasture turn-out. Alternatively, supplementation with folinic acid, rather than folic acid, can be helpful but is very expensive (nearly \$1000/month).

Since the use of sulfadiazine/ pyrimethamine suspensions produces rather poor results (one-third of affected horses fail to respond), demand for other drugs and alternative (holistic/nutriceutical) treatments has been high. In response, researchers and veterinarians next tried medications termed coccidiostats that are fed to

various farm animals as growth promotants. Coccidiostats are drugs that inhibit growth or kill protozoal organisms (coccidia) that compete for nutrients (and slow growth) in animals raised for food production. **Diclazuril** is a widely used feed additive for growing chickens and was the second drug to be widely used for treatment of EPM. Its advantage is that it is given for only 28 days, but the disadvantages are that it is not available for use in the United States. Your veterinarian can legally import the drug from Canada for use on your horse with appropriate FDA approval. However, it is very unpalatable, necessitating daily administration by a stomach tube to some horses. The cost of the medication alone is about \$600 (not including any fees for stomach intubation). Due to these disadvantages, diclazuril has been formulated into a pellet that can be top dressed on grain. Research efforts into its efficacy in this form are underway. Additionally, this formulation is not yet commercially available.

Next, Bayer Corporation is expected to release a **ponazuril** paste later this year. Ponazuril is a metabolite of **toltrazuril (Baycox®)**, that is, it is in the same family of drugs as diclazuril. This paste medication is recommended to be administered daily for 28 days. The anticipated cost is \$450-500. Although some anecdotal reports have praised these coccidiostats, a limited drug trial performed by researchers at the University of Kentucky found a similar treatment response with diclazuril as with sulfadiazine/ pyrimethamine suspensions (remember, EPM is a **bad disease**). Since protozoa are an important component of the normal large intestinal fermentation process that allows horses to digest hay, use of coccidiostats is not without risk of side effects. These include gastrointestinal upsets displayed as inappetance, colic, or diarrhea.

Blue Ridge Pharmaceuticals is also expected to release **nitazoxanide (NTZ)** paste later this year. Nitazoxanide is a "broad spectrum" anthelmintic (anti-parasite drug) that has been used to combat intestinal parasites of humans in developing countries and in patients with AIDS complicated by secondary protozoal infections. Similar to diclazuril and toltrazuril, it will be administered as a daily oral paste for 28 days. Although the price has not yet been released, cost to owners of horses enrolled in their open field study was \$895. Side effects with this drug have been slightly more

serious than with other medications, leading to recommendations for deworming with another anthelmintic prior to starting this treatment. Further, the risk of intestinal upsets in the early stages of use of this drug prompted the dose to be decreased in half for the initial few days of treatment.

Some veterinary clinicians are currently using one of the coccidiostats in combination with sulfadiazine/pyrimethamine suspensions. Arguments can be made both in support and against such treatment. The bottom line is that we don't know what the best treatment really is. Further, we won't likely have much better information in the near future, because none of the drug studies have compared the responses of horses with EPM to the different medications or to the natural course of EPM in horses that are not treated. In addition to the specific anti-protozoal drugs, a number of adjunctive therapies have been used on horses afflicted with EPM. For example, the anti-oxidant vitamin E is widely recommended, because it could be helpful for essentially any neurological disease in horses. In addition, because protozoa can hide from the immune system, use of various immunostimulants has been attempted but there is no information demonstrating improved responses with use of these agents. A good rule to remember is that whenever there are multiple treatments for the same disease, none of the treatments are highly effective (otherwise everyone would use the same treatment).

Finally, whenever we are faced with a disease as debilitating and frustrating as EPM in which a substantial number of patients fail to respond to treatment, individuals will promote use of alternative therapies and claim miracle cures in chronically disabled individuals. Although most of us trained in western medical societies typically disregard such therapies, I will admit that I am becoming more open minded to such approaches. However, before alternative approaches are pursued, it is first important to establish an accurate diagnosis of EPM and to consider alternative therapies as an adjunct treatment rather than the sole treatment for EPM. Next, ask the individual recommending alternative therapies about his or her training and credentials. Reputable individuals should have licensure within that state and, if not a veterinarian, they should work closely with your veterinarian in the management of the case. Also,

use common sense when considering these treatment options. I have yet to understand how acupuncture or a chiropractic manipulation can kill a protozoa hiding in a nerve cell; however, they may be adjunctive tools in management of pain and inflammation. Use of so-called holistic medications (herbs and nutraceuticals) is less clear cut. When we remember that many approved drugs are plant extracts, it is clear that herbs may have medicinal properties that have simply been undocumented by scientific studies. However, one certainty is that herbs are less rigidly controlled than medications approved and monitored by the FDA. Several studies have documented that the chemical agents suspected to impart medicinal qualities to some herbs vary widely in their content between different batches and sources of herbs.

In many ways, deciding to treat a horse for suspected EPM is like deciding whether or not to get on a roller coaster, only the ride may last 6-12 months. The point is that you must be ready for the ups and downs and unpredictable turns (both financial and emotional) that the treatment course will take because it's difficult to get off the roller coaster once you've bought the ticket and headed up the first steep hill! Also, have a clear picture as to what outcome is acceptable for you. Many horses can recover yet may not return to their previous level of athletic performance. *Submitted by Harold Schott, D.V.M., Ph.D., Diplomate ACVIM, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI. □*

Comparison of *Sarcocystis neurona* Isolates from US Horses

Sarcocystis neurona is a protozoan parasite that can cause neurological problems in infected horses. Most *Sarcocystis* parasites require 2 hosts to live. We know opossums have the parasite in their intestines. However, the entire lifecycle of the parasite is not completely known. That is, we don't know the specific history of how the parasite multiplies and what other host it needs to survive. Horses become infected by accidentally eating sporocysts shed in the feces of infected opossums. We suspect that pasture and feed stuffs may be routinely contaminated when there are large numbers of opossums prowling around farms at night.

Also, until recently, we haven't known whether the *Sarcocystis neurona* parasite from one horse is the same as the *Sarcocystis neurona* from another horse. We are especially interested in knowing whether the parasite in horses varies in different areas of the US. If the parasite varied in different places, it might cause different types of disease problems, it might affect our ability to diagnose the disease, and it might affect how horses respond to treatment. In a recent study at Michigan State University, *Sarcocystis neurona* samples that were cultured from 8 separate Michigan horses after they died of the disease were compared to *Sarcocystis neurona* from a California horse and *Sarcocystis* from a grackle.

Comparisons were made using several techniques. These are techniques that examine the molecular structure (DNA) and protein makeup of the various parasites. One test to compare proteins (SDS-PAGE analysis with silver staining) showed that *Sarcocystis* from the 8 horses appeared the same, but different from the grackle isolate. Also, one Michigan horse isolate had two proteins that were more prominent than those of the isolate from the Californian horse and the isolates from other Michigan horses. Using the Western blot test as a second way to compare the proteins, we found that all of the isolates of *Sarcocystis neurona* from horses appeared the same. Most of the opossum isolates appeared the same as those from the horses. However, the isolate from the bird looked different on this test.

Also, we compared the DNA from all of the isolates using a molecular test (polymerase chain reaction, PCR). DNA from each of the 8 equine-derived isolates and the grackle-derived isolate produced a positive test in the PCR. However, a second molecular test (restriction fragment length polymorphism, RFLP analysis) showed results characteristic for *Sarcocystis neurona* for only the horse samples. The grackle isolate had a test result characteristic of a separate species of *Sarcocystis*, *Sarcocystis falcatula*.

Finally, we examined all of the parasite isolates directly using a very high-powered microscope (electron microscope). All of the horse isolates showed similar structural features, while the bird isolate was different.

We conclude from this study that the 8 Michigan horse isolates are *Sarcocystis neurona*

species that are similar to the one from the horse from California. Additionally, although there are some small differences between the *Sarcocystis neurona* isolates from the horses, they share many similarities. We were able to show that the parasite from the grackle is a second species called *Sarcocystis falcatula*. This work should help in developing better diagnostic tests and vaccines to fight this deadly parasite. Submitted by Linda S. Mansfield, M.S., V.M.D., Ph.D., Departments of Large Animal Clinical Sciences and Microbiology, College of Veterinary Medicine, Michigan State University, East Lansing, MI. □

Diagnosis of EPM in Horses

The Western blot test is still the best test for use in diagnosis of equine protozoal myeloencephalitis (EPM) in living horses. In 1998, an improved western blot test was developed and released by the Clinical Parasitology Laboratory of the Animal Health Diagnostic Laboratory at Michigan State University. This test detects the presence of antibodies reacting against the causative parasite, *Sarcocystis neurona*, in the blood or the cerebrospinal fluid (CSF) of infected horses. CSF is another name for the fluids that bathe the brain and spinal cord. Currently, veterinarians can make a diagnosis of EPM caused by the parasite *Sarcocystis neurona* when they find symptoms of disease in the horse, along with positive blood and CSF tests. However, many times only the blood test is done. It is important to realize that a positive blood test indicates that the horse was infected, but does not give any clues as to whether the infection is active or whether the horse has fought it off but still has antibodies in the blood stream.

Therefore, it is important to apply the Western blot test in the proper way. The ability to diagnose correctly is much greater if the horse that is tested has neurological disease. The Western blot test is less meaningful in a normal horse. For example, the Western blot test has been used to check horses for infection with *Sarcocystis neurona* at pre-purchase examinations. Serum samples of many normal horses test positive for *Sarcocystis neurona*. In fact, this may mean that these horses are resistant to the parasite. Further information is needed to understand what this result means in a normal horse. The same is true for normal horses that test positive for *Sarcocystis neurona* in cerebrospinal fluid. This may be a false positive

test result. It may mean that the horse was successfully treated, but antibodies against the parasite are still present. There is beginning evidence to suggest that horses may harbor the parasite for long periods of time with little disease and then have signs of disease later when they are stressed or when their immune systems are not functioning well. Other interpretations can also be made. More work is needed to understand the biology of the *Sarcocystis neurona* organism in the horse before we can interpret these test results accurately. For these reasons, we do not recommend testing of normal horses at this time. Please contact the Clinical Parasitology Section, Animal Health Diagnostic Laboratory at Michigan State University with your questions about diagnosis of EPM. *Submitted by Linda S. Mansfield, M.S., V.M.D., Ph.D., Departments of Large Animal Clinical Sciences and Microbiology, College of Veterinary Medicine, Michigan State University, East Lansing, MI.* □

Control of EPM in Horses

Should I Use the New EPM Vaccine?

Some veterinarians and parasitologists have serious concerns about the new EPM vaccine that will soon come out on the market for horse owners. Mainly, these concerns center on the safety and efficacy of the vaccine. Although 890 apparently normal horses were given the vaccine with only a low number of side effects, there was no testing done to see how a horse with a history of previous

EPM reacts to the vaccine. Therefore, we have no idea of how a horse with *Sarcocystis neurona* either with or without active disease will react to the vaccine. Will there be autoimmune disease? Will there be exacerbation of low-level disease? We don't know.

Additionally, no tests were done to determine whether the EPM vaccine would actually work to protect horses against the parasite. At this time, it is difficult to test the efficacy of vaccines for *Sarcocystis neurona* in horses. There are no test systems worked out that can provide a useful answer about the efficacy of a new vaccine. So once again, we do not know whether this vaccine will work. More research needs to be done to make this possible. We do expect that some people will decide to try the new vaccine in their horses. Unfortunately, unless they participate in the study organized to examine the effects of vaccinating horses with the EPM vaccine, we will not learn much from their experience. However, we will make every effort to disseminate information about any possible adverse effects or positive results regarding the new vaccine in subsequent issues of this newsletter.



EPM Newsletter

Clinical Parasitology Section
Animal Health Diagnostic Laboratory
College of Veterinary Medicine
A12A Veterinary Medical Center
Michigan State University
East Lansing, Michigan 48824-1314
Phone: 517-353-2296

For additional information regarding EPM in horses please contact members of the EPM Research Group at Michigan State University including Drs. Linda S. Mansfield, Harold Schott, Susan Ewart, Judy Marteniuk, John B. Kaneene, Mary Rossano, Christine Corn, John Shelle, Jon Patterson, Alice Murphy, Ruth Vrable, and Nichole Grosjean.