

## Neurologic Disease: Current Topics In-Depth (Last Updated: 21-Nov-2003)

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### 2. Equine Protozoal Myeloencephalitis

Seroprevalence data has been collected from horses in many areas of the United States, and the results demonstrated that, in many areas, 50% of horses have been exposed to *Sarcocystis neurona*, the primary agent that causes equine protozoal myeloencephalitis (EPM) [8-12]. Little work has been performed regarding the prevalence of antibody to *N. caninum/N. hughesi* in horses; however, recent work found a seroprevalence of 23.3% in sera examined from two horses in slaughterhouses in the United States and none in horses in slaughterhouses in Argentina or Brazil [10].

Based on a recent national study conducted by the United States Department of Agriculture (USDA), the average incidence of EPM was  $14 \pm 6$  cases/10,000 horses/yr with the lowest incidence in farm/ranch horses ( $1 \pm 1$  cases/10,000 horses/yr). The incidence in other horses, in increasing frequency, was pleasure horses ( $6 \pm 5$  cases/10,000 horses/yr), breeding horses ( $17 \pm 12$  cases/10,000 horses/yr), racing horses ( $38 \pm 16$  cases/10,000 horses/yr), and competition/show horses ( $51 \pm 39$  cases/10,000 horses/yr).

Early reports suggested that young and old horses had an increased disease risk. Other investigators have corroborated this increased risk in young horses. Historically, EPM has been reported as a sporadic disease; however, clustering of cases may occur when all the risk factors for EPM are present. Important risk factors for development of EPM are opossums seen on the farm, presence of woods on the farm, time of the year, and occurrence of a stressful health event, such as illness or trauma, before development of the clinical signs of EPM [13]. When compared with winter, the risk of EPM increases when ambient temperature increases, with the highest risk of clinical disease in the fall. The National Animal Health Monitoring System (NAHMS) study [13,14] found the same seasonal risk; the NAHMS study also found a decreased risk if a creek or river was present on the farm and if feed was kept protected from wildlife access. Additionally, the NAHMS study found an increased risk if opossums were observed on the premises and an even higher risk if the opossums were seen frequently [14]. Additional risk factors identified in the NAHMS study were an increased risk with increased numbers of horses, purchased versus home-grown grain, use of wood chips or shavings as bedding, presence of rats and mice on the premises, and increased human population density [14]. A decreased risk was seen when there were woods within 5 mi of the premises and where surface water was the primary drinking source [4-6,14]. These findings demonstrate that management may play a role in the development of clinical EPM.

Etiology and Life Cycle - *S. neurona*, unlike most *Sarcocystis* spp, may aberrantly infect a large number of intermediate hosts. This wide host range is similar to that of *T. gondii*, which is phylogenetically close to *S. neurona* [15,16]. Sarcocysts of *S. neurona* have not been found in affected horses; thus, the horse is an aberrant, dead-end host [17,18]. *Sarcocystis neurona* cycles between the opossum and various other intermediate host species that have sarcocysts in their muscles. Intermediate hosts include nine-banded armadillos (*Dasypus novemcinctus*), striped skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), and sea otters (*Enhydra lutris nereis*) [19-22]. A study in Missouri and another study in Ohio both suggest that the domestic cat is also a natural intermediate host [23,24].

The life cycle of *S. neurona* has been completed in a laboratory setting in the striped skunk [21]; however, previous reports of antibodies to *S. neurona* in striped skunks (22 of 37 skunks) would

suggest that they are likely to be a natural intermediate host as well [25]. Muscle from captured wild raccoons and armadillos killed along the road were fed to laboratory-raised opossums. This resulted in the shedding of sporocysts infective for ponies, horses, raccoons, and IFN- $\gamma$  KO mice [19,22]. High seroprevalence of *S. neurona* antibodies in armadillos (100%) tested from three states and raccoons (58.6%) tested from four states further suggests that these species are natural intermediate hosts [22,24]. The variety of species suggests that many other species may be potential intermediate hosts for this organism. Opossums shed sporocysts in their feces after ingestion of the infected muscle of the intermediate hosts.

The life cycle of *N. caninum* or *N. hughesi* in horses is poorly understood; however, the definitive host of *N. caninum* is likely the dog [26], although it is not known if the dog is the definitive host of *N. hughesi*. Tachyzoites have been found in some horse tissues, including tissue cysts in two of the horses reported to have EPM caused by *Neospora* [27]. One case of neosporosis in a foal was determined to be a congenital infection, which does not occur with infection by *S. neurona* [28].

Several studies have induced experimental infection in horses using *S. neurona* sporocysts. These studies have been conducted in Kentucky, Florida, and Ohio. In all studies, horses that were infected developed mild to moderate neurological deficits, but the investigators were not able to isolate the parasite from the nervous tissue in any of the studies. The most severe signs (mild to moderate) were seen in horses stressed by transport [29]. All three studies attempted to mimic stress using dexamethasone; however, the clinical signs were less severe and seemed to improve over time [29-31]. Additionally, in all studies, regardless of the dose of sporocysts administered, some horses demonstrated an improvement in clinical signs without treatment [7,27,29-31]. This suggests that horses are capable of clearing large numbers of these organisms and may partially explain the high number of clinically normal horses with parasite-specific antibodies in the (CSF). After orally inoculating horses with  $1 \times 10^8$  *S. neurona* sporocysts, clinical signs of neurologic deficits were readily detectable, but parasites were not found in the CNS at 7 or 14 days post-infection [a]. This is unlike the *S. neurona* infection in the natural intermediate host, the raccoon, where parasites were readily detectable in the CNS at 7 days post-infection [32]. The life cycle of *S. neurona* in the horse remains enigmatic.

The organism seems likely to be transmitted through methods other than direct contact with opossum feces based on the estimated numbers of opossums in North America, the poor survival of opossums, and the limited individual range. Experiments performed by researchers in the 1980s suggested that birds may help disseminate sporocysts [33]. Secondary or vector transmission also was demonstrated by the recovery of sporocysts in the feces of budgerigars, canaries, mice, and chickens fed opossum feces. Recovered sporocysts were then fed to budgerigars to assess viability after transit through the digestive tract of those species [34]. Four of six budgerigars died, suggesting that sporocysts disseminated in this way may be transmitted to intermediate hosts [34]. Control of EPM may be difficult because of the apparent wide range of natural and aberrant intermediate hosts of *S. neurona*.

Insects such as flies and cockroaches may also be transport vectors for *S. neurona*. Earlier work demonstrated that flies may act as transport vectors for *T. gondii* [35,36]. Fatal pulmonary disease developed in psittacine birds that were fed cockroaches after the cockroaches had been fed opossum feces, suggesting that insects may play a role in the transmission of *S. neurona*; however, further investigation is necessary.

The pathogenesis of EPM is poorly understood, but it is assumed that horses ingest *S. neurona* and the course of infection and disease is similar to other species infected with *Sarcocystis* spp. Sporocysts of *S. neurona* are passed in the feces of the opossum and introduced into the feed and water supplies of intermediate hosts [37]. On reaching the gastrointestinal tract, sporocysts excyst release eight sporozoites, which penetrate the gut and enter arterial endothelial cells in various organs [37]. Meronts develop within host cells, resulting in cell rupture and merozoites release into the blood stream. This may be followed by a second round of merogony in vascular endothelial cells throughout the body [37]. In the appropriate intermediate host, a final round of merogony results in the formation of sarcocysts in various muscles [37]. The predator or definitive host subsequently ingests the infected muscle tissue to complete the life cycle [37]. At the

present time, sarcocysts of *S. neurona* have not been found in affected horses, indicating that the horse is likely to be an aberrant, dead-end host [37].

Mares suspected to have EPM have produced many normal foals. The earliest EPM case reported occurred in a 2-mo-old foal [38]. Assuming transplacental transmission does not occur, the minimum incubation period may be 8 wk. However, a recent case suggests the incubation period may be much shorter. Serum and CSF collected 4 days after onset of clinical signs were both negative for antibodies to *S. neurona*. Serum and CSF collected 3.5 wk later were both positive. This indicates that the parasite was ingested and then caused the clinical signs in the 10 - 12 days required to produce a detectable antibody response.

*Sarcocystis neurona* has been recovered from CNS lesions in several horses and subsequently, propagated in culture in the laboratory. Cultured merozoites have not induced clinical disease in the horse when administered to horses parenterally or introduced through the epidural space [39,40]. The merozoite stage of *Sarcocystis* spp. is not known to be transmissible to other animals either [40]. However, nude mice have been inoculated intraperitoneally with cultured merozoites and subsequently, developed evidence of *S. neurona*-associated encephalitis [41]. These were immunosuppressed strains of mice, and intraperitoneal injection would not likely be the normal route of infection with *S. neurona* in horses. It seems that at least three species of *Sarcocystis* are excreted in opossum feces; therefore, use of IFN- $\gamma$  KO mice that develop encephalitis in response to *S. neurona* infections would help to differentiate the strain of *Sarcocystis* spp. present [40]. The mechanism by which the merozoites enter the CNS of horses is currently unknown. The organism is believed to enter the CNS through infected leukocytes or directly through the cytoplasm of endothelial cells [40].

Diagnosis - Immunoblot analysis of serum and CSF provides antemortem information regarding exposure to *S. neurona* [42]. Other types of immunoassays are confounded by cross-reactivity with *S. fayeri* or other organisms that share antigens with *S. neurona*. Immunoblot testing of CSF samples has demonstrated > 90% specificity and sensitivity among approximately 300 neurologic cases that received postmortem examination [43]. More recent studies have questioned the specificity of these tests in clinical cases, and further investigation is needed.

Differential Diagnosis - Differential diagnoses for EPM include all diseases affecting the CNS, although the most common differential diagnoses are CVM and equine degenerative myeloencephalopathy (EDM) [4,44,45]. Both affect young horses (1 - 3 yr of age), but CVM occurs more often in males [4,46,47]. Equine herpes virus type-1 (EHV-1) myeloencephalitis often has an acute onset after an episode of fever, cough, and nasal discharge or after one or more abortions on a farm. This condition often affects more than one horse on a farm. EHV-1 has a rapid onset and often results in severe hindlimb weakness and ataxia along with bladder dysfunction. Urine dribbling may occur. Ataxia and weakness usually are symmetric and may result in recumbency. Some affected horses dog-sit. Cranial nerve involvement may be observed, but it is not common [48-50].

Another differential for EPM is polyneuritis equi, which can occur both acutely and insidiously. It is more common in mature horses and usually starts with hyperesthesia progressing to anesthesia. There is progressive paralysis of the tail, rectum, bladder, and urethra, leading to urine dribbling. Rear limb ataxia with gluteal atrophy may be present. Asymmetric cranial nerve deficits with involvement of cranial nerves V, VII, and VIII have been reported in 50% of the cases [51]. Verminous myeloencephalitis also should be considered, because the signs are extremely variable depending on the parasite's migratory pathway. Diffuse or multifocal brain and spinal cord lesions have been reported. The onset is usually sudden with rapid deterioration and death. The incidence of this disease is very low, perhaps owing to more intense parasite control [52]. West Nile Virus (WNV), first reported in the United States in 1999, has become the number one differential for EPM. The number of reported equine cases has increased from 25 in 1999 to 60 in 2000 to greater than 550 cases in 2001 [53]. In 1999, most WNV infections in the horse were diagnosed as EPM before a definitive diagnosis of WNV was determined [53]. Asymmetric

neurologic deficits with profound weakness and ataxia commonly seen in WNV infections is difficult to differentiate from EPM without the aid of ancillary diagnostic testing.

**Treatment** - Treatment has included the combination of potentiated sulfonamides (**trimethoprim-sulfas**) and **pyrimethamine**. The success rate of treated cases seems to be > 60 - 75%, with no complaints of anemia or thrombocytopenia. Previous work using the 1 mg/kg dose of **pyrimethamine** once a day did not result in anemia, but that dose was only administered for 10 days [54-57]. We recommend **sulfadiazine** at a dose of 20 mg/kg, q 24 h (once a day), PO administered for at least 5 mo, but the treatment must sometimes be extended.

Triazine derivative drugs used to prevent coccidiosis in other species in other countries have been used to treat EPM. These drugs (**diclazuril** and **toltrazuril**) were originally designed for use as herbicides. The response to therapy in horses with EPM was slightly better than the response documented for the standard therapy based on horses that had previously been treated with the standard therapy. One advantage to the use of these compounds is an appreciably shorter duration of therapy, because most treatment regimens are approximately 30 - 60 days. Recently, a metabolite of **toltrazuril** called **Ponazuril** [b], has been approved by the Food and Drug Administration (FDA) for use in the treatment of EPM. **Ponazuril** is administered at the rate of 5 or 10 mg/kg for 28 days, although some veterinarians recommend its use for between 30 and 60 days.

The prognosis for horses diagnosed with EPM seems to be similar regardless of the treatment used, because most reports suggest an approximate improvement rate of 60 - 75% when using the standard therapy [11,58-60]. It has been suggested that < 25% of affected horses may return to their original function [60]. A recent study with **diclazuril** resulted in approximately 75% improvement among severely affected horses and approximately 30% (11 of 36 horses) returned to their original level of performance or actually improved beyond the level of performance before illness [61].

A growing concern, regardless of what medication is used, is the percentage of horses which have a relapse in clinical disease after cessation of therapy, because some horses will relapse days, weeks, or even months after cessation of therapy. The mechanism of relapse is unknown but may be caused by the recrudescence of a truly latent stage of the parasite, the presence of a small persistent focus of infection, or perhaps the re-exposure of the horse to the parasite [62]. Anecdotal estimates of the relapse rates after standard therapy range from 10% to 28% of treated horses [11,58-60]. The relapse rate reported using **diclazuril** for the treatment of EPM was < 5% [61]. Anti-inflammatory medications are recommended when acute onset results in dramatic and progressive clinical signs [11,58-60]. The use of **flunixin meglumine** or **phenylbutazone** may be helpful. The usual dose of **flunixin meglumine** is 1.1 mg/kg, q 12 h, parenterally. IV administration of medical grade **dimethyl sulfoxide** [c] at a dose of 1.0 ml/kg (approximately 1 g/kg) in a 10% solution is administered once daily for 3 days in a row, and although not uniformly recommended, some clinicians use **dexamethasone** parenterally in severely affected horses (0.05 mg/kg, q 24 h) or sometimes empirically at 50 mg, q 24 h. The exacerbation of signs in stressed patients and the reports of horses with EPM showing a worsening of signs after the use of these medications suggest immunosuppression should be avoided [45]. Ancillary treatments may include padded helmets, slings, good supportive care, and a deeply bedded stall.

Because of the suspicion that protozoal infections occur more commonly in immunocompromised patients, immunomodulators or other therapies that may have a non-specific enhancement of the immune system may be helpful. Studies have shown that **levamisole** is a non-specific immunomodulator that affects T cell-mediated immunity, including delayed-type hypersensitivity, which also increases the phagocytic activity of macrophages [63]. The use of immunomodulators may have merit, but further investigation is necessary. Although unlikely, it is possible that these drugs may also enhance the immunopathologic effects associated with CNS infection.

Prolonged therapy with antifolate medications should be monitored for signs of bone marrow suppression with resultant anemia, thrombocytopenia, or neutropenia [55,56]. The combination of **trimethoprim-sulfamethoxazole** and **pyrimethamine** also has an effect on reproductive function in

pony stallions [64]. It may induce changes in copulatory form and agility, and it may also alter the pattern and strength of ejaculation [64]. Therefore, caution should be used when treating stallions for a neurologic disease believed to be EPM.

Supplementation with **folic acid**, **folinic acid**, and/or brewer's yeast has been recommended for treatment of presumed **folic acid** deficiency in horses treated with the standard therapy, particularly pregnant mares [56,65]. However, **folic acid** supplementation has been discouraged by some investigators because of poor absorption and the potential for toxic effects on the bone marrow [11]. Toxicity has been reported in newborn foals born to mares that were treated for EPM. These mares had been treated with anti-folate medications and, concurrently, supplemented with **folic acid** [66]. Therefore, particularly in pregnant mares, **folic acid** supplementation should not be used until controlled clinical trials can be performed to corroborate or refute these findings.

Some clinicians recommend the use of additional supplements such as **vitamin E** (6000 - 8000 IU/day, orally) and **thiamine** that may facilitate healing of nervous tissue when treating horses with EPM [11,67]. However, clinical trials have not been performed to establish the efficacy of this supplementation.

Prevention - Because of the nature of the horse business, prevention of clinical cases of EPM will be difficult. Another complicating factor is the widespread distribution of the parasite throughout many parts of the United States. Although a vaccine is currently available, it remains unproven. In light of the difficulties experienced in the development of effective vaccines for other protozoan parasites, development of an efficacious vaccine for EPM will most likely be in the distant future [68,69]. Closely monitoring high-risk age groups such as young horses and old horses for evidence of neurologic disease may help detect EPM early. Additionally, EPM may be the cause of the clinical signs that horses present for neurologic disease in the warmer months. Because many major horse competitions take place in the fall of the year, monitoring of horses subsequent to transport and competition may be helpful. Wildlife, such as opossums, and pests, such as mice and rats, should be denied access to feed by using rodent-proof containers and by storage in enclosed facilities. Excluding birds from facilities may help prevent some cases of EPM. Case histories from affected horses indicate that the development of clinical signs often occurs after some other health event. Close monitoring of broodmares close to foaling and horses that develop a major illness or injury is important, because it may aid the early diagnosis of EPM cases.

In addition to designing a prevention plan that minimizes risk factors associated with the definitive host, it is also important to consider the intermediate host's role in EPM. Several species of mammals have been reported to act as natural or laboratory intermediate hosts in the life cycle of *S. neurona*, and these animals only represent a threat after death. Therefore, veterinarians should encourage horse owners to pick up dead cats, armadillos, skunks, and raccoons on their property and dispose of the carcass. This will prevent opossums from eating the dead animals and excreting more sporocysts. Carcass retrieval should be done carefully with an inverted plastic garbage bag or some other similar tool. Cleaning the horse's stall, barn, and environment may also be helpful. However, a recent report suggests that all common disinfectants used in veterinary hospitals do not kill the parasite. The only effective way to kill the parasite is through the use of heat (> 60°C for 1 min). Therefore, feeding heat-treated feed and steam cleaning the horse's environment may help to reduce some cases of EPM.