Synopsis:
A review of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM)

Introduction

Protozoa are known to be the hunters and mainly feed off of bacteria. Within protozoa are four subgroups: ciliates, the flagellates, the sarcodina, and the apicomplexans. The apicomplexans are obligatory intracellular parasites which means that they must spend nearly all of their lifecycle within the host animal. All apicomplexans are parasitic and lack contractile vacuoles and locomotor processes (Abdullah et al., 2013). Among many of the protozoan apicomplexan parasites is the *Sarcocystis neurona* (Fig. 1); it is the most damaging and quite often fatal, of the equine parasites. The genus is named for the terminal developmental stage (sarcocyst) found in the intermediate host. The sarcocyst is the only developmental stage that is infectious for the definitive host. Equine Protozoal Myeloencephalitis (EPM) is an equine neurological disease that is caused by the protozoan parasite *Sarcocystis neurona*. *Sarcocystis neurona* affects the central nervous system of the equine, causing irreversible damage. The opossums are the primary host (whereas skunks, 9-banded armadillos, and raccoons are intermediate hosts), and the sexual reproduction of *S. neurona* happens within the digestive track of the opossums. The sporocyst is then carried to either the dead end host, which is the equine, or the to the intermediate hosts which then resides inside the skeletal muscle (where most sarcocystis infections stop). While the equine grazes, it ingests the opossums' feces, the *S. neurona* then migrates to the neural tissues and replicate continuously via schizogony leading to inflammation and necrosis of the brain, brainstem, and spinal cord (Elsheikha, 2011). The *S. neurona* causes lesions along the spinal cord and brainstem. Lesions on the central nervous system are multifocal areas of segmental hemorrhage (Fig. 2). Microscopically, non-suppurative myeloencephalitis characterized by mononuclear perivascular cuffing with necrosis and loss of

neurons, with infiltration of monocyte, basophils, lymphocytes, and some eosinophils, with schizonts and merozoites in neurons, glial cells and leucocytes in observed (Elsheikha, 2011). These lesions are irreparable damage to the equine's central nervous system and if the EPM is left untreated, ultimately death occurs to the equine.

Clinical Signs

Diagnosing EPM via clinical signs is one of the hardest diagnosis there is. This is in part due to the varieties of possible clinical signs having multiple diagnosis (i.e. sore back can be either skeletal muscle related, kissing spine disease, EPM, etc.). Potentially at risk horses show signs of lameness, muscle atrophy, disciplinary problems, back problems, shaking of the head, and training issues. Examples of training/performance problems that are indirectly affected by EPM are holding their head excessively high, pelvic sway, asymmetric stride length, unwillingness to work on their hind end when turning them, frequent bucking, excessive back soreness and head tossing. About 80% to 90% of horses with EPM have progressive neurologic disease manifested as ataxia, weakness, and conscious proprioceptive deficits of one or more limbs, indicating spinal cord involvement (Robinson, 2013). The significance of the site of *S. neurona* within the central nervous system is determined by the severity of the lesions and inflammation. Lesions created by the parasite tend to be focal and multi-focal. Although there are significant signs to spinal cord damage, cranial nerve damage can show more clinical signs than spinal cord damage due to EPM because of several factors. Head tilting and facial paralysis are two clinical signs, and horses may also exhibit seizure activity, dementia, head-shaking, amaurosis (central blindness), or narcolepsy-like activity (Robinson, 2013). The clinical signs in the horse are a gradual progression which makes early detection very difficult. Without early detection, survival rate decreases dramatically. In a longitudinal retrospective study of 251

horses with EPM, Saville et al, reported a 55.4% survival rate. Approximately 90% of horses in the study were treated for EPM, and 65% of treated horses showed some improvement in clinical signs (Sellon et al, 2011). Improvement of the treated horses that had very mild neurological deficits had a survival rate of 92%, moderate neurological deficits had a 72% survival rate and severe neurological deficits had a survival rate of 55.6%.

Diagnosis

The most confirmed and direct diagnosing of EPM is done by a necropsy. Due to the neurological progression and the high abundance of the antibody to *S. neurona* in horses, it makes diagnosing EPM very difficult antemortem. The first part of diagnosing EPM in a horse is to do a set of field test. These test are generally done by a vet and their staff. The horse is designed to stand and walk on all four legs with enough intensity that if someone tried to pull or push the horse sideways, it will not stagger and hold its ground. That is not the case with horses who have EPM. The first field test is done with two individuals and the horse. The horse is lead to walk forward by a vet technician or the owner, while the vet walks along the hind end pulling the tail towards him/her. The EPM horse will stagger its hind end towards the pull of the tail, while the healthy horse will pull its hind end away from the vet (resistance). The other field test is done while the horse is at a standing rest. The horse stands on all four legs and the vet picks up one of the front legs and sets it across the other front leg. A healthy horse will automatically move its leg back to normal position while the EPM horse will stand with its front legs crossed.

After the field test is completed and it's confirmed that some neurologic problem is evident within the horse, the next and final step is to pull cerebrospinal fluid (CSF) from the horse, along with a comprehensive metabolic panel (CMP) and complete blood count (CBC). The problem with conducting normal blood work, alone, is that most of the time, the pulled

blood will not show neurological abnormalities because blood count, serum and urinalysis will all show normal readings. Even with conducting the CSF, CMP, and CBC, seropositivity toward *S. neurona* can show either a positive EPM or a positive to a horse that has been exposed to the organism. CSF provide a "support system" in diagnosing EPM but not a definitive answer due to the probability of blood contamination when the test done. The contamination of blood can cause a false positive so therefore, CSF samples of less than 50RBCs/µl are used for the immunoblot test. Recent diagnostic testing have indicated that the immunoblot test is the most specific with a specificity rate of 89%. Even with a specificity rate of 89%, immunoblot test are better tests to rule out EPM rather than diagnose EPM. Other diagnostic tests for EPM include polymerase chain reaction (PCR) testing, the albumin quotient test, and the IgG index test.

There are two types of PCR testing: real time PCR and conventional PCR. Real time PCR is more sensitive and specific than conventional PCR, in addition to it being more time efficient. It identifies the disease causing pathogen and identifies the antibodies for that pathogen, which allows proper biomedical treatment to be used. While real time PCR testing can detect minute amounts of protozoan DNA, the unfortunate part about using real time PCR is that it creates multiple false positives when testing for *S. neurona*. Therefore, the best use of real time PCR is for postmortem diagnostics on brain tissue because a concrete ante mortem diagnosis of EPM relies on the detection of antibodies against the *S. neurona* in the serum along with CSF using the quantitative serological immunofluorescent antibody test (IFAT).

The albumin quotient test (Q_{alb}) and IgG Index were designed to detect contamination of the CSF sample with blood. For several years the calculation of Q_{alb} and IgG Index were recommended tests to use to assist in the interpretation of the IBT (the IBT was developed to detect the presence of *S. neurona* –specific antibodies in serum and cerebrospinal fluid).

Unfortunately, even with the Q_{alb} testing normal, false positives can still be indicated with the blood in the immunoblot.

Problems with diagnostic testing lie within the accuracies of each test and the variability within each test. Variation in test results can be anywhere from imprecision of the test to the technical inaccuracy of the technician conducting it. Evaluating the reliability of each diagnostic test also can play a role in accurate diagnosis of EPM. Parallel analysis of samples using two different assays allows calculation of the test's sensitivity and specificity (Granstrom, 1993). Due to so many diagnosing inaccuracies of EPM in antemortem horses, most of the diagnostic tests conducted today are considered test to rule out other diseases and parasite rather than directly diagnosing EPM. Furthermore, outbreaks of EPM doesn't happen like influenza or roundworms and strongyles. A group of horses in a pasture together can all acquire strongyles but a group of horses in the same pasture don't all acquire *S. neurona* and diagnosed with EPM, only one horse does.

Treatment and Recovery

Due to the progressive and damaging nature of *S. neurona* to the horse, treatment must be nearly immediate after diagnosis. What damage has been done already to the horse is irreversible, so acting quickly is important to stop any further neurological damage. Treatment appears to result in successful recovery in 70–75% of the affected horses, although, without postmortem confirmation it is somewhat difficult to know the true meaning of this comment (Dubey et al, 2001). Originally, a 12 week treatment of 10cc/day of sulfonamides (Trimethoprim/Sulfas) and pyrimethamine (which are dihydrofolate reductase inihibitors) were used for years with the diagnosis of EPM. Using the combination of Trimethoprim/Sulfas with pyrimethamine causes a sequential blockade of folate metabolism within the apicomplexan

protozoa. In today's pharmacokinetic studies, the initial recommended sufficient dose is 1mg/kg (1cc) per day for 30 days with a follow-up second 30 day dosing. If the horse is treated with just the sulfonamides and omit the trimethoprim, the recommended dosage is 20cc's per day, twice a day. Intermittent treatment therapies have also been applied but not recommended. Intermittent treatment consists of treating the horse with the drug once every two to four weeks, or daily for the first week of every month for a few months after initial treatment. The reason that it isn't recommended is that by doing intermittent treatments the *S. neurona* can mutate and form a resistance to the drug. Recent problems with treatment is with long time use and causing anemia in the horse. Additionally, problems with the response to recent therapy has caused an increase of the dose of sulfa/pyrimethamine. The increase in dosage shown not to be responsive in the 30 and 60 day treatments.

Treating with the sulfa/pyrimethamine will fight the parasite but the damage from the parasite causes dramatic inflammations. Using anti-inflammatory medications is highly recommended to be given along with the other drugs. Equine anti-inflammatory medications are flunixin meglumine (banamine) and phenylbutazone (bute). Intravenous (IV) administration of dimethyl sulfoxide (DMSO) at a dose of 1cc/day for three consecutive days in a 10% solution also helps with limiting the inflammation caused by *S. neurona*.

The level of recovery from EPM is based upon how much damage *S. neurona* has done. The length of time the parasite lived within its definitive host (the horse) will be a direct reflect to how many, and how devastating the lesions to the spinal cord and brain are. Neurologically, if the horse has aggressive debilitating effects, the ethical "recovery" would be euthanasia. If the effects are minimal, the horse has a good prognosis of returning to the performance arena. Ultimately it comes down to the owner of the horse and how much they know their horse can and

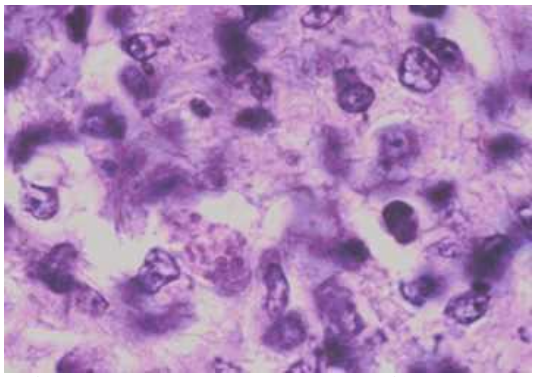
can no longer do. There are no published clinical studies of the rehabilitation of the EPM horse. So understanding your horse and understanding their new limitations due to the neurological damage from *S. neurona* is imperative to their recovery.

Prevention

Prevention strategies for EPM are more or less directed towards removing opossums' abilities to get into grains and horse feed. Preventions via vaccines are currently still being tested. A vaccine by Fort Dodge Animal Health has been developed and marketed but only for a conditional basis. The vaccine is a killed whole-parasite, and as a conditional licensed vaccine, it is proven to be safe, pure, and have a reasonable expectation of efficacy. The administration of this current vaccine results in an antibody response that is purported to inhibit parasite replication in vitro (Sellon, 2011). A study using 900 horses proved the safety of the compound but did not prove a complete resistance for acquiring EPM. Vaccinated horses still show a positive immunoblot for EPM. Simply put, the only prevention for EPM is monitoring where the horse grazes, ensuring excess grain spillage is removed where it could attract the intermediate hosts, and all tree fallen fruit is picked up. Grain storage areas should be sealed and away from hay storage areas. Opossums are omnivores, so they are highly attracted to grain and forage feeds, and not just the grains but the mice that also feed off of the grain. The sporocysts that come from the opossums are left where they were feeding, so ensuring tight security on grain tubes and containers are imperative to the prevention of EPM.

Figures

Figure 1. Microscopic image of *Sarcocystis neurona* within the spinal cord of an equine



Retrieved from http://instruction.cvhs.okstate.edu/jcfox/htdocs/clinpara/lst1_10.htm

Figure 2. Hemorrhagic lesions in the spinal cords of two horses with EPM. (A) The dura has been cut longitudinally and reflected away from the cord which shows presence of numerous multifocal hemorrhages. (B) Cross-sections of a spinal cord of a horse with EPM. Variable sized acute hemorrhages are present predominantly within the gray matter of the cord.

(Photos courtesy of Dr. A.N. Hamir, USDA, ARS, Ames, IA).

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